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Microbiological profiling of food additives and evaluation of their antibacterial efficacy

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Food additives are widely used to enhance the taste, texture, appearance and longevity of food items. The aim of this study is to determine the quality and antibacterial activity of different food additives and preservatives. In this study 8 categories of food additives were collected from different super shops in Dhaka city. Almost 90% samples were free from pathogenic microorganisms and a low number of total viable bacteria were found in all samples. Antibacterial activities of these samples were measured by agar diffusion method. Except food color, all remaining categories exhibited zone of inhibition against tested laboratory isolates between 8 mm and 22 mm in diameter. These findings indicate that adding food additives or preservatives in food item can reduce or prevent the growth of pathogenic microorganisms. Harmful effects of food additives to human health need to be evaluated before they are applied in food items.

Keywords: Food additives, Microorganisms, Antimicrobial activity.

INTRODUCTION

Microorganisms are present everywhere in nature and in different parts of human body. Like all other animals they need food for their survival hence compete with others for food and nutrients. Food can supply nutrients and energy that support the growth of microorganisms under favorable conditions. Significant growth those microorganisms can cause illness in human; others can cause spoilage of food (1). Commencement of enteric diseases (e.g., cholera, diarrhea, dysentery) in the developing countries principally occurs because of microbiologically contaminated food and water associated with unhygienic lifestyle (2, 4). In Bangladesh, cholera is a major public health issue due to poor sanitary practices (2-5). The major challenge for the food industry is to eliminate these undesirable changes in foods as a result of microbial contamination and to ensure quality of foods. According to FDA, food additives are substances that enhance stability and safety, maintain nutritional quality and improve texture, essence, and appearance of food (8). It includes preservatives, sweeteners, color additives, flavors and spices, flavor enhancers, nutrient supplements, emulsifiers, texturing agents, acidulants and enzymes (9).

Chemical additives like organic acids which include lactic, acetic and citric acid have been used to combat actions of these microorganisms (10). A large number of chemicals are available that can serve as food preservatives but food and drug administration (FDA) allowed a small number due to rules which should be strictly followed. They also ensure the acceptability and wholesomeness of the food, improve nutritional value, control appropriate pH, enhance flavor and give proper appearance (11-13). Sodium benzoate, Benzoic acid, Sodium sorbate, Potassium sorbate, Sodium nitrite are used as antimicrobial agents they inhibit the growth of bacteria, molds, insects and other microorganisms. Other commonly used food additives are food color, flavor and artificial sweeteners which are used to enhance flavor or impact desired color. Nowadays to meet the consumer expectations, various natural and synthetic flavors are used to enhance the taste of foods, colors (14). In order to avoid the risk of being diabetic patient, reduce the tooth decay and keep the food energy (calories) low, sweeteners are generally added to foods to replace sugar (11, 15). In order to enhance the shelf life of foods, chemical preservatives have also been used. The present study is designed to determine the microbiological quality and antimicrobial activity of some commonly used food additives.

MATERIALS AND METHODS

Sample collection and processing. Food color (red, yellow, green, blue); Flavor (vanilla, chocolate, strawberry, mango, orange), Sodium benzoate, baking powder, monosodium glutamate, cornflower, zero calories, saccharine were collected from different super shop of Dhaka City. 100 µl of each food color and flavor were spread on media and remaining sample were homogenized. In that case 25 g of each sample was mixed with 75 ml of buffer peptone water.

Estimation of total viable bacteria and fungi. For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1 ml of each sample was introduced onto the Nutrient Agar (NA) plate (Himedia Laboratories Pvt. Ltd Mumbai, India) and Sabouraud Dextrose Agar (SDA) plates (Himedia Laboratories Pvt. Ltd Mumbai, India), respectively by spread plate technique. Plates were incubated at 37°C for 24 h and at 25°C for 48 h for total viable bacteria and fungi, respectively (16, 17).

Estimation of *Escherichia coli, Klebsiella* spp., *Staphylococcus* spp. *Pseudomonas* spp., and *Bacillus* spp. 0.1 ml of each sample was spread onto the MacConkey agar plate (Himedia laboratories Pvt. Ltd Mumbai, India) for the enumeration of coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), Plates were incubated for 24 h at 37°C for coliforms. Likewise, *Staphylococcus* spp., *Pseudomonas* spp., *Bacillus* spp. were isolated onto Mannitol Salt Agar (MSA) plate (Himedia Laboratories Pvt. Ltd Mumbai, India) and *Pseudomonas* agar plate, Starch agar (Himedia laboratories Pvt. Ltd Mumbai, India) respectively by adding 0.1 ml of sample each, and all the

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plates were then incubated at 37°C for 24 h (16-18, 22).

Assay of determining anti-bacterial properties of samples through agar well diffusion method. Agar well diffusion method was performed to determine the antimicrobial activity of the samples. Individual bacterial pathogens (*Pseudomonas* spp., *Klebsiella* spp., *E. coli*, *Staphylococcus aureus* and *Bacillus* spp.) were spreaded properly over Muller Hinton Agar (MHA) (Oxoid, Ltd., Hampshire England) plates using sterile cotton swabs (15). Wells were made in MHA by cork borer. Each of the antibiotics was used directly on MHA, separately. Sample solutions were added in wells along with a positive control (Gentamycin disc: 10μg) and a negative control (Normal Saline). The presence of antimicrobial activity was determined by the production of a clear zone around the wells and the diameters of these zone were then measured (18, 23).

