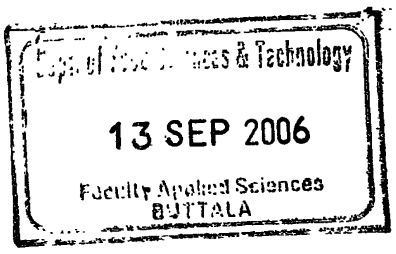
FORMULATION AND DEVALOPMENT OF A CEREAL BASED INFANT FOOD



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A research report submitted in partial fulfilment of the requirement for the Degree of Bachelor of Science in Food Sciences and Technology (Special)

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Declaration

The work described in this thesis was carried out by my self at the food Research unit, Department of Agriculture, Gannoruwa, Peradeniya under the supervision of Dr K.H Saranandha and Mrs K.M Somawathi. A report on this has not been submitted to any other university for another degree.

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AFFECTIONATELY DEDICATED TO
MY PARENTS AND TEACHERS

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ABSTRACT

In the current situation of Sri Lanka, most of mothers show an increasing trend to buy infant foods from market for their babies without preparing them at home and simultaneously since the crude birth rate is increasing at 1.3% annually, the demand is also increasing.

The above phenomenon demands infant foods to be imported from other countries both by government and private sectors to fulfil the market requirements. The situation has mainly arisen, due to lack of production of infant foods in Sri Lanka.

Corn, pumpkin, soya bean, and potato are some broadly cultivated crops in Sri Lanka with constant yields through out the year and contain lots of nutrients in the form of vitamins and minerals which are important to growth and development of infant.

The present research was conducted to utilize, tender corn to produce a semisolid base which contain 79.32% of moisture, 12.98% of carbohydrate, 4.6% of protein, 0.4% of fat, 2.0 % of fiber, 0.7% of ash. The 85% of semisolid base was formulated from 8.7% of pumpkin, 2% of potato and 4% of soya bean oil to reach the nutritional level up to Food and Drug Administration standards.

The major raw material of this product is tender corn which is harvested before contamination with aflatoxin hence the product is free from hazards of *Aspergillus*.

The product shelf life was evaluated at 4^0C-5^0C for three weeks with two types of packaging materials namely Triple Laminated Aluminium Foil and Polypropylene by the aerobic total plate count.

According to statistical analysis results (paired t test) Triple Laminated Aluminium Foil was selected as suitable packaging materials at 4^0C-5^0C for three weeks under Sri Lanka Standard 651(1989) Specification for Infants Formula.

The safety of the product for consumer was tested through determination of Coliform and *Salmonella* under SLS: 651, 1989. The results showed that the product is free from Coliform and *Salmonella* at 4^0C-5^0C for 3 weeks shelf life.

According to the trials and findings finally a good quality infant food was developed with 78.09% of moisture, 11.04% of carbohydrate, 4.06% of protein, 4.4% of fat, 1.76% of fiber and 0.65% of ash.

CONTENTS

ABBREVIATION

cal : Calory
et.al : and others
kg : Kilogram
g : Gram
mg : Milligram
IU : International Unit
ha : Hectare
U.S.A : United State of America
m : Meter
nm : Nanometre
mm : Millimeter
mt : Metric tone
hr : Hours
^{0}**C** : Centigrate
ml : Millilitre
PP : Poly propylene
F.D.A : Food and Drug Administration standards
M : Molarity
Con : Concentrated
UV : Ultraviolet
S.L.S : Sri Lanka Starnds
L : Liter
min : Minutes
% : Percentage
(-) : Nigligible Amounts
(...) : Data Not Available
kJ : Kilojole
mmol : Millimole
µg : Microgram
TLAF : Triple Laminated Aluminium Foil

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CHAPTER 1

1.1 Introduction

The term infant is defined as a child not more than 12 months of age and an infant formula is defined as a food intended for feeding of infants prepared from the milk of cows or other animals and /or other constituents of animal and or plant origin (SLS 561, 1989).

In general after 6 month of age, the body weight of a newborn becomes double. When the baby has reached their 12 months of age his body weight becomes three times of the birth weight. Along with their growth, the nutritional requirements also increase during this time period. Therefore this period which coincides with weaning is a crucial period in the growth and development of an infant. According to this phenomenon it is essential that we supply adequate nutritional requirements in the form of an infant formula in order to build up a healthy body (Wardlaw *et. al*, 2001).

Traditionally, the Sri Lankan mothers are used to nourish their babies with home made infant foods which typically consist of potato, eggs and rice. However mothers currently display an increasing trend to buy infant foods from market (Ministry of Healthcare, 2005).

However in the present context, Sri Lanka is not producing infant formula due to the lack of knowledge and technology. Therefore the government and private sector try to import infant formula to meet this demand. Infant formulae for infants up to 6 months and over 6 months are the two types which are certified by Sri Lanka government (SLS 561, 1989).

Most other developed countries are producing their own infant formula by using cereals, vegetables, meat extracts, milk and milk products as major ingredients. These ingredients are believed to provide the essential nutrients as well as vitamins and minerals which are important to the growth and development of an infant. Also most of lot of these crops are available in Sri Lanka through out the year under Yala and Maha season.

Considering the above situation, this study is focused on the formulation of an infant formula with locally available crops such as maize, pumpkin, potato and Soya bean oil.

1.2 Objectives

1.2.1 Overall objective:

Formulation and Development of a cereal based infant Food.

1.2. 2 Specific objectives:

i. Selection of suitable raw materials (ingredients) and their proportions.

ii. Selection of a suitable package.

iii. Shelf life evaluation.

CHAPTER 2

Literature Review

2.1 Nutrition of infants

Genetically acquired characteristics, parental quality of nutrition and adequacy of post natal diet determines the growth and development of an infant. Mammals produce milk to feed their offspring during their early life. Milk is a specifically and specially designed nutrition for infant mammals.

Growth and Development are two distinctive terms that go hand in case of normal children. Growth is an increase in size of the body or any part of the body. Where as, development is maturation of body tissues and organs so that all functions can be carried out. Generally, milk of a human is ideal food for its baby with in first 6 months for him growth and development. But after first 6 months of borne, the mother milk is not only enough to growth and development of infant. So, tripling the birth weight by the time of first birthday at second 6 months (at infant period) (Rao, 1999).

At the birth a babies possess 75% of water, 12% to 15% of fat and poorly developed muscle. First birth day body fat increases to about 20% to 25%. Water content decrease to 60%. Bones contain more of water and cartilage at birth. The total Calcium content of the skeleton at birth is 25% to 30%. Which is tripled by the end of first year (Rao, 1999).

The infant formula is defined as a food intended for feeding of infants prepared from the milk of cows or other animals and /or other constituents of animal and or plant origin (SLS 561, 1989).

One or more cereals such as wheat, rice, barley, oats, rye, maize, sorghum and buckwheat and /or legumes (pulses) and also sesame, arachis and Soya bean are used as basic ingredients for cereal based infant foods. In addition to that protein concentrates and other high protein ingredients, salt (NaCl), milk and milk product, eggs, meat, fats and oils, fruit and vegetables, sugar (nutritive

carbohydrate sweeteners), malt, honey, cocoa, potatoes, starches (enzyme modified starches and starches treated by physical means) are used as optional ingredients(SLS:1036,1995).

According to pediatric experts, solid foods may be offered to healthy baby at six months of age. Individual babies vary, so speak with infant's pediatrician about the best age for mother to introduce solid foods. The baby may be ready for solid foods if he or she:

- Can sit with support
- Can turn his or her head away when full
- Has doubled his or her birth weight (Organic infant formula with lipid, 2006)

2.2 The Requirement of Different Nutrients for Infants

The primary basis for the nutritional requirements of a breast fed infant (first 6 month of birth) is the amount of milk from mother ingested by a healthy breast fed infant. This nutrition composition would be sufficient for calculating nutritional need of a breast fed infant up to first 6 months, but the requirements for the next 6 month (infant period) is based on consumption of a formula and mixture solid or semi solid foods. The Requirements of different nutrients are briefly discussed below,

Table 2.1 Nutrients Requirements for Infant

Nutrients	Quantities
Energy (kg cal)	108/kg
Protein (g)	1.7/kg
Calcium (g)	0.5-0.6
Iron (g)	1
Retinal/Carotene (mg)	300/1200
Thiamine (mg)	54mg/kg
Riboflavin (mg)	65mg/kg
Niacin (mg)	710mg/kg
Vitamin B_6 (mg)	0.4
Folic acid (mg)	25
Vitamin B_{12} (mg)	0.2
Vitamin D (I.U)	200
Vitamin C (mg)	20

Source: Applied Science Series, A Text Book of Nutrition, (1999).

2.2.1 Energy Requirement

Infants have high skin surface. Infant body spend lot of energy to regulate their body temperature and metabolic activities. Their energy need of infants is 80 kcal to 130 kcal per kilogram of body weight. This energy is supplied by main nutrients such as carbohydrate, fat, protein. Half of this energy is used for maintaining the need of basal metabolism (Rao, 1999).

