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DETECTION THE PRESENCE OF BACTERIA IN VEGETERIAN BABY FOOD PRODUCTS IN SRI LANKAN MARKET

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***Abstract*— Vegetables are an important part of a healthful diet. They provide vitamins, minerals and fiber to help keep our body healthy. Vegetarian baby food known as without flesh foods (meat, seafood, poultry, wild game and their products) products are important components of a healthy and balanced diet which have the major source of fiber, iron, manganese and copper (Metals, 2019). However, vegetarian diet include a several of plant based foods such as grains, legumes, fruits, seeds and vegetables (Gracias and Mckillip, 2004).**

Vegetarian baby foods act major role of baby's health and nutrition before digestion of supplementary materials. These food materials have a high content of proteins, vitamins, lipids, carbohydrate and other required ingredients for baby (Rawat, 2015). According to the heart foundation, a well-balanced diet should include at least five portion of vegetables and fruits a day of variable types and that is known as a well-balanced diet (Sevcik and Rajchl, 2009).

***Index Terms*— Srilanken Market, Baby Food Products, Bacteria.**

I. INTRODUCTION

Vegetarian lifestyle is common choice of the people and it is becoming more popular among local families in Sri Lanka. Sri Lanka is an island which have many variation of climatic, climatic changes and temperature. With naturally occurring resources for a profitable agro industrial economy which also claims of an age old traditional agricultural base. Because of these reasons a wide range of vegetables and fruits flourish on our land.

Recently, because of busy life styles preparation of home meals have already led to the appearance of readymade baby foods products on the market. That is known as convenience foods and these foods are partially or fully cooked when packed. Vegetarian baby foods are becoming popular among working mothers in Sri Lanka which can be either local or imported products which is easy to cook and time serving method.

A. Why processed baby food products are important

The fresh and processed foods make up vital parts of the food supply and processed foo contributes to both food security and nutrition security that ensuring the food quality meets human nutrient needs (Calabretti et al., 2017).

All parents in the world wish they could feed their children homemade, various, healthy and tasty baby foods every day. In today worlds, most of busy mothers don't have the extra time to cook on a daily basis. So, when it comes to choose a brand for their baby food, they deeply anxious about the quality of the ingredients used in the meals. In Sri Lankan market prepared baby food products are very popular because it has more natural and shorter ingredients list.

B. Well-balanced nutrition of the pre prepared baby foods

The composition of commercial vegetarian baby food may contribute to present and future health benefits of young children. Since infants between 6 month and 3 years of age are rather limited in their food choices (Metals, 2019). Normally, the commercial baby food products serve as the important source of energy, basic nutrients, fiber, vitamins and minerals to establish their taste and eating patterns. When babies first begin eating they need specialized foods. That food must be nutritious and softly, it is better if the food is conveniently packed (Sevcik and Rajchl, 2009). Basically, the nutritive value of baby food deeply depends on the composition of the raw materials and the proportions of vegetable content (Calabretti et al., 2017). Sri Lankan farmers recently launched a brand new, colourful and attractive partnership to review the popular brands.

Additionally, to a package that has recently been designed by busy mothers who need more than the right amount. It is mainly made from corn, rice, soya and green gram, vitamin A, B, E and minerals and tymines which help children of the nation to be nourished to help their children's mind and body (Chakraborty et al., 2014).

C. Types of bacteria can identify in baby vegetarian food products

Infants are more sensitive than adults to food contaminants due to a higher rate of uptake by the gastrointestinal tract, an incompletely developed blood-brain barrier, an undeveloped detoxification system and high foo consumption relative to body mass (Toscano et al., 2013). Occasionally, vegetables can

become contaminated with harmful bacteria or viruses, which are also known as pathogens because food products have a rich source of nutrients and that can be an excellent medium for bacterial growth (Dashti, Jadaon and Dashti, 2009). Due to this bacterial growth, babies can be infected by bacteria or other infected pathogens and the major family of pathogens associated with food are members of Enterobacteriaceae. These bacteria are widespread in nature in soil, on plant surfaces and in digestive tracts of animals and are therefore present in many foods (Abdulamir et al., 2010).

