



A Quality Assessment of Commonly Vended Tomato, Plum and Mint Sauces in Kamoke, Pakistan

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Abstract: Sauces are food additives that are frequently consumed globally and especially with fast food in Pakistan but often their consumer quality related issues are noticed. That is why; current study was designed to examine selective physicochemical properties and microbial contamination in commonly vended tomato, plum and mint sauces in Tehsil Kamoke, District Gujranwala, Punjab, Pakistan. In this experimentation, total 15 samples were collected (5 samples of each plum, mint and tomato sauces) from a uniform distance of 2 km according to the map of Kamoke. For microbial culture based analysis, selective tests were done to mainly detect coliforms and results were statistically evaluated by applying one way ANOVA. The physicochemical parameters like pH and sugar content were found significant at 0.01% level for sauces of mint (4.32%) and for plum (15.54%), respectively. Among artificial sweeteners aspartame was totally absent in all samples but saccharine was present in some samples in greater amount than standard consumption values. The mint sauce samples were found contaminated (488CFU/ml) at 5% significance level. Similarly, for coliforms (472 to 568.6 CFU/ml) of selected locally vended plum, mint and tomato sauces were noted. Microbial contaminants included *Staphylococcus epidermis*, *Bacillus simplex*, *Escherichia coli*, *Salmonella spp*, *Arthrobacter dextranolyticus*, *Pseudomonas cidrella*, *Bacillus weihenstephanesis*, *Enterobacter aerogenus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. During sampling, it was observed that mostly sauces were exposed to high temperature and were placed uncovered. Vendors were unaware of personal hygiene, proper handling, storage and quality control standards for sauces which results in adulteration and contamination. It is recommended that to improve the quality of commonly vended sauces, their regular inspection along with the programmed general public awareness should be organized.

Keywords: Sauces, Quality Control, Adulteration, Contamination, Public Awareness.

1. INTRODUCTION

Sauces are gravies which serve as appetizers and as flavor enhancing agents so they are commonly served along with fried items and other cuisines in every region of the world. However, in poorly developed countries, these sauces are usually not prepared and served by following rules of food authority and general public hygiene. Though they are freshly prepared but usually served without addition of food preservatives, so have poor shelf life and they are sold for unlimited duration with improper storage which may result in foodborne diseases in consumers due to their intake [1].

Similarly, in currently selected region of Punjab Pakistan, these locally prepared and vended sauces are always in high demand to their good taste and economical price. But reported data indicated that almost half of the local vendors of sauces are located near to sewerage drains and garbage dumps, in much crowded places and such surrounding environment greatly promotes the microbial growth and foodborne pathogenesis [2]. The way tomato sauce is quite popular one around the globe but due to poor hygienic practice and handling by local vendors, often found loaded with microbial contamination, e.g., *E. coli*, *Listeria spp*, *Salmonella spp*, *Shigella spp*, *Vibrio spp*,

Staphylococcus spp, *Streptococcus spp*. along with the presence of other adulterants like paprika seeds, corn starch, sucrose and salt [3].

Moreover, these plum, tomato and mint sauces are often prepared by using dusty and poor quality ingredients like chili, onion, red and green tomatoes and coriander which may contain enteric pathogens causing disease outbreaks and health complications like diarrhea and malnutrition [4, 5]. In this regard, most commonly reported Gram negative bacterial genera include *Escherichia*, *Vibrio*, *Shigella*, *Salmonella*, *Kelbsiella* and a supportive factor is availability of higher temperature in surrounding environment which may not only alter the biochemical composition of sauces but also directly promotes the microbial growth [6]. Therefore, the current study was conducted to check quality of locally vended sauces of high demand in selected region because they may influence the health of consumers, if not prepared and served according to quality control standards.

2. MATERIALS AND METHODS

2.1. Study Area

This study was conducted in the Tehsil Kamoke (District Gujranwala, Punjab, Pakistan). The local food vendors along road sides are found quite common in selected region, that is why; total 15 samples (5 samples of each plum, mint and tomato sauces) were collected from 5 random locations of Tehsil Kamoke at a uniform distance of 2 km according to the map of this region. These samples were assigned with codes: Plum sauce samples (A, B, C, D, E), mint sauce samples (F, G, H, I, J) and tomato sauce samples (K, L, M, N, O).

2.2. Study Design

This experimental study was conducted for the evaluation of physicochemical properties and microbial contamination in commonly vended tomato, plum and mint sauces of Tehsil Kamoke.

