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# Oregon Agricultural College Experiment Station

Department of Dairy Husbandry

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## The Use of Pepsin as a Rennet Substitute in Cheddar Cheesemaking

BY

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CORVALLIS, OREGON

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## THE USE OF PEPSIN AS A RENNET SUBSTITUTE IN CHEDDAR CHEESEMAKING

In the late spring of 1916 the shortage of rennet extract for cheesemaking became acute. This was due to the cutting off by war conditions, of the supply of raw rennets from European sources. Prior to 1914, except for a negligible quantity, all rennets came from Europe,—a few from Denmark, but especially from the veal-eating sections of Austria-Hungary. As the shortage was more serious in the neutral nations contiguous to the warring powers than it had been in America, those nations passed strict embargo laws prohibiting the export of either raw rennets or extract. This had the effect of still further alarming the American cheesemaker, with resultant scouring for and cleaning up of all rennet extract stocks on the market. The small independent and cooperative factories were necessarily affected by such a condition more than the larger, better-managed plants and associations, and in a few instances those were forced temporarily to close their doors.

Rennet extract has in the past been employed exclusively as the milk coagulant in cheesemaking. Annually the United States uses for this purpose probably in excess of one hundred twenty thousand gallons. Since from twelve to thirty calf stomachs, depending upon their quality, are required for making one gallon of the extract, an enormous number of rennets must be secured from domestic sources if the shortage is to be entirely made up. The campaign for saving the stomachs of calves slaughtered in the United States, which was inaugurated by manufacturers of rennet extract, has met with a fair degree of success, one firm collecting during 1916 over 1,000,000 rennets. Nevertheless, with this supply, the normal output of the extract has been cut to about 80%. With cheese factories being built or enlarged to supply a prosperous export trade and increased home consumption, the demand for extract has increased and made absolutely necessary the use of a substitute in order to eke out the available supply and to leave enough for the manufacture of those foreign types of cheese for which a substitute cannot be satisfactorily used. The western maker has been further affected in securing an adequate supply by transportation difficulties and by the fact that having secured greater cooperation from eastern than western cheesemakers in collecting rennets, the manufacturer has felt obliged to supply the eastern trade first.

### History of the Use of Pepsin

As soon as the seriousness of the rennet shortage was realized, the use of pepsin, which had been tried out experimentally in cheesemaking in a limited way, was proposed. The substance had previously been used in comparatively small quantities for medicinal purposes in the treatment of digestive troubles and certain pathologic conditions of the stomach and throat.

Pepsin is the active ferment present in the gastric juice and is responsible for the changing of proteids into peptones during the process of digestion. It was first recognized as such and named in 1836 by

Schwann, and by Wasmann in 1840. It is present in the gastric juice of all mammals including man. Until the last few years it has been manufactured for commercial purposes from the stomach of the sheep, calf, or hog. The supply at the present time comes practically entirely from the stomach of the pig. It has never been satisfactorily isolated; its presence being known only by its effects. Evidence would indicate it to be of a proteid nature. The commercial product, known also as pepsin, is sold usually in the form of amber colored, translucent flakes or granules and of 1:3000 strength, of such concentration that one part of pepsin under standard conditions will digest 3000 parts of coagulated egg albumen. It has been isolated in as strong as 1:25000 strength, but the technique becomes so complex that commercially and for practical purposes the 1:3000 strength seems best. Like other digestive ferments, pepsin, which is a product of a living organism, has the property of bringing about certain changes without in itself being affected by the change. Warmth and a certain degree of acidity are required for its action.

Both being extracts of the digestive juices from an animal stomach, it is to be expected that pepsin and rennet would have similar properties. Van Slyke is inclined to the belief that each extract contains two, rather than one, enzyme, the one, *rennin*, which has the power to coagulate milk and the other, *pepsin*, which breaks down the proteids of milk and curd into simpler proteins. The fact that pepsin will not curdle sweet milk while rennet will, can then be easily explained by the relative amounts of *rennin* and *pepsin* each contains. This distinction between the two; namely, that commercial pepsin contains a greater amount of *pepsin* than *rennin* and that rennet extract contains more *rennin* than *pepsin* is the one now accepted.

