STUDIES WITH FAT-SPLITTING ENZYMES ON HOMOGENIZED AND UNHOMOGENIZED MILK

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Introduction

The interest of the milk distributors in soft-curd milk has grown tremendously in the past few years. This stimulated interest has largely been caused by the increased use of evaporated milk, which is a soft-curd milk, in infant feeding. It is the desire of the milk distributors to place on the market a fresh milk having digestion characteristics as suitable for infants as has the canned variety. Washburn (1), Wallace (2), and Weisberg (3) have reported that the curd tension of milk could be reduced by homogenization. Findings of these, and other workers, showing the possibility of producing soft-curd milk with the homogenizer is bringing homogenized milk to the attention of milk dealers and research workers.

It has been shown that homogenized milk has certain definite nutritional advantages in increasing the digestibility of milk. That the medical profession is alert to recognize the possibilities of soft-curd milk, produced by homogenization and other methods, is evidenced by the approval of "Soft-Curd Certified Milk" by the American Association of Medical Milk Commissions and its endorsement by physicians and pediatricians in many places. The Council on Foods of the American Medical Association in showing its approval of soft-curd milk published a report concerning the nutritional significance of the curd tension of milk (4).

The work that has been done on homogenized milk has been concerned chiefly with the nature of the curd formed when the milk is subjected to the action of the enzyme, pepsin, and with a measure of the rate of pro-

tein digestion when the homogenized milk is subjected to the action of proteolytic enzymes. No work has been reported on fat digestion. It is generally believed that homogenization is an aid to fat digestion because the total fat surface of the milk is increased tremendously.

The present investigation is planned to determine in-vitro the influence of homogenization on the extent of fat digestion when the milk is subjected to the action of various fat-splitting enzymes. Since homogenized milk is receiving considerable attention at the present time, it seems desirable that we should know the effect of the processing upon the relative digestibility of the fat.

Review of Literature

Doan and Welch (5) point out that the value of soft-curd milk lies in its superior digestibility. They found that milk with small soft curds is more quickly digested by the gastric juices. According to Doan (6) any explanation of the effect of homogenization on the curd tension of milk hinges, without question, on the increased dispersion of the fat which introduce more points of weakness in the coagulum. This is in line with Weisberg's theory (3). The increase in adsorbed protein may not participate in the coagulation in the same manner as it would in the motile state. It has also been noted that the curd from homogenized milk is more highly hydrated than from unhomogenized milk.

Doan and Welch (5) carried out an investigation in which they compared the digestibility of hard-curd milk with that of soft-curd milk. They used the enzyme, pepsin, as the digestive agent. The digestibility comparisons of the two milks were made at the following pH levels: 6.0, 5.0, 4.0, 3.0, 2.0. The data indicated that soft-curd milk would be more readily digested and eliminated from the stomach at any pH.

Espe and Dye (7), using dogs, found that milk of low curd tension was eliminated faster from the stomach than milk of double that tension. The same results were obtained with humans and calves. Doan and Welch (5) carried out some experiments in which they fed soft-curd and hard-curd milks to rats to determine the relative digestibility of the two milks. The data indicated that soft-curd milk forms a definite large curd lump in the digestive system of the rat but that it is softer and looser than that formed by hard-curd milk. Apparently in rats soft-curd milk is elimi-

nated more rapidly from the stomach and digests faster than hard-curd milk.

Doan and Welch (5) also carried on an investigation as to the comparative digestibility of hard-curd and soft-curd milks on humans. It was concluded from the study that the curds formed in the stomach after the ingestion of one pint of hard-curd milk are noticeably larger and tougher than those obtained with soft-curd when equal quantities of milk were consumed. There was little doubt that the amount of curd remaining in the stomach at definite intervals of time following ingestion, is less with soft-curd milk than with hard-curd milk, indicating that the former is passed through the pylorus and eliminated from the stomach more rapidly than the latter.

Dorner and Widmer (8) have shown that homogenization caused raw milk and cream to become distinctly rancid after a few hours. The development of rancidity increased as the size of the fat globules decreased. This rancidity was caused by a lipase apparently always present in raw cow's milk. Halloran and Trout (9) state that the titratable acidity of raw milk was always raised by homogenization. Pasteurization of the milk before homogenization prevented both the rise in acidity and the development of rancid flavor.

Doan (10) has shown that the fat-splitting enzyme, lipase, occurring naturally in cow's milk may be inactivated by heating to 148° F. by the flash method, or to 138° F. with a holding period of 15 minutes, or 132° F. with a holding period of 30 minutes.

Investigation

A. Object of the Investigation:

It is pointed out in a review of the literature that homogenization of cow's milk is an aid to protein digestion of that milk. The object of the present investigation is to determine what effect homogenization has upon fat digestion of cow's milk.

B. Milk and Its Treatment:

The milk used for this work was supplied by the Virginia Polytechnic Institute herd. The milk was obtained in a ten-quart tinned can as it came into the receiving room in the morning of the day on which digestion determinations were to be made.

The homogenizer used was a fifty-gallon per hour capacity unit furnished through the courtesy of the Cherry-Burrell Corporation. The machine was of stainless steel construction throughout the working parts.

Before homogenization, the milk was heated in a water bath to 138° F. and held at that temperature for 15 minutes to inactivate the lipase naturally present in the milk. A sample of the milk was taken at this point and held for the unhomogenized sample. The remainder of the milk (2 gallons) was poured into the supply tank of the homogenizer while still at a temperature of 138° F. and homogenized at 2500 pounds pressure. Samples were taken as the homogenized milk came from the delivery pipe. Thus homogenized and unhomogenized samples of the same batch of milk were obtained. If digestion determinations were to be made immediately, the samples were cooled to 98.6° F., but if as much as one hour elapsed before the digestions could be begun the samples were cooled to 45° F. and held at that temp-

erature until the digestions were begun.

C. Experimental Procedure:

In beginning the work a general procedure was followed in carrying out the fat digestion determinations. Certain difficulties were encountered, and in an attempt to overcome these difficulties the procedure was modified several times. The general procedure is given below, and deviations from this will be pointed out as the description of the work progresses.

The enzymes used were steapsin powder, prepared by the Pfanstiehl Chemical Company, Waukegan, Illinois; gastric lipase, prepared by The Armour Laboratories, Chicago, Illinois; and Ricinus lipase.

Ricinus lipase was prepared in the laboratory by following the procedure used by Longnecker and Haley (11). The lipase material was prepared from large size castor beans* by hulling and macerating them, extracting the fat as completely as possible with low boiling petroleum ether (20-40° C.) and finally drying, pulverizing, and sifting the finished product through a 50-mesh sieve.

General Procedure:

Digestion mixtures, each of which consisted of 1.6 milliliters of 5 % rennin solution and 3 milliliters of 0.2 % steapsin solution (or gastric lipase solution) were placed in separate rubber test tubes and tempered to 98.6° F. Into two of the digestion tubes containing the digestion mixture were pipetted 50 milliliters of homogenized milk that was tempered to 98.6° F., and into two other digestion tubes containing the digestion mixture were pipetted 50 milliliters of the same milk unhomogenized also tempered to 98.6° F. The samples were then placed into a 98.6° F. water bath for 10 minutes to allow

* Courtesy of Baker Castor Oil Company, Newark, New Jersey

for coagulation to take place. After coagulation had taken place, 2 milliliters of 0.2 % steapsin (or gastric lipase solution) were added. At this stage of the procedure one digestion tube containing homogenized milk and one containing unhomogenized milk were both heated to 160° F. for 5 minutes and cooled immediately. The heat treatment inactivated the enzyme in the control samples.

The heated and unheated samples were placed in a water bath at 98.6° F. for 2 hours. The water of the bath was agitated with a motor driven agitator. The waves of water against the side of the rubber test tube were desirable in our attempt to simulate peristalic action of the stomach and intestines.

The extent of digestion was measured by using a modification of the method used by Longnecker and Haley (11). The method used was as follows: after the 2-hour digestion period, the contents of the digestion tubes were placed in 30 milliliters of a hot alcohol-ether mixture (25 milliliters of 95 % alcohol and 5 milliliters of ethyl ether), and the free fatty acids in the digestion mixture were titrated with one-tenth normal sodium hydroxide using phenolphthalein as the indicator. The difference between the number of milliliters of one-tenth normal sodium hydroxide required for the control and that required for the digested sample indicated the extent of digestion. The measure of digestion is recorded in milliliters of one-tenth normal sodium hydroxide throughout this work.

In some cases the pH of the control and digested samples was determined by the glass electrode method. The lowering of the pH through digestion indicates the extent of digestion. This observation was made in order to compare the titratable acidity and the pH.

