

Rapid Method for the Detection of Fake Protein Addition in Milk

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Abstract

The purpose and aim of this research work is rapid detection of fake protein or added protein in milk, In 3rd world, especially in Pakistan, where total protein criteria for milk acceptance norm exists at Processor end, chemicals containing NH₂ or N are added in fresh milk to top up the total protein by middle man to mask the adulteration. When milk is coagulated the casein protein form the coagulant while all other proteins including the Whey protein and chemicals they get separate in the form of filtrate. Naturally, whey proteins (Globulin & Albumin) have a specific ratio to casein protein. Whey proteins are 20% of the total protein. To detects the presence or absence of any fake chemical that produces its impact on milk protein, a rapid and easy method has been established by simple filtration and titration method. To set this method as standard and make it as a benchmark, different commercial milk samples of reliable selected sources were chosen (including Farm Milk samples, Prema, Gourmet milk AA milk, Nestle Milk) and did the comparative study of total and whey protein against the adulterated samples.

Keywords

Total Protein; Whey Protein; Haleeb Milk; Competitors

Introduction

Milk is the complex composition of Fats, Carbohydrates, proteins and solid contents. The main role of milk is to provide the proper nourishment and healthy body. From all of this milk protein contains more essential amino acids than any other natural food (*Jud Heinrichs* 1855). Milk protein consists of two parts namely (i) Total protein that contains casein and (ii) Whey protein (*Frank.K* 2003). The percentage of casein and whey protein varies in cow and buffaloes milk. In case of cow's milk 80% is casein and remaining 20% is whey protein whereas in buffaloes 87.50% casein and 12.50% whey (*A.Hethmankova* 2012).

Concentration of protein in milk is 33 g/L in which casein have its part 26g/L (79.5% of total protein) (*F. Lara* 2005). Furthermore casein is composed of alpha s₁, alpha s₂, beta casein and kappa casein which is

respectively 30.6, 8.0, 28.4 and 10.1 % of total protein. While total whey protein in milk is 6.38g/L (19.5% of total protein). Whey protein is composed of alpha lactalbumin, BSA, immunoglobulin and protease peptone which is 9.8, 1.2, 2.1 and 2.4% of total whey protein respectively (*H.V.Petit* 2001).

Experiments show that addition of ammonium sulphate, ammonium hydroxide and ammonium carbonate increases total protein %age. This increases proportion in whey protein not in casein protein. The addition of these chemicals causes to increase the whey protein level in milk which fortunately raised the total protein percentage (*Rashmi. A* 2013). Physiochemical result shows that due to the addition of these fake chemicals in milk produce the impact in appearance of milk as well, that resembles with the proper whey protein color and it takes much less time in filtration process as compare to pure protein (*Ahmed* 2013).

Material and Method

The purposed study will be conducted for determination of fake protein in milk.

Material

Titration flask 100 ml, Beaker 100 ml. Beaker 500ml, Burette, pH Meter, filter paper, Hot plate

Reagents

Sodium Hydroxide 0.1N (4.0gm NaOH in 1000ml Distill water),

Phenolphthalein Indicator 1% (1.0 gm in 100ml Ethanol),

Formalin (37% Formaldehyde)

Citric Acid.

Ammonium sulphate.

Procedure

1. Take 10ml of thoroughly mixed sample in a titration flask.

2. Neutralize the sample with 0.1N NaOH using phenolphthalein (1%) as Indicator up to 8.20 pH Value.
3. Add 2ml of Formalin solution (37%).
4. Wait for 5 minutes.
5. Again Titrate with 0.1N NaOH up to 8.20 pH Value. Note the volume (V1).
6. Also repeat above for blank reading without sample (V2)
7. Now again take sample volume 100 ml. from same sample
8. Heat sample up to 90-95 °C.
9. Add 1g citric acid to clot milk.
10. Stir clock wise slowly with glass rod.
11. Now filter clotted milk through filter paper whatman No 2 or common filter paper sheet.
12. Take 10 ml sample from filtrate.
13. Titrate with NaOH 0.1N to Neutralize up to 8.20 pH Value.
14. Add 2ml of Formalin solution (37%).
15. Wait for 5 minutes.
16. Repeat titration up to 8.20 pH Value for Whey protein %
17. Perform calculation as below