It has also been held that both pepsin and rennet contained but the one enzyme which had the property of producing both the changes credited to *rennin* and *pepsin*. To prove this, Dr. Alfred Vivian, then of the University of Wisconsin, made several cheese, using commercial pepsin as the coagulating agent. The results were reported in the June 26, 1903, number of "Hoard's Dairyman." One vat was set using 1 1-7 ounces of scale pepsin for each 1000 pounds of milk; while a check vat was run using Hansen's rennet extract at the rate of three ounces for each 1000 pounds of milk. The vat set with pepsin coagulated in seven minutes, while 16 minutes was required with rennet. The pepsin curd acted similar to the rennet curd and the chemical compounds formed during ripening were the same as those formed in a high rennet cheese. Later the cheese developed the characteristic flavor of a high rennet cheese. Two judges scoring the cheese placed the pepsin cheese over the control sample. Vivian states that three ounces of a five percent solution is equal in coagulating power to the same amount of a standard rennet extract. At that time also pepsin was more expensive than rennet and variable in strength. While but few tests were run, he concluded that as good cheese can be made from pepsin as from rennet.

In 1904, Dean of the Ontario (Canada) Agricultural College conducted a series of experiments designed to compare pepsin with rennet as a milk coagulant. As a result he secured a better quality of cheese with pepsin, but suffered one-half pound lower yield for each 1000 pounds

of milk; and one-half pound greater loss in weight during the curing process. Pepsin was used at the rate of six and two-thirds ounces of a five percent solution. In the control sample three and one-third ounces of rennet extract was used. The flavor and total score on the rennet and pepsin cheese was 35.5 and 38.5, and 90.7 and 91.1 respectively,

#### The Use of Pepsin in Oregon

The shortage of rennet in this State did not become serious until June, 1916. In order to give proper directions for the use of pepsin in the face of impending rennet shortage this Station began in May of that year a series of experiments, preliminary report of which was made in June, 1916. These have been carried on continuously under practical factory conditions, in the College factory since that time. A majority of the brands of commercial pepsins have during that time been used, including those manufactured and sold by the Cudahy, Eli Lilly, Parke-Davis, Stearns, Jones, and Digestive Ferments companies.

In each case the pepsin was weighed and made up in 5% solution using lukewarm water, the temperature of which at no time was allowed to exceed 105° F. The solution of pepsin was made up the evening before the day of its use. Four ounces of either the 5% solution of pepsin or rennet extract was used as the standard amount of coagulant for each 1000 pounds of milk. Each was diluted before addition to the milk by thirty times its volume of cold water. The recorded results are of batches of milk of as near the same quality and run under as nearly the same conditions of manufacture as was possible. The milk was not divided, but one batch was set with rennet and the other with pepsin. As it is delivered daily, however, there should be very little difference in the quality of factory milk on successive days.

#### The Effect of Pepsin on Time of Coagulation, Time of Making, and Condition of Curd

Tables I and II give in detail the results secured from twelve representative batches of cheese using commercial pepsin and rennet extract as the coagulating agents. Pepsin was used at the rate of four ounces of a 5% solution in water (1.5 ounce of dry pepsin) to one thousand pounds of milk. Four ounces of rennet was used for each 1000 pounds of milk. The milk was set at practically the same degree of acidity as determined by the Mann's acid test and set and cooked at the same temperatures. From 1000 to 2000 pounds of milk was made up in each batch.

As will be noted from inspection of Tables I and II, the average time elapsing between addition of rennet and first signs of coagulation, when the milk tested .195 of 1% acid was eight minutes, while the time required for coagulation when pepsin in 5% solution was used on milk of the same percent acid averaged 9.75 minutes, or a 22% longer period. This would indicate that commercial pepsin contains less *rennin* than does rennet extract.

More time was also required for the pepsin curd to become sufficiently firm for cutting. Twenty percent longer time was required for the pepsin curd than the rennet curd to firm, the average time being 32.4

and 27 minutes respectively for each. This difference was noticed with all pepsin curds run. While the difference between the two curds is slight, due to the many factors which may cause changes in a rennet curd it is impossible for an expert to distinguish between a pepsin and a rennet curd with any degree of certainty. The feel, taste, and appearance are the same. A maker who is working each day with the two, however, will notice that a longer time is required for the pepsin curd to firm, that it tends to be softer and more spongy, that it is more brittle, and that it tends to break up more easily during the working process. This fact makes necessary its more careful handling during the time from cutting to drawing the whey, if losses of fat in whey are to be kept at a minimum.

There are two other points brought out by the table: (1) the time from cutting to dipping, or time required for firming the curd; and (2) the time required to complete the cheesemaking process. For the latter the time from setting to salting is more indicative of the actual time rather than the time from setting to hooping, because the time elapsing between salting and hooping is more or less variable, depending upon the convenience of the cheesemaker. Practically the same time was required to firm either type of curd, and very nearly the same to finish a batch of cheese, the average showing 21 minutes or 9% in favor of the pepsin curd. Table IV shows that on the average a pepsin curd will contain more moisture than a rennet curd. It would naturally follow that acid development in the curd from the time of drawing whey would be more rapid and the process hastened by this shortening of the period between dipping and milling of the curd. This effect on the complete time required for making is counterbalanced to a degree, in that rennet contracts the curd more rapidly than pepsin. This is indirectly brought out by the fact that approximately the same time is required to firm the curd, when pepsin is assisted in its action by a slightly higher cooking temperature. With these two factors tending to equalize each other, there is no particular advantage of rennet over pepsin so far as time is concerned.