In beginning the experimental work, several digestion determinations were

made on unhomogenized milk to test the procedure. After this preliminary study, work was begun on homogenized milk. These preliminary determinations were made with the idea in mind of making such changes in the procedure followed as to give an ideal method to use throughout all the digestion determinations.

D. Presentation of Results:

The following data were obtained by experimental laboratory methods at the Virginia Polytechnic Institute. The data obtained are presented in table that follows. As nearly as possible, the explanation of the data of each experiment immediately precedes the presentation of that data on the data sheet.

Experiment I shows the extent to which the fat of unhomogenized milk was digested using the enzyme, gastric lipase, as the fat-splitting agent. Determinations were made at various pH levels, (a) pH 6.3, which is the pH of normal milk plus the digestion mixture; (b) pH 4.5; and (c) pH 2.3. In varying the pH levels an attempt was made to approximate the acid condition of the stomach during the process of digestion. Experimental determinations showed that the addition of 2 milliliters of normal hydrochloric acid would lower the pH of the milk plus digestion mixture to about pH 4.5, and that the addition of 4 milliliters of normal hydrochloric acid would lower the pH of the milk plus digestion mixture to about pH 2.3 Data in Experiment I show that there was some digestion, as measured by the increase in titratable acidity, in the pH 6.3 medium, but at the lower pH levels the extent of fat breakdown was markedly decreased and is in one instance, a negative value. In every case the pH was not lowered correspondingly to the increase in titratable acidity, but in general there was a relationship between the lowering of the pH and the increase in titratable acidity.

In using the enzyme, steapsin, Experiment II shows that there was more digestion at pH 6.3 than at the lower pH levels.

It was thought that by increasing the concentration of the enzyme from a 0.2 % solution to a 0.4 % solution the extent of digestion would be increas-

	.1 N NaOH required for titrating the digested and control samples	pH at the end of the digestion period	Extent of Digestion: Increase in titratable acidity .1 N NaOH	Extent of Digestion: pH lowering
	mls.		mls.	
Experiment I - Digestion of Unhomogenized Milk at Various pH Levels When a 0.2 % Gastric Lipase Solution Was Used.				
A. Unhomo. milk, rennin, gastric lipase (pH 6.3)				
a. Sample	25.0	6.05	5.5	0.25
Control	19.5	6.3		
b. Sample	21.1	6.1	9.3	0.32
Control	11.8	6.42		
B. Unhomo. milk, rennin, gastric lipase, 2 mls. N HCl (pH 4.5)				
a. Sample	26.9	5.0	-2.5	0.05
Control	29.4	5.05		
b. Sample	28.2	4.55	1.4	0.00
Control	26.8	4.55		
C. Unhomo. milk, rennin, gastric lipase, 4 mls. N HCl (pH 2.3)				
a. Sample	48.3	3.65	2.4	0.25
Control	45.9	3.9		
b. Sample	47.8	3.2	2.4	0.25
Control	45.4	3.45		
Experiment II - Digestion of Unhomogenized Milk at Various pH Levels When a 0.2 % Steapsin Solution Was Used.				
A. Unhomo. milk, rennin, steapsin (pH 6.3)				
a. Sample	20.0	6.1	9.5	0.4
Control	10.5	6.5		
B. Unhomo. milk, rennin, steapsin, 2 mls. N HCl (pH 4.5)				
a. Sample	26.6	4.55	1.1	-0.25
Control	25.5	4.3		
C. Unhomo. milk, rennin, steapsin, 4 mls. N HCl (pH 2.3)				
a. Sample	46.1	3.025	-0.3	1.525
Control	46.4	4.55		
Experiment III - Digestion of Unhomogenized Milk at Various pH Levels When a 0.4 % Gastric Lipase Solution Was Used.				
A. Unhomo. milk, rennin, gastric lipase (pH 6.3)				
a. Sample	34.0	5.9	17.6	0.5
Control	16.4	6.4		
b. Sample	34.5	5.9	17.8	0.5
Control	16.7	6.4		
c. Sample	31.7	5.9	15.3	0.5
Control	16.4	6.4		
d. Sample	31.8	6.35	11.0	-0.35?
Control	20.8	6.0		
B. Unhomo. milk, rennin, gastric lipase, 2 mls. N HCl (pH 4.5)				
a. Sample	35.7	5.05	4.5	-0.1
Control	31.2	4.95		
C. Unhomo. milk, rennin, gastric lipase, 4 mls. N HCl (pH 2.3)				
a. Sample	51.1	3.85	2.1	-0.1
Control	49.0	3.75		

ed considerably. Experiment III shows that the amount of digestion was markedly increased in the pH 6.3 medium when the increased concentration of gastric lipase solution was used. The samples digested in the lower pH media showed only slight digestion.

Because the enzymatic curds of the samples digested in the pH 6.3 media of Experiments I, II, and III were disintegrated at the end of the digestion period, it seemed a possibility that the enzyme used was contaminated with a proteolytic enzyme, possibly trypsin. From this observation it was thought advisable to determine what effect the gastric lipase solution or the steapsin solution would have on the curd tension of unhomogenized milk. It is known that trypsin will reduce the curd tension of milk. The curd tension measurements were made by following a modified procedure of that used by Flora. (12). The modification consisted of pipetting 1.6 milliliters of a 5 % rennin solution, and 10 milliliters of a 0.2 % gastric lipase solution or 10 milliliters of a 0.2 % steapsin solution into a beaker and tempering to 95° F. The milk samples were tempered to 95° F. and added to the beaker containing the rennin and enzyme solutions by means of a 100 milliliter pipette which was held vertically and centered over the beaker. The milk was blown from the pipette with the tip of the pipette above the surface. The beaker and its contents were allowed to set exactly 10 minutes in a 95° F. water bath. The control with no enzyme had 10 milliliters of distilled water added to keep the dilution constant. The curd tension measurements were made with the Submarine Signal curd tension apparatus. Experiment IV shows that the curd tension of the samples containing the gastric lipase solution was markedly lowered.

In making curd tension measurements, Flora (12) used a 0.45 % pepsin so-

lution as the coagulant. The medium in which the pepsin was dissolved was one-tenth normal hydrochloric acid. To make further observations on the effect of gastric lipase on the curd tension of unhomogenized milk, a 0.45 % pepsin solution made up with one-tenth normal hydrochloric acid was used on one sample, and a 0.45 % pepsin-0.8 % gastric lipase solution made up with one-tenth normal hydrochloric acid was used on the other sample. Also, the same enzyme solutions made up to the same concentrations, but using distilled water as the medium instead of one-tenth normal hydrochloric acid were used to determine the effect of gastric lipase on the curd tension of unhomogenized milk. Experiment V showed that gastric lipase did not lower the curd tension when the solvent of the enzymes was one-tenth normal hydrochloric acid, but when the solvent was distilled water gastric lipase caused a very marked curd tension reduction. This is evidence that the factor in the gastric lipase that was responsible for the curd tension reduction was not active when exposed to the acid medium.

In order to make still further studies on the effect of gastric lipase on the curd tension of unhomogenized milk, three different gastric lipase solutions were made up: (a) in a 1 pH medium, (b) in a 3 pH medium, and (c) in a 5.67 pH buffer medium. The solutions were allowed to set over-night, and the next morning the pH of each solution was adjusted to pH 7. Pepsin and rennin were both used as coagulants. It may be seen from Experiment VI that gastric lipase in these solutions causes no reduction in curd tension except in the case where the medium was the pH 5.67 buffer solution. This evidence tends to indicate that both the 1 pH and 3 pH media inactivated the factor present in the gastric lipase that was responsible for the reduction in curd tension, while this factor was not inactivated at a pH of 5.67.

experiment IV - The Effect of a 1.143 % Gastric Lipase Water Solution on the Curd Tension of Unhomogenized Milk.

A. Samples:

a. 100 mls. milk, 1.6 mls. rennin, 7 mls. water
b. 100 mls. milk, 1.6 mls. rennin, 7 mls. gastric lipase solution

c. 100 mls. milk, 1.6 mls. rennin, 7 mls. water
d. 100 mls. milk, 1.6 mls. rennin, 7 mls. gastric lipase solution

Experiment V - An Observation on the Effect of a 0.45 % Pepsin in .1 N HCl; also a Solution of 0.45 % Pepsin-0.8 % Gastric Lipase in .1 N HCl on the Curd Tension of Unhomogenized Milk.

Also, an Observation on the Effect of the Same Enzymes Made Up to the Same Concentration With Distilled Water on the Curd Tension of Unhomogenised Milk.