Table 2.2 Energy production of main nutrients

Nutrients	kcal per g
Carbohydrate	4
Fat	9
Protein	4

Source: (SLS 561, 1989).

2.2.2 Carbohydrates

Carbohydrates are simple sugars or polymers of sugars like starch. These sugars may be glucose, fructose, galactose, etc. These units may be bonded in pairs or small groups. There may be complex structure like starch built from thousands of sugar units. These are classified as

1. Monosaccharides
2. Disaccharides
3. Oligosaccharides
4. Polysaccharides

Lactose is the main carbohydrate in an infant diet which accounts to about 40% of calories from human milk. Lactose helps in the absorption of calcium and prosperous and in maintaining a normal intestinal microflora. Due to this, some carbohydrates like sucrose, cane syrup and dextrimaltose are added to in preparation of infant formulas. Mainly sucrose is easily available and cheap and has an advantage over lactose it is easily digestible and absorbed more rapidly but less than glucose and maltose (Rao, 1999).

2.2.3 Fat

Lipids include fat, oils, and other fat like substances. They are composed of fatty acids and glycerol. Fats are organic compounds of carbon, hydrogen, oxygen. Compared to carbohydrates fats have a very lower proportion of oxygen. Lipids also contain phosphates, carbohydrates or other nitrogenous compounds.

Fatty acid may be saturated or unsaturated depending on the number of hydrogen atoms attached to each carbon atom. In case of saturated fatty each of the carbon atoms has two hydrogen atoms attached to it. In an unsaturated fatty acid one hydrogen atom is missing from each of the tow adjoining carbon atoms due to which doubled bond is formed between to carbon atoms. These are classified as

1. Simple lipids
2. Compound lipids
3. Derived lipids

About 40 to 50 per cent calories in human milk and in most formulas are supplied by fat. Fat provides essential fatty acids for infants. About 5% of the calories are derived from linoleic acid in breast fed infant. So, addition of vegetable fat or fish oil which is rich in linoleic acid is desirable to when formulation of infant foods. Cholesterol is also essential for synthesis of bile salt and for the development of central nervous system. But the desirable level of cholesterol in and infant diet is not known (Rao, 1999).

2.2.4 Protein

Proteins are made up of amino acids as units in various proportions and arrangements. The possess carbon, hydrogen, oxygen, nitrogen and in few sulphur. Some protein have prosperous, small amount of iron, copper etc. The presence of nitrogen makes protein different from carbohydrate and fats. Proteins on average have 16% nitrogen. Protein molecules are larger than carbohydrate and lipid molecules and from colloidal solution. These are classified as

1. Simple proteins
2. Conjugated proteins
3. Derived proteins (Rao, 1999).

2.2.5 Vitamins

Vitamins define as a group of potent organic compounds other than carbohydrate, protein, fat, but are necessary in minute amounts and are necessary for maintenance, growth and reproduction. These can not be synthesized by the organism.

The vitamins are broadly divided in to tow groups fat soluble and water soluble. Within the classes the vitamins differ widely in their properties, functions and distribution. About 15 vitamins are isolated in pure state from natural foods. 15 Vitamins are standardized in infant formula by the Food and Drug Administration (FDA). These vitamins are discussed below,

a) Fat soluble vitamins
    1. Vitamin A
    2. Vitamin D
    3. Vitamin K
    4. Vitamin E
b) Water soluble vitamins
    I. Vitamin B complex
        1. Vitamin B, or thiamin
        2. Riboflavin
        3. Niacin and Niacinamide
        4. Pyridoxine or Vitamin B_6
        5. Pantothenic acid
        6. Folic acid
        7. Biotin
        8. Choline
        9. Para Amino Benzoic acid
        10. Inositol
        11. Vitamin B_{12} or Cobalamine
        12. Lipoic acid
    I. Vitamin C or Ascorbic acid
    II. Vitamin P or Bioflavanoids
    III. Carbitine and Aaurine (Rao, 1999).

2.2.6 Minerals

The infant needs may minerals for proper growth. Most of time, these minerals are not rich in raw materials that is used to produced of infant formulas. Therefore, Minerals should be fortified.

E.g.: Infant foods based on animal milk should be fortified with iron.

Infant foods based on Soya bean should be fortified with iodide (Rao, 1999).

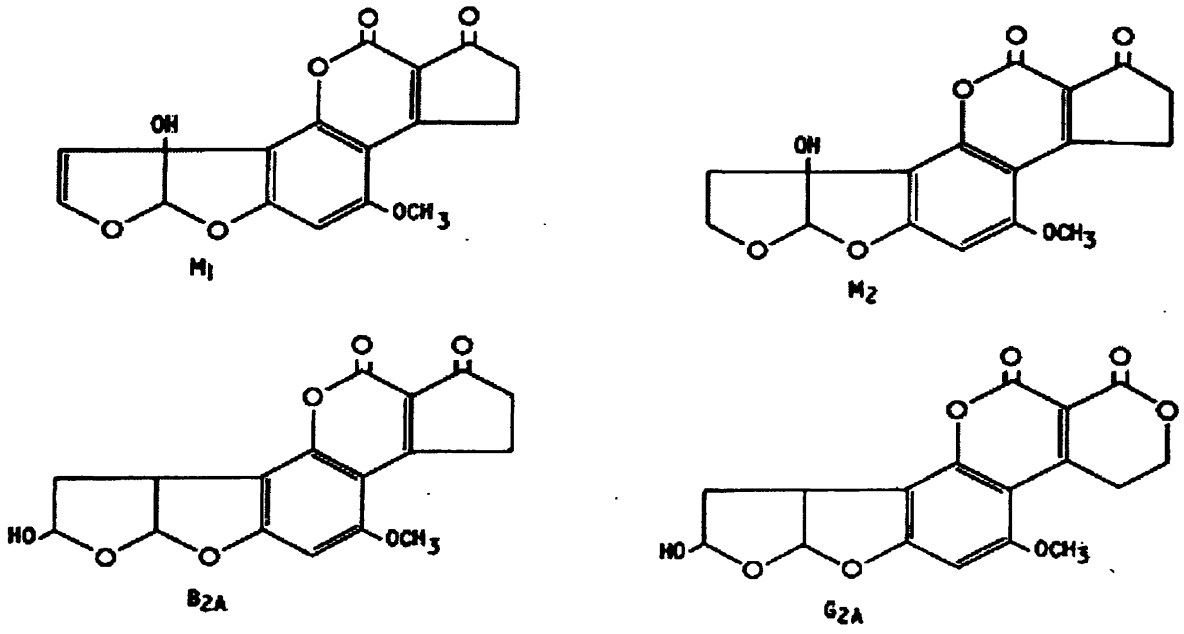
2.3 Aflatoxins

2.3.1 Natural occurrence

Food products contaminated with Aflatoxins include cereal (maize, sorghum, pearl millet, rice, and wheat), oilseeds (groundnut, soybean, sunflower, cotton), spices (chillies, black pepper, coriander, turmeric, zinger), tree nuts (almonds, pistachio, walnuts, coconut) and milk (Reddy and Waliyar, 2006).

2.3.2 Physical and chemical properties

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *Aspergillus parasiticus* on variety of food products. Among 18 different types of aflatoxins identified, major members are aflatoxin B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is normally predominant in amount in cultures as well as in food products. Pure AFB1 is pale-white to yellow crystalline, odorless solid. aflatoxins are soluble in methanol, chloroform, actone, acetonitrile. A. flavus typically produces AFB1 and AFB2, where as A. parasiticus produce AFG1 and AFG2 as well as AFB1 and AFB2. Four other aflatoxins M1, M2, B2A, G2A which may be produced in minor amounts were subsequently isolated from cultures of *Aspergillus flavus* and *Aspergillus parasiticus*. A number of closely related compounds namely aflatoxin GM1, parasiticol and aflatoxicol are also produced by *Aspergillus flavus*. aflatoxin M1and M2 are major metabolites of aflatoxin B1 and B2 respectively, found in milk of animals that have consumed feed contaminated with aflatoxins (Reddy and Waliyar, 2006).



Figures 2.1 Structure of aflatoxins M_1, M_2, B_{2A} and G_{2A}.

Aflatoxins are normally refers to the group of difuranocoumarins and classified in two broad groups according to their chemical structure; the difurocoumarocyclopentenone series (AFB1, AFB2, AFB2A, AFM1, AFM2, AFM2A and aflatoxicol) and the difurocoumarolactone series (AFG1, AFG2, AFG2A, AFGM1, AFGM2, AFGM2A and AFB3). The Aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of AFB1 > AFG1 > AFB2 > AFG2 as illustrated by their LD_{50} values for day-old ducklings. Structurally the dihydrofuran moiety, containing double bond, and the constituents liked to the coumarin moiety are of importance in producing biological effects. The Aflatoxins fluoresce strongly in ultraviolet light (ca. 365 nm); B1 and B2 produce a blue fluorescence where as G1 and G2 produce green fluorescence (Reddy and Waliyar, 2006).