Data were kept on the condition of the curd at drawing the whey, at milling, and at hooping. No abnormality was noted at any period in either of the two types.

TABLE I. EFFECT OF PEPSIN ON TIME OF COAGULATION, TIME OF MAKING AND CONDITION OF CURD

Conditions under which set										Time of making					Condition of Curd
Acid in Milk	Fat	Solids Not Fat	Strength Pepsin	Amount Pepsin	Setting Temp.	Cooking Temp.	Coagu-lation	Setting to Appln. Heat	Time to Cook- ing	Cutting to Dipping	Dipping to Milling	Setting to Salting	Setting to Hooping		
.195	...	...	5% solu.	4 oz.	86° F.	103° F.	10"	35"	27"	57"	1' 7"	1' 55"	3' 52"	4' 37"	Good
.195	...	...	5% solu.	4 oz.	86°	104°	10"	31"	22"	35"	1' 7"	1' 55"	3' 43"	4' 33"	Good
.195	...	...	5% solu.	4 oz.	86°	104°	9"	32"	22"	30"	1' 2"	2' 10"	4' 4"	4' 49"	Good
.195	4.4	8.68	5% solu.	4 oz.	86°	104°	9"	32"	22"	30"	1' 2"	1' 45"	3' 34"	4' 4"	Good
.195	4.7	8.69	5% solu.	4 oz.	86°	104°	10"	36"	20"	50"	1' 20"	1' 55"	4' 6"	4' 51"	Good
.195	4.5	8.7	5% solu.	4 oz.	86°	104°	10"	37"	20"	40"	1' 10"	2' 5"	4' 2"	4' 47"	Good
.195	4.4	8.78	5% solu.	4 oz.	86°	103°	10"	32"	20"	30"	1' 0"	1' 40"	3' 32"	4' 32"	Good
.195	4.3	8.74	5% solu.	4 oz.	86°	103°	10"	29"	20"	35"	1' 5"	2' 10"	3' 59"	4' 39"	Good
.195	4.4	8.88	5% solu.	4 oz.	86°	104°	11"	34"	26"	25"	1' 1"	1' 50"	3' 40"	4' 25"	Good
.195	4.4	8.76	5% solu.	4 oz.	86°	103°	10"	35"	25"	30"	1' 5"	1' 35"	3' 20"	4' 15"	Good
.195	4.3	8.81	5% solu.	4 oz.	86°	103°	10"	29"	20"	35"	1' 5"	2' 0"	3' 49"	5' 14"	Good
.195	4.2	...	5% solu.	4 oz.	86°	103°	8"	27"	20"	35"	1' 5"	1' 55"	3' 42"	4' 37"	Good
Average															
.195	4.4	8.75	5% solu.	4 oz.	86°	103.5°	9.75"	32.4"	22"	36"	1' 5.7"	1' 55"	3' 47"	4' 29"	Good

TABLE II. EFFECT OF RENNET EXTRACT ON TIME OF COAGULATION, TIME OF MAKING, AND CONDITION OF CURD

Conditions under which set										Time of making					Condition of Curd
Acid in Milk	Fat	Solids Not Fat	Rennet rate per 1000 lbs. Milk	Set-ting Temp.	Cook- ing Temp.	Setting to Coagu-lation	Setting to Appln. Heat	Time to Cook- ing	Cutting to Dipping	Dipping to Milling	Setting to Salting	Setting to Hooping			
.2	4.5	8.9	4 oz.	86°	103°	8"	23"	27"	30"	1' 7"	2' 55"	4' 45"	5' 25"	Good	
.2	4.5	8.65	4 oz.	86°	102°	8"	28"	34"	30"	1' 14"	2' 35"	4' 42"	5' 52"	Good	
.195	4.4	8.73	4 oz.	85°	103°	9"	27"	30"	40"	1' 25"	2' 5"	4' 22"	5' 2"	Good	
.2	4.5	8.9	4 oz.	86°	105°	8"	25"	20"	50"	1' 20"	2' 30"	4' 25"	5' 13"	Good	
.195	4.5	...	4 oz.	85°	105°	8"	30"	30"	30"	1' 10"	2' 5"	4' 5"	5' 10"	Good	
.2	4.6	8.82	4 oz.	86°	104°	8"	22"	25"	30"	1' 5"	2' 10"	4' 52"	5' 37"	Good	
.195	4.4	8.83	4 oz.	86°	103°	8"	25"	20"	40"	1' 10"	2' 5"	3' 55"	4' 55"	Good	
.195	4.4	8.88	4 oz.	86°	103°	8"	29"	23"	40"	1' 13"	2' 20"	4' 17"	5' 17"	Good	
.2	4.3	8.41	4 oz.	86°	103°	8"	28"	20"	35"	1' 33"	2' 0"	3' 53"	4' 43"	Good	
.195	4.5	...	4 oz.	86°	103°	8"	30"	20"	35"	1' 5"	1' 40"	3' 25"	4' 20"	Good	
.195	4.6	8.8	4 oz.	86°	100°	8"	30"	20"	48"	1' 18"	1' 15"	3' 18"	3' 58"	Good	
.195	4.6	8.97	4 oz.	86°	100°	8"	28"	15"	35"	1' 0"	1' 50"	3' 33"	4' 23"	Good	
Average															
.197	4.48	8.79	4 oz.	85.8°	102.8	8"	27"	23.7"	37"	1' 13"	2' 7"	4' 8"	5' 0"	Good	