A. Enzymes made up with .1 N HCl:

a. 100 mls. milk, 10 mls. pepsin solution
b. 100 mls. milk, 10 mls. pepsin-lipase solution

c. 100 mls. milk, 10 mls. pepsin solution
d. 100 mls. milk, 10 mls. pepsin-lipase solution

e. 100 mls. milk, 10 mls. pepsin solution
f. 100 mls. milk, 10 mls. pepsin-lipase solution

B. Enzymes made up with distilled water:

a. 100 mls. milk, 10 mls. pepsin solution
b. 100 mls. milk, 10 mls. pepsin-lipase solution

c. 100 mls. milk, 10 mls. pepsin solution
d. 100 mls. milk, 10 mls. pepsin-lipase solution
e. 100 mls. milk, 10 mls. pepsin-lipase solution

Experiment VI - Three Different 0.4 % Gastric Lipase Solutions Were Made: (a) in 1 pH Medium, (b) in 3 pH Medium, (c) in 5.67 pH Medium. After Standing Over-night All Three Solutions Were Standardized at pH 7. The Effect of These Solutions on the Curd Tension of Unhomogenized Milk When Using Pepsin or Rennin as the Coagulant.

A. Samples:

a. 100 mls. milk, 10 mls. pepsin in distilled water
b. 100 mls. milk, 10 mls. pepsin in distilled water

c. 100 mls. milk, 10 mls. pepsin solution, 1 pH gastric lipase solution
d. 100 mls. milk, 10 mls. pepsin solution, 1 pH gastric lipase solution

e. 100 mls. milk, 10 mls. pepsin solution, 3 pH gastric lipase solution
f. 100 mls. milk, 10 mls. pepsin solution, 3 pH gastric lipase solution

.1 N NaOH required for titrating the digested and control samples	pH at the end of the digestion period	Extent of Digestion		Curd Tension
		Increase in titratable acidity .1 N NaOH	pH lowering	
mls.		mls.		gms.
				14
				4
				14
				4
				42
				44
				33
				33
				48
				63
				28
				4
				24
				4
				4
				13
				14
				13
				15
				13
				15

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Since the factor present in gastric lipase that was responsible for the reduction of the curd tension of unhomogenized milk seemed to be inactivated in the above described solutions, and since this factor was suspected of being trypsin, it was desirable to make fat digestion determinations on unhomogenized milk using these same enzyme solutions as the digestive agent. Experiment VII shows that digestion was very slight, indicating that possibly the activity of the fat-splitting enzyme had been reduced also. The activity of the enzyme solution made up in the buffer medium, pH 5.67, was greater than the activity of the other two enzyme solutions. It is to be remembered that this solution also lowered the curd tension of unhomogenized milk, indicating the possible presence of trypsin.

In order to study further the activity of gastric lipase when made up in solutions of different pH values, three 0.4 % gastric lipase solutions were made up in (a) 1 pH medium, (b) 3 pH medium, and (c) distilled water. After standing over-night the pH of each gastric lipase solution was adjusted to pH 6.3. The activity of these enzyme solutions on unhomogenized milk may be seen from Experiment VIII. There was some digestion, but the extent of digestion was not as great as it was expected to be. In this experiment three control determinations were made; however the one used in determining to what extent digestion had taken place was the same as had been used in all previous determinations; namely, the one that was heated to 160° F. for 5 minutes. Since it was thought that possibly this heat treatment was not sufficient to completely inactivate the enzyme, a control was carried out that was subjected to a temperature of 180° F. for 15 minutes. Still another control was carried out that had the 10 milliliters of gastric lipase solution replaced with 10 milliliters of water. These control determinations were made for study with the idea in

	.1 N NaOH required for titrating the digested and control samples	pH at the end of the digestion period	Extent of Digestion: Increase in titratable acidity .1 N NaOH	Extent of Digestion: pH lowering	Curd Tension
	mls.		mls.		gms.
g. 100 mls. milk, 10 mls. rennin in distilled water					17
h. 100 mls. milk, 10 mls. rennin in distilled water					17
i. 100 mls. milk, 10 mls. rennin solution, 1 pH lipase-pepsin solution					21
j. 100 mls. milk, 10 mls. rennin solution, 1 pH lipase solution					16
k. 100 mls. milk, 10 mls. rennin solution, 5.67 pH lipase-pepsin solution					8
l. 100 mls. milk, 10 mls. rennin solution, 5.67 pH lipase- pepsin solution					9
Experiment VII - Digestion of Unhomogenized Milk Using the Gastric Lipase Solutions Described in Experiment VI.					
A. Unhomo. milk, rennin, 1 pH gastric lipase solution					
a. Sample	9.7	6.6	1.2	0.0	
Control	8.5	6.6			
Control - no lipase, 10 mls. water	4.8	6.85			
B. Unhomo. milk, rennin, 3 pH gastric lipase solution					
a. Sample	11.1	6.5	1.3	0.1	
Control	9.8	6.6			
Control - no lipase, 10 mls. water	4.0	6.75			
C. Unhomo. milk, rennin, 5.67 pH gastric lipase solution					
a. Sample	33.6	6.025	12.1	0.325	
Control	21.5	6.35			
Control - no lipase, 10 mls. water	4.4	6.8			
Experiment VIII - Digestion of Unhomogenized Milk Using 0.4 % Gastric Lipase Solutions Made Up Differently: (a) in 1 pH Medium, (b) in 3 pH Medium, (c) in Distilled Water. After Standing Over-night the Solutions Were Standardized to pH 6.3.					
A. Unhomo. milk, rennin, 1 pH gastric lipase solution					
a. Sample	14.8	6.5	6.8	0.08	
Control - heated 160° F. for 5 min.	8.0	6.58			
Control - heated 180° F. for 15 min.	6.1	6.7			
Control - no lipase, 10 mls. water	4.3	6.75			
B. Unhomo. milk, rennin, 3 pH gastric lipase solution					
a. Sample	30.0	6.0	7.5	0.1	
Control - heated 160° F. for 5 min.	22.5	6.1			
Control - heated 180° F. for 15 min.	11.0	6.55			
Control - no lipase, 10 mls. water	5.5	6.58			
C. Unhomo. milk, no rennin, gastric lipase in distilled water solution					
a. Sample	27.0	5.9	8.4	0.3	
Control - heated 160° F. for 5 min.	18.6	6.2			
Control - heated 180° F. for 15 min.	8.6	6.55			
Control - no lipase, 10 mls. water	10.8	6.7			

mind of possibly changing the procedure followed on the control sample to one that would give a sample more nearly a true control.

To show conclusively that the gastric lipase, and steapsin being used were contaminated with some proteolytic enzyme, digestion determinations were carried out on skim milk. To measure the extent of protein digestion the Walker Test for casein was used (13). The test consisted of titrating the sample at the end of the digestion period with one-tenth normal sodium hydroxide to the end-point of phenolphthalein; then adding 10 milliliters of a 40 % solution of formaldehyde. The reading of the burette was now taken and the alkali solution was again added until the same degree of pink color appears as was obtained from the first titration. The reading of the burette was again taken, and the difference between the two readings measured the extent of protein digestion. In Experiment IX the samples under "A" were carried through the digestion procedure but with no enzyme added to them. It can be seen that digestion on these samples was negligible, while there was considerable protein break-down of all other samples that had either gastric lipase, or steapsin solutions added to them. It was concluded that a proteolytic enzyme was present.

The next step was to inactivate, if possible, the proteolytic enzyme without, at the same time, reducing the activity of the fat-splitting enzyme. Gastric lipase solutions were prepared (a) in a 1 pH medium, (b) in a 3 pH medium, and (c) in a 8.7 pH medium. The three solutions set over-night and were standardized to pH 6.3 the next morning. Protein digestion determinations were carried out on skim milk using these solutions. Experiment X-A and E show that when the digestion procedure was carried out with no enzyme, protein digestion was negligible, indicating that the digestion obtained when either gastric lipase or steapsin solutions were added was because of the action of the protein-

splitting enzyme. Under "B" and "F" of Experiment X which show the activity of gastric lipase and steapsin solutions respectively, when made up with 1 pH media, it may be seen that there was no digestion and even negative values were obtained. In each of these determinations there appeared at the end of the digestion period heavy curd particles on the bottom of the flask which, no doubt, accounts for the negative values. It may be seen that there had been protein digestion under "C" and "G" of Experiment X, when the activity of the gastric lipase and steapsin solutions respectively were made up with 3 pH media. Also, under "D" and "H" of Experiment X it can be seen that there had been digestion where the enzyme solutions were made up with 8.7 pH media. This experiment tends to show that the proteolytic enzyme was inactivated when allowed to set over-night in a 1 pH medium, but media of 3 pH and 8.7 pH did not inactivate it on standing over-night.