2.3. Physicochemical Analysis

2.3.1. Ash content determination by microwave oven method

For this, in 1 g of each sauce sample 2 ml of H_2O_2

was added and this mixture was microwaved at each power (low, high and medium) up to 3 minutes to dry. Later on, allowed to cool down and then 1.5 ml of distilled water was added into dried sample and mixed it well. This prepared sample was added into labeled ependroff and it was centrifuged at 5000 rpm for one hour then the weight of obtained pellet was calculated [7] by following Equation (1):

$$\% \text{ of ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (1)$$

2.3.2. Evaluation of pH and electrical conductivity (EC)

The pH and electrical conductivity of all samples were determined in triplicate manner with digital pH and EC meter [8, 9].

2.3.3. Evaluation of acidity

To determine acidity by titration method, 10ml of each sauce sample were titrated against NaOH (0.1 N) solution after the addition of 2-3 drops of phenolphthalein indicator until the color of solution was turned into purple and calculations were done [11] by using Equation (2):

$$\text{Acidity} = \frac{\text{Amount of NaOH} \times \text{Conc. of NaOH} \times 1.5}{\text{Amount of sample}} \quad (2)$$

2.3.4. Detection of artificial sweeteners

2.3.4.1. Brix test:

A thick fine layer of each sample was placed on the refractometer and reading of percentage sugar was noted from scale. On the basis of results of this test, saccharine test was further performed only for those samples for which < 5% reading was noted which were an indication that artificial sweeteners were might be present [12].

2.3.4.2. Saccharine test:

This test was performed only for those sauces which have < 5% sugar content and for this purpose, 1g sauce sample was dissolved 100 ml distilled water and placed for 1hr. shaking. After that the prepared solution was centrifuged at 8400 rpm speed for 10 minutes at 20 °C. The obtained supernatant was used to measure optical density by spectrophotometer and further calculations were done [12] by employing Equation (3):

$$\text{Percentage} = \frac{\% \text{ of Saccharine} \times 100}{\text{specific absorbance} \times \text{wt}} \quad (3)$$

2.3.4.3. Aspartame test:

To perform aspartame test, 20 g of each sample were dried in oven then 20 ml chloroform and 5 ml acetate buffer were added into each sample and placed in shaker for 45 minutes. After that filtration of each prepared solution was done and obtained filtrates were dried on hot plate. After drying, 5 ml ninhydrin solution was added into each sample containing flask and again dried them on hot plate. No color was appeared in all which indicated that samples had no aspartame content [12].

2.4. Detection of Microbial Contamination

For the detection of microbial contamination, following tests were performed:

2.4.1. By using nutrient agar

Nutrient agar plates were prepared in triplicates for all 15 samples and spreading of 1ml of each sauce sample was done and plates were incubated at 37 °C for 48 hrs. After that plates were examined for probable bacterial colonies identification by observing morphological features and colony forming units (CFU/ml) were also determined [13]. For microbial colonies probable identification and confirmation, following biochemical tests were performed:

2.4.1.1. Catalase test:

On a clean glass slide, small amount of sample from each obtained microbial colony on nutrient agar was placed then 2 drops of H₂O₂ were poured on it and changes were noted [14].

2.4.1.2. Gram staining:

Separate microbial smear was prepared for each detected strain. It was air dried and heat fixed then its staining was done according to the manual [15].

2.4.1.3. Amylase test:

Each detected microbial contaminant was isolated on separate nutrient agar plate and 2 to 3 drops of 10% iodine solution were added on it and waited

for 10 minutes to note observations and the results were noted that either microbe is amylase producer or not [16].

2.4.2. Coliforms detection by using EMB agar

To detect the presence of fecal microbial contamination, eosin methylene blue (EMB) agar plates were prepared in triplicates for all 15 samples and spreading of 1ml of each sauce sample was done and plates were incubated at 37 °C for 48 hrs. After that plates were examined for probable bacterial colonies identification by observing morphological features and colony forming units (CFU/ml) were also determined [17].

3. RESULTS AND DISCUSSION

3.1. Physicochemical Analysis

3.1.1. Ash content

The sample K of mint sauce had the lowest ash contents whereas J and M samples of plum sauce and in case of tomato sauce, sample O had highest ash contents (Table 1). The presence of ash was indicator of amount of total solids. So the higher percentage of ash/total solids presented greater amount of contamination and vice versa [18].