### The Effect of Pepsin on Loss of Fat in Whey

A large loss of fat in the whey from the use of pepsin not only affects the profit and loss page of the ledger, but also the quality of the cheese in cases where the loss is excessive or where the butterfat accumulates in the mechanical openings of the cheese. Table III gives the percent fat in the whey from twenty-five vats of milk set with rennet and pepsin. The samples of whey for testing were taken from the whey drawn at the time of dipping and do not, therefore, include the richer drippings from the curd during the milling, salting, and pressing operations.

TABLE III. SHOWING EFFECT OF PEPSIN ON LOSS OF FAT IN WHEY

	Average												
Rennet	.21	.25	.24	.21	.27	.21	.25	.24	.25	.21	.20	.25	
Pepsin	.15	.17	.21	.23	.25	.21	.23	.23	.17	.20	.17	.20	.28
	.22	.24	.26	.20	.24	.27	.27	.27	.25	.27	.22	.23	.20
													.218
													.26
													.24

Despite the fact that the pepsin curd was handled more carefully than the rennet curd, there was a 10% greater loss of fat than in the case of the rennet curd. Since the finer any curd is cut or the more it is broken up, the greater is the loss of fat, such a condition is then to be expected. The fat globules are held in the curd by the coagulum. Wherever the curd is cut the fat globules on the cut surface are released, by the friction and movement during the stirring of the whey. While the release of butterfat into whey is inevitable, the quantity released can be lessened by agitating the whey in such manner as to cause a minimum breaking up of the curd cubes.

In a factory manufacturing 100,000 pounds of cheese yearly, assuming the above figures indicative of the fat losses with either coagulant used, in the average factory, there would be lost in a year from milk set with rennet 1684 pounds butterfat, and 1854 pounds, or 170 pounds more, if pepsin were used. Assuming the whey fat to be worth 40 cents a pound there would be \$68.00 worth more fat lost through the use of pepsin than rennet. In case a whey separator is used this loss can be cut down to a minimum. If it is assumed that the whey separator will skim to .01 of 1%, that whey fat is worth 40 cents a pound, and that milk fat is worth 12 cents more a pound than butterfat in whey cream, the loss of fat in a year's time will amount to \$20.40 more with pepsin than with rennet.

The figures given in Table III were taken from batches of milk handled carefully during the making process. Undoubtedly the losses in both instances, and especially with the pepsin curd, would be greater if the curd had been handled as roughly as it is in the average factory. Especial care was taken in uniformly and thoroughly mixing the pepsin solution with the milk and in so agitating the curd cubes until they became sufficiently firm as to prevent their being broken.

### Effect of Pepsin on the Moisture Content of Cheese

In Table IV is given the moisture content of twelve batches each of rennet- and pepsin-made cheese. All tests were made from the finished article ten days after paraffining.



TABLE IV. THE MOISTURE CONTENT OF RENNET AND PEPSIN CHEESE

% Moisture	Average																						
Rennet...	37.13	36.64	38.60	36.65	38.88	34.97	34.63	40.17	36.40	34.79	38.18	39.27	37.18										
Pepsin...	37.43	38.25	38.02	41.53	40.01	34.41	36.69	39.14	37.73	36.49	38.17	39.04	38.08										

Moisture control in cheese is far more complex than it is in butter, the cheesemaker being guided as to relative amounts through the feel and condition of the curd. In case butterfat has been removed, the cheesemaker will unconsciously endeavor to secure the soft velvety condition of curd—which is due to butterfat—through the incorporation of more water. Where loss of fat with pepsin is excessive as compared with rennet, other conditions remaining the same there will usually be slightly more moisture incorporated. This is probably accentuated by the longer time required to cook a pepsin curd, if equal temperatures are used, unless the maker uses care to secure a proper cook before drawing the whey. This is due to the greater force and tenacity with which rennet binds together the curd particles, and with which it contracts the curd during the cooking process. Since the contraction of curd during cooking is brought about by the combined action of rennet, heat, and acid in the milk, it can be hastened, where pepsin is used, by utilizing to a greater degree the last two named factors: that is, setting with slightly higher acidity in the milk and cooking at a slightly higher temperature. In cooking, however, care must be taken to use no higher temperature than is necessary to secure sufficient firmness when the acidity of the whey indicates it is ready to draw.