Experiment XI shows the activity of the enzyme solutions prepared in Experiment X after they had set at a temperature of 35° F. for 4 days. It is to be remembered that these solutions were standardized to pH 6.3 after standing over-night at the mentioned pH levels, and it was at pH 6.3 that they stood for 4 days. Protein digestion of skim milk was carried out, and also the fat digestion determinations were made on unhomogenized whole milk. It was observed that there was still no protein digestion of skim milk when the gastric lipase solution was used that had been prepared in a 1 pH medium. There was some digestion when gastric lipase solutions were used that were prepared in 3 pH and 8.7 pH media, but the activity of the enzymes had been reduced since there was not as much digestion as was obtained with these same enzyme solutions in Experiment X.

Since the proteolytic enzyme had been inactivated in the gastric lipase

	Walker's test for protein .1 N NaOH mls.	Extent of protein digestion .1 N NaOH mls.
Experiment IX - Digestion of Skim Milk Using 0.4 % Gastric Lipase Solution and 0.4 % Steapsin Solution.		
A. Skim milk, no rennin, no enzyme, 10 mls. water		
a. Sample	10.7	0.3
Control	10.4	
b. Sample	10.3	-0.1
Control	10.4	
c. Sample - 0.4 mls. N NaOH (pH 7.5)	10.4	0.0
Control	10.4	
B. Skim milk, no rennin, 10 mls. gastric lipase solution		
a. Sample	20.4	10.0
Control	10.4	
b. Sample	21.5	11.1
Control	10.4	
C. Skim milk, no rennin, 10 mls. steapsin solution		
a. Sample	19.5	9.1
Control	10.4	
b. Sample	19.0	8.6
Control	10.4	
c. Sample - 0.4 mls. N NaOH (pH 7.5)	21.0	10.6
Control	10.4	
Experiment X - Digestion of Skim Milk Using 0.4 % Gastric Lipase Solutions Prepared Differently: (a) in 1 pH Medium, (b) in 3 pH Medium, (c) in 8.7 pH Medium. Also Digestion of Skim Milk Using 0.4 % Steapsin Solutions Prepared Differently: (a) in 1 pH Medium, (b) in 3 pH Medium, (c) in 8.7 pH Medium. All These Enzyme Solutions Stood Over-night in the Media in Which They Were Prepared, and Then Standardized to pH 6.3.		
A. Skim milk, no rennin, no enzyme, 10 mls. water		
a. Sample	9.1	-0.4
b. Sample	9.5	0.0
c. Sample - 0.4 mls. N NaOH (pH 7.5)	8.4	-1.1
a, b, c. Control	9.5	
B. Skim milk, no rennin, 10 mls. 1 pH gastric lipase solution adjusted to pH 6.3		
a. Sample	6.5	-3.0
b. Sample	6.5	-3.0
c. Sample - 0.4 mls. N NaOH (pH 7.5)	9.4	-1.0
a, b, c. Control	9.5	
C. Skim milk, no rennin, 10 mls. 3 pH gastric lipase solution adjusted to pH 6.3		
a. Sample	20.0	10.5
b. Sample	20.0	10.5
c. Sample - 0.4 mls. N NaOH (pH 7.5)	18.5	9.0
a, b, c. Control	9.5	
D. Skim milk, no rennin, 10 mls. 8.7 pH gastric lipase solution adjusted to pH 6.3		
a. Sample	19.0	9.5
b. Sample	19.0	9.5
c. Sample - 0.4 mls. N NaOH (pH 7.5)	16.3	6.8
a, b, c. Control	9.5	

Continued on next page

	.1 N NaOH required for titrating the digested and control samples	Increase in titratable acidity	Walker's test for protein	Extent of protein digestion
		.1 N NaOH	.1 N NaOH	.1 N NaOH
	mls.	mls.	mls.	mls.
E. Skim milk, no rennin, no enzyme, 10 mls. water				
a. Sample			9.6	0.1
b. Sample			9.1	-0.4
c. Sample - 0.4 mls. N NaOH (pH 7.5)			9.7	0.2
a, b, c. Control			9.5	
F. Skim milk, no rennin, 10 mls. 1 pH steapsin solution adjusted to pH 6.3				
a. Sample			6.9	-2.6
b. Sample			10.1	0.6
a, b. Control			9.5	
G. Skim milk, no rennin, 10 mls. 3 pH steapsin solution adjusted to pH 6.3				
a. Sample			16.5	7.0
b. Sample			17.1	7.6
c. Sample			16.6	7.1
a, b, c. Control			9.5	
H. Skim milk, no rennin, 10 mls. 8.7 pH steapsin solution adjusted to pH 6.3				
a. Sample			17.4	7.9
b. Sample			16.6	7.1
c. Sample			17.6	8.1
a, b, c. Control			9.5	
Experiment XI - Digestion of Skim Milk and Unhomogenized Whole Milk Using the 0.4 % Gastric Lipase and 0.4 % Steapsin Solutions That Were Prepared in Experiment X. For This Experiment the Enzyme Solutions Were Allowed to Stand 4 Days at a Temperature of 35° F.				
A. Skim milk, no rennin, 10 mls. 1 pH gastric lipase solution adjusted to pH 6.3				
a. Sample			7.5	-3.0
b. Sample			8.1	-2.4
c. Sample - 0.4 mls. N NaOH (pH 7.5)			10.1	-0.4
a, b, c. Control			10.5	
B. Skim milk, no rennin, 10 mls. 3 pH gastric lipase solution adjusted to pH 6.3				
a. Sample			15.1	4.6
b. Sample			14.9	4.4
c. Sample - 0.4 mls. N NaOH (pH 7.5)			16.1	5.6
a, b, c. Control			10.5	
C. Skim milk, no rennin, 10 mls. 8.7 pH gastric lipase solution adjusted to pH 6.3				
a. Sample			15.0	4.5
b. Sample			15.8	5.3
c. Sample - 0.4 mls. N NaOH (pH 7.5)			15.2	4.7
a, b, c. Control			10.5	
D. Unhomo. whole milk, rennin, 10 mls. 1 pH gastric lipase solution adjusted to pH 6.3, 0.4 mls. N NaOH (pH 7.5). Control was heated to 180° F. for 15 minutes				
a. Sample	11.9	3.8		
Control	8.1			
b. Sample	12.9	5.3		
Control	7.6			

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and steapsin solutions that were prepared in 1 pH media, it was desirable to carry out fat digestion determinations on unhomogenized milk using these enzyme solutions. Any increase in titratable acidity would be the result of fat break-down alone rather than a combination of fat break-down and protein break-down. Some digestion was obtained as shown in "D" and "E" of Experiment XI, but the activity of the fat-splitting enzyme probably was reduced in the low pH media.

In Experiment XII gastric lipase and steapsin solutions were made up with 1 pH media. The solutions stood 24 hours and then the pH was adjusted to 6.3. The activity of these enzyme solutions was observed using unhomogenized milk as the substrate. Both enzyme solutions gave some digestion, but evidently the activity of the fat-splitting agent was reduced in the 1 pH media.

Experiment XIII was to show the activity of Ricinus (castor bean) lipase on homogenized and unhomogenized milk. The Ricinus lipase solution was made up with a pH 4.7 buffer solution because the optimum activity of this enzyme is pH 4.7 to pH 4.8 (11). Sufficient normal hydrochloric acid was added to lower the pH of the milk plus digestion mixture to pH 4.8. It was noticed that an acid curd developed at the lowered pH value, which made the measurement of the true increase in titratable acidity difficult. Samples were digested in which normal hydrochloric acid was added to lower the pH to 5.2 to avoid the formation of an acid curd. Also some of the digestion determinations were carried out without any rennin to observe if any appreciable difference in the extent of digestion would result. From the data obtained there was some indication that greater digestion was obtained without rennin; however the difference was so small that definite conclusions are drawn with reservation. The difference in extent of digestion between homogenized and unhomogenized milk was so small