3.1.2. Estimation of acidity

In the current study, the overall amount of acidity ranged from 2 to 9% (Table 1). The minimum concentration of acidity for plum sauce samples was noted as 2.43% while the maximum obtained value was 9.8%. Similarly, the mint sauce samples showed acidity concentration in range from 2.73 to 9.13% whereas for tomato sauce samples 2.56 to 9.63% range of acidity was observed. The higher acidity level was an indicator of less microbial growth while lower acidity concentration is considered as a promoting factor for microbial growth and may result in low quality of sauces for consumption [19].

3.1.3. Measurement of pH

The observations regarding pH of different sauces showed that maximum acidic value was obtained for sample of tomato sauce whereas maximum basic nature and statistically significant value at 0.01%

Table 1. Tabulated observations of physicochemical parameters sauce samples.

Samples	Ash content (%)	Acidity (%)	pH	Conductivity (mv/cm)	Sugar (%)
Plum	10.40 ± 2.04 (5)	5.26 ± 1.34 (5)	3.40 ± 0.04 (5)	22.33 ± 3.93 (5)	15.54 ± 2.05 (5)***
Mint	5.19 ± 1.07 (5)	6.98 ± 1.15 (5)	4.32 ± 0.07 (5)***	27.53 ± 3.73 (5)	3.35 ± 0.65 (5)
Tomato	7.61 ± 1.94 (5)	6.05 ± 1.28 (5)	3.68 ± 0.19 (5)	21.27 ± 6.64 (5)	6.05 ± 1.32 (5)

* All values are in mean ± SEM (n) and the results were found significance at 0.1% (***) level.

level was noted for mint sauce (Table 1). While the pH of plum sauces was in the range of 3.31 to 3.52. The detection of low pH was an indication of bacterial food contamination barrier but higher acidic pH of some sauce samples was also showing that these were already rich with microbial load, that's why; high concentration of hydroxide ions which occurred by high temperature. However; the sauce samples with high pH values were found more prone to microbial contamination [20].

3.1.4. Determination of electrical conductivity

The electrical conductivity reading was found maximum for mint sauce which was 27.53 mv/cm while minimum value of EC was 21.27 mv/cm recorded for tomato sauce (Table 1). The higher level of conductivity in eatables cause various diseases in humans like frequent urination, constipation, stomach pain, vomiting etc. whereas low level of electrolyte also results in such as thirst, fatigue, muscles weakness, loss of appetite, etc. That is why; standard values should be maintained but variations in electrical conductivity mainly occur due to the presence of impurities [5].

3.1.5. Determination of sugar percentage

According to the ANOVA results significant sugar content was found 15.54% in plum sauce at 0.01% level (Table 1) because addition of sugar is normally done to prepare this sauce and to reduce the microbial growth. Moreover, the brix test results for mint sauce showed minimum percentage of 3.35% which was an indication of no adulteration of any sugary content but of poor shelf life [10, 14].

3.1.6. Analysis of artificial sweeteners

In this test saccharine was detected at 217nm and

aspartame was detected at 280nm. Saccharine and aspartame test had significant non polar characteristics because they had azo and aromatic rings. These artificial sweeteners are the alternative of sugar which is mostly added in the locally vended foods. Normal quantity of artificial sweeteners is safe to use but high quantity is harmful for the health of consumers [21].

In current study, aspartame was not found in any sample of sauces while for the saccharine detection, following results were obtained and in mint and some tomato samples, its adulteration was found which might be done to improve its taste and for better shelf life but it is considered harmful for health of consumers, if it is found more than 2.3 milligrams per pound and currently detected amounts were higher than standard value [22].

Table 2. Percentage of saccharine of plum, mint and tomato sauces.

Sample (s)	Observations
A, C, D, F, G, J, M	No saccharine
B	0.2872%
E	0.2221%
H	0.4271%
K	0.3463%
N	0.6267%
I	0.4258%
L	0.3706%
O	0.2967%