#### Effect of Pepsin on Yield of Cheese

This is an item of prime economic importance and is one of the first questions asked by the cheesemaker contemplating the use of pepsin. A slight variation in yield would be a factor which would require serious consideration.

TABLE V. SHOWING YIELDS FROM RENNET AND PEPSIN CURDS

	Rennet		Pepsin	
	Fat in milk	Yield per 100 lbs. milk	Fat in milk	Yield per 100 lbs. milk
	%		%	
	4.45	11.8	4.5	11.7
	4.3	12.2	4.3	12.6
	4.3	12.2	4.3	11.5
	4.5	12.5	4.5	12.2
	4.7	12.3	4.7	12.4
	4.7	12.9	4.7	12.8
	4.6	11.9	4.6	11.9
	4.5	11.6	4.5	11.6
	4.6	11.9	4.6	11.9
	4.6	11.3	4.6	11.3
	4.5	11.6	4.5	11.3
	4.5	11.3	4.5	11.5
	4.5	11.7	4.5	11.6
	4.6	11.9	4.6	11.6
	4.6	11.5	4.6	11.6
	4.4	11.6	4.4	11.2
	4.5	11.3	4.5	11.0
	4.7	11.8	4.7	11.6
	4.6	11.8	4.6	11.8
	4.4	12.2	4.4	11.6
	4.5	11.6	4.5	11.4
	4.5	11.8	4.5	11.6
	4.5	12.0	4.5	12.0
	4.5	12.2	4.5	12.0
	4.6	12.0	4.6	12.0
Average	4.55	11.87	4.55	11.74

Table V, giving the averages of 50 samples of milk, one half set with rennet, and the other half with pepsin, shows the yield to be slightly higher with rennet than with pepsin. This difference amounts to but .13 of one pound for each one hundred pounds of milk set, so that it is practically nothing. There is a tendency to incorporate more moisture in pepsin than in rennet cheese, but this tendency to increase yield, is cut to slightly less than that of rennet by the greater loss of fat. When used correctly there should be but very little difference between the yields of pepsin and rennet coagulated curd.

#### The Effect of Age on Pepsin

At the beginning of the experiment there was used a small sample of scale pepsin which had been held in the laboratory at ordinary room temperatures for at least four years. There was sufficient pepsin for running but two batches of milk. Table VI gives the average of these two samples as compared with two batches using fresh pepsin which were run under practically identical conditions.

TABLE VI. SHOWING VALUE OF OLD VS. FRESH PEPSIN IN CHEESEMAKING

Stage of Process	Old Pepsin	Fresh Pepsin
Acidity of milk when set	.20 of 1%	.197 of 1%
Cooking temperature	104.5° F.	103.5° F.
Setting to coagulation	7.5 min.	9.5 min.
Setting to cutting	27.5 min.	35.0 min.
Cutting to dipping	1' 17"	1' 5"
Setting to salting	4' 10"	3' 45"
Acid at dipping	.17 of 1%	.17 of 1%
Acid at salting	.47 of 1%	.50 of 1%
Score: Flavor	39.75	40.25
Total	90.75	90.50

The data in Table VI show conclusively that when kept in a dry place the dry form of pepsin will keep indefinitely without loss of strength. This keeping quality, in addition to its weight, is one of the chief advantages of pepsin.

There is at present no published data available on the keeping qualities of the liquid pepsins found on the market, other than that they will keep in good condition at least three months. There is apparently no reason, however, why the same preservatives that are used in rennet extract will not serve equally well in the preservation of pepsin.

#### Pepsin and Rennet Cheese Scores

The influence any coagulant will have on the flavor, body, and texture of cheese is most important, for upon these factors depend the market qualities of the product. Since the Oregon make is almost entirely of the home-trade type, and texture in that grade is not emphasized to the degree that it is with the export, the scores on flavor and body will be of most importance.

