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ASSESSMENT OF IMPROPERLY STORED TETRA PACK FOODS FOR THEIR MICROBIOLOGICAL QUALITY AND SAFETY

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Abstract:

The improper storage of Tetra Pack foods poses significant risks to their microbiological quality and safety, potentially compromising consumer health. Samples were subjected to controlled storage conditions mimicking improper handling, followed by microbiological analysis to quantify total viable counts (TVC), This study assessed the microbiological quality and safety of seven Tetra Pack food samples (Mango Juice, Milk, Soya Milk, Buttermilk, Flavoured Milk, Tomato Puree, and Coconut Milk) exposed to environmental conditions for 8 days. Samples were analyzed periodically on the 2nd, 4th, 6th, and 8th days to evaluate contamination levels. Total colony counts increased progressively over time, with 45 bacterial isolates identified. Among these, 4 were gram-positive cocci (9%), including *Staphylococcus aureus* (75%) and coagulase-negative Staphylococci (25%); 21 were gram-negative rods (47%), and 20 were gram-positive rods (44%). Predominant pathogens included *Enterobacter aerogenes*, *Kluyvera ascorbata*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Bacillus* species. Specific pathogens varied by product: Mango Juice contained *E. aerogenes*, *Bacillus coagulans*, and *S. aureus*; Milk harbored *K. ascorbata*, *Bacillus simplex*, and *P. aeruginosa*; and Tomato Puree showed *S. enterica*, *B. coagulans*, and coagulase-negative Staphylococci. Additionally, five crunchy snacks and five beverages were tested for non-permitted food colors. Metanil yellow was detected in three snacks, and fast red dyes were found in two beverages, indicating the use of unauthorized additives. These findings highlight the risks of improper storage and adulteration in Tetra Pack foods, emphasizing

the need for stringent quality control measures to ensure food safety and prevent public health hazards.

Keywords: Tetra Pack, Microbial contamination, Spoilage, Food poisoning,

Introduction:

Plastic-coated paper cartons, commercially known as Tetra Paks, are widely used in the food industry for packaging beverages such as fruit juices, milk, yogurt, and other liquid or semi-solid foods. These cartons provide a significant microbial shelf life compared to other packaging methods, making them ideal for perishable products (Tetrapak International, 2011). The packaging consists of five layers coated with aluminum or plastic, which protect the contents from moisture, shear stress, and air or flavor escape. For short shelf-life products like dairy, low-density polyethylene is used as a barrier coating, while aluminum foil combined with polyethylene is used for longer shelf-life products. The outer plastic coating ranges from 12-20 g/m², and the inner coating ranges from 15-60 g/m².

Liquid foods are packaged under highly sterile conditions to ensure safety and longevity. Advances in food processing technologies have further extended the shelf life of products packaged in these cartons, allowing perishable items like milk and fruit juices to be stored without refrigeration (Beth Terry, 2007). Despite these advancements, food poisoning remains a concern, often caused by bacterial contamination, which may not alter the taste or appearance of the product (Cecilia *et al.*, 2019).

Food poisoning is a significant global health issue, caused by the ingestion of contaminated food containing bacteria, viruses, chemicals, or toxic metals like lead or cadmium. Bacterial contamination is the most common cause, with pathogens such as *Salmonella*, *Staphylococcus aureus*, *Clostridium botulinum*, *Escherichia coli*, *Shigella*, and *Campylobacter* being major culprits. According to the World Health Organization (WHO), around 600 million people fall ill annually due to contaminated food, resulting in approximately 425,000 deaths, including 125,000 children (Cecilia *et al.*, 2019).

Health and Economic Impacts

Microbial contamination not only poses serious health risks but also leads to significant economic losses in the food industry. Food spoilage caused by microorganisms during storage and distribution affects food quality and shelf life, while pathogenic microorganisms can cause severe infections. Proper sealing and sterilization of food in impermeable containers are essential to prevent microbial colonization (Adams and Moss, 2008).

Common Foodborne Bacterial Illnesses

Common foodborne bacterial illnesses include salmonellosis, caused by *Salmonella* species (primarily *S. enterica* and *S. bongori*), presenting with diarrhea, fever, and headache, and preventable through proper cooking and hygiene (Jay, 2000). *Staphylococcus aureus*, caused by enterotoxin-producing strains, leads to sudden vomiting and diarrhea, with prevention relying on proper food handling and refrigeration (Adams and Moss, 2008). *Clostridium botulinum*, producing a neurotoxin, causes severe symptoms like vomiting, respiratory paralysis, and can be fatal, necessitating proper canning and cooking practices (Jay, 2000).

