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Microbiological analysis of popular spreads used in restaurants inside Dhaka city, Bangladesh

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Spreads are used widely for making the fast foods more amazing and tasty. Varieties and cross combinations of ingredients are used to make many flavours of fast foods. Different restaurants prepare their signature spreads for attracting people but this can cause opposite result if not prepared using high quality raw materials and proper hygienic conditions are not maintained. Current study was conducted on ten different types of spreads (pesto, cilantro, queso, tomato sauce, peanut butter, honey, mustard, cream cheese, chocolate sauce and butter) which are used by the local restaurants in Dhaka city, Bangladesh. Almost all the samples (nine out of ten) harbored total viable bacteria exceeding the standard limit. Four spread samples showed high fungal count (10^2 cfu/gm). Four samples showed to be positive (>10^1/ml recommended acceptable count) for *Escherichia coli* and *Klebsiella* spp. which indicates that these spread cannot be recommended for public consumption. Among all the samples examined, only chocolate sauce showed acceptable result without the presence of coliforms, *Staphylococcus* spp. and *Pseudomonas* spp. This finding suggests proper maintenance of sanitation in spread preparation and selling area. A proper guidelines and monitoring can help keep up the quality of food additives.

Keywords: Spread, Contamination, Microbiological quality, Hygiene.

INTRODUCTION

Snacks are very popular among people of the modern age as they need less input of labour, time saving and give better taste. The use of different kinds of spreads has made the taste and appeal of the snacks better than before. There are many kinds of commercial and local spreads and people are using their innovative ideas to make new recipes for more variation in spreads. Some such popular spreads are mustard, chocolate sauce, tomato sauce, cream cheese, pesto, queso, honey, butter, cilantro, peanut butter, honey mustard, honey-garlic, chilli-garlic, tomato salsa etc. All of these spreads are made from natural organic products which provide many health benefits (like maintaining blood lipid level in hypercholesterolemia patients, inhibiting the risk of breast cancer initiation, controlling urinary tract infections etc.) by providing fibre, minerals, unsaturated fatty acids, proteins etc. which made them very popular among the health conscious people (1-9). Due to the nutritional component, these spread can harbour the contaminating bacteria in them. The quality of each of the raw materials used for preparation of spreads also determines the final quality of the spread (10). Contamination may arise also during cultivation, irrigation water, harvesting, processing, storage, transporting process, from insects/animals or rodents, food handlers, cross contamination etc. (11-15). *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., *L. monocytogenes* and *E. coli* O157:H7 (in Cilantro spread), *Streptococcus* sp., (in queso), *Staphylococcus* sp., verotoxin producing *E. coli* (in butter), *Salmonella* sp. (peanut butter), *Shigella* sp., coliforrms (in butter), *Mycobacterium paratuberculosis*, *Yersinia enterocolitica* (in cream cheese), *Clostridium perfringens* (in pesto) etc. All of these bacteria are responsible for different food borne diseases if consumed (16-18). Bacteria could be very common in the spreads if they were not prepared in appropriate hygienic condition (19-22). The quality of the ingredients and the equipment used during the preparation of the spreads are also important factors of contaminations (19-22). Spreads are very commonly consumed with different food items and the risks associated with the spreads due to microbial contamination may cause public health hazards resulting in outbreaks of gastrointestinal diseases. The aim of the current study was to determine the microbiological quality of some popular spreads available in different restaurants in Dhaka city, Bangladesh.

MATERIALS AND METHODS

Study area and sample processing. Spreads have become very popular item for preparation of fast foods in restaurants all over the world. Due to changes in tastes, new varieties of spreads are being prepared with innovative recipes and combinations of ingredients. Ten different popular spreads including tomato sauce, tamarind sauce and mayonnaise were collected from different restaurants in Dhaka city, Bangladesh for microbiological quality analysis. The study included one sample from each category. These samples were collected during the time span of March 2019 to April 2019. They were collected aseptically and transferred to the microbiological laboratory for microbiological quality analysis as soon as possible. After homogenization, the samples were serially diluted from this homogenate to10^{-5} dilution (23-24).