3.2. Microbial Analysis

3.2.1. Analysis of microbes by nutrient agar

For the detection of microbial contamination, first of all, general purpose medium (nutrient agar) was used and it was observed that maximum colony forming units were found in mint sauce which was 488 CFU/ml at 5% level of significance as compared to plum and tomato sauces, 407 CFU/ml and 360 CFU/ml, respectively. Whereas ANOVA results for the colony size were not found significant (Table 3). The probable identification showed that *Staphylococcus epidermidis*, *Bacillus simplex*, *Arthrobacter dextranolyticus*, *Pseudomonas cidrella* and *Bacillus weihenstephanesis* were present in currently studied local sauce samples (Figure 1). Moreover, the presence of *Staphylococcus epidermidis* showed the poor handling and hygiene practice by sellers which may result in food poisoning. Whereas the presence of *Bacillus simplex* indicated the prevailing improper storage practice and the detection of *Arthrobacter dextranolyticus* highlighted the usage of adulterants and poor grade ingredients. *Pseudomonas cidrella* indicated the sauce samples were not stored at ideal temperature and it is mainly transmitted in food items from infected hands of labor and handlers. Similarly, the presence of *Bacillus weihenstephanesis* was a clear indication of improper washing of used vegetables and fruits which were used to prepare sauces [25].

3.2.2. Gram staining

All detected microbial strains were examined via gram staining to detect either they are gram positive or negative as confirmatory test. Gram positive bacterial contaminants were *Staphylococcus epidermidis*, *Bacillus simplex*, *Arthrobacter dextranolyticus* and *Bacillus weihenstephanesis* whereas *Pseudomonas cidrella* was gram negative [26].

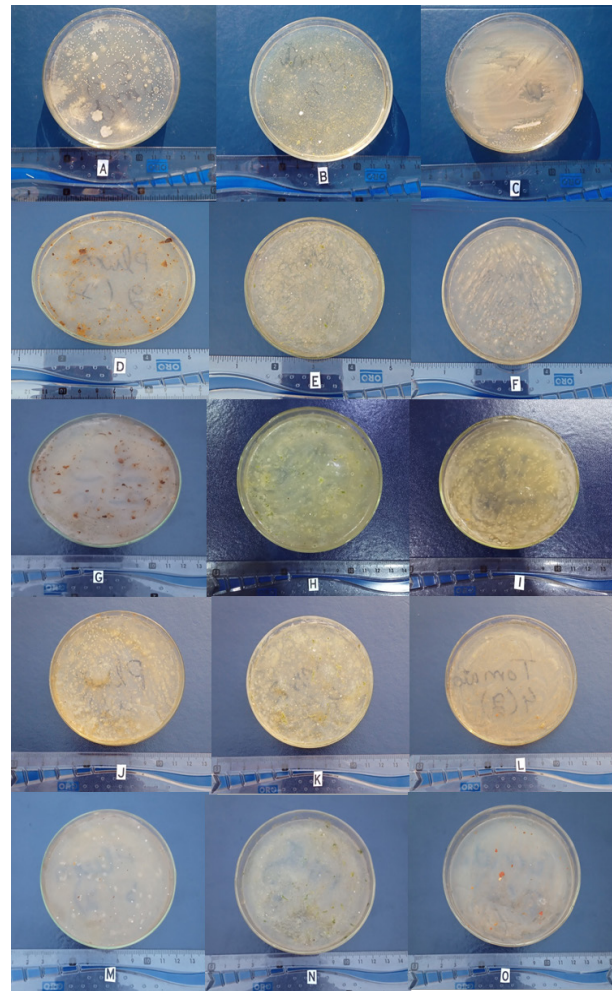


Fig.1. Morphological view on nutrient agar of microbial contamination detected in sauce samples and CFU/ml was calculated by using these plates: A, B, C, D, E (Plum sauce samples); F, G, H, I, J (mint sauce samples) and K, L M, N, O (tomato sauce samples).

3.2.3. Catalase test

The catalase test was also performed to detect catalase producers. Some samples including: A, D, E, F G, H and M, were catalase positive which indicated the presence of possible enteric bacterial

Table 3. Colonial diameter and CFU/ml on selected media for selected local sauces.

Sample	Microbial colonial diameter on nutrient agar (mm)	CFU/ml of nutrient agar	Microbial colonial diameter on EMB agar (mm)	CFU/ml of EMB agar
Plum	1.35 ± 0.47 (5)	407.4 ± 35.538 (5)	1.46 ± 0.17 (5)	472 ± 74.79 (5)
Mint	1.24 ± 0.21 (5)	488 ± 33.098 (5)*	1.76 ± 0.33 (5)	568.6 ± 74.66 (5)
Tomato	1.50 ± 0.59 (5)	360.8 ± 30.578 (5)	1.98 ± 0.43(5)	547.2 ± 108.12 (5)

* All values are in mean ± SEM (n) and ANOVA was applied and the results were found significance at 5% (*) level.