Enterohemorrhagic *E. coli* (EHEC), particularly strain O157:H7 , results in diarrhea and abdominal cramps, with prevention requiring thorough cooking of meat and avoiding cross-contamination (Buchanan and Doyle, 1997). Shigellosis, caused by *Shigella* species, presents with abdominal pain and bloody diarrhea, preventable through hygiene and proper cooking (CDC, 2011). Campylobacteriosis, caused by *Campylobacter jejuni* from poultry, causes fever, nausea, and diarrhea, with prevention emphasizing proper poultry cooking and food handling hygiene (Quinn *et al.*, 2001).

Detection and Control of Pathogens

Detection of foodborne pathogens involves culturing samples on selective media and identifying the organisms through biochemical tests or molecular techniques like PCR. Prevention strategies include proper cooking, refrigeration, and hygiene practices. Education of food handlers and consumers is also crucial in reducing the risk of foodborne illnesses (WHO, 2008).

Plastic-coated paper cartons like Tetra Paks have revolutionized food packaging by extending the shelf life of perishable products. However, microbial contamination remains a significant challenge, leading to foodborne illnesses with severe health and economic

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consequences. Proper food handling, cooking, and storage practices, along with advancements in detection technologies, are essential to ensure food safety and prevent outbreaks of foodborne diseases.

Methodology:

Sample collection and Processing:

A total of seven Tetra Pack food samples were collected and were processed for the isolation of bacterial pathogens. The samples included mango juice, milk, soy milk, buttermilk, flavoured milk, tomato puree and coconut milk. The Tetra Packs were punctured to a pinhole size opening and were exposed for 8 days. The first sample was collected from the day the Tetra Pack was punctured and was serially diluted to determine the load of microbes in the food. Likewise, the samples were collected on 2^{nd}, 4^{th}, 6^{th} and 8^{th} day.

Enumeration of Tetra Pack food samples by total plate count:

Total viable aerobic bacteria of Tetra Pack food samples were enumerated by standard plate count (SPC) method. 1 gm of food sample was taken and it was serially diluted up to 10^{-7} dilutions with 9 mL sterilized peptone water. 0.1 ml of the diluted sample suspension from 10^{-7} dilution was spread platted on nutrient agar plates and were incubated at 37°C for 24 hours. After incubation, the plates having well-paced colonies (30–300) were used for counting and the colonies were counted by a colony counter. Total viable aerobic count per mL or per gram was calculated by multiplying the average number of colonies per plate by reciprocal of the dilution and expressed as colony forming units (cfu) per mL or per g of sample.

$$\text{CFU} = \frac{\text{Total number of colonies}}{\text{Dilution factor X Volume of the sample}}$$

Isolation of bacteria:

The food samples from the Tetra Pack were also inoculated on Nutrient Agar (NA), Mannitol Salt Agar (MSA), Blood Agar (BA) and MacConkey Agar (MAC) for the bacterial isolation. The plates were incubated at 37°C for 24 hours. Next day the colonies were picked up and gram staining, motility, catalase and oxidase were done. The bacterial isolates were identified as per the standard protocol.

Detection of non-permitted colours (metanil yellow and fast red) in food beverages:

About 0.1 gm of samples (crunchy snacks) was taken in the test tube and then about 1 ml of propanol was added to dissolve the sample. 5-10 drops of HCL was added to the sample and then the color of the sample was observed. Presence of pink colouration indicates the presence of metanil yellow. For the detection of fast red colour 1 ml of the sample was taken in a clean test tube. 2ml of isopropanol and hydrochloric acid were added and kept undisturbed. The presence of non-permitted colour is indicated by the dispersion of the colour to the acetic phase.

Results:

A total of seven Tetra Pack food samples were collected which included Mango Juice, Milk, Soya Milk, Buttermilk, Flavoured Milk, Tomato Puree and Coconut Milk which were processed for the presence of bacterial pathogens.

Enumeration of Tetra Pack food samples by total plate count:

Total viable aerobic bacteria of Tetra Pack food samples were enumerated by standard plate count (SPC) method. (Table 1)

Table 1: Standard Plate Count (CFU/ml)				
Name of the sample	**Day 2**	**Day 4**	**Day 6**	**Day 8**

Mango Juice	109	190	184	TNTC
Milk	145	153	217	284
Soya Milk	138	162	245	TNTC
Butter Milk	225	296	TNTC	TNTC
Flavoured Milk	102	149	234	TNTC
Tomato Puree	108	408	TNTC	TNTC
Coconut Milk	189	336	TNTC	TNTC

Legend: TNTC- Too Numerous To Count

The Tetra Pack food samples were pierced and were exposed to the environment for a total period of 8 days. The samples were collected periodically on 2nd, 4th, 6th and 8th day to assess the level of contamination leading to food poisoning. It was observed that the total colony counts gradually increased as the number of days.

