SWEET (UNADJUSTED OR UNPASTEURIZED MILK)									
No.	Date 1916	pH	Acidity Per cent	R _x	Strength	Coag. 1 : 30,000 Min.	Coag. in Min.	MILK ADJUSTED Acidity Per cent	pH
1.....	9/ 7	5.277	0.22	4415 No. 2	1 : 3000	3.75	3.75	0.294	...
2.....	9/ 8	6.581	0.16	4416 No. 4	1 : 3000	2.75	2.0		
				4893 No. 1	1 : 10,000	0.9	0.5	0.19	6.21
				4893 No. 2		2.8	1.5		
3.....	9/15	6.56	0.135	5532	1 : 3000	Inert	4.0		
				5528 No. 2	1 : 3000	Inert	1.75	0.17	6.34
				5470	1 : 3000	Inert	2.0		
4.....	9/18	6.33	0.1425	Elix. Pepsin	1 : 3000	Inert	1.25		
				4468		Inert	2.0		
				Elix. Pepsin		Inert	1.5	0.1975	5.857
				Elix. Pepsin		3.61	1.0		
5.....	9/19	6.49	0.1475	Scale, No. 1	1 : 3000	Inert	0.75		
				Elix. 5446		Inert	2.75	0.205	6.04
				Elix. 5447		Inert	3.5		
6.....	9/20	6.41	0.15	5579	1 : 3000	30	1.5		
				5580		Inert	8.25	0.2075	6.17
7.....	9/22	5.70	0.1525	4422 No. 6	1 : 3000	Inert	6.25	0.2075	5.784
				4420 No. 3	1 : 3000	Inert	1.0		
8.....	10/ 2	6.3	0.14	5520 No. 7	1 : 3000	Inert	2.25	0.19	5.5
				No. 8	1 : 3000	Inert	2.5		
				4420 No. 3	1 : 3000	Inert	1.0		
				4422 No. 6	1 : 3000	Inert	1.0		
9.....	10/ 4	...	...	5808 No. 2	1 : 3000	30	14.5	0.20	...
				5627	1 : 3000	20	6.5		
10.....	10/ 6	...	0.13	5579	1 : 3000	30	9.25		
				Sol. No. 1		Inert	Less than 1	0.20	...
				No. 2		Inert	Less than 1		
				No. 3		Inert	Less than 1		
				No. 4		Inert	Less than 1		
11.....	10/20	...	0.145	4420 No. 3	1 : 3000	Inert	1.75	0.18	...
				No. 4		Inert	2.25		

EXPERIMENT 2—This test illustrates the question of dilution. When the 1 : 10,000 pepsin was diluted with 7 parts milk sugar, it was apparently inert when tested on sweet milk of 0.16 per cent acidity, but when the same milk was brought to an acid concentration of 0.19 per cent lactic acid the pepsin dilution was found to test 1/8 as strong as the original.

EXPERIMENT 3—In this assay the three scales were apparently inert in milk of 0.135 per cent acidity, also one fluid drachm of the elixir failed to coagulate one quart of milk. When the acidity of the milk was brought to 0.17 per cent, coagulation readily took place.

STRENGTHS OF MIXTURES USED

- EXP. 2. Rx 4893 No. 1 = 1 part 4893 + 2 parts Milk Sugar
 Rx 4893 No. 2 = 1 part 4893 + 7 parts Milk Sugar
 EXPS. 3, 4 AND 10. Elixir Pepsin = 1 fluid drachm contains 2.5 grs. 1 : 3000 Pepsin.
 EXP. 6. Rx 5580 = 1 part 1 : 10,000 Pepsin + 7 parts Milk Sugar.
 EXP. 11. Rx 4420 No. 4 = 1 part 4420 No. 3 + 4 parts Milk Sugar

CONCLUSIONS

The foregoing experiments seem to indicate that the substance which gives the rennetic property to pepsin is not the same substance which gives this property to the calf rennin. It has been shown that it is necessary to activate the pepsin by means of acid before it shows its rennetic power in its proper degree. The adjustment of the milk to a definite acid concentration also aids the coagulation by calf rennet, but the great difference between pepsin and calf rennin in this respect is that calf rennin never fails to coagulate *sweet milk* or to give comparable results upon dilution, while pepsin fails more often than it produces coagulation on perfectly sweet milk. Another great difference is that pepsin acts best on very ripe milk, even to an acidity of 0.2 per cent or more, while rennin is not favored by such a high acid concentration.