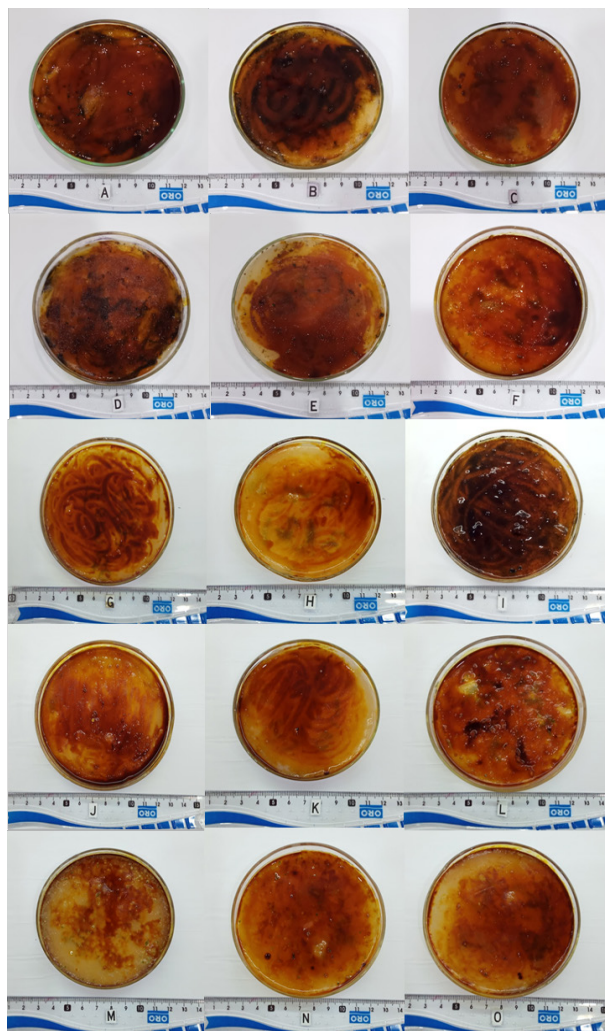


Fig. 2. Pictorial observations of amylase test for detection of starch. The samples A, D, E, F, G, J, M, L, O showed positive results while samples B, C, H, I, K, N showed negative results.

strains while some samples including sample J, B, K, N, C, I, L and O were catalase negative which indicated their absence (Figure 3). Catalase test is performed only for currently obtained the gram positive bacteria. Samples which were catalase positive were enterobacteria [30]. The contamination of enterobacteria in food items like locally vended sauces occurs via skin, nails and GIT infections etc. of food handlers. But the major concern is presence of such pathogenic bacteria in food products may further cause several diseases such as infections of bones and joints [27].

3.2.4. Amylase test

The results of amylase test indirectly helped not only to detect type of microbial strains as

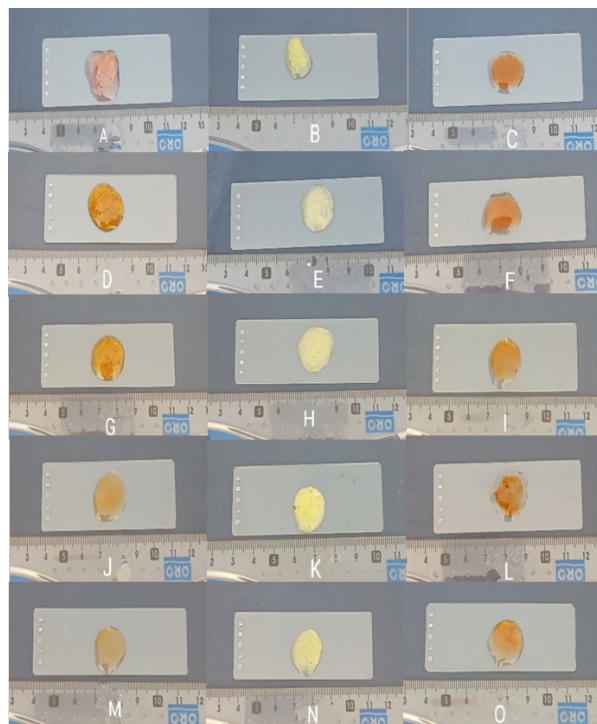


Fig. 3. Observations of catalase test. The samples A, D, E, F, G, H, M showed positive results whereas sample B, C, I, J, K, L, N, O were showed negative results.

contaminants but also indicated biochemical quality of sauces. The samples of sauces A, D, E, F, G, J, M, L and O showed positive results, i.e., microbial contaminants were not amylase producers so starch immediately appeared deep blue in culture plates while other samples of sauces B, C, H, I, K and N showed negative results in which starch was not found because color of iodine was not changed as starch content was already catalyzed by amylase producers (Figure 2). The presence of high level of amylase in edibles than normal range may harm the health of consumers but still unchecked amount of starch is usually added by local vendors in sauces to improve their consistency which results in more amylase production by microbes or contaminants because mostly these starch rich sauces are not stored according to the quality standards [23].