Prevalence of bacterial isolates from Tetra Pack food samples:

A total of 7 samples were collected from Tetra Pack foods and were processed, of which 45 bacteria were isolated and identified. Among the isolates, 4 were found to be gram-positive cocci in clusters (9%) followed by 21 gram-negative rods (47%) and 20 were gram-positive rods (44%).

Prevalence of gram-positive cocci:

Among 4 gram-positive cocci in clusters, 3 (75%) were found to be *Staphylococcus aureus* and 1 (25%) was found to be Coagulase negative Staphylococci (CoNS).

Prevalence of gram-negative rods:

A total of 21 different bacterial species were identified from Tetra Pack food samples. The prevalence of bacterial isolates was as follows- *Kluyvera ascorbata, Enterobacter aerogenes, Leminorella grimontii, Salmonella enterica* and *Pseudomonas aeruginosa.* (Table 2)

Table 2: Prevalence of gram-negative bacteria (n =21)

S.No	Name of the organisms	Percentage of isolation
1	*Kluyvera ascorbate*	7(33%)
2	*Enterobacter aerogenes*	6(28%)
3	*Leminorella grimontii*	4(19%)
4	*Pseudomonas aeruginosa*	2(10%)
5	*Salmonella enterica*	2(10%)

Prevalence of gram-positive rods:

A total of 20 gram-positive rods were identified from Tetra Pack food samples. The prevalence of bacterial isolates was as follows- *Bacillus simplex, Bacillus validus, Bacillus firmus, Panibacillus alginolyticus* and *Bacillus coagulans.* (Table 3)

Table 3: Prevalence of gram-positive bacteria (n =20)

S.No	Name of the organisms	Percentage of isolation
1	*Bacillus simplex*	5(25%)
2	*Bacillus validus*	4(20%)

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3	*Bacillus firmus*	3(15%)
4	*Paenibacillus alginolyticus*	4(20%)
5	*Bacillus coagulans*	4(20%)

Table 4: Distribution of bacterial pathogens among Tetra Pack food sample:

S.No	Name of the samples	Type of bacteria found
1	Mango juice	*Enterobacter aerogenes, Bacillus coagulans, Staphylococcus aureus*
2	Milk	*Kluyvera ascorbate, Bacillus simplex, Pseudomonas aeruginosa*
3	Soya milk	*Enterobacter aerogenes, Bacillus firmus*
4	Butter milk	*Bacillus validus, Kluyvera ascorbate, Staphylococcus aureus*
5	Flavoured milk	*Enterobacter aerogenes, Bacillus simplex*
6	Tomato puree	*Salmonella enterica, Bacillus coagulans, CoNS*
7	Coconut milk	*Leminorella grimontii, Paenibacillus alginolyticus, Kluyvera ascorbata*

The predominant bacterial pathogens found in mango juice were *Enterobacter aerogenes, Bacillus coagulans, Staphylococcus aureus.* Milk showed the presence of *Kluyvera ascorbata, Bacillus simplex and Pseudomonas aeruginosa.* Soya milk showed the presence of *Enterobacter aerogenes and Bacillus firmus.* Butter milk showed the presence of *Bacillus validus, Kluyvera ascorbate,*

Staphylococcus aureus. Bacterial pathogens found in flavoured milk were *Enterobacter aerogenes* and *Bacillus simplex.* Tomato puree showed the presence of *Salmonella enterica, Bacillus coagulans* and *CoNS.* Coconut milk showed the presence of *Leminorella grimontii, Paenibacillus alginolyticus* and *Kluyvera ascorbate.* (Table 4)

Detection of non-permitted colours (metanil yellow and fast red) in food beverages:

Five crunchy snacks were tested for the presence of metanil yellow and five beverages were tested for the presence of fast red dyes which are considered as non-permitted food colours. Three out of five crunchy snacks showed positive results for the presence of metanil yellow and two out five beverages showed positive results for the presence of fast red dyes in the food samples which confirmed the addition of non-permitted colours in the foods. (Table 5)