1) Description of foodborne bacteria

a) Escherichia coli

E.coli is generally non-pathogenic character and is a catalase positive, oxidase negative, gram negative and non-spore forming rods (Kornacki and Marth, 1982). A neutral pH is optimal for growth of E.coli and to prevent food poisoning include educating food workers in safe food handling techniques and proper personal hygiene, properly heating food to kill pathogens and holding foods under appropriate conditions to avoid bacterial manipulation (Vidic et al., no date).

b) Salmonella spp

Most species of genus Salmonella, normally identified as human pathogens because they differ characteristics and the severity of the illness they cause (Rajwar, Srivastava and Sahgal, 2015). The one of the most important causes of food borne illness can caused from salmonella. They are gram negative, non-spore forming rods which are faculatively anaerobic which catalase positive and generally motile with peritrichous flagella. Salmonella are heat sensitive and readily destroyed by pasteurization temperatures (Hiranyada et al., 2018).

c) Listeria monocytogenes

Listeria monocytogenes is a gram positive, non-spore forming, rod shaped, facultative intercellular bacterium. Its ability to grow at temperatures as low as 00C permits multiplication at typical refrigeration temperatures, greatly increasing its ability to evade control in human foodstuffs (Sevcik and Rajchl, 2009).

d) Bacillus cereus

Bacillus cereus is a gram positive, rod shaped, facultative anaerobic, motile, beta hemolytic, spore forming bacterium commonly found in soil and food (Molnar et al., 2010).

e) Shigella spp

Shigella is a genus of bacteria that is gram negative, facultative anaerobic, non-spore forming, nonmotile, rod shaped and genetically closely related to E.coli. Shigella is one of the leading bacterial causes of diarrhea (Villalobo and Torres, 1998).

f) Staphylococcus aureus

This bacteria is a gram positive, round shaped bacterium that is a member of the Fimicutes, and it is a usual member of the microbiota of the body (Kikuchi et al., 2002).

g) Clostridium spp.

Clostridium is a genus of gram positive bacteria. This genus includes several significant human pathogens, including the causative agents of botulism and tetanus. They are obligate anaerobes capable of producing endospores (Gracias and Mckillip, 2004).

When babies ready to eat low moisture content of dehydrated vegetarian food products they act as inhibitory factors with respect to any bacterial spores or vegetative that have survived drying or processing. These bacteria cannot act any direct role and grow in their spoilage (Biology, 2000). The quantity of the products are considered as an indicator of the hygiene standards of their production, processing and control. However, the remanufactures baby foods are considered a type of food that is very susceptible to infants for bacterial infections (Weaver et al., 2014).

To find out the pathogenic bacteria or other microorganisms, it is important to observe typical decomposition organisms in order to decrease losses during production. Bacteria and other germs are naturally present on the food and food surface (eg: fresh vegetables and fresh fruits) or the production of food products (Gunaratne and Corke, 2015).

In food industry, they has different categories of microbiological contamination:

- Pathogenic bacteria
- Spoilage organisms
- Bacterial toxins
- Indicator organisms.
- Viruses

D. Food borne hazards in Sri Lanka

As a developing country, food or water borne diseases are the key issues in food safety assurance in Sri Lanka. Bacterial diarrhea and hepatitis A, salmonella infections are common sources of food borne diseases in our country (Lavindi, 2018). Listeria monocytogenes can contaminated with the highest incidence in different vegetarian market food items.

Table 1: Number of food poisoning cases reported by medical officers of health in Sri Lanka.

Year	Food poisoning cases
2008	1763
2009	1103
2010	1671
2011	1277

Table 1 represented the occurrence of L.monocytogenes in different food items in the market. 38% of all tested samples were contaminated with the highest incidence found among vegetables.