Observation and Calculations

$$\text{Total Protein}\% = (V1 - V2) \times 1.94$$

$$\text{Whey Protein} = (V1 - V2) \times 1.94$$

$$\text{Whey Protein } \%$$

$$\text{Whey } \% \text{ of total protein} = \frac{\text{Whey Protein } \%}{\text{Total Protein } \%} \times 100$$

$$\text{Total Protein } \%$$

Whereas: 1.94 is the Factor of formalin

V2 = Blank reading of Formalin Solution

Results

TABLE 1 PRODUCT: FRESH AND FARM MILK

Sr No.	Product	ANIMAL NO.	DOP	TOTAL PROTEIN (%)	WHEY PROTEIN (%)	Whey % of Total
1.	FARM MILK	383	14-12-11	3.1	0.38	13%
2.	FARM MILK	412	14-12-11	3.7	0.8	21%
3.	FARM MILK	125	14-12-11	3.3	0.8	23%
4.	FARM MILK	405	14-12-11	3.5	0.8	22%
5.	FARM MILK	451	14-12-11	3.3	0.7	20%
6.	FARM MILK	432	14-12-11	2.5	0.4	15%
7.	FARM MILK	363	14-12-11	3.3	0.6	18%
8.	FARM MILK	452	14-12-11	3.1	0.6	19%
9.	FARM MILK	418	14-12-11	3.1	0.4	13%
10.	FARM MILK	1024	14-12-11	3.8	0.8	20%
11.	FARM MILK	—	01-12-11	3.1	0.58	18.7%
12.	FARM MILK	—	13-12-11	2.91	0.58	20%
13.	FARM MILK (HFL)	—	01-12-11	2.52	0.50	20%

TABLE 2 REFERENCE SAMPLES (ADULTERATED)

SR No.	PRODUCT	DOP	DOE	BATCH	TOTAL PROTEIN (%)	WHEY PROTEIN (%)	WHEY % OF T.P
1.	Reference sample	16-11-11	16-02-12	269	2.91	1.16	40%
2.	Reference sample	17-11-11	17-02-12	275	2.52	1.16	46%
3.	Reference sample	17-11-11	17-02-12	276	2.91	1.16	40%
4.	Reference sample	18-11-11	18-02-12	277	2.71	0.97	35%
5.	Reference sample	19-11-11	19-02-12	278	2.32	1.16	50%
6.	Reference sample	19-11-11	19-02-12	280	3.1	1.16	37%
7.	Reference sample	16-11-11	16-02-12	281	3.1	0.97	31%
8.	Reference sample	16-11-11	16-02-12	282	3.1	1.16	37%
9.	Reference sample	16-11-11	16-02-12	283	3.1	0.97	31%
10.	Reference sample	16-11-11	16-02-12	284	2.91	0.97	33%
11.	Reference sample	06-12-11	06-03-12	316	2.91	1.35	47%
12.	Reference sample	08-12-11	08-03-12	317	3.1	1.35	44%
13.	Reference sample	08-12-11	08-03-12	318	3.1	0.97	31%
14.	Reference sample	09-12-11	09-03-12	319	2.91	0.97	33%
15.	Reference sample	09-12-11	09-03-12	320	3.1	0.58	19%
16.	Reference sample	28-11-11	28-02-12	301	3.1	0.97	31%
17.	Reference sample	29-11-11	29-02-12	302	3.1	1.35	44%
18.	Reference sample	29-11-11	29-02-12	303	2.91	0.97	33%