Table: 2.3 Chemical and physical properties of Aflatoxins

Aflatoxin	Molecular formula	Molecular weight	Melting point
B1	C17 H12O6	312	268-269
B2	C17 H14O6	314	286-289
G1	C17 H12O7	328	244-246
G2	C17 H14O7	330	237-240
M1	C17 H12O7	328	299
M2	C17 H14O7	330	293
B2A	C17 H14O7	330	240
G2A	C17 H14O8	346	190

Source: Properties of aflatoxin and it producing Fungi, (2006)

2.3.3 Chemical reactions of aflatoxins

The reaction of aflatoxins to various physical conditions and reagents have been studied extensively because of the possible application of such reactions to the detoxification of aflatoxins contaminated material (Reddy and Waliyar, 2006).

2.3.4 Heat resistance of aflatoxins

Aflatoxins in dry state are very stable to heat up to the melting point. However, in the presence of moisture and at elevated temperatures there is destruction of aflatoxin over a period of time. Such destruction can occur either with aflatoxin in oilseed meals, aflatoxin in roasted peanuts or aflatoxin in aqueous solution at pH 7. Although the reaction products have not been examined in detail it seems likely that such treatment leads to opening of the lactone ring with the possibility of decarboxylation at elevated temperatures (Reddy and Waliyar, 2006).

2.4 Maize (*Zea mays*)

2.4.1 Introduction

Maize is cultivated in many Asian, African and Latin American countries, under both irrigated and rainfed conditions. *Zea mays* is the botanical name of the crop which was included in the poaceae family of the plants. As a "Food Crop", maize was firstly discerned by the Latin America (this is only cereals originating from the Americans) in the early centuries, and now it has become one of the major commercial crops in the world especially in African and American countries.

The primary consumers of maize were the rural farming population in many countries and it was also consumed by the livestock sector in developed as well as developing countries. Therefore, maize is one of the major trade commodities of the international markets.

Maize requires well drained, deep soil. The optimum soil pH is in the range of 5.3-6.0. It grows in localities not particularly suitable for other cereals.

In Sri Lanka Maize is grown mainly (in the Dry Zone) as a rainfed crop during Maha. It is cultivated in both settled and shifting (Chena) types of highland as a pure as well as mixed crop. This requires at least 500mm-600mm of rain fall evenly distributed during the growing season. Since very recent past, the Yala cultivation under irrigated condition has been expanding (Sanker and Dayananda, 1998).

Table: 2.4 The recommended varieties of Sri Lanka, days of maturity and yield

Verities	Days for maturity	Yield (kg / ha)
Aruna	90-100	4400
Bhadra	105-115	4,428-4,500
Muthu	110-115	5300
Ruwan	105-110	4300

Source: Department of Agriculture, Sri Lanka (2006)

2.4.2 Major Producing Districts

Ampara (23%), Anuradhapura (21%), Monaragala (16%), and Badulla (16%) are the major producing districts which account for 75% of the national production. the next important districts and areas are respectively Matale (5%), Batticaloa (4%) and Mahaweli H (3%).

In term of the average extent cultivated during 1991-1995, Anuradapura district take priority by accounting for 25% of the total land area. The other important districts in terms of the extent cultivated are respectively Ampara (17%), Badulla (17%) and Monaragala (17%) (Sanker and Dayananda, 1998).

2.4.3 Seasonality of production

Maize is seasonal crop which mainly cultivated in Maha season under rainfed conditions. However, Yala cultivation is possible only with irrigation, especially in the dry zone. Maha cultivation commences during October-December period under the North-East monsoon in major producing areas. nearly 95% of the extent and production are recorded during Maha season (Sanker and Dayananda, 1998).

2.4.4 Major import/export countries

The major importing countries were China and india. Among the other import countries South Africa, Argentina, U.S.A and Thailand were prominent Export from Sri Lanka was very small. The highest quantity of exports was nearly 5mt. recorded in 1990. The major exporting countries were Singapore, Maldives, Canada and Germany (Sanker and Dayananda, 1998).

2.4.5 Consumption

The per capita consumption of maize has declined over time. The consumer Finance and socio-economic Survey 1981/1982 has indicated that the per capita consumption of maize was 42.3 grams per month and it has declined to 21.9 grams by 1986/1987. According to the house hold income and expenditure survey conducted by

the department of censes and statistics, the per capita consumption of maize was 18.8 grams per month in 1990/1991. Considering the higher protein content than rice or wheat, the declining trend in its consumption pattern is an issue that needs more attention to be paid (Sanker and Dayananda, 1998).

2.5 Soya bean (*Glycine max*)

2.5.1 Introduction

Soya been is a legume which provides protein of high biological value. Asia is the place of the origin, but USA is the major producer at present. Soya flour is widely used in the bakery industry. Soya oil is used for industrial as well as cooking purposes.

Soya can be grown in most agro-climatic regions, but the dry done and the drier parts of the intermediate zone are the preferred areas. Any soil with a pH of 6-7 is adequate and excessive moisture is not suitable.

In Sri Lanka, Soya has become a popular dry zone crop cultivated in paddy fields in yala season. PB-1and Bossier are the recommended varieties by the Department of Agriculture and the former is more popular due to it's early maturity and high yield. The potential yields of both are between 2,500-2,800 kg/ha (Sanker and Dayananda, 1998).

2.5.2 Major Producing Districts

North Central and Central Provinces produce more than 90% of soya production in the country. Anuradhapura district and Mahaweli H areas account for 71% of the extent cultivated and 72% of the total quantity produced. Then Matale, Nuwara Eliya and Polonnaruwa districts are respectively important for both the extents cultivated and quantities produced, which are 21% in each category.

Mahaweli H area is the foremost (37%) in production although it is second to Anuradhapura in term of the extent cultivated, which is 28%, due to the higher level of productivity there (Sanker and Dayananda, 1998).

2.5.3 Seasonally of production

As revealed by production statistics there is no large seasonal bias in production. However, 54%of the production is from yala season. In mid-1980s and in 1990 over 80% of the production has been from Maha season and this trend has change later on (Sanker and Dayananda, 1998).

2.5.7 Major Import/Export countries

Soya is being imported mainly from India, China, Argentina, U.S.A, and Malaysia (Sanker and Dayananda, 1998).

2.5.8 Consumption

According to the available limited data, the per capita consumption of Soya bean has slightly increased between the period 1978/1979 and 1981/1982. The increase is high in the urban sector followed by the estate sector (Sanker and Dayananda, 1998).

2.6 Soya bean oil

2.6.1 History of Soya bean oil in Sri Lanka

Soya bean oil, which is obtained from the seeds of the plant *Glycine max* presently one of the dominant oils in the world oils and fat market. The Ceylon oils and Fats Corporation ventured in to production and marketing of edible Soya bean oil with the introduction of its new solvent extraction plant in 1982. The plant was installed by Costruzioni Meccaniche Bernadini, It has been consisted 50mt of feed per hours (Wijesundera).

2.6.2 Extraction of Soya bean oil

Mechanical expellers such as the once used for the expression of coconut oil from copra are not suitable for the extraction of oil from soya bean because the oil content in Soya bean is relatively low (18-20%). Soya bean oil is therefore obtained by solvent extraction. The extraction procedure consists of three main stages (Wijesundera).

a) Preparation of the beans:-

The soya beans are successfully passed through a magnetic separator and a plane shifter to remove stray metal and other extraneous before feeding in to breaker rolls where they are crushed in to smaller pieces for optimum operation of the solvent extraction process, it is desirable to maintain the moisture content of the beans at 9.5-10.0%, and pre preparatory flaking is accomplished most satisfactorily at about 70^0C. The desired conditioning of the broken beans is achieved by means of a steam-jacketed cooker (Conditioner). The beans are then conveyed to flaking rolls which produces flakes about 0.2mm thick.

b) Solvent extraction

In general solvent extraction involves washing of the oil seed with and appropriate solvent such as hexene so that the oil is dissolved in the solvent to form a mixture called miscella, which drains from the meal. The extractor at the Ceylon oil and Fats Corporation employs a continuous counter-current percolation process.

c) Reclamation of solvent from oil and meal

Miscella from the extractor contain approximately 20% oil is filtered and are successively fed in to a pre-heater and distiller and finally in to oil stripper, miscella entering the stripper contain 90-95% oil. Inside the Stripper live steam introduced in to it at the bottom flows upward counter-current to the cascading flow of oil. The finished oil product leaves the stripper at about 99.8% or higher oil content and is pumped to storage tanks.

The wet spent flakes leaving the extractor contain approximately 35%hexane, 7-8% water and 0.5-1.0% oil. These are fed in to the desolventizer-toaster unit where the solvent hexane is stripped off by means of steam. This unit also carries out an operation known as toasting which serves to inactivate trypsin inhibitors and improve the palatability of the meal for animals (Wijesundera).

