



## Evaluation of the Chemical and Microbial Quality of Raw Milk Sold at the Markets in Hodeidah City-Yemen

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**Abstract:** This research aimed to evaluate the chemical and microbial quality of raw milk sold at the markets of Hodeidah city. Ten raw milk samples were collected from different markets, in addition two samples of brand pasteurized milk for comparison. The specific gravity, total solids, pH, acidity, fat percentage and protein percentage were estimated. The total number of bacteria, total coliform bacteria, yeasts and molds was stimated as microbial quality.

The results showed that 60% of the samples had a specific gravity below normal limits as a result of adulteration of milk with water, while one sample obtained a high specific gravity (1.036), which is an indication of the addition of powdered milk. There was significant difference among the different samples in Total Solid (TS) and sample S7 was the lowest (9.47%). The pH of some samples decreased below the normal limits for raw milk, in addition to the increase in titratable acidity as a result of microbial activity in the milk. Samples S4 and S7 had low fat percentages, this may be due to the nature of the milk or part of the milk fat was removed. Protein percentages were within normal limits for milk, except for sample S3, which was (4.82%) as a result of the possible addition of powdered milk. Microbial analysis results showed a high number of total bacteria in raw milk samples compared to pasteurized milk. Coliform bacteria were also present in 60% of the samples, in addition yeasts and molds were presence of in high numbers.

**Keywords:** Raw milk, Chemical Quality, Microbiological Quality, Hodeidah Markets

### 1. Introduction

Milk is considered one of the best foodstuffs with high nutritional value because it contains all nutritional elements and is considered one of the important foods in human nutrition at all stages of life (Xulu et al., 2019). Cow milk constitutes 85% of the commercial level in all countries of the world, followed by buffalo milk (11%), and then goats and sheep at 2% each. Cow's milk contains varying percentages of known nutritional elements, in addition to a good percentage of vitamins and mineral elements (Eckles et al., 1951). Milk quality is determinate according to a set of standard criteria such as the amount of impurities, specific gravity, percentage of fat and protein, in addition to the total number of bacteria and pathogenic bacteria (FAO, 2008). Milk production process is one of the factors affecting its chemical and microbial quality, Sometimes, milk is adulterated by adding water, removing part of its components, or adding some substances that prevent the deterioration of milk or preserve its chemical properties (Hempfen et al., 2004). Also, lack of care of the milk during the milking process can lead to a rapid change in its characteristics and thus rapid spoilage, therefore, producing milk with acceptable standards is a challenge for farmers and livestock breeders, which is what



associations and official bodies seek to do by awareness and training them on methods of proper production of milk and thus the possibility of marketing it (Tola, 2002).

This research aimed to evaluate the chemical and microbial quality of raw milk sold at the markets in Hodeidah city.

## 2. Materials and Methods

**Milk samples:** Milk sellers display raw milk in the main markets in metal containers and then divide it into plastic bags without conducting any treatments. Therefore, ten samples of raw milk collected from different markets in Hodeidah city, randomly, in addition to samples of pasteurized milk manufactured by local companies. The samples were placed in a refrigerated container and transported to the laboratory to conduct the necessary chemical and microbial analysis.

**Chemical analysis:** Specific gravity and pH were carried out using the method described by Awal *et al.*, (2016), while the total acidity in the milk was measured using the titration method according to what was mentioned by Aggarwal and Sharma (1961). Milk fat was estimated using Gerber method (Goss, 1953), while total solids (TS) was estimated according to the method described by AOAC (2003). Protein estimation was carried out using the Kjeldahl method according to Pearson (1977).

**Microbial analysis:** Total number of bacteria, coliforms, yeasts, and molds was determined according to the method described by APHA (1998).

**Statistical analysis:** The data for the various tests were analyzed using the analysis of variance (ANOVA) method, and the Duncan multinomial test was used at the 5% probability level to determine the significant differences between the means, using the statistical analysis program STAT SAS, 1995. .

## 3. Results and Discussion

### Chemical composition

#### Specific gravity (g/cm<sup>3</sup>)

Specific gravity is defined as the relationship between the gravity of a substance and the gravity of water at the same temperature. Therefore, it is a result of milk components balance, and any imbalance in these components leads to a change in the milk gravity value.