	.1 N NaOH required for titrating the digested and control samples	pH at the end of the digestion period	Extent of Digestion: Increase in titratable acidity .1 N NaOH	Extent of Digestion: pH lowering
	mls.		mls.	
E. Unhomo. whole milk, rennin, 10 mls. 1 pH steapsin solution adjusted to pH 6.3, 0.4 mls. N NaOH (pH 7.5). Control was heated to 180° F. for 15 min.				
a. Sample	11.4		5.0	
Control	6.4			
b. Sample	12.7		5.9	
Control	6.8			
Experiment XII - Digestion of Unhomogenized Milk Using 0.4 % Gastric Lipase Solution That Was Made Up in a 1 pH Medium, Let Stand 24 Hours, and Adjusted to pH 6.3; and 0.4 % Steapsin Solution That Was Made Up in a 1 pH Medium, Let Stand 24 Hours, and Adjusted to pH 6.3.				
A. Unhomo. milk, rennin, gastric lipase solution; control was heated to 180° F. for 15 min.				
a. Sample	11.1		4.0	
Control	7.1			
b. Sample - 0.4 mls. N NaOH (pH 7.5)	9.5		3.9	
Control	5.6			
B. Unhomo milk, rennin, steapsin solution, control heated to 180° F. for 15 min.				
a. Sample	9.0		2.0	
Control	7.0			
b. Sample - 0.4 mls. N NaOH (pH 7.5)	9.4		3.9	
Control	5.5			
Experiment XIII - Digestion of Homogenized and Unhomogenized Milk Using 0.4 % Ricinus Lipase Made Up in pH 4.6 Buffer Solution.				
A. Homo. and unhomo. milk, rennin, Ricinus lipase solution; control was heated to 180° F. for 15 min				
a. Homo. sample - 1.8 mls. N HCl (pH 4.8)	18.0	4.88	0.7	-0.13
Control	17.3	4.75		
b. Unhomo. sample - 1.8 mls. N HCl (pH 4.8)	18.7	4.85	2.5	-0.05
Control	16.2	4.80		
c. Homo. sample - (pH 6.3)	7.7	6.15	0.3	-0.05
Control	7.4	6.10		
B. Homo and unhomo. milk, no rennin, Ricinus lipase; control was heated to 180° F. for 15 min.				
a. Homo. sample - 1.8 mls. N HCl (pH 4.8)	22.0		3.0	
Control	19.0			
b. Unhomo. sample - 1.8 mls. N HCl (pH 4.8)	23.8		1.9	
Control	21.9			
c. Homo. sample - 1.3 mls. N HCl (pH 5.2)	14.5		1.3	
Control	13.2			
d. Unhomo. sample - 1.3 mls. N HCl (pH 5.2)	13.9		0.6	
Control	13.3			
Experiment XIV - Digestion of Homogenized and Unhomogenized Milk Using 0.8 % Steapsin Solution.				
A. Homo. and Unhomo. milk, no rennin, 5 mls. steapsin solution				
a. Homo. sample - 1.3 mls. N HCl (pH 5.2)	24.1		5.6	
Control	18.5			
b. Unhomo. sample - 1.3 mls. N HCl (pH 5.2)	26.0		8.2	
Control	17.8			

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that no significance was attached to the finding. Even at the pH of 5.2 difficulties with curd formation were encountered.

From Experiment XIV, when an 0.8 % steapsin solution made with distilled water was used without rennin, there can be seen a definite trend toward greater digestion where the substrate was unhomogenized milk than when the substrate was homogenized milk. There was a wider difference in the extent of digestion between homogenized and unhomogenized milk when the digestion took place in a pH 6.3 medium.

In Experiment XV the amount of enzyme added was increased from 5 milliliters of an 0.8 % enzyme solution that was used in Experiment XIV and preceding experiments to 10 milliliters of an 0.8 % enzyme solution. The procedure followed with the control was changed in this experiment. Two drops of toluene were added to both the control and the digestion samples after the milk and digestive agents were added. The digestion sample was placed in the 98.6° F. water bath and the control was titrated immediately with one-tenth normal sodium hydroxide using phenolphthalein as the indicator. This titration value is the value recorded for the control. In this procedure the toluene prevents bacterial growth, and any increase in titratable acidity that results in the digestion sample is therefore a result of digestion.

In every digestion determination of Experiment XV, in which gastric lipase or steapsin solution was used, there was greater digestion of unhomogenized milk than of the same milk homogenized. When the Ricinus lipase solution was used digestion was extremely slight. It was thought that possibly the make-up of Ricinus lipase in a water solution was reducing its activity. To avoid the water solution, 100 milligrams of the enzyme were weighed into the digestion tubes. Results of Experiment XV-E show that no digestion took place; e-

	.1 N NaOH required for titrating the digested and control samples	Increase in titratable acidity .1 N NaOH
	mls.	mls.
c. Homo. sample - 1.3 mls. N HCl (pH 5.2)	29.4	8.5
Control	20.9	
d. Unhomo. sample - 1.3 mls. N HCl (pH 5.2)	29.6	9.1
Control	20.5	
e. Homo. sample - 1.3 mls. N HCl (pH 5.2)	29.8	8.1
Control	21.7	
f. Unhomo. sample - 1.3 mls. N HCl (pH 5.2)	27.3	2.0 ?
Control	25.3	
g. Homo. sample - (pH 6.3)	17.4	6.0
Control	11.4	
h. Unhomo. sample - (pH 6.3)	18.9	10.2
Control	8.7	
i. Homo. sample - (pH 6.3)	27.8	7.3
Control	20.5	
j. Unhomo. sample - (pH 6.3)	33.5	20.0
Control	13.5	
k. Homo. sample - (pH 6.3)	27.8	9.6
Control	18.2	
l. Unhomo. sample - (pH 6.3)	29.2	17.1
Control	12.1	
Experiment XV - Digestion of Homogenized and Unhomogenized Milk Using Steapsin, Ricinus Lipase, and Gastric Lipase Solutions.		
A. Homo. and unhomo. milk, 10 mls. 0.6% steapsin solution; control was heated to 180° F. for 15 min.		
a. Homo. sample	49.9	26.7
Control	23.2	
b. Unhomo. sample	54.3	39.8
Control	14.5	
B. Homo. and unhomo. milk, 10 mls. 0.8 % steapsin solution. 2 drops of toluene were added to both the digestion sample and the control, and the control was titrated immediately		
a. Homo. sample (pH 6.3)	49.8	37.3
Control	12.5	
b. Unhomo. sample (pH 6.3)	54.8	42.5
Control	12.3	
a. Homo. sample (pH 6.3)	44.3	18.7
Control	25.6	
b. Unhomo. sample (pH 6.3)	44.8	25.8
Control	19.0	
a. Homo. sample (pH 6.3)	37.3	13.3
Control	24.0	
b. Unhomo. sample (pH 6.3)	48.5	26.7
Control	21.8	

Continued on next page

	.1 N NaOH required for titrating the digested and control samples	Increase in titratable acidity .1 N NaOH
	mls.	mls.
a. Homo. sample - (pH 6.3)	42.0	18.8
Control	23.2	
b. Unhomo. sample - (pH 6.3)	49.1	32.3
Control	16.8	
a. Homo. sample - 1 ml. N NaOH (pH 9.6)	48.9	23.8
Control	25.1	
b. Unhomo. sample - 1 ml. N NaOH (pH 9.6)	46.7	37.2
Control	9.5	
a. Homo. sample - 1. ml. N NaOH (pH 9.6)	56.5	39.3
Control	17.2	
b. Unhomo. sample - 1 ml. N NaOH (pH 9.6)	54.6	40.1
Control	14.5	
C. Homo. and unhomo. milk, 10 mls. 0.8 % gastric lipase solution. Toluene was added to both the control and digestion sample and the control was titrated immediately		
a. Homo. sample - (pH 6.3)	47.0	18.0
Control	29.0	
b. Unhomo. sample - (pH 6.3)	58.0	33.0
Control	25.0	
D. Homo. and unhomo. milk, 10mls. 0.8 % Ricinus lipase solution. Toluene was added to both the digestion sample and the control and the control was titrated immediately		
a. Homo. sample - (pH 6.3)	10.6	-2.2
Control	12.8	
b. Unhomo. sample - (pH 6.3)	14.1	0.9
Control	13.2	
c. Homo. sample - 1 ml. N HCl (pH 5.5)	13.3	-5.0
Control	18.3	
d. Unhomo. sample - 1 ml. N HCl (pH 5.5)	13.9	-3.9
Control	17.8	
E. Homo. and unhomo. milk, 100 mgs. Ricinus lipase. Toluene was added to both the control and digestion sample and the control was titrated immediately		
a. Homo. sample - (pH 6.3)	10.5	-3.5
Control	14.0	
b. Unhomo. sample - (pH 6.3)	10.6	-3.4
Control	14.0	
c. Homo. sample - (pH 5.7) 0.8 mls. N HCl	11.5	-7.5
Control	19.0	
d. Unhomo. sample - (pH 5.7) 0.8 mls. N HCl	9.7	-4.0
Control	13.7	

ven negative values were obtained.