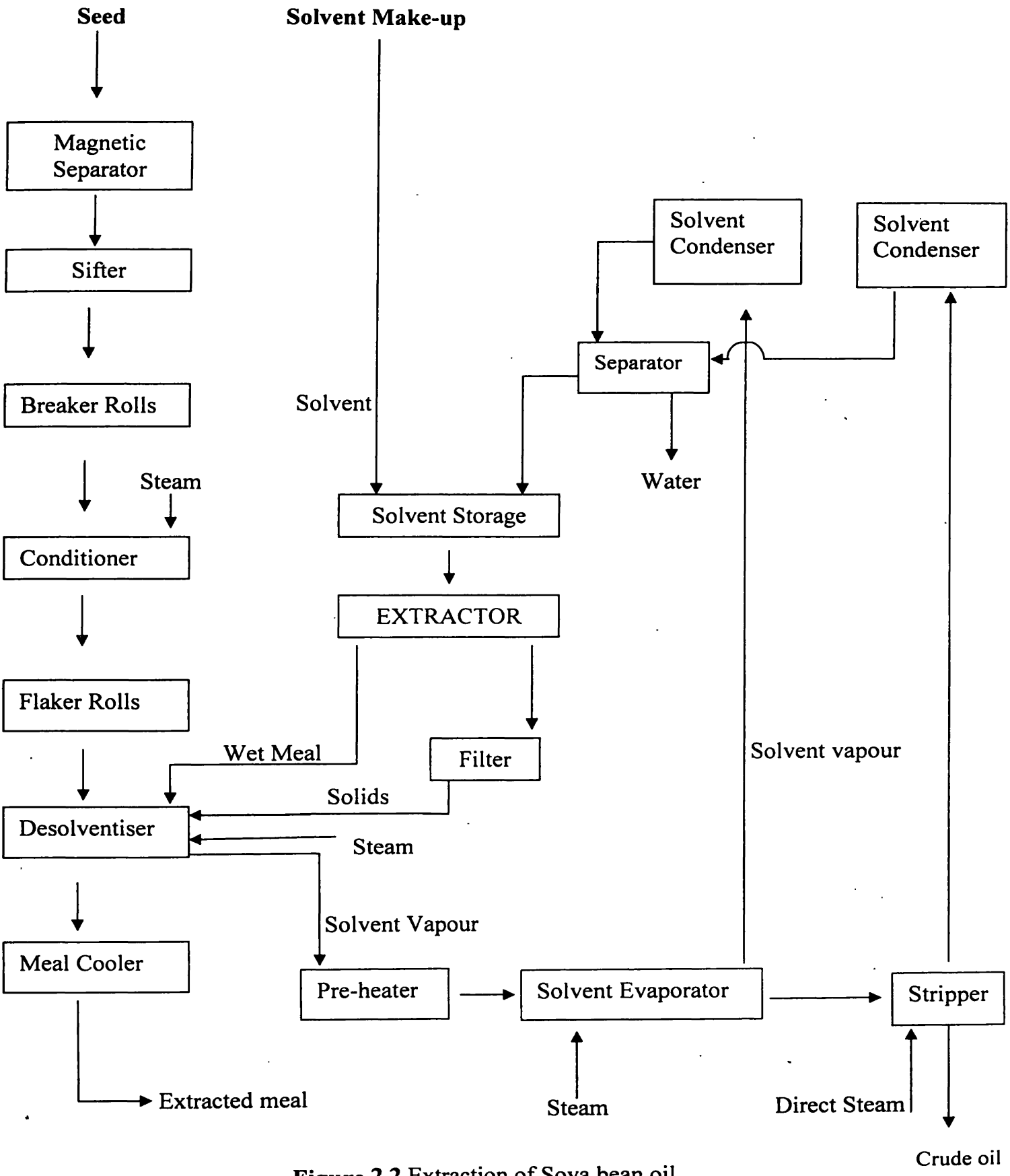


Figure 2.2 Extraction of Soya bean oil.

Source: Manual of chemical industry of Sri Lanka.

2.7 Pumpkin (*Cucurbita maxima*)

2.7.1 Introduction

Pumpkin (*Cucurbita maxima*) is a native of South America and is cultivated through out India, Sri Lanka and most tropical rejoins of the world. In Sri Lanka it is best grown in the dry zone especially after the rains.

Propagation is by seeds and required high temperature of 25^0C to 30^0C and a low humidity. Soils with high organic contents are ideal for normal growth. Pumpkin grows well in altitude below 2000m. A.N.K and local strains are cultivated. Harvesting of fruits can be done in 80-140 days of sowing or planting. The average yield 15-25 MT/ha (Henagedara *et. al*, 2000).

2.7.2 Major producing districts

In the period 1993-1997, over 1/3 of total area cultivated was in the two district; Anuradhapura (18%) and Kurunegala (16%) and the balance in the district of Matale, Hambantota, Monaragala and Ratnapura, above 34%. During the same period 35% of total production come from Kurunegala (19%) and Anuradhapura (16%). Hambantota, Matele and Moneragala are the major producing districts, which account for over 30% of the national production. Since the pumpkin is the rainfed crop, about 65% of annual production is grown in Maha season (Henagedara *et.al*, 2000).

2.7.3 Consumption

Per capita consumption is 2.79kg per annum, which indicates a marginal increase compared to the 1980s level. There is no significant change in consumption by urban, rural and estate dwellers. There is also no close relationship between income and consumption (Henagedara *et.al*, 2000).

2.8 Potatoes (*Solanum tuberosum*)

2.8.1 Introduction

The potato (*Solanum tuberosum*) was originally discovered by the indigenous inhabitants of maya and Inka in Andies mountains in South America. It was introduced to European countries in the 16 centenary as a food commodity for the people.

Potatoes were first introduced to this country in 1850s, grown well in the upcountry wet, intermediate and dry zones at temperatures between 24^0C and 32^0C as well as in the Puttalama and Jafna districts during Maha season.

There two groups of varieties, commercial and local. Arka, Vekero, Cardinal, Desiree and Isna are the commercial verities with 3.5-month maturity (Henagedara *et.al*, 2000).

2.8.2 Consumption

The per capita consumption of potato has increased from 1.3kg in 1973 to 3kg in 1981/1982 and it is slightly declined by 1986/1987. The consumption level was higher in the urban sector followed by estate and rural sectors. Generally the consumption level has increased with the increase of the income level (Henagedara *et.al*, 2000).

CHAPTER 3

Materials and methodology

3.1 Preparation of Semisolid Corn Base

3.1.1 Materials

Tender corn (Aruna)
Knife
Cutting board
Grinder
Sieve (No-40)
Electrical balance

3.1.2 Methodology

- The corn seeds were separated by using a knife from fully matured tender corn pods.
- Then 500g of seeds were grinded for 5-8 minutes with 100ml of potable water by using grinder and slurry was obtained.
- The slurry was drained out with 900ml of potable water through No-40 sieve.
- The washed out solution was heated up to 70^0C-75^0C while stirring and a low thick paste was obtained.
- 850g of paste was measured.

3.2 preparation of pumpkin paste

3.2.1 Materials

Pumpkin fruit (local strain)
Knife
Cutting board
Grinder
Electrical balance

3.2.2 Methodology

- The good quality fully mature pumpkin fruit was selected and washed with chlorinated water
- Peel was removed
- 100g of pumpkin flesh was measured.
- The measured quantity was cut in to small pieces.
- The pieces were grinded by using a grinder into a paste and 80g of pumpkin paste was measured.