19.	Reference sample	29-11-11	29-02-12	304	3.1	1.55	50%
20.	Reference sample	30-11-11	30-02-12	305	3.1	1.35	44%
21.	Reference sample	30-11-11	30-02-12	306	3.29	0.58	31%
22.	Reference sample	30-11-11	30-02-12	305	3.1	1.35	44%
23.	Reference sample	30-11-11	30-02-12	306	3.29	0.58	31%
24.	Reference sample	01-12-11	01-03-12	307	3.1	0.77	25%
25.	Reference sample	02-12-11	02-03-12	308	3.1	0.77	25%
26.	Reference sample	02-12-11	02-03-12	309	3.1	0.97	31%
27.	Reference sample	03-12-11	03-03-12	310	3.1	0.97	31%
28.	Reference sample	03-12-11	03-03-12	311	3.29	0.38	13%
29.	Reference sample	04-12-11	04-03-12	312	3.1	0.97	31%
30.	Reference sample	04-12-11	04-03-12	313	3.1	0.77	25%
31.	Reference sample	05-12-11	05-03-12	314	3.29	0.97	29%
32.	Reference sample	05-12-11	05-03-12	315	2.9	1.16	40%
33.	Reference sample	24-11-11	24-02-12	291	2.91	0.97	335
34.	Reference sample	23-11-11	23-02-12	290	2.91	1.35	46%
35.	Reference sample	24-11-11	24-02-12	292	3.1	1.16	37%
36.	Reference sample	24-11-11	24-02-12	293	3.1	1.35	44%
37.	Reference sample	26-11-11	26-02-12	296	3.1	1.35	44%
38.	Reference sample	23-10-11	23-01-12	250	3.3	1.16	35%
39.	Reference sample	29-10-11	29-02-12	256	3.1	0.388	13%
40.	Reference sample	31-10-11	31-02-12	257	3.1	1.6	50%
41.	Reference sample	31-10-11	31-01-12	258	3.1	1.6	50%
42.	Reference sample	05-11-11	05-02-12	262	3.1	1.6	50%
43.	Reference sample	03-11-11	03-02-12	259	2.9	1.6	55%
44.	Reference sample	24-10-11	24-02-12	252	2.9	1.4	48%

TABLE 3 PRODUCT: COMPETITOR UHT MILK

Sr No.	Product	DOP	DOE	Batch No.	Total Protein	Whey Protein	Whey % of Total
1.	NESTLE	20-10-11	12-01-12	129315802	3.1	0.39	13%
2.	NESTLE	03-12-11	25-02-12	13371580114	2.91	0.39	14%
3.	NESTLE	11-11-11	03-02-12	13151581	3.1	0.58	19%
4.	NESTLE	10-11-11	02-02-12	131415802	3.1	0.48	16%
5.	NESTLE	11-11-11	03-02-12	131515802	3.1	0.58	19%
6.	NESTLE	01-12-11	23-02-12	133515801	3.1	0.58	19%
7.	NESTLE	01-11-11	14-01-12	130515801p	3.1	0.58	18.7%
8.	NESTLE	14-10-11	14-01-12	1226158121L	2.9	0.38	13%
9.	NESTLE	30-11-11	22-02-12	133415801	2.9	0.38	13%
10.	NESTLE	25-11-11	17-02-12	132915802	2.9	0.38	14%
11.	NESTLE	06-11-11	19-01-12	131015801	3.1	0.58	19%
12.	NESTLE	04-12-11	26-02-12	133815801	3.1	0.58	19%
13.	NESTLE	12-12-11	05-03-12	134615801	3.1	0.47	16%
14.	NESTLE	18-11-11	10-02-12	132215801	2.9	0.56	19%
15.	NESTLE	17-11-11	09-02-12	132115801	3.2	0.49	15%
16.	GOOD MILK	24-11-11	24-02-12	1898	2.13	0.97	46%
17.	GOOG MILK	24-09-12	24-12-11	1620	2.32	0.97	41%
18.	OLPER	16-09-11	15-12-11	5585HO	2.91	1.16	40%
19.	OLPER	13-11-11	11-02-12	5858	3.1	0.39	13%
20.	OLPER	15-11-11	15-02-12	5873	2.9	0.39	14%
21.	OLPER	03-11-11	01-02-12	5816	2.8	0.8	28%
22.	OLPER	29-11-11	27-02-12	5926	2.9	0.582	20%
23.	OLPER	20-09-11	18-12-11	5735	2.9	0.38	14%
24.	OLPER	23-10-11	21-01-12	5775	3.1	1.358	44%
25.	OLPER	02-12-11	01-03-12	5941	2.73	0.54	20%
26.	OLPER	10-12-11	09-03-12	5976	2.73	0.51	19%
27.	OLPER	29-11-11	27-02-12	5930	2.64	0.39	15%
28.	OLPER	12-12-11	11-03-12	5983	2.8	0.47	17%
29.	OLPER	27-11-11	25-02-12	5914	2.8	0.52	19%
30.	OLPER	13-12-11	12-03-12	5985	2.7	0.58	21%
31.	OLPER	14-12-11	12-03-12	5949	2.61	0.46	18%
32.	OLPER	03-12-11	02-03-12	5943	2.74	0.58	21%