The results in table (1) showed that 60% of the samples obtained a specific gravity lower than the normal limits for gravity in raw milk (1.028-1.032 g/cm<sup>3</sup>), according to both FAO (1988) and Tamime (2009), where the gravity values for these samples ranged between 1.023-1.012 g/cm<sup>3</sup>. Sample S7 obtained the lowest value (1.012 g/cm<sup>3</sup>), the low specific gravity of the milk is considered one of the indicators of the milk adulteration process by adding water to the milk. The results agreed with what was found by Gemechu and Tesfaye, (2016), some raw milk samples had a gravity below normal limit. While the results obtained were less than Karmaker *et al.*, (2020) and Gemechu *et al.* (2015). The results of the study agreed with what was reported by Eckles *et al.*, (1951) stated that the process of diluting milk with water leads to a decrease in its specific gravity, as farmers and livestock breeders often resort to adding water to milk in order to increase its quantity (Omoro *et al.*, 2005). On the other hand, the specific gravity in sample S3 was high (1.036), and according to what O'Connor, (1995) stated, a high specific gravity in the milk is considered an indication of the addition of skim milk powder because the fat percentage was normal. While specific gravity of pasteurized milk samples P1 and P2 obtained 1.030 and 1.031 g/cm<sup>3</sup>, respectively. These results agreed with what Karmaker *et al.*, (2020) who found pasteurized milk (1.028-1.032 g/cm<sup>3</sup>), which is within the normal limits for liquid milk.

#### Total Solid (TS%)

Total solids (TS) express the total solid content of milk as a percentage and include fat, sugar, protein, and salts. Results in table (1) showed significant differences between fresh milk samples in the percentage of total solids, as solids decreased in the samples that obtained low values in specific gravity, where sample S7 obtained the lowest percentage of total solids (9.47%). It is the same sample that obtained the lowest specific density value (1.012).

Results obtained agreed with the findings of Karmaker *et al.*, (2020) and Hossain *et al.*, (2011) that adding water to milk led to a decrease in the percentage of total solids. While sample S3 obtained the highest value that



exceeding the normal limits, which reinforces the hypothesis of adding powdered milk to liquid milk (Zelalem and Ledin, 2001).

While, there were no significant differences between the pasteurized milk samples, the results were identical to what was found by Hossain *et al.*, (2011).

### **pH**

The pH accurately indicates the condition of the milk, as whenever the values are within normal limits, this means that the milk is fresh.

Results in table (1) indicated that there were significant differences between the pH values of the milk samples, which ranged between 6.37-6.77. Both O'Connor, (1995) and FAO, (1999) indicated that the pH of fresh cow's milk ranges between 6.6-6.8.

Decrease of pH milk below 6.6 is considered an indication of an increase in the number of bacteria in the milk (O'Connor, (1995). This was confirmed by the results of the microbial analysis (Table 2), as in the samples whose pH was lower than 6.6, the numbers of bacteria, yeasts, and molds were high. The results of the study agreed with what was stated by many researchers, that the decrease in pH values in milk is due to an increase in the number of bacteria and their multiplication (Huque *et al.*, (2018) and Saxena and Poonam. (2013).

### **Acidity (%)**

Titration acidity is an indicator of the freshness of milk and bacterial activity (Hossain *et al.* 2011). Popescu and Angel, (2009) also indicated that high-quality liquid milk has an acidity of no more than 0.14. The results in table (1) indicate that there are significant differences in acidity values between the milk samples. Sample No. S9 obtained the highest value (0.23%), which is an indication of the high number of bacteria.

While sample S2 obtained the lowest value (0.14%), which means that the milk is good and fresh. Referring to the microbial analysis table, the microbial content of sample S2 was very low, which suggests this possibility. The results of the study agreed with the findings of Karmaker *et al.*, (2020) in that milk samples whose acidity was high had high microbial content.

The titratable acidity of milk is expressed in terms of percentage of lactic acid, as fresh milk does not contain any appreciable amount of lactic acid, so the increase in acidity is a rough measure of the age and activity of the bacteria. Within a short period after milking, acidity increases due to increased bacterial activity and increased amount of lactic acid in the milk, and this depends on the cleanliness of production and the temperature at which the milk is kept (Saxena and Poonam, 2013).

Determining the acidity level in milk is an important factor in judging the quality of milk, as acidity affects the taste. When the acidity reaches about 0.3%, the taste of the milk becomes acceptably acidic. At 0.4% acidity, the milk is clearly sour, and at 0.6% the milk curdles as a result of the increased acidity. (sour taste) and at acidity exceeding 0.9%, milk is spoiled (Srairi *et al.*, 2006).