In carrying out Experiment XVI, a batch of milk was heated to 100° F., a part of the batch was held out for the unhomogenized batch, and the remainder was homogenized at 100° F. This temperature was not sufficient to inactivate the lipase naturally present in cow's milk. Digestion determinations were made on these homogenized and unhomogenized milk by pipetting 50 milliliters of each of the milks into a flask and digesting them for 4 hours at room temperature. It can be seen from Experiment XVI-1-A that homogenized milk increased in titratable acidity over the unhomogenized sample. Also, regular digestion determinations were made on the homogenized and unhomogenized milk using an 0.8 % steapsin solution as the digestive agent. The unhomogenized sample showed greater digestion.

Then in Experiment XVI-1-C the fat from the above described homogenized and unhomogenized milk was extracted using a modification of the Mojonnier fat extraction method (15). Seventy-five milliliters of milk were placed in a separatory flask. To this was added 7.5 milliliters of ammonium hydroxide, 50 milliliters of 95 % alcohol, 125 milliliters of ethyl ether, and 125 milliliters of petroleum ether with vigorous shaking after the addition of each reagent. The ether layer containing the fat was allowed to separate by gravity, and then was poured off into a fat extraction dish. The ether was evaporated, and the fat was dried in an oven at 100° C. under a 25 inch vacuum for 5 minutes, and then cooled in a dessicator.

In making the digestion determinations using the extracted fat as the substrate, 100 milligrams of the enzyme, which in this case was Ricinus lipase, were weighed into each of four 50 milliliter erlenmeyer flasks. Into each of two flasks was pipetted 1 milliliter of fat from homogenized milk, and into

each of the other two flasks was pipetted 1 milliliter of fat from the unhomogenized milk. Then 0.6 milliliters of acetic acid were pipetted into each of the 4 flasks, and the flasks were shaken vigorously for 3 minutes to emulsify the mixture. The 0.6 milliliters of acetic acid lowered the pH of the resulting mixture to pH 4.7, which is the optimum pH for the activity of Ricinus lipase. There was no significant difference in extent of digestion of the fat from the homogenized and unhomogenized milks. This is shown in Experiment XVI-1-C.

In contrast to the milk used in the digestion determinations of Experiment XVI-1, a batch of milk was heated to 143° F. for 30 minutes. A part of the batch was held out for the unhomogenized sample and the remainder was homogenized at 143° F. The heat treatment was sufficient to inactivate the lipase naturally present in the milk. Samples of this batch, homogenized and unhomogenized, were digested at room temperature for 4 hours with no digestive agents added. The results, as shown under Experiment XVI-2-A, reveal small but not significant difference in extent of digestion.

Also, regular digestion determinations were made on homogenized and unhomogenized milk using an 0.8 % steapsin solution as the digestive agent. The results given under Experiment XVI-2-B show that the unhomogenized sample gave the greater digestion.

The fat was extracted from the homogenized and unhomogenized milk, and digestion determinations were carried out following the same procedure described above, except 100 milligrams of steapsin were used in the place of the 100 milligrams of Ricinus lipase, and 0.6 milliliters of one-tenth normal sodium hydroxide were added in place of the 0.6 milliliters of one-tenth normal acetic acid. The results of Experiment XVI-2-C showed slightly more digestion of the fat extracted from unhomogenized.

Experiment XVI - D' ɜstion of ɑomogenized and Unhomogenized Milk; Also Digestion of Fat Extracted From the Same Homogenized and Unhomogenized Milk.

	.1 N NaOH required for titrating the digested and control samples	Increase in titratable acidity .1 N NaOH
	mls.	mls.
1. Milk was heated to 100° F. and homogenized.		
A. Homo. and unhomo. milk, no enzyme added, digested at room temperature 4 hours		
a. Homo. sample	21.1	5.7
Control	15.4	
b. Unhomo. sample	10.8	0.2
Control	10.6	
B. Homo. and unhomo. milk, 10 mls. 0.8 % steapsin solution		
a. Homo. sample	40.2	16.1
Control	24.1	
b. Unhomo. sample	49.3	32.3
Control	17.0	
C. Extracted fat (1 ml.) from homo. and unhomo. milk, 100 mgs. Ricinus lipase, 0.6 mls. acetic acid (pH 4.7)		
a. Homo. fat sample	13.7	12.1
Control	1.6	
b. Unhomo. fat sample	13.7	12.5
Control	1.2	
2. Milk was heated to 143° F. for 30 minutes and homogenized. 500 mls. of the milk were taken after the heat treatment for the unhomogenized sample, and the remainder was homogenized.		
A. Homo. and unhomo. milk, no enzyme added, digested at room temperature for 4 hours		
a. Homo. sample	10.8	0.4
Control	10.4	
b. Unhomo. sample	11.2	0.9
Control	10.3	
B. Homo. and unhomo. milk, 10 mls. 0.8 % steapsin solution		
a. Homo. sample	37.1	22.8
Control	14.3	
b. Unhomo. sample	47.7	33.9
Control	13.8	
C. Extracted fat (1 ml.) from homo. and unhomo. milk, 100 mgs. steapsin, 0.6 mls. .1 N NaOH		
a. Homo. fat sample	8.0	7.0
Control	1.0	
b. Unhomo. fat sample	11.3	10.3
Control	1.0	
3. To 3 liters of the heated milk in "2" were added 1½ grams steapsin. 500 mls. were held for the unhomogenized sample, and the remainder was homogenized.		
A. Homo. and unhomo. milk, no additional enzyme was added		
a. Homo. sample	30.2	13.0
Control	17.2	
b. Unhomo. sample	22.4	8.7
Control	13.7	

To 3 liters of the unhomogenized milk that was heated to 143° F. for 30 minutes and cooled to 98.6° F. were added 1½ grams of steapsin. The enzyme was stirred well into the milk. Five hundred milliliters of the milk-enzyme mixture were held for the unhomogenized sample and the remainder was homogenized at 2500 pounds pressure. Digestion determinations were made by placing 50 milliliters of the homogenized and 50 milliliters of the unhomogenized milk-enzyme mixtures in digestion tubes and digesting them with no additional enzyme in a 98.6° F. water bath. The results shown in Experiment XVI-3-A reveal that there was slightly more digestion with the homogenized milk-enzyme mixture than with the unhomogenized milk-enzyme mixture.

In Experiment XVII a batch of milk was heated to 138° F. for 15 minutes to inactivate the lipase naturally present in the milk. Four liters of this milk were cooled to 98.6° F. and 250 milligrams of gastric lipase were added and stirred well into the milk. Five hundred milliliters of the milk-enzyme mixture were held for the unhomogenized sample and the remainder was homogenized at 2500 pounds pressure. Digestion determinations were made on samples of these milk-enzyme mixtures by pipetting 50 milliliters of each into digestion tubes and digesting in a 98.6° F. water bath for 2 hours with no additional enzyme. It is seen from Experiment XVII-1-A that negative digestion values were obtained for these homogenized and unhomogenized milk-enzyme mixtures. Then 50 milliliters of the same milk-enzyme mixtures were placed in digestion tubes, and 10 milliliters of an 0.8 % steapsin solution were added. The tubes were then placed in a 98.6° F. water bath for 2 hours. Digestion was considerably greater on the unhomogenized sample than on the homogenized sample.

To 4 liters of milk previously heated to 138° F. for 15 minutes were added 250 milligrams of gastric lipase. The enzyme was stirred well into the milk,

	.1 N NaOH required for titrating the digested and control samples mls.	Increase in titratable acidity .1 N NaOH mls.
Experiment XVII - Digestion of Homogenized and Unhomogenized Milk Using Steapsin and Gastric Lipase. Toluene Was Added to Both the Control and Digestion Samples, and the Control Was Titrated Immediately.		
1. To 4 liters of milk were added 250 mgs. of gastric lipase. 500 mls. were held for the unhomogenized sample and the remainder was homogenized.		
A. Homo. and unhomo. milk, 10 mls. water, no additional enzyme added		
a. Homo. sample	11.5	-3.6
Control	15.1	
b. Unhomo. sample	6.7	-5.0
Control	11.7	
B. Homo. and unhomo. milk, 10 mls. 0.8 % gastric lipase solution		
a. Homo. sample	48.8	20.0
Control	28.8	
b. Unhomo. sample	58.8	34.5
Control	24.3	
2. To 4 liters of milk were added 250 mgs. steapsin. 500 mls. were held for the unhomogenized sample and the remainder was homogenized.		
A. Homo. and unhomo. milk, 10 mls. water, no additional enzyme added		
a. Homo. sample	11.3	-3.4
Control	14.7	
b. Unhomo. sample	11.2	0.8
Control	10.4	
B. Homo. and unhomo. milk, 10 mls. 0.8 % steapsin solution		
a. Homo. sample	44.8	20.0
Control	24.8	
b. Unhomo. sample	53.6	30.4
Control	23.2	
Experiment XVIII - Digestion of Homogenized and Unhomogenized Milk Using Ricinus Lipase. Toluene Was Added to Both the Control and Digestion Samples, and the Control Was Titrated Immediately.		
1. Plain homogenized and unhomogenized milk.		
A. Homo. and unhomo. milk, 10 mls. 0.8 % Ricinus lipase solution		
a. Homo. sample (pH 6.3)	12.3	0.2
Control	12.1	
b. Unhomo. sample (pH 6.3)	12.9	0.1
Control	12.8	
c. Homo. sample - 1.8 mls. N HCl (pH 4.8)	18.2	-5.4
Control	23.6	
d. Unhomo. sample - 1.8 mls. N HCl (pH 4.8)	17.9	-4.5
Control	22.4	
2. To 4 liters of milk were added 500 mgs. Ricinus lipase. 500 mls. of the mixture were held out for the unhomogenized sample and the remainder was homogenized.		