3.3 preparation of potato paste

3.3.1 Materials

Potatoes
Knife
Cutting board
Grinder
Electrical balance

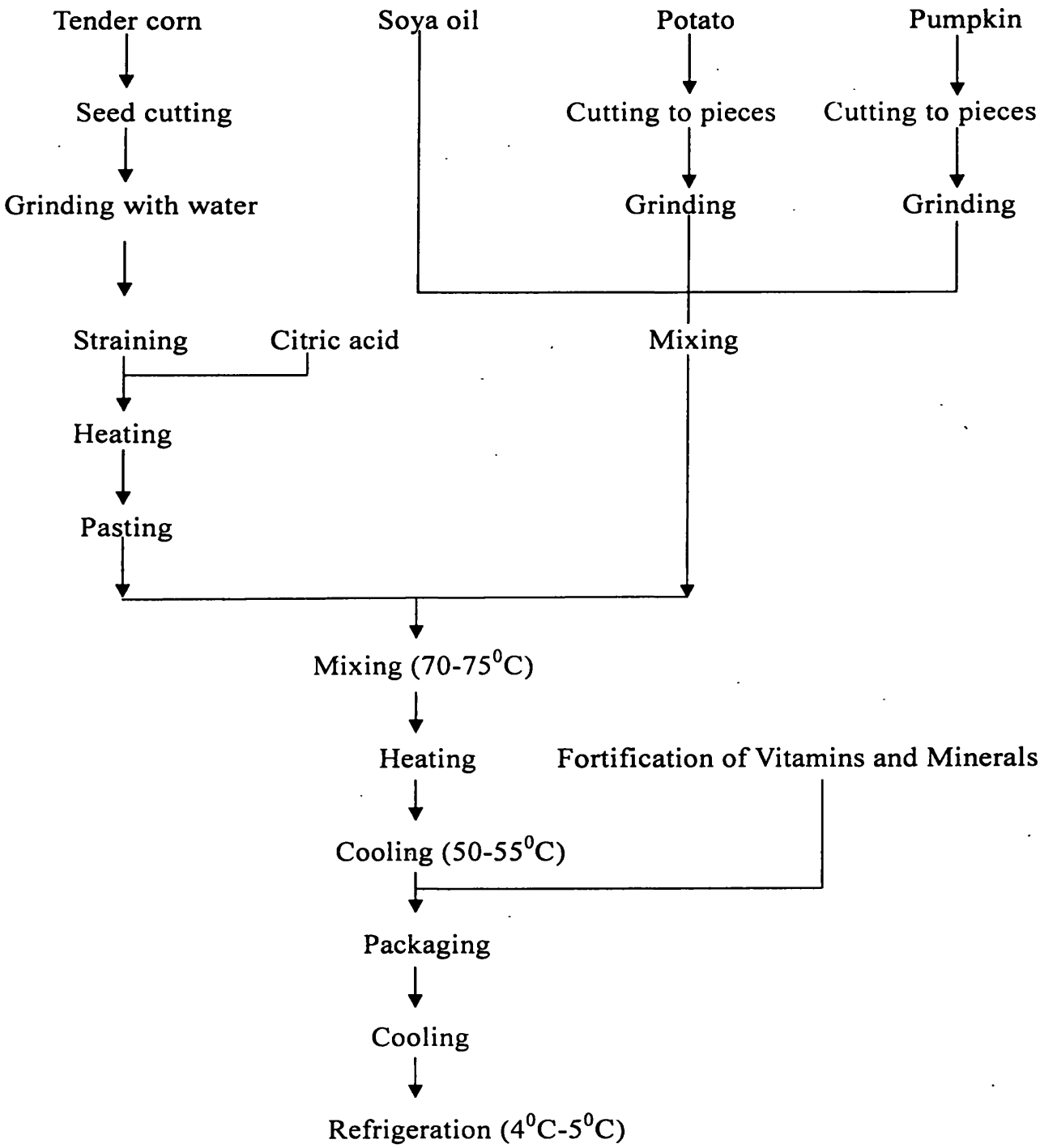
3.3.2 Methodology

- The fully mature potato tubers were selected and washed from chlorinated water.
- Peel was removed.
- 100g of potato was measured.
- The measured quantity was cut in to small pieces.
- The pieces of potatoes were grinded by using grinder and 20g of paste was measured.

3.4 Preparation of infant formula

- The prepared mixtures of pumpkin paste, potato paste and Soya bean oil were mixed with 850g of semisolid corn base.

- Then the above mixture was heated up to 70^0C- 75^0C while stirring.
- Then a suitable amount of vitamins and minerals was added to paste and stirred thoroughly and the mixture was cooled to 50^0C-55^0C.
- The 513mg of $CaCl_2$, 4.7mg of Zinc acetate, 0.6mg of $CuSO_4$, 1105.8mg of $MgCl_2$, 200mg of vitamin E and 25mg of vitamin-C are added to product.
- The product was packed and cooled to room temperature.
- Finally the product was stored at 4^0C-5^0C a refrigerator (Domestic fridge).



Figures 3.1 Processing flow diagram of the infant food.

Figures 3.2 View of the infant food.

3.5 Proximate Analysis of Semisolid Corn Base

3.5.1 Determination of Moisture (Oven-Drying method)

3.5.1.1 Apparatus

Moisture dish made from stainless steel
Oven maintained at 105 ± 1^{0}C
Electronic Balance
Desiccators
Food Sample

3.5.1.2 Methodology

- The moisture dish was cleaned and dried at 110 ^{0}C for 1hour in oven to remove any adhering moisture.
- Then 5g of semisolid corn base sample was weighed accurately into the moisture dish and weight (W_2) by using electrical balance which was pre weighted. (W_1)
- This food sample was placed in an oven at 105 ^{0}C for 4 hrs and was cooled in desiccators and weighed.

- The above process was repeated (drying, cooling, and weighing at 30 min intervals) until the difference between the two consecutive weighing not exceeded 1mg.
- Finally, the lowest weight of moisture dish with food sample was weighed.(W_3)

3.5.1.3 Calculation of Moisture percentage

$$\text{Moisture percentage} = \frac{W_1 - W_3}{W_2 - W_1} \times 100$$

Total solid percentage = $100 - W_3$

3.5.2 Determination of Total Fat

3.5.2.1 Apparatus

Beaker (100ml)
Electronic Balance
Measuring cylinder
Water bath (maintained at 20-80 ^{0}C)
Separation funnel

3.5.2.2 Chemicals

Con. Hydrochloric acid
Ethanol
Ether
Petroleum ether
Water
Food Sample

3.5.2.3 Methodology

- 2g (W_1) of sample was weighed into 50 ml of beaker and 2ml of 95% ethanol and 10ml of HCl (prepared by mixing 25ml of con HCl with 1 ml of water) were added then that contents were mixed thoroughly.

- The beaker was kept in the water bath at 70-80 ^{0}C for 30min-50min and cooled.
- 10 ml of ethanol was added to above mixture and transferred in to a separation funnel. The beaker was washed with 25ml of ether in three portions of washing and it was added to the funnel.
- 20ml of petroleum ether was added to the funnel which was stoppered with a cork and shaken vigorously for about 5 minutes.
- The funnel was stood until a clear of pet ether could be observed. The upper ether layer was taken in to a clean, previously weighed dried flask (W_2) and it was dried by using water bath until a constant weight was obtained. (W_3)

3.5.2.4 Calculation of Total Fat percentage

$$\text{Total Fat percentage} = \frac{(W_3) - (W_2)}{(W_1)} \times 100$$

Weight of the sample-(W_1)

Weight of the empty flask-(W_2)

Weight of the flask with fat- (W_3)

3.5.3 Determination of Protein

3.5.3.1 Apparatus

Kjeldahl flask

Distillation unit

Burette

Titration flask

Beakers (500ml)

Petridis

Electronic balance

Stand

Measuring cylinder (100ml)

3.5.3.2 Chemicals

0.002M HCl
Con H_2SO_4
Ethyl alcohol
Sodium sulphate
Sodium hydroxide
5% Boric acid
Catalyst tablets (Selenium)
Methylene blue
Methyl red
Food sample

3.5.3.3 Methodology

- Preparation of $NaSO_4$ solution:
  50g of NaOH and 8g of Na_2SO_4 were added to 100ml of distilled water.
- Preparation of Boric acid solution
  4g of boric acid was dissolved in 100ml of distilled water
- Preparation of indicators:
  0.5g of methyl red was dissolved in 25ml of ethanol and 0.5g of methylene blue was dissolved in 20ml of ethanol. Then t portion of methylene blue and ethanol mixture were mixed one portion of methyl red and ethanol mixture. It is used as the indicator.

- 0.03g of defatted food sample was taken in to a kjeldahl flask Then 1.0500g of kjeldahl tablets and 10ml H_2SO_4 were added to the above flask. After that, the kjeldahl flask was placed on kjeldahl digestion unite at 4h for digestion.
- Then the flask was cooled for1h.
- Then defatted sample was taken in to round bottom flask. 5ml of Boric acid sample was taken in to titration flask and added 3 drops of indicators. The kjeldhal unit was arranged.

- After that the kjeldhal unit was heated by using steam generator. Then 8 ml of Na_2SO_4 solution was added to sample which was boiled from steam generator. Then the NH_3 was trapped in to the boric acid solution until increase to 15ml volume in titration flask.
- Finally it was titrated with 0.02M HCl colour change was appeared and got end point reading.

3.5.3.4 Calculation of Protein percentage

$$\text{Percentage of N} = \frac{(\text{A-B}) \times \text{molality of HCl} \times 14}{\text{Weight of food sample} \times 1000} \times 100$$

Protein percentage = N% × 6.25

3.5.4 Determination of Total Ash

3.5.4.1 Apparatus

Muffle furnace
Silica dish
Electronic balance
Desicator
Bunsen burner
Steam bath
Filter papers
Electronic burner

3.5.4.2 Chemicals

Hydrochloric acid (2M and 10M)
Water
Food sample

3.5.4.3 Methodology

- 5g (W_2) of sample was weighed in to clean and dry silica dish. (W) Then sample was ignited slowly by using Electronic burner, until no more fumes were evolved.
- Then the sample was placed in Muffle furnace at 500^0C for 4 hours until the sample was free from black carbon particles.
- The process was repeated as igniting, cooling, weighing at half hour intervals until the difference between two successive weighing was less than 1mg.
- Record the lowest mass (W_1)

3.5.4.4 Calculation of Total Ash percentage

$$\text{Total ash percentage} = \frac{W_1 - W}{W_2} \times 100$$

Mass of the empty dish -W
Mass of the dish with Ash-W_1
Mass of the sample- W_2

3.5.5 Determination of Fiber

3.5.5.1 Apparatus

Beaker (250ml)
Desiccator
Electronic balance
Filer paper (watmann 52)
Heating mantle
Litmus paper
Oven (maintained at 100 ± 2^0C)
Reflux condenser
Round bottom flask
Sintered crucible
Suction pump

3.5.5.2 Chemicals

Con. Sulphuric acid
Ethanol
Sodium hydroxide
Food sample

3.5.5.3 Methodology

- 25g (W_1) of food sample was measured in to round bottom flask which was defatted by using ethanol ether. Then 200ml of dilute H_2SO_4 was measured in to above round bottom flask. After that the round bottom flask was placed on heating mental and boiled for 30 min.