TABLE VII. SHOWING THE EFFECT OF PEPSIN ON SCORE OF CHEESE

Rennet Cheese Scores				Pepsin Cheese Scores			
Flavor	Body	Texture	Total	Flavor	Body	Texture	Total
39.	14.	12.5	90.5	41.	13.	13.5	92.5
41.	14.	14.	94.	40.5	12.75	13.5	91.75
38.	14.	11.5	88.5	38.	14.	12.	89.
41.	14.	13.5	93.5	41.5	12.5	12.75	92.25
39.5	12.5	14.	91.	41.	13.5	13.5	93.
41.	13.5	13.5	93.	40.	12.	13.	90.
41.	12.5	14.5	93.	40.25	13.	13.5	91.75
41.5	12.	12.5	91.	39.	13.	12.5	89.5
41.	10.5	13.	89.5	41.25	12.5	13.5	92.25
41.5	12.5	14.	93.	40.5	12.5	13.5	91.5
41.	13.5	12.5	92.	39.	13.	13.	90.
41.5	12.	13.25	91.75	39.	12.	13.	89.
40.	13.	13.25	91.25	39.	12.5	13.5	90.
39.5	12.5	13.5	90.5	41.5	13.5	13.5	93.5
40	11.5	13.5	90.	40.	12.75	13.	90.75
41.	12.	12.5	90.5	40.5	13.75	13.25	92.5
40.	13.25	12.	90.25	40.75	13.75	12.5	92.
41.25	13.	12.5	91.75	41.5	13.	14.	93.5
41.5	12.	12.5	91.	41.5	12.25	13.25	92.
40.	14.	14.5	93.5	40.	13.	12.	90.
41.	13.5	14.	93.5	40.	13.	13.5	91.5
39.5	14.	14.25	92.75	41.25	13.	13.5	92.75
36.5	13.75	14.5	89.75	39.5	12.	12.5	89.
39.	13.75	14.5	92.25	38.5	14.	12.75	90.25
40.	13.5	14.25	92.75	37.	13.5	13.5	89
Average							
40.25	12.99	13.38	91.62	40.08	12.95	13.12	91.17

The cheese scores recorded in Table VII were made by two members of the College Dairy department. The cheese were scored together so that in no case did the judges know the coagulant used. The scoring was done one month after date of make so as to note any effect of either coagulant on rate of curing. An inspection of the table will show that there is no difference between the scores of these cheese. There is a point, however, not shown by the score. There was more criticism of the pepsin than rennet cheese for crumbliness, although where this defect was not present the body was very good. Samples showing such fault were those stirred longer than normally in the whey.

The score on flavor shows that at least up to the time the cheese is a month old, pepsin neither hastens nor retards ripening.

#### Grades of Pepsin Used in Cheesemaking

Pepsin is sold on the market in powdered, granular, scale, and spongy forms. The composition and strength of the different forms are the same; the difference in form resulting from the way in which they are precipitated and mechanically separated from each other. The first three are secured in essentially the same manner. The pepsin extract is partially evaporated in vacuo to reduce its moisture content to the proper degree. When sufficiently concentrated it is poured while in a thick sirupy condition over glass plates and allowed to dry in a drying cupboard. When dry, it is scraped from these plates with a glazier's knife. The larger flakes or scales are sold as scale pepsin, the finely broken portion as granulated, and the remainder ground finely and sold as powdered pepsin. There is a slight objection by the trade, however, to these types, because when being scraped from the glass plate minute

splinters of glass are occasionally raised with the scales. This objection holds with both the granular and powdered types of pepsin.

Spongy pepsin is a type which is dried in such form that each particle resembles a miniature sponge. While made in the same strength as the other types, it has the decided advantage of presenting much greater surface area to the solvent when in the process of solution. This aids considerably in its speed of solution. With powdered pepsin there is a tendency for a sticky layer to form on the outside surface, which prevents the contact of the water with the powder on the inside. This stickiness causes the powder to agglutinate in lumps as soon as it is added to water, from which condition it is difficult to secure solution. Both the granular and scale types are more readily dissolved than the powdered, the spongy, however, being the best of the four in this respect.

The differences between the four types being physical only, it will be seen that weight for weight their strength is the same. Although the strength may be made to vary during the process of manufacture by adjusting the proportion of pepsin enzyme in the extract, that commonly sold on the market for cheesemaking and medicinal purposes is a 1:3000 strength as determined by the form of test described in the U. S. Pharmacopoeia. If a more concentrated pepsin than this is sold, its strength will be indicated on the label of the container.

#### The Cost of Rennet and Rennet Substitutes

The cost of any coagulant for 1000 pounds of milk is comparatively small, but in the aggregate an appreciable item. When pepsin was first placed on the market the difference between the cost of rennet and pepsin as coagulants was very marked. Pepsin, like rennet has now doubled in price so that the differences are not now so apparent. Table VIII gives some idea of the relative economy of five coagulants, the prices quoted being those holding at the present time.