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and 500 milliliters of the milk-enzyme mixture were held for the unhomogenized sample and the remainder was homogenized at 2500 pounds pressure. Digestion determinations were made on samples of these milk-enzyme mixtures by pipetting 50 milliliters of each into digestion tubes and digesting in a 98.6° F. water bath for 2 hours with no additional enzyme. From Experiment XVII-2-A it may be seen that there was no significant difference in the extent of digestion between the two milks. Then 50 milliliters of the same milk-enzyme mixtures were placed in digestion tubes, and 10 milliliters of an 0.8 % gastric lipase solution were added. The tubes were then placed in a 98.6° F. water bath for 2 hours. Digestion was considerably greater in the unhomogenized sample than in the homogenized sample.

In Experiment XVIII-1 the regular digestion procedure was followed using an 0.8 % Ricinus lipase solution on homogenized and unhomogenized milk samples. In no case was there a definite trend in any direction.

Then to 4 liters of milk previously heated to 138° F. for 15 minutes and cooled to 98.6° F. were added 500 milligrams of Ricinus lipase. Five hundred milliliters of the milk-enzyme mixture were held for the unhomogenized sample, and the remainder was homogenized at 2500 pounds pressure. Digestion determinations were made on samples of these milk-enzyme mixtures by pipetting 50 milliliters of each into digestion tubes and digesting in a 98.6° F. water bath for 2 hours with no additional enzyme. It may be seen from Experiment XVIII-2-A that no significant difference in extent of digestion was revealed. Then 50 milliliters of these same milk-enzyme mixtures were placed in digestion tubes, and 10 milliliters of an 0.8 % Ricinus lipase solution were added. The tubes were then placed in a 98.6° F. water bath for 2 hours. No difference in extent of digestion could be observed in the two milks.

To 4 liters of milk previously heated to 138° F. for 15 minutes were added 100 milligrams of steapsin. The enzyme was stirred well into the milk, and 500 milliliters of the milk-enzyme mixture were held for the unhomogenized sample and the remainder was homogenized. Digestion determinations were made on samples of these milk-enzyme mixtures by pipetting 50 milliliters of each into digestion tubes and digesting in a 98.6° F. water bath with no additional enzyme. The extent of digestion of the two milks was determined after a 1 hour digestion period and again after a 2 hour digestion period. Experiment XIX-1A and B shows that there was digestion of the homogenized sample, but not of the unhomogenized sample.

Then to 200 milliliters of plain homogenized milk previously heated to 138° F. for 15 minutes were added 5 milligrams of steapsin which was the same ratio of enzyme to milk as the 100 milligrams to 4 liters of milk described above. Also 5 milligrams of steapsin were added to the same milk unhomogenized. Fifty milliliters of each of these milks were pipetted into digestion tubes and digested in a 98.6° F. water bath for 2 hours. Experiment XIX-2A shows a slightly greater digestion in the homogenized milk than in the unhomogenized milk.

In Experiment XX, as in Experiment XVII-2, 250 milligrams of steapsin were added to 4 liters of milk previously heated to 136° F. for 15 minutes. After stirring the enzyme well into the milk, 500 milliliters of the mixture were held for the unhomogenized sample and the remainder was homogenized at 2500 pounds pressure. Digestion determinations were carried out by pipetting 50 milliliters of the milk into separate digestion tubes and digesting in a 98.6° F. water bath. Digestion periods were for 1 hour, 2 hours, and 4½ hours. Results as shown in Experiment XX-1-A, B, and C were not such that any significance could be attached. Then, part of the same milk, before any enzyme was added, was

	.1 N NaOH required for titrating the digested and control samples	Increase in titratable acidity .1 N NaOH
A. Homo. and unhomo. milk, 10 mls. water, no additional enzyme added	mls.	mls.
a. Homo. sample (pH 6.3)	12.8	0.4
Control	12.4	
b. Unhomo. sample (pH 6.3)	12.4	0.0
Control	12.4	
c. Homo. sample - 1.8 mls. N HCl (pH 4.8)	17.8	-4.2
Control	22.0	
d. Unhomo. sample - 1.8 mls. N HCl (pH 4.8)	20.0	-1.4
Control	21.4	
B. Homo. and unhomo. milk, 10 mls. 0.8 % Ricinus lipase solution		
a. Homo. sample (pH 6.3)	12.0	-0.4
Control	12.4	
b. Unhomo. sample (pH 6.3)	10.9	-1.7
Control	12.6	
c. Homo. sample - 1.8 mls. N HCl (pH 4.8)	16.4	-5.1
Control	21.5	
d. Unhomo. sample - 1.8 mls. N HCl (pH 4.8)	18.4	-5.8
Control	24.2	
Experiment XIX - Digestion of Homogenized and Unhomogenized Milk Using Steapsin. Toluene Was Added to Both the Control and Digestion Samples, and the Control Was Titrated Immediately.		
1. To 4 liters of milk were added 100 mgs. steapsin. 500 mls. of the mixture were held out for the unhomogenized sample and the remainder was homogenized.		
A. Homo. and unhomo. milk, no additional enzyme added; 1 hour digestion period		
a. Homo. sample	15.5	1.0
Control	14.5	
b. Unhomo. sample	14.0	0.0
Control	14.0	
B. Homo. and unhomo. milk, no additional enzyme added; 2 hour digestion period		
a. Homo. sample	16.0	1.5
Control	14.5	
b. Unhomo. sample	14.0	0.0
Control	14.0	
2. To 200 mls. of homogenized milk were added 5 mgs. of steapsin which was the same ratio of enzyme to milk as the 100 mgs. of steapsin to 4 liters of milk in "1" above. Also 5 mgs. of steapsin were added to 200 mls. of unhomogenized milk.		
A. Homo. and unhomo. milk, no additional enzyme added; 2 hour digestion period		
a. Homo. sample	17.0	3.3
Control	13.7	
b. Unhomo. sample	15.5	1.5
Control	14.0	

Experiment XX - Digestion of Homogenized and Unhomogenized Milk Using Steapsin. Toluene Was Added to Both the Control and Digestion Samples, and the Control Was Titrated Immediately.

	.1 N NaOH required for titrating the digested and control samples mls.	Increase in titratable acidity .1 N NaOH mls.
1. To 4 liters of milk were added 250 mgs. of steapsin. 500 mls. of the mixture were held out for the unhomogenized sample and the reaminder was homogenized.		
A. Homo. and unhomo. milk, no additional enzyme; 1 hour digestion period		
a. Homo. sample	16.0	0.8
Control	15.2	
b. Unhomo. sample	14.2	0.0
Control	14.2	
B. Homo. and unhomo. milk, no additional enzyme; 2 hours digestion period		
a. Homo. sample	13.7	-1.5
Control	15.2	
b. Unhomo. sample	13.8	-0.4
Control	14.2	
C. Homo. and unhomo. milk, no additional enzyme; 4½ hours digestion period		
a. Homo. sample	14.2	-1.0
Control	15.2	
b. Unhomo. sample	16.1	1.9
Control	14.2	
2. To 300 mls. of homogenized milk were added 18.75 mgs. of steapsin which was the same ration of enzyme to milk as the 250 mgs. of steapsin to 4 liters in "1" above. Also 18.75 mgs. of steapsin were added to 300 mls. of unhomogenized milk.		
A. Homo. and unhomo. milk, no additonal enzyme; 1 hour digestion period		
a. Homo. sample	16.5	3.1
Control	13.4	
b. Unhomo. sample	14.7	1.2
Control	13.5	
B. Homo. and unhomo. milk, no additional enzyme; 2 hours digestion period		
a. Homo. sample	15.9	2.5
Control	13.4	
b. Unhomo. sample	10.3	-3.2
Control	13.5	
C. Homo. and unhomo. milk, no additional enzyme; 4½ hours digestion period		
a. Homo. sample	12.0	-1.4
Control	13.4	
b. Unhomo. sample	13.1	-0.4
Control	13.5	

homogenized and the rest was used for the unhomogenized sample. To 300 milliliters of each of the homogenized and unhomogenized samples were added 18.75 milligrams of steapsin, which is the same ratio of enzyme to milk as was the 250 milligrams to 4 liters of milk used above. After stirring well, 50 milliliters of each of the milks were placed in separate digestion tubes and digested in a 98.6° F. water bath for 1 hour, 2 hours, and 4½ hours. As shown in Experiment XX-2-A and B there was a slight trend toward greater digestion of the homogenized milk, but the difference was not wide enough to be conclusive.