- After that the boiled sample was filtered through the sintered crucible by using reflux condenser which was pre weighted. Thus the sample was filtered with not water again until the acid condition had been removed.

- Now the rested sample was boiled with 200ml of 1M NaOH solution for 3min then the sample was filtered by using suck pump. Thus the sample was filtered with not water again until the alkaline condition had been removed (Litmus papers were used for find to acid and alkaline condition).

- The rested sample was dried with sintered crucible from oven and weighted (W_3) which was cooled in dedicator. Those processes repeat until a constant mass was obtained.

- Finally ash the contents of the crucible at 600^0C in a muffle furnace and weighted which was cooled dedicator that repeat until a constant mass was obtained. (W_4)

3.5.5.4 Calculation of Fiber percentage

$$\text{Fiber percentage} = \frac{W3-W4}{W1} \times 100$$

Mass of the sample -W_1

Mass of the crucible with sample after oven dried- W_3

Mass of the crucible with sample after incineration-W_4

3.6 Determination of Aflatoxins

3.6.1 Apparatus

Thin Layer Chromatogram

Electronic balance

Capillary tube

Rotary evaporator

Measuring cylinder

Solvent chamber

3.6.2 Chemicals

$CHCl_3$

CH_3OH

$AgNO_3$

Water

Food sample

3.6.3 Methodology

- 5g of defatted food sample was taken and dissolved in 50ml of a 1:1 mixture of $CHCl_3$ and CH_3OH.
- A Thin Layer Chromatogram was run using the remain solvent using a solvent mixture of C_2H_5OH:CH_3OH:H_2O (96:3:1).

- After developing the chromatogram, the Thin Layer Chromatogram plate was dried and sprayed with $AgNO_3$.
- Finally, the blue fluorescence spots were observed by using UV lamp.

3.6 Proximate Analysis of Final Products

The determination of moisture, total fat, protein, total ash, fiber and aflatoxins of the final product were carried out previously mentioned procedures as respectively 3.5.1, 3.5.2, 3.5.3, 3.5.4, 3.5.5 and 3.6.

3.8 Determination of water activity (Salt liquefaction test)

3.8.1 Apparatus

Test tube with lid
Spatula
Fridge

3.8.2 Chemicals

NaCl (0.75)
KBr (0.80)
KCl. (0.84)
Product sample

3.8.3 Methodology

- Small sample of the product was taken and put in to the bottom of test tube. The test tube was placed horizontally.
- The vary small amount of chemical was put in the test tube (but the chemical and food is not attached together)
- The test tube was sealed quickly using a lid.
- This steps was fallowed for each test tube
- The test tubes were placed at 4^0C-5^0C and room temperature.

3.9 Microbiological testing

All the analysis methods are based on SLS 651:1989 which is the specification for INFANT FORMULAE (First revision)

3.9.1 Aerobic plate count

3.9.1.1 Preparation of Culture media

Materials and Apparatus:-

Distilled water(1000ml)
Nutrient Agar (28g)
Laboratory glass wear
Magnetic stirer
Autoclave
Electric balance

Procedure:-

- 28g of Nutrient Agar was dissolved in a Flask with 1L of water by using a Magnetic stirrer.
- The prepared medium was placed in the Autoclave and sterilized at 121 ± 1^0C for 20 minutes.
- Then warm sterilized (45 ± 0.5^0C) media transferred to 30 number of pettry dishes with having 15ml to each and kept for solidify on the lamina flow

3.9.1.2 Preparation of test sample

Materials and Apparatus:-

Food sample (10g)
Meshearing cylinder
Electric balance
Pipettes
Rotary blender
Distilled water

Procedure:-

- 10± 0.1g of food sample was weighed aseptically in to a sterile blender jar and 90ml of distilled water was added to make up the 10^{-1} dilution.
- The weighed sample was blended in 15000-20000 revolution at 2-2.5 minutes by using rotary blender and stomacher was operated for 10 minutes.
- Finally, the blended sample was placed at 15minute to settle down of large particles.
- The cerial dilution was carried out up to 10^{-3} by using up going liquid portion of above sample

3.9.1.3 Inoculation of culture media

Materials and Apparatus:-
Initial suspension
First decimal dilution sample
Second decimal dilution
Petri dishes
Pipettes
Incubator

Procedure:-

- 1ml of final suspension (10^{-3} dilution) was transferred to steriled petri dish by using a steriled pipette.
- Same procedure was carried out for 30 samples of product.
- Finally, the petri dishes were incubated for 72±3 hours at 31±1 ^{0}C.

3.9.1.4 Counting of microbes

- Microbial colonies were counted by using electric colony counter.

3.9.2 Coliform

3.9.2.1 Preparation of Culture media

Materials and Apparatus:-

Distilled water(1000ml)
MacConkey broth (40g)
Flask
Ignition tubes
Test tubes
Magnetic stirer
Autoclave
Electric balance

Procedure:-

- 40g of MacConkey broth medium was dissolved in a Flask with 1L of distilled water during the boiling by using Magnetic stirer
- 10 ml of culture media was transfered to each sterile test tube. Then ignition tube was placed in the each test tube without trapping air bubbles.
- The prepared medium was placed in the Autoclave and sterilized at $121\pm1^{0}C$ for 20 minutes.

3.9.2.2 Preparation of test sample

- The procedure was same as the analysis of Aerobic plate count.

3.9.2.3 Detection and Enumeration of Microbes

Materials and Apparatus:-
Initial suspension
First decimal dilution sample
Second decimal dilution
Pipettes
Incubator

Procedure:-

- 1ml of initial suspension (10^{-1} dilution) was transferred to above three sterile test tubes by using a sterile pipette.
- Then, 1ml of first decimal dilution sample (10^{-2} diluent) was transferred to another three steriled test tubes by using another steriled pipette.
- This procedure was followed to other decimal dilution sample (10^{-3} diluent.)
- The test tubes were incubated for 72±3 hours at 31±1 ^{0}C.
- Finally the numbers of samples were counted which initial colour was change.

3.9.3 *Salmonella*

3.9.3.1 Preparation of Culture media

Materials and Apparatus:-

Distilled water(1000ml)
Brilliant green (0.5g)
Conical flask
Magnetic stirer
Autoclave
Electric balance

3.9.3.2 Preparation of test sample

- Representative food samples were used

Procedure:-

- The 0.5g of Brilliant green sample was dissolved in a conical flask with 1L of steriled distilled water by using Magnetic stirer
- 25g of food sample was added to 225ml of sterile distiled water which was containing 1ml of Brilliant green solution.
- The medium was placed at room temperature for 60±10 min.

- The mixture was placed at 37^0C for 16-20hr.
- The xylose lysine desoxycolate (XLD) media was inoculate from 1ml of above sample and incubation at 37^0C for 24hr.
- Finally, black colonies were counted by using electric colony counter.
- Same procedure was carried out for 10 samples of product.

CAPTER 4

Results and Discussion

4.1 Determination of semisolid corn base preparation

Starch is the reserve carbohydrate of corn plant and occurs as granules in the cells of corn seeds in plastids, separated from the cytoplasm (Meyer, 1960). When cells of tender corn seeds were grounds, the starch granules come out from ruptured cells and made slurry. These starch granules are washed out with water from above slurry during the draining procedure.

The starch granules of corn contain both amylose and amylopectine molecules. In addition to that waxy or glutinous starch can be consisted.

When washed out solution which was prepared from ruptured corn seeds are heated, it form a paste due to gelatinization. Generally gelatinization is a major change of carbohydrates on cooking. When starch granules were mixed with cold water, no apparent changes occure at initially. But when the water is heated, the viscosity of the mixture increased and if the concentration of the starch is sufficiently great, it is known as gelatinization (Meyer, 1960).

Amylase is believed to form gels more readily. Because the linier shape of amylase molecules are allows the formation of a three dimensional network ease. But the ability of a natural starch to form paste differ considerably with,

- The variety of starch.
  The corn starch show different behaviour patterns. Therefore, corn starch easy to make a good paste than this is one reason usage of corn starch in this product.
- pH of solution.
  The pH at which gelatinization is measured is most important in hear when preparation of corn base the pH must be controlled at 6.
- Temperature and length of heating.
  In hear, the gelatinization temperature is 70-75^{0}C and time is measured at 15min.
- Size of the granules.

4.2 Formation of colour in semisolid corn base

The carotinoids are a group of yellow, orange, and orange-red fat soluble pigments widely distributed in nature. This carotene is a mixture of three isomers such as α,β, γ- carotene(Meyer,1960).