RESULTS AND DISCUSSION

Food preservatives play a vital role in prevention of food spoilage (19). Here different types of food additives are used to check their microbial quality and as well as antimicrobial activity. Total viable bacteria were observed in case of food color in a range between 7.2×10^2 cfu/ml and 9.3×10^2 cfu/ml and food color is free from pathogenic microbes. In case of food flavor, the presence of the highest number of total viable bacteria was recorded for lemon (4.4×10^2 cfu/ml) and the lowest for vanilla 2×10^1 cfu/ml. Pathogenic microorganisms were absent in food flavor. On the other hand total viable bacteria count for baking powder, corn flower and saccharine were 3×10^1 cfu/ml, 5×10^1 cfu/ml and 8.5×10^1 cfu/ml respectively. Presence of *Staphylococcus* spp. and *Pseudomonas* spp. were found in baking soda. A small number of fungal species was found in corn flower and saccharine.

Surprisingly antibacterial activity was found in the tested food additives. Mango flavor show antibacterial activity against *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp. and *Staphylococcus* spp. In this study, chocolate flavor has no antimicrobial activity. Vanilla flavored display a significant range of antibacterial activity against all tested isolates. Strawberry, Lemon and Orange flavor showed a limited zone of inhibition against the bacterial isolates except *Klebsiella* spp. Saccharine, corn flower and monosodium glutamate shows antibacterial activity against *E. coli* and *Pseudomonas* spp. Previous study reported that the presence of microorganisms in preservatives which support our present study (21, 22). Nevertheless, to our knowledge, the microbiological analysis of food additives in Bangladesh has been first time reported in our study. So very few report are available about this particular

Table 1. Microbial analysis of 'Food Color'

Sample Name	Microbial load (cfu/ml)				
	TVBC	TFC	*Staphylococcus* spp.	TCC	*Pseudomonas* spp.
Red	9.1×10^2	0	0	0	0
Yellow	8.6×10^2	0	0	0	0
Green	7.2×10^2	0	0	0	0
Blue	9.3×10^2	0	0	0	0

Table 2. Microbial analysis of 'Food Flavours'

Sample Name	Microbial load (cfu/ml)				
	TVBC	TFC	*Staphylococcus* spp.	TCC	*Pseudomonas* spp.
Vanilla	2×10^1	0	0	0	0
Chocolate	2.1×10^2	0	0	0	0
Strawberry	4.1×10^2	0	0	0	0
Mango	3.6×10^2	0	0	0	0
Lemon	4.4×10^2	0	0	0	0
Orange	2.5×10^2	0	0	0	0

Table 3. Microbiological analysis of other additives

Sample Name	Microbial load (cfu/ml)				
	TVBC	TFC	*Staphylococcus* spp.	TCC	*Pseudomonas* spp.
Baking Powders	3×10^1	0	2×10^1	0	4×10^1
Corn flower	5×10^1	7×10^1	0	0	0
Monosodium glutamate	0	0	0	0	0
Zero calories	0	0	0	0	0
Sodium benzoate	0	0	0	0	0
Saccharine	8.5×10^2	10	0	0	0

TVBC = Total viable bacterial count; TFC = Total fungal count; TCC = Total coliform count.

Table 4. Antimicrobial activity of food flavors

Flavors	Organisms					
	E. coli	*Bacillus* spp.	*Pseudomonas* spp.	*Klebsiella* spp.	*Salmonella* spp.	*Staphylococcuss* spp.
Mango	0	8 mm	12 mm	0	14 mm	12 mm
Chocolate	0	0	0	0	0	0
Vanilla	16 mm	22 mm	20 mm	20 mm	22 mm	14 mm
Strawberry	15 mm	12 mm	10 mm	0	10 mm	12 mm
Lemon	10 mm	9 mm	11 mm	12 mm	8 mm	10 mm
Orange	8 mm	10 mm	7 mm	0	0	0

Table 5. Antimicrobial activity of other food additives

Preservatives	Organisms				
	E. coli	***Bacillus* spp.**	***Pseudomonas* spp.**	***Klebsiella* spp.**	***Staphylococcus* spp.**
Sodium Banzoite	0	0	0	0	0
Baking flower	0	0	0	0	0
Saccharine	0	0	12 mm	0	0
Corn flower	15 mm	0	15 mm	0	0
Zero calories	12 mm	0	0	0	0
Monosodium glutamate	18 mm	0	12 mm	0	0

study. A recent report has shown to extend the shelf-life of fruit juice sodium benzoate is effective but in our study we did not found any antibacterial activity of this sample (20, 23).

Maintenance of food quality and stability during the storage condition is one of the critical point and challenges for food industry (20). In this regard, both natural and artificial preservatives play a major role in foods to slow down spoilage, increase shelf life and preserve color and essence. Apart from these they inhibit the action on spoilage microorganisms, thereby preventing food poisoning and related illnesses. Preservatives make the environment unfavorable for microbes to survive by lowering the pH, enhancing salt content, and bringing down water activity (21). In addition, newer alternatives, including natural antimicrobials derived from plants, animals, and microbes have been explored for potential application in foods. Food additives are completely monitored by codification worldwide. In the US, Food and Drug Administration (FDA) evaluates the safety of unapproved food additives to determine whether they should be approved. The evaluation includes the amount of the substance that would normally be consumed as well as short and long-term health effects and other safety considerations.

CONCLUSION

This study has examined the quality and antimicrobial activity of food additives. Additives help to assure the availability of wholesome, appetizing and affordable foods that meet consumer's demands from season to season. Food additives have widely been applied in food industry to prevent the food borne pathogen and maintain the organoleptic properties of food. Due to some side effects of artificial preservatives, natural preservatives have gained interest. Sometimes the reaction of preservatives can be life-threatening. It is suggested that consumers should try to consume preservatives as less as possible with their diet. Food manufacturing companies should take proper care and follow the recommended guidelines and limit during the application of additives and preservatives.

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