In Experiment XXI a series of digestion determinations were made on fat that was extracted from homogenized and unhomogenized milks. The enzymes used were steapsin and Ricinus lipase. Data under Experiment XXI show that no definite trend could be established. In some determinations digestion was greater with the fat extracted from homogenized milk, in other determinations digestion was greater with the fat extracted from unhomogenized milk, and in some determinations the extent of digestion is practically the same for the fat extracted from the two milks.

Experiment XXI - Fat Extracted From Homogenized Milk and Fat Extracted From Unhomogenized Milk Was Digested Using Ricinus Lipase and Steapsin. Toluene Was Added to Both the Control and Digestion Samples, and the Control Was Titrated Immediately.

	.1 N NaOH required for titrating the digested and control samples	Increase in titratable acidity .1 N NaOH
	mls.	mls.
1. One ml. of fat was pipetted into each of the digestion flasks.		
A. Homo. and unhomo. fat, 100 mgs. Ricinus lipase, 0.6 mls. .1 N acetic acid (pH 4.7)		
a. Homo. fat sample	26.8	25.9
Control	0.9	
b. Unhomo. fat sample	24.0	23.1
Control	0.9	
B. Homo. and unhomo. fat, 100 mgs. steapsin, 0.6 mls. .1 N NaOH		
a. Homo. fat sample	10.2	7.1
Control	3.1	
b. Unhomo. fat sample	11.9	8.8
Control	3.1	
2. Fat was weighed into each of the digestion flasks.		
A. Homo. and unhomo. fat, 100 mgs. Ricinus lipase, 0.6 mls. .1 N acetic acid (pH 4.7)		
a. Homo. fat sample - 1.088 grs.	23.6	22.7
Control	0.9	
b. Unhomo. fat sample - 1.041 grs.	23.2	22.3
Control	0.9	
c. Homo. fat sample - 1.023 grs.	27.7	27.0
Control	0.7	
d. Unhomo. fat sample - 1.053 grs.	27.7	26.8
Control	0.9	
e. Homo. fat sample - 0.914 grs.	27.6	26.7
Control	0.9	
f. Unhomo. fat sample - 1.018 grs.	28.6	27.7
Control	0.9	
g. Homo. fat sample - 1.017 grs.	26.1	25.3
Control	0.8	
h. Unhomo. fat sample - 1.075 grs.	24.8	24.0
Control	0.8	
B. Homo. and unhomo. fat, 100 mgs. steapsin, 0.6 mls. .1 N NaOH		
a. Homo. fat sample - 1.040 grs.	4.6	2.7
Control	1.9	
b. Unhomo. fat sample - 1.034 grs.	11.8	9.3
Control	2.5	
c. Homo. fat sample - 1.087 grs.	14.0	11.0
Control	3.0	
d. Unhomo. fat sample - 1.080 grs.	12.7	9.4
Control	3.3	
e. Homo fat sample - 1.154 grs.	15.3	13.0
Control	2.3	
f. Unhomo. fat sample - 0.954 grs.	12.6	10.5
Control	2.1	
g. Homo. fat sample - 1.104 grs.	14.2	12.5
Control	1.7	
h. Unhomo. fat sample - 0.996 grs.	14.2	12.2
Control	2.0	

Discussion

The pH levels of 4.5 and 2.3 employed in some of the preliminary digestion determinations were chosen in order to approximate the acid condition of the human stomach during the process of digestion. These preliminary digestion determinations revealed greater digestion at pH 6.3, which was the pH of normal milk plus digestion mixture, than at either of the lower pH levels. The decreased digestion at pH 4.5 was probably, partly at least, caused by the fact that at this hydrogen-ion concentration the casein was precipitated giving a curd that persisted throughout the digestion period. It was believed that some fatty acids were liberated inside the curd, but that they were not titratable. The fact that negative digestion values were obtained in some instances may be explained by stating that the curd tied up some of the fatty acids that were liberated during the digestion process, as well as some constituents of the sample that were responsible for the initial acidity.

Early in this investigation it was suspected that both the gastric lipase and steapsin being used were contaminated with some proteolytic enzyme. Since trypsin will reduce the curd tension of milk, it was desirable to determine what effect, if any, a gastric lipase solution would have on the curd tension of milk. It was shown that this enzyme solution lowered the curd tension of milk; thus giving more evidence for the belief that trypsin was present. In order to prove to our own satisfaction that some proteolytic enzyme was present in both gastric lipase and steapsin, it was desirable to determine if any digestion would be obtained with skim milk as the substrate using water solutions of these enzymes. Since digestion of skim milk was obtained when either gastric lipase or steapsin was used, it was concluded that a proteolytic enzyme, possibly trypsin, was present.

It was found possible to inactivate the proteolytic enzyme in 1 pH and 3 pH media, but this finding was of no value in this work, because the activity of the fat-splitting enzyme was also reduced in the 1 pH and 3 pH media.

This investigation showed that there was more digestion of unhomogenized milk than of homogenized milk when either gastric lipase or steapsin was used. This fact was surprising because it was believed that the increased fat surface of homogenized milk would be the factor responsible for greater digestion of homogenized milk. Since nothing in the literature explains directly why there was more digestion of unhomogenized milk than of homogenized milk, a suggestion is offered that possibly may explain the phenomenon. Clayton (16) and others have found that there is a protein film adsorbed on the fat globule both when the globule exists in its natural size and also in its reduced size after homogenization. In order for the fat-splitting enzyme to reach the fat, it must first penetrate the adsorbed protein film. Once the enzyme penetrates the protein film of the unhomogenized milk there is a larger fat globule upon which the enzyme is free to act than is the case when the enzyme penetrates the film of the much smaller fat globule of the homogenized milk.

Another possible explanation lies in the fact that it was learned in this work that the homogenized sample coagulated sooner after the enzyme was added than did the unhomogenized sample. Trypsin, which was suspected of being present, was probably responsible for the coagulation. After coagulation had taken place in a sample the fat-splitting enzyme was able to liberate free fatty acids that would be titratable only from the surface of the coagulum. The fact that the fat surface, upon which the enzyme could liberate free fatty acids that would be titratable, was reduced sooner by coagulation in the

homogenized sample than in the unhomogenized sample may account for the greater digestion of the unhomogenized milk.

Very slight, if any, digestion was obtained on either homogenized or unhomogenized milk when the enzyme, Ricinus lipase, was used in a water solution. Apparently the activity of this enzyme was greatly reduced in water solution. The enzyme was very active when used in the dry form to digest extracted fat in Experiment XXI.

The digestion determinations using fat extracted from homogenized milk and fat extracted from unhomogenized milk as the substrate revealed no difference in the extent of digestion of the fat from the two milks when either steapsin or Ricinus lipase was used. In the early digestion determinations with extracted fat as the substrate, one milliliter of the fat was pipetted into each of the digestion flasks. Differences in the extent of digestion of the fat extracted from the two milks being studied were obtained. Since the fat incorporated more or less air, it was thought that possibly these differences could be accounted for in that the pipette was not delivering the same amount of fat each time. When the fat was accurately weighed into the digestion flasks no trend of difference could be established in the extent of digestion between the fat extracted from homogenized milk and that extracted from unhomogenized milk.

Summary and Conclusions

1.

The enzymes, gastric lipase and steapsin, used in this investigation were contaminated with some proteolytic enzyme, possibly trypsin.

2.

It was found possible to inactivate the proteolytic enzyme in gastric lipase and steapsin by making solutions of the enzymes in a 1 pH medium, or a 3 pH medium. The finding was of no importance in this work because the activity of the fat-splitting enzyme was reduced at the low pH levels.

3.

There was greater digestion of unhomogenized milk than of homogenized milk when the enzymes, gastric lipase or steapsin, were used.

4.

The activity of Ricinus lipase in water solution was greatly reduced.

5.

There was no difference in the extent of digestion of fat extracted from homogenized milk and of fat extracted from unhomogenized milk when the fat was accurately weighed into the digestion flasks. The enzymes, Ricinus lipase and steapsin, were used.

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