E. Detection of microorganisms in the vegetarian food products

Vegetarian baby food samples can be tested with test tools for bacteria and the general screening tests for microbiology are performed by Polymerase Chain Reaction (PCR), ELISA tests, feed disks and agar trays. The conventional methods implemented in food analysis consist of sample homogenization and subsequent culturing of the microorganisms on agar plates followed by biochemical identification.

1) Susceptibility testing for bacteria

The susceptibility test was performed to identify significant bacterial isolates and to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infection (Buller and Court, 2013).

a) Susceptibility testing methods

BROTH DILUTION TESTS

This is the oldest antimicrobial susceptibility testing method which can performed by two ways, using macro dilution or micro dilution methods. These methods involve the preparation of twofold dilutions of antibiotics in a liquid growth medium dispensed in test tubes (Hudzicki, 2016).

b) Antimicrobial gradient method

This is the commercial method that provide micro-dilution panels, instrumentation and automated reading of plates which intended to reduce technical errors and time wastage (Use, 2012).

c) Disk diffusion method

This is the routinely used method for determining antimicrobial resistance. Muller Hinton agar is considered to be the best media for this method. Because it shows the acceptable batch to batch reproducibility for susceptibility testing and gives satisfactory growth of nonfastidious pathogens (Buller and Court, 2013).

d) Agar dilution method

Agar dilution method involves the incorporation of varying concentrations of antimicrobial agent into an agar medium by using serial twofold dilutions, followed by the application of a defined bacterial inoculum to the agar plates. This method has the ability to rest multiple bacteria and the potential to improve the identification of minimum inhibitory concentration (Use, 2012). The culture media which used for the bacterial culturing is represented in the Table 2.

Table 2: Bacterial Culture Media

Bacteria	**Culture media**
E.coli	LB medium/ MacConkey agar
Listeria monocytogenes	Brain Heart Infusion
Salmonella	Bismuth Sulfite Agar
Clostridium spp.	Sulfite Polmyxin Sulfadizine Agar
Staphylococcus aureus	Blood Agar
Shigella	MacConkey Agar/Xylose Lysine Deoxycholate Agar
Bacillus cereus	Nutrient Agar

After culturing the bacteria, they are inoculated to extract the DNA.

2) DNA Extraction methods to identify the inoculated bacterial DNA

a) Wizard Genomic DNA purification kit

This wizard genomic DNA purification kit provides a simple and solution based method for isolation of DNA from white blood cells, tissue culture cells, animal tissues, plant tissues an gram negative or gram positive bacteria. DNA purified with this system is suitable for a variety of applications digestion with restriction endonucleases and membrane hybridizations. For isolation of DNA from bacteria, there is a four main steps.

Purification
RNase digestion
Salt precipitation
Isopropanol precipitation

b) MiniBEST bacteria genomic DNA extraction kit

This kit is designed to purify genomic DNA from variety of bacteria including both gram positive and gram negative. For this method, used special lysis buffer in combination with DNA preparation membrane to efficiently purify gnomic DNA from bacteria.

c) DNA extraction via modified heat shock/boiled-cell method

Using this method can break the cells and release the DNA because of lysing agents containing different chemicals like lysozyme, proteinase K, TWEEN20, sodium hydroxide/ sodium dodecyl sulfate, guanidine isothiocyanate and Triton X-100 as chemical agents. The physical factors including for this is heating, cooling, freezing, microwave irradiation, beads beating, magnetic field capturing, binding to glass beads and etc…

3) Polymerase Chain Reaction (PCR)

Gene amplification and sequencing have led to the discovery of new pathogens as gents of disease and have enabled us to better classify microorganisms from culture. For bacteria, mycobacteria and fungi, many gene targets have been

recognized as useful tools for identification. After determining the most efficient method of DNA extraction, the PCR is conducted for DNA extracted from samples enriched and standard bacteria (Atawodi, 2016).

PCR is a molecular biology technique used to amplify a minute amount of DNA and the key ingredients of PCR reaction contain Taq polymerase, template DNA, primers, $MgCl_2$ and nucleoids. There are three basic steps of the PCR including denaturation, annealing and the extension (Brasileira *et al.*, 2013).