### **Fat (%)**

The results in table (1) indicated that there were significant differences in fat percentages between the samples, ranging between 1.9 and 4.5%. Commercially and nutritionally, milk fat is considered the most important component of milk (Hossain *et al.*, 2011).

According to the Yemeni standard specification for raw milk (12/2003), cow's milk should not be less than 3% fat, which means that samples S4 and S7 are less than the normal limits for milk fat. This could be due to the nature of the milk (low fat) or that it was conducted partial milk fat defatting (Karmaker *et al.*, 2020).

The results of the study agreed with what was found by Samia *et al.*, (2009) in samples of cow's milk in Sudan showed a decrease in the percentage of fat in some samples. The results of the study also coincided with what was found by Dey and Karim, (2013) that the decrease in the density of the milk may be due to the addition of water or the removal of part of the milk fat. On the other hand, the percentage of fat in the pasteurized milk samples was within the limits of the standard specification for pasteurized milk. Similar results in pasteurized milk were found by both Hossain *et al.* (2011) and Huque *et al.*, (2018).

### **Proteins (%)**

The results in table (1) showed that there are no significant differences between the milk samples in protein percentage, except for sample S3. The increase in the protein percentage in that sample (4.82%) is due to the possibility of adding skim milk powder, which is evident through the high density of the milk (1.036).



The protein percentages in the rest of the samples ranged between 3.30-3.58, according Hossain et al., (2011), the amount of protein in raw milk ranged between 3.03-3.57%. The results of the study agreed with what was found by Abd Elrahman et al., (2009) in that the protein content in local raw milk was 3.48%. Fikrineh et al. (2012) also reported that the protein content in samples of milk produced on local farms was 3.46%. The protein percentage in pasteurized milk samples P1 and P2 was 3.56 and 3.60, respectively. These values were consistent with what Karmaker et al., (2020) found in pasteurized milk samples in Bangladesh.

**Table 1:** Chemical analysis results of raw milk samples.

Samples	Specific gravity (g/cm <sup>3</sup> )	TS %	pH	Acidity %	Fat %	Proteins %
S1	1.021 a	11.40 a	6.64 a	0.15 c	4.2 a	3.40 a
S2	1.023 a	11.52 a	6.80 a	0.14 d	4.0 a	3.30 a
S3	1.036 a	14.18 b	6.72 a	0.15 d	4.5 a	4.82 b
S4	1.013 a	10.0 c	6.50 a	0.16 c	2.5 b	3.37 a
S5	1.023 a	11.50 a	6.45 a	0.18 c	3.5 b	3.58 a
S6	1.017 a	11.20 a	6.77 a	0.15d	4.5 a	3.46 a
S7	1.012 a	9.47 d	6.56 a	0.16d	1.9 b	3.43 a
S8	1.030 a	12.65 e	6.75 a	0.15 d	4.2 a	3.32 a
S9	1.031 a	12.88 e	6.37 a	0.20 a	4.4 a	3.42 a
S10	1.031 a	12.31 f	6.61 a	0.15 b	4.1 a	3.50 a
P1	1.030	11.48	6.61	0.16	3.5	3.56
P2	1.031	11.46	6.70	0.15	3.6	3.60
ST	1.028-1.032	11-13	6.4-6.8	0.13-0.17	3-5	3.40

**S1 – S10:** raw milk samples, P1 and P2: pasteurized milk samples, ST: Standard specification. Similar letters in the column of the table mean that there are no significant differences, and different letters mean that there are significant differences at the 5% level.

#### 4. Microbial analysis

##### Total count of bacteria (TCB)

Raw milk is a good medium for the growth of microorganisms due to its high water content and diversity of nutrients in addition to its close to neutral pH, which makes it one of the best media for the growth and multiplying microbes (Soomro et al., 2002).

The results of the microbial analysis (Table 2) showed that the total count of bacteria in the milk samples was high, ranging between  $7.5 \times 10^3$  to  $2.2 \times 10^7$  cfu/ml. The reason for the increase in the total number of bacteria in the milk is due to the poor health conditions during milking, as the increase in the number of bacteria can be due to several reasons, the most important of which are: the animal being infected with mastitis, the animal's udder being dirty, the animal's environment and the milking place being unclean, the milking tools and the milker's hands being unclean and milk don't cooling after milking immediately (Fadaei, 2014). Similar results were obtained by Huque et al., (2018) who indicate the high total number of bacteria in raw milk was between  $2.31 \times 10^5$  -  $2.45 \times 10^5$  cfu/ml). also, similar results were found by Iknomov et al., (1956) who stated that the number of bacteria in milk depends on milking and hygiene techniques. On the other hand, pasteurized milk samples showed a decrease in the total count of bacteria, due to the effect of pasteurization and packaging under sterile conditions. Microbial contamination not only reduces the quality of milk, but also threatens the health of the community when consumed this type of milk (El-Leboudy et al., 2017)