Considering the above facts, together with the additional notation that in pepsin these two properties, the milk-curdling and proteid-hydrolyzing, have never been isolated, so that we have in the one case an

enzyme of high curdling power with no proteolytic strength, and in the other a high proteolytic power with no coagulative strength leads me to accept the theory that these two activities are closely bound in a single molecule, and that under the proper conditions of temperature, etc., it is only necessary for this large molecule (pepsin) to come in contact with milk and acid, or protein and acid when the respective activities of milk coagulation or protein hydrolysis takes place.

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A METHOD FOR THE DETERMINATION OF ALCOHOL

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The fact that so many methods have been suggested for the determination of alcohol is indicative of much to be desired in the speed and ease with which determinations can be made. The specific gravity method with which accurate results can be attained and which is most popular cannot in general be applied to liquids other than pure alcoholic solutions. This paper has been prepared suggesting a method which does not require in many cases the distillation necessary to specific gravity determinations, in the hope that the results may be of value in analytical chemistry.

Frary¹ and others have observed that potassium fluoride or potassium carbonate when added to alcoholic solutions will cause the alcohol to separate from the remainder of the solution, that is, that aqueous solutions of potassium fluoride or potassium carbonate are immiscible with alcohol. Frary has suggested a method based on these observations for the determination of ethyl alcohol by the use of potassium fluoride. The method is to add sufficient of the salt (carefully weighed out) to an alcoholic solution in a glass-stoppered cylinder to cause the alcohol to separate, add an in-

¹ "Studies in Chemistry, No. I," University of Minnesota, 1912.

indicator (phenolphthalein) which colors the alcoholic solution and then gradually add water with frequent shaking till the two layers just disappear and the two liquids become completely miscible. From the weight of liquid (which is then determined) and the weight of the salt, Frary finds the percentage of alcohol from a table which he has prepared. He finds that the separation of alcohol by means of potassium fluoride is apparently quite independent of ordinary laboratory temperature conditions.

There are various objections to this method. The potassium fluoride must be perfectly dry and pure. Also, after the analyst has made such a titration as is suggested above, made the weighings and used the table he has consumed much time. It seemed at the outset of the present work that, if the salting out of the alcohol was quantitative, the entire volume of alcohol could be separated and that the process would be much simpler if the percentage by volume were read off directly.

METHOD OF DETERMINATION

The determinations were made at first in graduated tubes of 15 cc. capacity which had been prepared by cutting off 50-cc. gasometer tubes and later in centrifuge tubes of the same capacity. The tubes were all graduated to 0.1 cc. and before use were carefully calibrated with water. In every case the readings were estimated to 0.01 cc. In a determination, 10 cc. of alcoholic solution at 15.6° were used where the concentration was less than about 50 per cent, but where the concentrations of alcohol were greater than this, 5 cc. were taken and the volume made up to 10 cc. in the tube. Potassium fluoride was then added till the tube read about 13 cc. It was found by trial that this gave sufficient of the salt to saturate the solution. A small crystal of malachite-green was dropped in which served to color the alcohol layer so that the volume could be easily read. Various substances could be used for this purpose such as a small piece of phenolphthalein, iodine, etc., but the malachite green proved quite satisfactory giving no color to the aqueous potassium fluoride solution. The tubes were then closed with a tight-fitting stopper and shaken vigorously for perhaps 2 minutes. If the potassium fluoride is dry the solution warms up but when placed in the centrifuge for 2 or 3 minutes, cools down to about room temperature. The alcohol separates in the upper layer while the excess of solid salt settles to the bottom of the tube. From the volume of alcohol observed and the volume of alcoholic liquid used for the determination, the percentage of alcohol by volume is readily found. Since one cc. of this alcohol changes about 0.001 cc. per degree Centigrade at room temperatures, the volume can be corrected to 15.6° if so desired. It was found by experiment that the potassium fluoride did not precipitate quite the last traces of alcohol and when the readings were made from the bottom of the meniscus it was necessary to add 0.15 cc. to observed readings to allow for this and the amount of alcohol adhering to the sides of the tube.