Total bacterial and total fungal count. 0.1 ml diluted sample from 10^{-3} dilution of each samples was spread over nutrient agar and Sabouraud Dextrose Agar (SDA) medium and incubated overnight at 37°C and 25°C respectively (23).

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Determination of *Pseudomonas* spp., *Escherichia coli, Klebsiella* spp. *Staphylococcus* spp. 0.1 ml sample was spread on *Pseudomonas* agar (OXOID, UK) for the determination of *Pseudomonas* spp., MacConkey agar (OXOID, UK) for identification of *Escherichia coli*, *Klebsiella* spp. and MSA (Mannitol Salt Agar, OXOID, UK) for determination of *Staphylococcus* spp., respectively and incubated overnight at 37^{o}C. All the suspected isolates were then confirmed by biochemical tests (23).

Biochemical identification of the isolates. Isolated bacteria were identified by standard biochemical test. Biochemical characteristics of the isolates were determined by Triple Sugar Iron (TSI), Methyl Red (MR), Voges-Proskauer (VP), motility, oxidase, catalase, indole and citrate utilization tests were done and then compared with standard charts to identify the bacteria (23).

RESULTS AND DISCUSSION

People are attracted to fast foods nowadays more than previous time and different types of spreads are an inevitable part of preparing fast food items like sandwiches, burgers, rolls etc. Thousands of spreads are being consumed with fast food items daily. It is important to maintain the quality of the spreads by careful selection of high grade raw materials, proper hygiene practiced by the personnel who prepares spreads, utensils and machines used during preparation etc. If all of these are not maintained properly, people will be at risk and may suffer from serious health problems upon consumption of the contaminated spreads. Locally produced food items like spreads have been reported with the presence of pathogenic microorganisms and threat to public health (24-28). Locally prepared spreads that are used in fast food items and also served as dipping item have more chance of getting contamination with pathogenic microorganisms than commercial spreads. The personnel who are involved in processing the locally customized spreads have no training on the microbiological safety and sanitation play a major role in increasing the chance of contamination (29). The equipment used during preparation of sauces is another important factor in introducing microorganisms (30).

The spread samples collected from different restaurants in Dhaka city showed varied counts of total viable bacteria (Table 1) ranging from 10^2 cfu/gm (in chocolate sauce) to 10^8 cfu/g (in butter). Except chocolate sauce and peanut butter, all other spreads showed higher bacterial count exceeding the limit of 10^4 cfu/g suggested by the Standardization organization for G.C.C. (GSO). GSO/FDS 1016/2014 (31). Total fungal count was observed within the margin of 10^1 cfu/gm to 10^5 cfu/g. The recommended limits are; total viable bacteria = 10^4, Yeast and mold = 10^2, *E. coli* = 10, *Staphylococcus aureus* = 10^2 per g (30). Higher counts of these bacteria were found in pesto, tomato sauce, peanut butter, cream cheese. *Staphylococcus* spp. and *Pseudomonas* spp. were found only in pesto (3.3×10^3 cfu/gm and 2.2×10^2 cfu/gm), tomato sauce (2.2×10^3 cfu/gm and 1.2×10^2

Table 1: Microbiological analysis of sauces (cfu/gm).