- Denaturation happens at 94^0C which breakdown the hydrogen bonds and denatures that into single stranded form.
- At the annealing stage the temperature is decreased to 50-65^0C and under this low temperature the DNA primers are attach to complementary sequence.
- Extension happens at 72^0C and at this step the reaction temperature is raise to make new strand of DNA by the Taq polymerase enzyme.

Polymerase Chain Reaction can changed the way of performing microbiological analyses towards the detection of specific microbial DNA as target, because some pathogens like many Salmonella and Campylobacter strains may be viable but non-culturable (Profile, 2017). This incident caused for their detection can leads to a false negative result and a failure in pathogen detection. PCR technique is widely employed in food safety analysis. Moreover many kits have been developed to facilitate food testing which is speed and simplicity of utilization (Brasileira *et al.*, 2013).

PCR based detection of pathogens requires a pre-enrichment step to increase the number of cells for detection of DNA from dead bacteria. For achieve this problem can add cell membrane impermeable dye to PCR reagents which can penetrate only into dead cells that ensures only food scontaminated with living bacterial cells produce an amplicon (Atawodi, 2016).

Classic methods to identify bacteria are based on characteristics observed in known strains with predictable biochemical and physical properties under optimal growth conditions. The gene target that is most commonly used for bacterial identification is 16S rRNA an~1500 base pair gene that codes for a portion of the 30S ribosome.

a) Primers used to identify the food borne pathogens

PRIMERS TO IDENTIFY E.COLI BACTERIA

The primers which are used to identify E.coli is presented in the below table.

Table 3: Primers used to identify E.coli

Name of the primer	**Primer sequence (5'-3')**	**Product size (bp)**
ECP79F	GAAGCTTGCTTCTTTGCT	79-96
ECR620R	GAGCCCGGGGATTT CACAT	602-620
ECB75F	GGAAGAAGCTTGCTTCTTTGCTG	75-97
ECA75F	GGAAGAAGCTTGCTTCTTTGCTGAC	75-99
ECR619R	AGCCCGGGGATTTCACATCTGACTTA	594-619

According to the above primer table, there are three set of primer pairs are designed and tested. The primer pairs and the optimal temperature used for experiments are shown in the table. This probe targeted a 16s rRNA gene sequence conserved in the domain bacteria and occurring near the center of the PCR products generated by all primer pairs. The selective amplification of E.coli occurred only when the annealing temperature in the PCR was elevated to 72°C, which is 10°C higher than the optimum for the primers (Oh *et al.*, 2009)

Table 4: Optimal temperatures and excepted PCR product size for the primer pairs

Primer pairs	Optimal melting temperature (^{0}C)	Base pair length (bp)
ECP79F-ECR620R	55	541
ECB75F-ECR620R	59	545
ECA75F-ECR619R	60	544

PRIMERS TO IDENTIFY LISTERIA SPECIES

There are six sets of primers targeted against 16S rRNA and virulence genes such as iaq, hly and prf was evaluated in separate PCR assays. The iaq gene encodes the protein p60, which is common to all Listeria species. Based on the iaq DNA sequence comparison, primer combination for the specific identification by PCR of all serotypes of L.monocytogenes (primers MonoA and MonoB) (Kumar and Grover, 2015).

PRIMERS TO IDENTIFY SALMONELLA SPP

The table 5 represented the primers which are used to identify Salmonella spp in food products (Paião, Arisitides and Murate, 2013).

Table 5: Primers used for identification of Salmonella spp

Primers	Length	Primer sequence (5'-3')	Amplification product (bp)
Inv-A forward	22	CGG TGG TTT TAA GCG TAC TCT T	796
Inv-A reverse	21	CGA ATA TGC TCC ACA AGG TTA	
IE-1 forward	20	AGT GCC ATA CTT TTA ATG AC	316
IE-2 reverse	19	ACT ATG TCG ATA CGG TGG G	
Flic-C forward	20	CCCGCTTACAGGTGGACTAC	432
Flic-C reverse	20	AGCGGGTTTTCGGTGGTTGT	

PRIMERS TO IDENTIFY CLOSTRIDIUM SPP

The specific detection of the Clostridium spp is based on PCR amplification of the 16S rRNA gene using oligonucleotide primers Clos58-f AAAGGAAGATTATACCGCATAA and Clos780-r AATCTTGCGACCGTACTCCCC which PCR product size is 722bp (Dore and Popoff, 2005).