ANALYTICAL RESULTS

Tables I and II are given to show the results obtained by this method of analysis. The first series of determinations was made on pure aqueous alcoholic solutions, the concentrations of which had been carefully determined by the specific gravity method at 15.6°, Table I. A one per cent alcoholic solution just shows the presence of alcohol by this method.

TABLE I—ALCOHOL DETERMINATIONS IN AQUEOUS SOLUTIONS BY POTASSIUM FLUORIDE METHOD CORRECTED TO 15.6° C.

No.	ALCOHOL USED—		Cc. 100 PER CENT ALCOHOL		Difference	PER CENT ALCOHOL FOUND
	Strength	Actual Cc.	In volume used	As Found		
1.....	95.34	5 cc.	4.77	4.71	—0.06	94.2
2.....	82.0	5 cc.	4.10	4.11	+0.01	82.2
3.....	75.71	5 cc.	3.79	3.87	+0.08	77.4
4.....	67.69	5 cc.	3.38	3.40	+0.02	68.0
5.....	57.84	5 cc.	2.89	2.84	—0.05	56.8
6.....	41.84	5 cc.	2.09	2.07	—0.02	41.4
7.....	30.4	10 cc.	3.04	3.09	+0.05	30.9
8.....	18.48	10 cc.	1.85	1.88	+0.03	18.8
9.....	12.4	10 cc.	1.24	1.28	+0.04	12.8
10.....	5.0	10 cc.	0.50	0.53	+0.03	5.3
11.....	1.26	10 cc.	0.12	0.15	+0.03	1.5

Values read from bottom of meniscus and 0.15 cc. added.

Table II shows the analysis of various substances for alcohol. In nearly all cases the alcohol was determined directly, but in several analyses precipitates were formed when the fluoride was added. In such cases the alcohol was distilled and the determinations made on the distillates. Whiskey was successfully analyzed after clarifying with alumina cream.

TABLE II—ALCOHOL DETERMINATION IN DIFFERENT SUBSTANCES CORRECTED TO 15.6° C.

No.	SUBSTANCE ANALYZED	Cc. Used	100% ALCOHOL FOUND BY		% Alcohol Found by		Difference Per cent
			K ₂ F ₂ METHOD	% by vol.	Sp. Gr. Method		
1	Alcohol (denatured).....	5	4.7	94.0	...	...	...
2	Beer(a).....	10	0.43	4.3	4.1	...	+0.2
3	Beer(b).....	10	0.45	4.5	4.2	...	+0.3
4	Cider(a).....	10	0.50	5.0	5.28	...	—0.28
5	Hair tonic(a).....	5	1.45	2.0	29.4	...	—0.4
6	Tincture of Iodine(a).....	5	4.55	91.0	...	...	...
7	" " ".....	5	4.73	94.6	95.0	...	—0.4
8	" " ".....	10	4.74	47.4	47.5	...	—0.1
9	" " ".....	10	2.35	23.5	23.75	...	—0.25
10	Tincture of Iron(b).....	5	4.73	94.6	95.0	...	—0.4
11	" " ".....	10	4.74	47.4	47.5	...	—0.1
12	Vanilla Extract(a).....	5	1.35	27.0	27.65	...	—0.65
13	Whiskey(b).....	5	2.50	50.0	...	...	...
14	Whiskey(a).....	5	2.50	50.0	50.0	...	...

(a) After distillation. (b) Before distillation.

CONCLUSIONS

I—A method has been proposed for the easy, rapid determination of ethyl alcohol which, if care is observed in reading the tubes, gives a fair degree of accuracy. The potassium fluoride used is inexpensive and need not be purified. It is best to dry this substance but small amounts of moisture in it do not affect the results.

II—The method cannot be used for solutions containing less than 1 or 2 per cent of alcohol or where other organic liquids are present such as acetone, essential oils, etc.

It might be pointed out, however, that in a solution such as the tincture of iodine the determination is very simple. The iodine solution is placed in the tube, the potassium fluoride added, the tube shaken, centrifuged, and the volume of the alcohol (which contains the iodine in solution) read. The iodine in the solution seems to have little effect on the volume of the alcohol.