33.	OLPER	16-12-11	14-03-12	5953	2.8	0.49	18%
34.	NOOR PUR	25-11-11	23-02-12	322	1.6	0.19	13%
35.	NOOR PUR	11-11-11	04-2-12	310	1.7	0.58	34%
36.	NOOR PUR	09-11-11	07-01-12	282	1.7	0.77	45%
37.	NOOR PUR	06-11-11	05-03-12	340	1.6	0.19	13%
38.	DAIRY PURE	13-11-11	13-02-12	C	1.6	0.8	50%
39.	Nestle by Marketing	16.12.11	09.03.12	1350158010	3.20	0.60	19%
40.	Olper by marketing	6.12.11	05.03.12	5951	3.16	0.83	26%

TABLE 4 PRODUCT: COMPETITOR PASTEURIZED MILK SAMPLE

Sr No.	Product	DOP	DOE	Batch No.	Total Protein	Whey Protein	Whey % of Total
1	PREMA	15-12-11	21-12-11	—	3.298	0.388	12%
2	GOURMET	15-12-11	18-12-11	—	2.716	0.388	15%
3	NURPUR	15-12-11	21-12-11	—	2.134	0.582	28%
4	PREMA	13-12-11	19-12-11	—	3.1	0.58	19%
5	GOURMET	14-12-11	17-12-11	—	2.9	0.582	20%

Discussions

When whey protein or artificial protein is added to raw milk, the ratio between total protein and whey protein gets disturb which is indicator of tempering of milk. Whey proteins and fake proteins gets pass into filtrate once milk protein (casein) is coagulated.

We have done this study on farm collected milk and do the comparison between different milk samples available in market (shown in table 1-4). From all these we observed the following below results.

Total protein of farm milk by Rapid Method: 3.10% (100%). Whey protein after acidification: 0.58% (18%). After addition of Ammonium sulphate 0.50% in above farm milk. Total protein by Rapid Method was 14.74% (which is more 375% More than actual protein) and Whey protein after acidification was 16.49% (114%). Similarly after addition of Ammonium sulphate 0.10% in above farm milk. The results found were, total protein by Rapid Method 4.65% (which is 48% more than actual) whereas whey protein after acidification 3.88% (83 %).

From the above result in the tablet form we can concluded that Farm milk Doodah meets the pure milk protein proportions according to the standard values standards. If we added 0.50% Fake chemical (Aluminum Sulphate) in Farm milk then almost 12% total protein increased and it will be also increased in whey protein 114% with same rapid test method. Same activity performed with reduce dosage of Aluminum sulphate 0.1% in above Farm milk and 1.55% protein increased and also increase whey protein 83% with same method.

By summarizing all the result we can conclude that if we added whey powder to re-constituted milk it is detectable through above mentioned testing protocol.

ACKNOWLEDGEMENT

Mr. Faisal Imran Hussain Malik, the CEO of Haleeb Foods Limited, joined with the aim of turnaround of the company (HFL), which was in severe crises due to bad quality of incoming fresh milk, caused by adulteration. Mr. Malik introduced the concept of "Sell Right" through emphasizing on development of adulteration detection in milk.

The above research, "detection of fake protein addition by rapid method", not only helped to improve the quality of Haleeb foods products but also impacted on overall business results enabling the fastest turnaround in cooperate sector.

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