TABLE VIII. SHOWING THE COST OF COAGULATING 1000 LBS. MILK WITH RENNET AND RENNET SUBSTITUTES

Coagulant	Price	Rate per 1000 lbs. milk	Will coag. lbs. milk	Cost per 1000 lbs. milk	Yearly Cost 100,000 lbs. Factory
Rennet Extract	\$7.50 per gal.	4 oz.	32,000	23.4c	\$212.73
Powdered Rennet	\$31.40 per lb.	½ oz.	34,000	91.2c	\$829.09
Spongy Pepsin	\$4.75 per lb.	¼ oz.	64,000	7.4c	\$ 67.27
Liquid Pepsin	\$4.15 per gal.	4 oz.	32,000	13.0c	\$118.18
Rennet Pepsin	\$5.50 per gal.	4 oz.	32,000	17.2c	\$156.36

As will be seen from the above the use of pepsin has decided advantages economically. Whether or not the extra care required in weighing and dissolving the pepsin, as well as in handling the curd, costs more than the saving, is a problem each factory manager must decide for himself. At present prices in the West, rennet is three times as expensive as pepsin. This is in part due to transportation costs, for both products are manufactured either in the East or Middle West. One pound of dry pepsin is sufficient to coagulate 64,000 pounds of milk, while it requires two gallons or eighteen pounds of rennet extract to coagulate that amount. This difference in cost would not be so great

in eastern factories, where transportation does not play so important a part in the cost of the product.

### Summary

1. It requires a slightly longer time to coagulate milk of .2 of 1% acidity with pepsin of 5% solution using four ounces per 1000 pounds of milk than with rennet used at the same rate. The time for the completion of the cheesemaking process is practically the same with either coagulant.

2. The loss of fat in the whey is greater with pepsin than with rennet, it being 10% greater under the conditions of manufacture described herein.

3. A pepsin-curd cheese will ordinarily be slightly more moist than a rennet-curd cheese. This variation will range from .5 of 1% to 2% and will average practically 1%.

4. The yields per 100 pounds of milk are nearly the same whether rennet or pepsin is used as the curdling agent. The yield was slightly in favor of the rennet-curd cheese.

5. When kept in a tightly stoppered bottle or in a dry place dry pepsin will maintain its strength indefinitely.

6. Pepsin has no effect on the flavor and body of cheese. The texture of a pepsin-curd cheese is somewhat inferior to that of a rennet-curd cheese.

7. The type of pepsin has no effect upon its strength or value in cheesemaking. Spongy pepsin is more desirable than other types because it is more rapidly soluble.

8. At present prices it is much cheaper to coagulate milk with pepsin than with rennet.

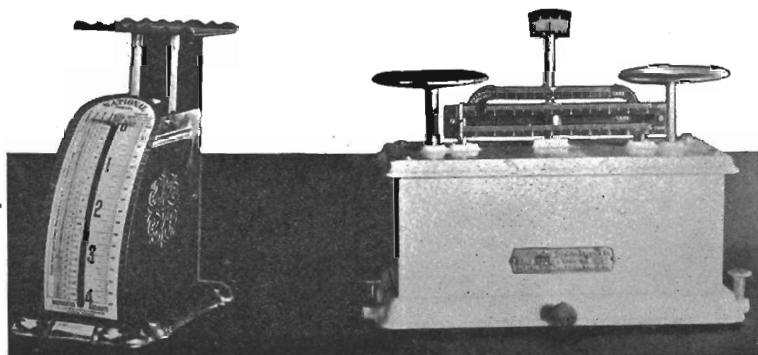
9. Pepsin is a satisfactory substitute for rennet in cheddar cheesemaking.

### Directions for the Use of Pepsin in the Cheese Factory

Where rennet is not obtainable or is excessive in price pepsin will serve as a satisfactory substitute. Whether the liquid or dry form shall be used will depend on the maker. Provided the dry pepsin is properly weighed, completely dissolved, and used in the correct proportions, it will give equally as good results as the liquid. It is cheaper than the liquid but has the disadvantage of having to be made up each day before being used. This objection is a serious one with some makers. There is the additional disadvantage that in the rush of the day's work the amount is sometimes hurriedly and incorrectly weighed.

In using dry pepsin the solution should be made the day before, or at least three or four hours before, it is to be used. Solution is greatly hastened if lukewarm water is used. The temperature, however, should not exceed 105° F., since a higher temperature tends to weaken the enzyme responsible for the curdling, while a much higher temperature will kill it. Dry pepsin should always be weighed, for a measured ounce only approximates an ounce in weight, and will vary considerably

with the form of pepsin used. There are several types of scales suited for the purpose. A postal scale is probably quickest, although it will not stand up very long under the hard usage given the average creamery scale.