However, the tender corn seeds contain number of carotinoides such as zeaxanthin, cryptoxanthin, xanthphyll, α-carotene, β-carotene, γ-carotene, k-carotene, keo- cryptoxanthin, hydroxyl –alpha-carotene. These are affected to light yellow colour of the product given through corn base. The colour of the product can be changed due to the amount of carotene content of different varieties. Bright yellow colour is given from the verity due to high amount of carotene than other (Meyer, 1960).

In addition, fully mature pumpkin is also a carotene rich crop. It effect the improvement of colour of the product. Also tender corn seed and pumpkin are not promote browning reaction due to absence of phenolic and poly phenolic compounds as it's constituents.

However, when ground potato promotes browning reaction. The amount of potato slurry added is low, also mixing and heating process are done quickly to control activation of enzyme which is responsible for browning reaction. So that yellow colour of the product was maintained.

There was no appeared fluracens in testing of aflotoxin to 10 samples of semisolid corn base and 5 samples of final products.

4.3 Prevention of Aflatoxins semisolid corn base

Aspergilus is grown in soil where the crop is cultivated. Seed can be contaminated with the fungus while it is grown. Contamination takes place during harvesting and drying process mainly. Aflatoxin causes damage to brain and liver cells damage in infants. Due to that reason, corn is not used to produce infant formula which is cultivated in Sri Lanka. In addition to that, traditionally the corn is harvested at dry season in Sri Lanka. The environmental factors such as

temperature and humidity are favourable for reproduction of *Aspergilus.* Therefore, the contamination is easy. Also, the corn pods are properly sealed with outer leaf sheaths. Therefore, the contamination of *Aspergilus* spores is avoided in tender corn hence can be used to produce infant foods.

There was no appeared blue fluorescence in testing of Aflatoxins to 10 samples of semisolid corn base and 5 samples of final products

4.4 Proximate Analysis of semisolid corn base

After proximate analysis of the semisolid corn base following composition was obtain.

Table: 4.1 composition of semisolid corn base

Name of nutrients	Amount %
Protein	4.6
Fat	0.4
Fiber	2.0
Moisture	79.32
Ash	0.7

According to proximate analysis79.32% of moisture content and 20.68% of total solid content were recorded in semisolid corn base. The summations of of protein, fat, fiber, and ash contents are 7.7%. So the semisolid corn base contain 12.98% of carbohydrates (the vitamins and minerals content of tender corn are lass than 1g so the vitamins and minerals content is a negligible factor for the calculation)

4.6% of protein, 0.4% of fat and 12.98% of carbohydrates content are measured in semisolid base. Generally, the tender corn contains 4.7 % of protein, 0.9 % of fat and 24.6% carbohydrate. (Appendix III) The insufficient extraction of corn seed, growth condition of crops and maturity level of pods may be cause for such differences.

According to FDA recommendation, the infant formula must be consisted 1.8-4.5g of protein and 3.3-6.0 of fat content per 100kcal.

Therefore 8.7g of pumpkin, 2.0g of potato and 4.0g of Soya bean oil were added to 85g of semisolid base as ingredients to reach FDA standard.

4.5 Proximate Analysis of final product

The final product composition was given in table

Table 4.2 Final product composition

Name of nutrients	Amount %
Protein	4.06
Fat	4.4
Fiber	1.76
Moisture	78.09
Ash	0.65

According to proximate analysis78.09% of moisture content and 21.91% of total solid content are present in final product. The summations of amount of protein, fat , fiber, and ash is 10.87% So, the final product contain 11.04% (21.91%-10.87%) of carbohydrates (the vitamins and minerals content of the product which made from plant materials is less than 1g so the vitamins and minerals content is a negligible factor to this calculation).

The total calory value is 100 given from 16.24kcal from 4.06% of Protein, 39.6 kcal from 4.4% of Fat and 44.16kcal from 11.04% of carbohydrate. Due to this above raw material formulation is similar to FDA recommendation (Appendix I).

4.6 Determination of minerals

The vitamins and minerals contents of the product were standardized by adding major nutrients. Generally, minerals are not destroyed during product processing steps. Therefore, the mineral content of the product can be assumed as the values shown in the table (Appendix IV).

Table 4.3 Mineral content of raw materials and final product

Name of minerals	Minerals content per100 g			
	Corn	pumpkin	potato	Final product
Calcium (mg)	9	10	10	8.7
Phosphorus(mg)	121	30	40	106.26
Magnesium(mg)	40	38	30	38
Iron(mg)	1.1	0.11	0.48	0.953
Zinc(mg)	-	0.26	0.53	0.03
Manganese(μg)	-	0.05	0.13	6
Copper(μg)	-	0.05	0.6	7
Iodine(mg)	...	...	...	...
Sodium(mg)	51	5.6	11	44.66
Potassium(mg)	151	139	247	145.80
Chloride(mg)	34	4	16	29.56

Source: Applied science series, A Text Book of Nutrition, (1999).

If all minerals are come to the product as 100%, the following amounts 513mg of $CaCl_2$, 4.7mg of Zinc acetate, 0.6mg of $CuSO_4$, and 1105.8mg of $MgCl_2$ must be added to product to reach FDA recommendation

These fortified amounts of minerals can be changed. Due to changes of mineral content in raw materials in insufficient extraction of corn seeds, growth condition of crops, diseases, genetic factors and others.

4.7 Determination of vitamins

During processing the product, lot of vitamins destroy through grinding, mixing and heating. Destruction behaviour of vitamins during processing is given below,

Table 4.4 Destruction behaviour of vitamins

Name of vitamins	Distraction condition
Vitamin A	Stable to ordinary cooking and alkalies condition.
Vitamin D	Stable to heat and alkalies condition.
Vitamin K	Stable to acid condition/ destroy to alkalies, light and oxidizing condition.
Vitamin E	Stable to heat and acid condition/ destroy to alkalies condition.
Thiamine	Stable to 120^0C in acid condition/ destroy to alkalies condition.
Riboflavin	Stable to acid condition/ destroy to alkalies and bright light.
Vitamin B6	Stable to heat and acid condition.
Vitamin B12	Destroy to alkalies condition.
Niacin	Stable to heat, acid, alkal, light, oxidation, autoclaving condition.
Folic acid	Stable to 100^0C in acid condition.
Pantothenic	Stable to 120^0C for 30min in natural solution.
Biotin	Stable to heat, light, acid and 120^0C for 30min aqueous condition.
Vitamin C	Destroy to $50\text{-}55^0$C.
Choline	Stable to 100^0C in acid condition.
Inositol	...

Source: Applied science series, A Text Book of Nutrition, (1999).

In this product, the maximum temperature level reached was 70^0C- 75^0C at pH-6.under this condition, Vitamin K, Vitamin E, Thiamine, Riboflavin, Vitamin B_6, Vitamin B_{12}, Niacin, Folic acid, Pantothenic, Biotin, Choline like vitamin can be remained. However, vitamin A, D, C are subjected to destruction. The vitamin content of the final product can be assumed as shown in table which is calculated through general composition of raw materials (Appendix III).

Table 4.5 Vitamin content of raw materials and final product.

Name of vitamins	Vitamins content per100 g			
	Corn	pumpkin	potato	Final product (Assumed content)
Carotinoids(μg)	32	50	24	-
Vitamin D	...	...	...	-
Vitamin K	-	-	-	...
Vitamin E	...	...	...	...
Thiamine(μg)	0.11	0.06	0.1	147.7
Riboflavin(μg)	0.17	0.04	0.01	179.5
Vitamin B6	-	-	-	-
Vitamin B12	-	-	-	-
Niacin(μg)	0.6	0.5	1.2μg	573.5
Folic(μg)	-	13	7μg	11.19
Pantothenic	...	...	...	...
Biotin	...	...	...	...
Choline (mg)	-	136	100	13.38
Inositol	...	...	...	...
Vitamin C(mg)	-	2	17	-

Source: Applied science series, A Text Book of Nutrition, (1999).

Assuming available vitamins are coming to the product 100%, 200mg of vitamin E and 25mg of vitamin C are added to product to reach FDA recommendations. After mixing and heating of ingredients, the vitamins and minerals content of the sample should be predetermined through test sample and

calculated formulated amount of vitamins and minerals from out side to reach FDA standard.

4.8 Determination of water activity

Table 4.6 Result of salt liquefaction test

Name of chemicals	Room temperature	4^0C -5^0C
NaCl	Liquefied	Liquefied
KBr	Liquefied	Liquefied
KCl	Not liquefied	Not liquefied

The water activity is depending on the amount of free water in food. If amount of free water is high the water activity is also high.

If water activity of product is higher than used chemical's wateractivity, the chemical can liquefy (because of the free water molecules have absorbed to the chemical, therefore chemical can liquefy).

If water activity of product is lower than used chemical's water activity, the chemical can not liquefy (because of the free water molecules have not absorbed to the chemical, therefore chemical can not liquefy).

According to that theory and observation the water activity of the product is between 0.80-0.84 at room temperature as well as 4^0C-5^0C.