PRIMERS TO IDENTIFY STAPHYLOCOCCUS AUREUS

The 16s rRNA gene is part of all bacteria and the sequence of 16s rRNA gene are indicted variable regions because the use of the 16srRNA DNA fragment, amplified by using specific oligonucleotides. Staphylococcus aureus is the only known bacterium that produces coagulase enzymes, which can determine by the coa gene. The previous studied proved that, coa gene is directly related to the coagulase test (Biology, 2000).

Table 6: Primers to identify Staphylococcus aureus

Name of the primer	**Primer sequence (5'-3')**	**Product size (bp)**
Coaf	ATAGAGCTGATGGTACAGG	674
Coar	GCTTCCGATTGTTCGATGC	

PRIMERS TO IDENTIFY BACILLUS CEREUS

To identify the Bacillus cereus, the gene motB is used and that encode flagella motor protein MotB classified as an outer membrane protein which is going to use in this experiment. The target gene to be detected was a fragment of the motB gene, and the PCR primer set is called BCFomp1/ BCRomp1. The size of the PCR products for these primers is 575 bp (Molnar *et al.*, 2010).

PRIMERS TO IDENTIFY SHIGELLA SPP

The use of PCR to amplify a specific virA gene fragment serve as a highly specific and sensitive method to detect virulent bacteria of the genus Shigella and enteroinvasive Escherichia coli. Amplification of a 215bp DNA band was obtained by using isolated genomic DNA of Shigella. Moreover, a multiplex PCR with specific virA and bacterium restricted (16S ribosomal DNA) primers generated an amplification product of 755bp for all bacteria tested and an additional 215bp product for Shigella (Wang *et al.*, 2015).

Table 7: Primers to identify Shigella spp

Name of the primer	**Primer sequence (5'-3')**	**Product size (bp)**
virAF	CTGCATTCTGGCAATCTCTTCACATC	215
virAR	TGATGAGCTAACTTCGTAAGCCCTCC	

4) Agarose gel electrophoresis

This method is used in biochemistry, molecular biology, genetics and clinical chemistry to separate a mixed population of DNA in a matrix of Agarose. There are some factors which affect migration of nucleic acid in gel (Reddy and Raju, 2014). They are,

- Concentration of the gel- The larger molecules are resolved better using a low concentration gel while small molecules separate better at high concentration gel.
- Size of the DNA fragment being electrophoresed- Smaller molecules travel faster than larger molecules in gel and double stranded DNA moves at a rate that is inversely proportional to the number of base pairs.
- Conformation of the DNA molecule- The movement of the DNA may be affected by the conformation of the DNA molecule (Anderson, Wright and Meksem, 2013).

II. CONCLUSION

The baby food market is expanding in Sri Lanka, especially as more women enter the workforce. This trend was confirmed because that the availability of baby food products that are sweet in taste, cereals with added sugar and that vegetarian

food products promote an environment that enhances sweet taste preferences (Liyanawickramasinghe *et al.*, 2018). The nutritional impact of the baby vegetarian processed foods are nutritionally important to infants' diet (Kim *et al.*, 2011).

Infants are within a vulnerable age group and have a restricted diet compared to other age groups (Rajwar, Srivastava and Sahgal, 2015). Therefore, it is recommended that foods are monitored to ensure safe use. The published surveys of raw fruits and vegetables demonstrate that there is potential for a wide range of these products to become contaminated with microorganisms (Weaver *et al.*, 2014) . Almost the infant baby vegetarian food products have been contaminated with pathogens either from the environment, from human or animal faeces or through storage, processing and handling could potentially cause diseases (Sevcik and Rajchl, 2009).

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