3.2.5. Coliform detection

To detect the microbial contamination of sewerage water (coliforms) selective medium, eosin methylene blue (EMB) agar was used and detected microbial strains included *Enterobacter aerogenus*, *Klebsiella pneumonia*, *Pseudomonas aerogenus*, *Escherichia coli* and *Proteus mirabilis* (Figure 4). The diameters of their colonies were measured

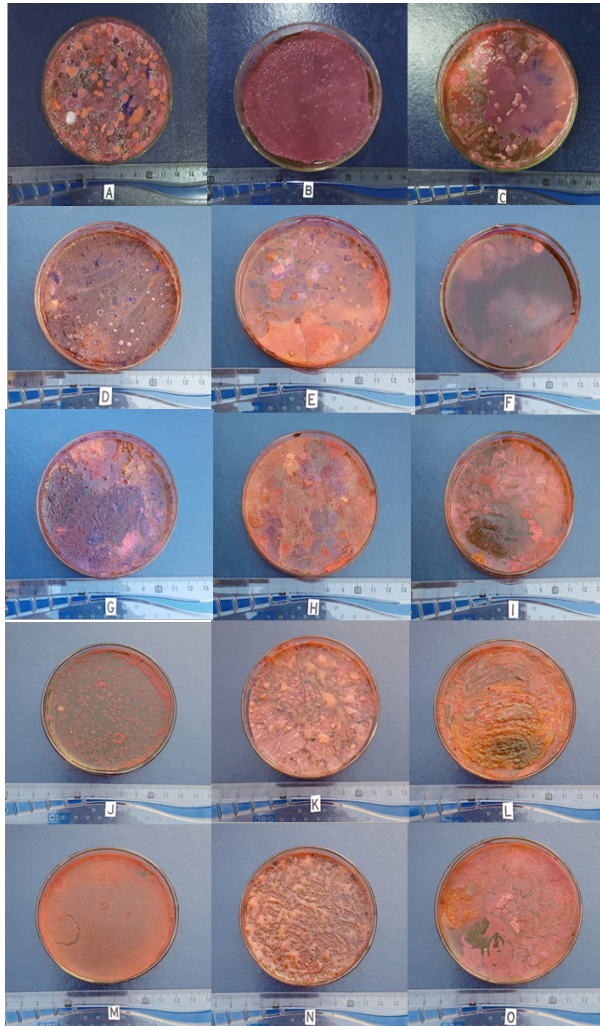


Fig. 4. Morphological view on EMB agar of coliforms contamination detected in sauce samples and CFU/ml was calculated by using these plates: A, B, C, D, E (Plum sauce samples); F, G, H, I, J (mint sauce samples) and K, L M, N, O (tomato sauce samples).

which were not found significant statistically. While the maximum coliforms were detected 568.6 CFU/ml in samples of locally vended mint sauce than plum and tomato sauces (Table 3). It means the used utensils, water added to sauces and hands of venders were might be contaminated coliforms either due to seepage of sewerage in to clean water supply which is utilized for dish washing and for preparation of local sauces or simply due to poor hygiene practice of venders [28]. Moreover, the intake of such coliform contamination having local sauces may result in skin, eye pneumonia, urinary tract and several other infections [24, 29].

4. CONCLUSIONS

It was concluded that among the selected commonly vended sauces, tomato sauce was overall more inappropriate for consumption than plum and mint sauces. Moreover, low grade ingredients were also used. As an outcome, consumption of these sauces may result in foodborne pathogenesis i.e., the way harmful ratio of artificial sweeteners was detected in tomato sauce. Similarly, contamination of several microbial pathogens was also detected in all selected samples of locally vended sauces. Thus organized steps of food authority of regular inspection and other public and private sector departments are required for the general public awareness and to train local venders about handling, preparation and storage of these sauces [31]. In this way, the quality of locally vended food items can be ensured.

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6. CONFLICT OF INTEREST

All authors have no conflict of interest.

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