##### Coliform Total (CT)

The presence of coliform bacteria in foods is considered an indicator of the occurrence of some form of unwanted fecal contamination (Saha and Ara 2012). Results in table (2) indicated that 60% of the samples were contaminated with coliform bacteria, as they exceeded the standard limits for this type of bacteria in raw milk (100 cfu/ml), where sample S9 was the highest in the number of coliform bacteria ( $1.8 \times 10^5$  cfu/ml). Similar results were found by Hossain et al., (2011) who reported that the numbers of coliform bacteria in raw milk ranged between  $4.5 \times 10^3$  and  $2.03 \times 10^6$  cfu/ml. Poor animal hygiene, contaminated water, unsanitary milking



practices, improper washing of milking equipment and tools, in addition to lack of attention to the cleanliness of the milkers' hands can lead to a high number of coliform bacteria in the raw milk (CDFA, 2008).

While the pasteurized milk samples were free of coliform bacteria, similar results were found by Hossain et al., (2011) and Huque et al., (2018) they reported the absence of coliform bacteria in pasteurized milk. CDFa, (2008) indicated that coliform bacteria are not supposed to be present in milk after pasteurization due to their sensitivity to the pasteurization temperature. If present, it is evidence of contamination after pasteurization.

### Yeasts and Molds

Contamination of raw milk with yeasts and molds can occur in the primary production stages (farm and milk equipment) or in the manufacturing environment, and this type of contamination often leads to uncontrolled fermentation in the milk and thus its spoilage (Lavoie and Touchett, 2012).

Results in table (2) indicated that there were significant differences in the numbers of yeasts and molds in the samples, where sample S2 was the lowest in numbers of yeasts and molds (10 cfu/ml), while sample S9 was the most contaminated with yeasts and molds ( $1.2 \times 10^6$  cfu/ml), the study agreed with what Karmen and Vengust, (2008) who found regarding the increase in the number of yeasts and molds in raw milk sold in local markets. Exposure of milk to air for different periods, in addition to the lack of poor hygiene practices during milking and mixing old milk with new milk, can lead to an increase in the number of yeasts and molds in raw milk (Kuma et al., (2015)). On the other hand, the pasteurized milk samples were free of yeasts and molds, as evidence of the efficiency of the pasteurization process and the control of sanitary conditions during manufacturing.

**Table 2:** Microbial analysis results of raw milk samples

Samples	TCB (cfu/ml)	CT (cfu/ml)	Y & M (cfu/m)
S1	$18 \times 10^6$	$5 \times 10^4$	$2.5 \times 10^5$
S2	$7.5 \times 10^3$	0	0
S3	$3.6 \times 10^6$	$2 \times 10^4$	$10^4$
S4	$1.5 \times 10^7$	$7 \times 10^4$	$6.5 \times 10^5$
S5	$1.6 \times 10^6$	0	$1 \times 10^3$
S6	$1.1 \times 10^6$	$1.3 \times 10^5$	$3.6 \times 10^5$
S7	$1.4 \times 10^7$	$1.6 \times 10^5$	$1.7 \times 10^5$
S8	$2 \times 10^7$	0	$1.8 \times 10^3$
S9	$1.6 \times 10^7$	$1.8 \times 10^5$	$1.2 \times 10^6$
S10	$2.2 \times 10^7$	$1.3 \times 10^4$	$7.6 \times 10^5$
P1	50	0	0
P2	35	0	0
ST	$10^4$ good milk $10^5$ acceptable milk	100cfu/ml	$10^3$

**S1 – S10:** raw milk samples, P1 and P2: pasteurized milk samples, ST: Standard specification.

### 5. Conclusion

The results of the study showed that most of the samples of raw milk sold in the markets of Hodeidah city were adulterated, either by adding water, adding powdered milk, or removing part of the fat.

The study also showed that the microbial load of the milk was high, especially coliform bacteria, which means there are health risks for this type of milk. While the pasteurized milk samples were within normal limits, chemically and microbially. Therefore, it is necessary to apply health requirements during the milking process, in addition to keeping the milk in refrigerated condition to reduce microbial activity. Boiling the milk before consuming it is considered an important factor in eliminating microbial risks.

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