4.9 Final product labelling

Under FDA standard, the final product labels should be similar to below table.

Table 4.7 Final product label

NUTRITION INFROMATION		
Servings per package: (insert number of servings)		
Serving size:g(or ml or other units as appropriate)		
Name nutrients	Quantity per servings	Quantity per 100g(or 100ml)
Energy	kj (cal)	kj (cal)
Fat, total(insert claimed fatty acids)	g	g
Carbohydrates-(suger)	g	g
Sodium	mg(mmol)	mg(mmol)
insert any other nutrient or biological active sub tenses to be declared	g, mg, μg (or other units as appropriate)	g, mg, μg (or other units as appropriate)

Source: Australia and New Zealand food standards code, (2000).

4.10 Selection of suitable package

The aerobic plate counts were obtained for the two packages, after storage at 4^0C-5^0C for three weeks in 10^{-3} dilution for 30 samples of package. (Appendix V and Appendix VI) The results are given below.

```
Two-sample T for TLAF vs pp

        N   Mean   StDev   SE Mean
TLAF   30   8.03    1.63      0.30
PP     30   9.70    1.73      0.31 P-Value = 0.000   DF = 57
```

H_0: There was no significant difference in microbial colony counts between TLAF and PP at 5% significant levels.

H_1: There was a significant difference in microbial colony counts between TLAF and PP at 5% significant levels.

According to above result, H_0 is rejected due to the p valve is lees than 0.05 at 5% significant level. So, H_1 is accepted and there was a significant difference in microbial colony counts between TLAF and PP at 5% significant levels.

Based on results of colony counting, more than 10^4 of microbe cells have been consisted for 1g of product in 3 samples from 30 samples which was packed in TLAF and more than 10^4 of microbe cells have been consisted for 1g of product in 9 samples from 30 samples which was packed in PP. Under SLS: 651, 1889 (Appendix VI) the product was rejected which is packed in PP cup.

According to above results and SLS: 651, 1889 recommendation the most suitable packaging a material is TLAF for this product.

4.11 Determination of Coliform

Coliform was determined in the product after storage at 4^0C for three weeks in 10^{-3} dilution under SLS: 651, 1889. (Appendix VI)The results is presented below,

Table 4.8 The results of determination of Coliform

Name of test	Number of tested sample	Positive answer	Negative answer
Coliform	5	-	5

According to that result the product is free and safe from Coliform.

4.12 Determination of *Salmonella*

Salmonalla was determined in the product after storage at 4^0C for three weeks under SLS: 651, 1889 (Appendix VI).There were no black colonies in all petri dishes. According to that result the product is free and safe from *Salmonalla.*

4.13 Product preparation for serving

The unopened pack of product was dip at 50^0C to 55^0C in hot water bath for 5-8min, warmed product get out and cut in to pieces. Then, the pieces are ready for serving.

CHAPTER 5

5.1 Conclusion

The textural properties and colour of the final product can be attributed to the raw materials used in the formulation process. Therefore no addition of colouring agents or no texture development ingredients was required in the formulation.

The product contains 11.04% of carbohydrate, 4.06% of protein and 4.4% of fat which were obtained by mixing ingredients in a ratio of 85% of tender corn, 8.6% of pumpkin, 2% of potato and 4% of soya bean oil. The above composition tallies with up lift Food and Drug Administration standards for infant foods.Vitamins and minerals fortification was carried out to the formulation to FDA standards.

The final product is prasented in a triple laminated aluminium foil pouch which can retain the quality of the product for an estimated period of 3 weeks under refrigerated condition (4^0C-5^0C).

5.2 Further studies and recommendation

1. Determination of dosages of vitamins and minerals required to fortify the product.
2. Reduction of water activity.
3. Improvement of shelf life.
4. Conduction of sensory evaluation.

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APPENDIX I

Food and Drug Administration standards for Major nutrition and Vitamins in infant foods

Major nutrients

Nutrient per 100kcal	Recommended level
Protein (g)	1.8-4.5
Fat (g)	3.3-6.0
Linoleic acid (mg)	300 or more

Source: Food and Drug Administration, Department of Health and Human (2002)

Vitamins

Name of vitamins	Recommended level
Vitamin A	250-750IU
Vitamin D	40-100IU
Vitamin E	0.7 IU
Vitamin K	4μg
Thiamine	40μg
Riboflavin	60μg
Vitamin B_6	35μg
Vitamin B_{12}	0.15μg
Niacin	250μg
Folic	4μg
Pantothenic	300μg
Biotin	1.5μg
Vitamin C	8mg
Choline	7mg
Inositol	4mg

Source: Food and Drug Administration, Department of Health and Human (2002)

APPENDIX II

Food and Drug Administration standards for Minerals in infant foods.

Minerals

Name of minerals	Recommended level
Calcium	60mg
Phosphorus	30mg or more
Magnesium	6mg or more
Iron	0.15-3.0mg
Zinc	0.5mg or more
Manganese	5μg or more
Copper	60μg or more
Iodine	5-75μg
Sodium	20-60mg
Potassium	80-200 mg
Chloride	55-150 mg

Source: Food and Drug Administration, Department of Health and Human (2002)

APPENDIX III

Major nutrients content of raw materials.

Name of food stuff	Moisture (g)	Protein (g)	Fat (g)	Minerals (g)	Fiber (g)	Carbohydrate (g)	Energy Kcal
Maize-tender	67.1	4.7	0.9	0.8	1.9	24.6	125
Soya bean	8.1	43.2	19.5	4.6	3.7	20.9	432
Pumpkin	92.6	1.4	0.1	0.6	0.7	4.6	25
potato	74.7	1.6	0.1	0.6	0.4	22.6	97

Source: Applied science series, A Text Book of Nutrition, (1999).

Vitamins content of raw materials.

Name of food / Name of vitamins	Maize-tender	Soya bean	Pumpkin	potato
Carotene (µg)	32	426	50	24
Vitamin D(mg)	...	...	...	..
Vitamin E	....	...	...	..
Vitamin K	...	..	..	..
Thiamine (mg)	0.11	0.73	0.02	0.10
Riboflavin (mg)	0.17	0.39	0.01	0.01
Vitamin B6 (mg)	-	-	-	-
Vitamin B12	....	-		
Niacin (mg)	0.6	3.2	0.2	1.2
Folic acid (µg)	-	100	13.0	7.0
Pantothenic	....	....	....	...
Biotin	....	...	...	...
Vitamin C (mg)	6	-	2	17
Choline (mg)	-	-	136	100
Inositol	...	...	...	...

Source: Applied science series, A Text Book of Nutrition, (1999).

APPENDIX IV

Minerals content of raw materials

Name of food / Name of minerals	Maize-tender	Soya bean	Pumpkin	potato
Calcium (mg)	9	240	10	10
Phosphorus (mg)	121	960	30	40
Magnesium (mg)	40	175	38	30
Iron (mg)	1.1	10.4	0.11	0.48
Zinc (mg)	-	4.4	0.26	0.41
Manganese (mg)	-	2.35	0.05	0.18
Copper (mg)	-	1.38	0.05	0.18
Iodine (mg)	...	...	....	....
Sodium (mg)	51.7	-	5.6	11.0
Potassium (mg)	151	-	139	247
Chloride (mg)	34	-	4	-

Source: Applied science series, A Text Book of Nutrition, (1999).

Composition of Soya bean oil

Nutrients	Quantity per 100g
Energy	884 kcal
Protein	0g
Total fat	100g
Cholesterol	0g
Carbohydrate	0g
Dietary fiber	0g
Na	0g

Source: New turkey brand product.

APPENDIX V

Result of aerobic plate colony counts for Triple Laminating Aluminium Foil (TLAF) and Polypropylene (PP) package.

Numbers of colonies in TLAF	Numbers of colonies in PP
8	10
9	11
10	9
9	6
7	8
8	11
6	12
9	9
8	9
11	10
8	7
11	11
8	10
5	13
12	8
9	9
6	11
8	9
8	9
7	8
9	11
7	14
6	10
8	9
7	9
6	9
8	10
9	12
8	8
6	9

APENDIX VI

Statistically analysis result of aerobic plate colony counts

Two-Sample T-Test and CI: TLAF, PP

```
Two-sample T for TLAF vs PP

       N  Mean  StDev  SE Mean
TLAF  30  8.03   1.63     0.30
PP    30  9.70   1.73     0.31


Difference = mu (TLAF) - mu (PVC)
Estimate for difference:  -1.66667
95% CI for difference:  (-2.53414, -0.79920)
T-Test of difference = 0 (vs not =):T-Value =-3.85
P-Value = 0.000  DF = 57
```

Microbial limits SLS: 5651, 1889

Test organism	Limits per gram			
	n	c	m	M
Aerobic plate count	5	1	10^4	10^5
Coliforms	5	1	10	100
Salmonella	10	0	0	-

n is the number of samples to be tested.
c is the maximum allowable number of samples yielding values between m and M.
m is limit below which a count is acceptable for any samples.
M is limit below which a count is acceptable for any samples.

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