Table 5: Detection of non-permitted colours (metanil yellow and fast red) in food beverages

Samples	Chemicals added	Results
Crunchy Snack 1	Propanol + HCl	Appearance of pink colour
Crunchy Snack 2	Propanol + HCl	Absence of pink colour
Crunchy Snack 3	Propanol + HCl	Appearance of pink colour
Crunchy Snack 4	Propanol + HCl	Absence of pink colour
Crunchy Snack 5	Propanol + HCl	Appearance of pink colour
Beverage 1	Propanol + HCl + Acetic acid	Absence of pink colour
Beverage 2	Propanol + HCl + Acetic acid	Appearance of pink colour
Beverage 3	Propanol + HCl + Acetic acid	Appearance of pink colour
Beverage 4	Propanol + HCl + Acetic acid	Absence of pink colour
Beverage 5	Propanol + HCl + Acetic acid	Absence of pink colour

Discussion:

This study investigated the microbial contamination of various packaged food products and the presence of non-permitted food colorings in snacks and beverages. Paperboard, a common packaging material (Rhim, 2010), can harbor microorganisms due to its biodegradable nature and the conducive environment of paper production (Välsänen, 1991). While some countries have established microbial limits for food packaging, a global consensus on acceptable levels is lacking. This is a concern, as food packaging plays a critical role in food safety.

The study examined several products. Mango juice revealed the presence of *Enterobacter aerogenes*, *Bacillus coagulans*, and *Staphylococcus aureus*. Juice contamination can occur at any stage of processing, with spoilage organisms and pathogens posing a risk (Bello, 2014). Yeasts and molds are common juice contaminants due to their acid tolerance, while inadequate processing can introduce pathogenic bacteria like *E. coli* O157 and *Salmonella* (Song *et al.*, 2010).

Milk, a nutritious medium for microbial growth (Aebi *et al.*, 2015), showed contamination with *Kluyvera ascorbate*, *Bacillus simplex*, and *Pseudomonas aeruginosa*. Contamination sources in milk include milking equipment, air, soil, and unhygienic handling (Afroz *et al.*, 2013). Psychrotrophic bacteria like *Pseudomonas* spp. can cause spoilage, while antibiotic-resistant bacteria pose a public health threat (Sharma and Malik, 2012). Soy milk, a protein-rich beverage, contained *Enterobacter aerogenes* and *Bacillus firmus*. Its nutrient content makes it susceptible to microbial spoilage (Momoh *et al.*, 2011;), with coliform bacteria indicating hygienic standards.

Buttermilk, a fermented dairy product, showed the presence of *Bacillus validus*, *Kluyvera ascorbate*, and *Staphylococcus aureus*, while flavored milk contained *Enterobacter aerogenes* and *Bacillus simplex*. Psychrotrophic bacteria can impact flavor in dairy products. Tomato puree, a widely consumed product (Valadez *et al.*, 2012), was contaminated with *Salmonella enterica*, *Bacillus coagulans*, and CoNS. Contamination can occur during harvesting, handling, and processing (Garg *et al.*, 2013; Wogu and Ofuase, 2014).

Coconut milk contained *Leminorella grimontii*, *Paenibacillus alginolyticus*, and *Kluyvera ascorbate*. While coconut products have reported health benefits (Khoramnia *et al.*, 2013), contamination with bacteria like *Staphylococcus* spp. and *E. coli* is a concern (Thapaliya *et al.*, 2014; Shin *et al.*, 2016; Jensen *et al.*, 2019).

Finally, the study investigated the use of non-permitted food colorings. Three out of five crunchy snacks tested positive for metanil yellow, and two out of five beverages contained fast red dyes. Synthetic food colors, while offering advantages in cost and stability (Rezaei *et al.*, 2014; Naseem *et al.*, 2015; Purba *et al.*, 2015), can pose health risks (Bachalla, 2016), highlighting the need for regulation and monitoring.

Conclusion:

In conclusion, this study examined the microbial contamination of Tetra Pack packaged fruit juices and other foods after opening and exposure to the environment. While Tetra Pack's aseptic packaging is a significant food preservation innovation, the study revealed that these products are susceptible to contamination once opened. Various bacteria, including some potentially harmful ones like Staphylococcus aureus and Salmonella enterica, were detected in the samples after 8 days of exposure. This highlights the importance of proper handling and storage of Tetra Pack products after opening to minimize the risk of foodborne illnesses. The study suggests that predictive microbiology and risk assessment could be valuable tools for improving food safety measures and ensuring the quality of packaged foods for consumers.

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