Samples	Total Viable Bacterial Count (TVBC)	Total Fungal Count (TFC)	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas* spp.	*Staphylococcus* spp.
Pesto	5.0×10^5	1.3×10^3	0	0	2.2×10^2	3.3×10^3
Cilantro	2.0×10^5	1.8×10^1	4.5×10^2	8.0×10^1	0	0
Queso	4.2×10^5	2.7×10^1	0	0	0	0
Tomato sauce	1.0×10^6	4.0×10^4	0	0	1.2×10^2	2.1×10^3
Peanut Butter	1.2×10^3	6.3×10^4	0	0	0	0
Honey	5.0×10^4	1.3×10^3	1.4×10^1	0	0	0
Mustard	2.0×10^6	1.8×10^3	0	8.0×10^4	0	0
Cream cheese	4.2×10^7	2.7×10^4	2.0×10^2	3.5×10^2	2.0×10^4	1.0×10^1
Chocolate sauce	1.0×10^2	4.0×10^1	0	0	0	0
Butter	1.2×10^8	6.3×10^5	2.0×10^4	3.4×10^4	0	0

Note: Recommended limit for spreads: Total viable bacteria= 10^4 cfu/g, Yeast and mold= 10^2 cfu/g, *E. coli*= 10 cfu/g, *Staphylococcus aureus*= 10^2 cfu /g (30).

Tables 2: Confirmative biochemical tests for the isolates.

Assumed Organisms	TSI			H_2S reaction	Indole test	MR Test	VP test	Citrate test	Catalase	Oxidase Test
	Slant	Butt	Gas							
Escherichia coli	Y	Y	+	-	-	-	-	+	+	-
Pseudomonas spp.	Y	Y	+	+	-	+	-	+	+	+
Klebsiella spp.	Y	Y	+	-	-	-	+	+	+	-
Staphylococcus spp.	Y	Y	-	-	-	-	-	+	+	-

Note: TSI=Triple Sugar Iron, Y=Yellow (Acidic), MR=Methyl Red, VP=Voges-Proskauer.

cfu/gm) and cream cheese (1.0×10^1 cfu/gm and 2.0×10^4 cfu/gm). Highest growth for *Escherichia coli* (2.0×10^4 cfu/gm) and *Klebsiella* spp. (3.4×10^4 cfu/gm) were found in butter spreads. Cilantro, honey, cream cheese and butter are not acceptable for consumption as these bacteria have exceeded the recommended limit (Standardization organization for G.C.C. (GSO), Standard no. GSO/FDS 1016/2014) (31). All of the isolates were presumptively confirmed after biochemical identification by conducting several biochemical tests like TSI, MR, VP, citrate test, catalase, oxidase and indole test (Table 2).

Other researchers individually studied different spreads and found different results. Chocolate spreads studied by Amevor, *et al.,* 2018 (32) showed quite satisfactory results with fungal count and total bacterial count within the FDA recommended limit. Butter studied by Gazo, *et al.,* 2018 (33) showed huge microbial load similar to our study. Spreads are one of the most popular food additives and used in lots of amount daily throughout the world. So the awareness should be developed in general population regarding the pathogens causing health hazards to the consumers due to the consumption of these contaminated sauces and the food laws should be followed during preparation of such food additives. Standard Operating Procedures should also be followed properly to restrict any unwanted contaminants or microorganisms into the preparation process. Also the use and storage in appropriate environmental condition according to the required suggestions should be followed. Using a big jar/bottle of spreads and using many times with contaminated spoons or not covering properly might invite pathogenic microbes into the spread where they will grow further and cause spoilage of the spreads. A disposable system or an individual small pack may be an alternative approach to reduce those kinds of contaminations.

CONCLUSION

Spreads are found in lots of flavor and are very popular among young generations. But this nutritious substance can also be harmful for the health if it harbors adequate amount of pathogenic bacteria and may cause different health injuries. Pathogenic bacteria can be introduced from variety of sources and can be reduced by decreasing the pH upon the addition of organic acids (34). But overdose of these preservatives can cause health problems also. So different regulatory bodies including both government and non government organizations should work together to address these urgent public health issues. Checking the quality of all the ingredients used for spread production, maintaining preparation procedure, using hygienic equipments, proper storage condition and safe usage while using for long time should be maintained properly to reduce the risk of microbial contamination of the spreads as well as the foodborne diseases of the consumers.

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