TYPES OF BALANCES SUITABLE FOR WEIGHING PEPSIN

For factory work it is most convenient to use pepsin at the rate of one quarter ounce a thousand pounds of milk. If it is desired to use the solution at the same rate as rennet, one ounce of the dry pepsin is dissolved in 16 liquid ounces, or one pint of water. A clean bottle should be used for this purpose. In preparing the solution add the pepsin to the water, rather than water to the pepsin. If water is added to the weighted quantity of pepsin in the bottle there is a decided tendency for the latter to form a very thick, sticky mass, making its solution a tedious process.

The ability of pepsin to curdle milk depends, as is true with rennet, but to a much greater degree, upon the acidity of the milk. Rennet may be used to coagulate milk of low acidity, as in the making of Swiss or Brick cheese, although it requires a longer time for its action. Pepsin cannot be used for such purposes unless added in excessive quantities. It is desirable, therefore, to ripen milk to be set with pepsin to a slightly higher degree of acidity than if it were to be set with rennet. The ideal acidity for its use is .2 of 1%,—corresponding to two and one-half spaces on the average Marschall Rennet Test,—although by allowing a longer time for the coagulating and firming of the curd it may be set at as low as .19 of 1% acid. Where a lower degree of acid than .2 of 1% is allowed at the time of addition of coagulant, better coagulation is secured if the temperature is raised from 86° F. to 88° F.

Pepsin solution must be more carefully and thoroughly mixed with the milk than rennet extract. The solution after being diluted with cold water in the same proportions as rennet extract is ready to be added to the milk. A good method for its addition is to pour it slowly in the vat following the dipper or rake used to agitate the milk. It is good practice to time the mixing in of the solution, three minutes being used to secure its uniform distribution throughout the entire bulk of milk. After the solution is well mixed in, a cloth cover, in order to maintain a uniform temperature, should be placed over the vat. When the curd

is sufficiently firm, it is cut in exactly the same manner as a rennet curd. From cutting to dipping, however, and especially for the first fifteen minutes of cooking, it should be handled more carefully than a rennet curd. While a pepsin curd has apparently the same appearance, feel, and taste as an ordinary curd it does not stick together with the same tenacity as a good rennet curd. For this reason the curd cubes are more easily broken during cooking—a fact borne out by the greater fat losses in the whey. For the first ten or fifteen minutes it is best to stir the curd by hand or by means of some type of agitator which breaks the curd no more than necessary. It is during the first fifteen minutes that the curd cubes begin to harden and "heal over," so that if handled carefully, especially at this time, the fat loss in the whey may be held at a minimum. The remainder of the cheese-making process, as well as ripening is carried on in identically the same manner as with rennet.

#### Causes of Difficulties in the Use of Pepsin

Deviation from rules, or carelessness are responsible for many of the difficulties in the use of pepsin. Its disadvantage of having to be made up each day has led many careless makers to measure rather than weigh the amount to be used, with the result that an imperfect or too rapid coagulation is secured. Again the strength of the pepsin may have been weakened by dissolving it in alkali water; by too great dilution with water; by mixing it in a dipper containing soap, washing powder, or cheese color; or by exposure, either at the time of mixing or later, to excessive heat or light.

The condition of the milk affects the action of pepsin in the same manner that it affects the action of rennet, though in some cases to a greater degree. Such abnormal action may be brought about by the addition of such preservatives as salt, borax, or formalin to the milk; by watered, colostrum, or pasteurized milk; by the presence of abnormal bacterial ferments; by milk from cows late in the lactation period, or from sick or diseased animals. Milk which has stood in rusty cans or vats, because of the large amount of iron it will dissolve, or milk from cans containing washing powders or other alkaline substances, is difficult of coagulation.

A perfect coagulation may also be hindered by an incorrect temperature of the entire vat or parts of the vat. The former is often caused by the use of an incorrect thermometer and the latter by lack of thorough mixing of the milk before and during the addition of pepsin. The stirring in of pepsin for too long or too short a time, or shaking the vat or otherwise agitating the curd after it has begun to curdle will give an uneven and imperfect coagulation. Three minutes should be sufficient to secure a thorough and uniform admixture of pepsin solution to milk.

The most notable difference between pepsin and rennet is their action on sweet milk. To act best with pepsin the milk should show .2 of 1% acid as determined by the Mann's acid test, or two and one half spaces by the Marschall Rennet test. If set at much less than this degree of acidity the time of coagulation is greatly lengthened and the curd is often too soft for cutting and handling. Setting of the milk when of too light acidity may be due to an inaccurate neutralizer solution or a faulty Marschall Rennet test.