"DEVELOPMENT OF COWPEA-RICE LADOO - A PROTEIN RICH PRODUCT"

PROJECT WORK

SUBMITTED TO DEPARTMENT OF PG STUDIES IN FOOD SCIENCE AND NUTRITION,

BESANT WOMEN'S COLLEGE, MANGALORE

IN THE PARTIAL FULFILMENT OF REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

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CERTIFICATE

This is to certify that the project work entitled **"*DEVELOPMENT OF COWPEA-RICE LADOO - A PROTEIN RICH PRODUCT*"** is an authentic record of independent research work done by ***Ms. NIREEKSHA .V.(Reg. no 189044802)*** under my supervision during the period of **January 2020 to April 2020**, submitted to Mangalore university for the partial fulfilment for the award of the degree of ***Master Of Science In Food Science And Nutrition*** and the present work has not been previously formed the basis for the award of degree, diploma, fellowship, associateship or other titles.

Place: MANGALORE
DATE: 10 SEPTEMBER 2020

RESEARCH GUIDE

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(Accredited by the NAAC with 'A' Grade)

CERTIFICATE

This is to certify that the project work entitled ***"DEVELOPMENT OF COWPEA-RICE LADOO - A PROTEIN RICH PRODUCT",*** submitted to the ***Department of PG Studies in Food Science and Nutrition,*** by ***Ms. NIREEKSHA .V.(Reg. no 189044802)*** towards the partial fulfilment of the degree of ***Master Of Science In Food Science And Nutrition,*** is a faithful record of original work carried out by her in the academic year **2019-2020.**

Place: MANGALORE

DATE: 10 SEPTEMBER 2020

HEAD OF THE DEPARTMENT

DECLARATION

I, ***Nireeksha .V. ,*** hereby declare that project work entitled **"*DEVELOPMENT OF COWPEA-RICE LADOO - A PROTEIN RICH PRODUCT"*** is a bona fide record of research work done by me under the supervision of ***DR. ASHA RAI M.G.,*** **Head , Department of PG Studies In Food Science and Nutrition, Besant Women's College, Mangalore.**

The information depicted in the current report is the result of my own work, except where the reference is made. The information provided in the report is authentic as per my knowledge.

The results embodied in the project work have not been submitted to any other university or institution for the award of any degree, diploma, associateship, fellowship or similar titles.

Place: MANGALORE
Date: 10 SEPTEMBER 2020

NIREEKSHA .V.

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ABSTRACT

Protein-energy malnutrition (PEM) is one of the major concern in kids below the age of 5 years, but the protein intake in the diet of young adults remain unnoticed. As a result, there are chances of them suffering from protein deficiency which will lead to other deficiencies as well. So it becomes crucial that all age groups are equally analysed because the young adult contributes the majority economic stability of the country as most of them fall into working groups. The development of the plant-based protein-rich product which acts as a complete protein equivalent to animal protein by cereal-pulse combination can help cope up the macro-nutrient deficiency to some extent. The use of the least number of ingredients helps to formulate an affordable protein-rich product. The protein profile which is the major component of product standardization claims the 50:50 and 60:40 to have high protein content which was further taken to a sensory evaluation in order to select the best proportion for ladoo based on consumer preference. The proximate analysis claimed the ladoo to be high in energy (537.76kcals) and protein (10.80g) along with carbohydrates (109.03g) per 100gms of the sample. The physiochemical analysis depicted the pH to be almost neutral, the total acidity within the range. The shelf life study was predominantly based on sensory evaluation claiming the product to have a shelf life of 21 days (3 weeks) as the product deteriorated in the fourth week. The product hygiene was within acceptable ranges on the basis of microbial analysis and the packaging was done using bagasse containers made out of sugarcane fibre as it is biodegradable in nature.

CHAPTER 1
INTRODUCTION

The need for developing a product arises when the nutritional status of the population requires rectification. Usually to develop a product first the underlying deficiencies must be detected by collecting data on the nutritional status of the population. After knowing the lacking nutrient, the natural food sources of that particular nutrient must be analysed in terms of its nutritional composition. A combination of two or more food groups would be considered to be beneficial because it will compensate for the nutrient which is deficient in one particular group. All these criteria will lay the foundation for formulating a better product. In the current case, the population (especially the young adults) are lacking in quality protein, so keeping in mind the food habits of all the age groups and all classes of the society; a four-ingredient ladoo from the staple and cheap yet nutritious ingredients such as rice, cowpea, jaggery, and ghee is designed. Therefore, considering the drawbacks of limiting amino acids, the cereal-pulse combination of rice-cowpea is used in order to uplift the protein value so that all age groups of the community can consume it and improve their protein status.

1.0 INTRODUCTION

Major calorie requirement of an individual is met mainly by the energy and the protein supplied through the diet. In the Indian scenario major portion of the meal is comprised of cereals and the remaining part is comprised of the pulses, roots and tubers; vegetables, fruits, milk and its products in the decreasing order of their consumption (Sheela *et al.,* 1999). More than half of the population in the country consume vegetarian diet due to cultural practises and beliefs. Hence the Indian diets, lack protein from the animal source. Apart from this, the proportion of energy and protein is not well balanced in the Indian diet which may be due to economic reasons as pulses and animal foods are expensive which cannot be affordable on daily basis by all the classes of the society (Minocha *et al.,* 2017). But in the current scenario, there is drop in the calorie consumption of the Indian population. This drop is aiding in the imbalance of energy and protein as they are the major contributors of the diet, along with other nutrients. There is noticeable fluctuation in protein and energy, that leads to protein-energy malnutrition (PEM) which is very evident in lower classes of the society especially kids below the age of 5. In case of adults the diet lacking in protein will be the underlying cause of various other micronutrient deficiencies. Due to this, asymptomatic cases of PEM are seen in adults belonging to all socio-economic groups (Swaminathan *et al* 2012). Usually the PEM is concerned only with kids and so government focuses on this group whereas the other age group such as young adults (20-40years) who might be protein deficient but are asymptomatic go unnoticed. The young adults who are at the most active stage of their life, if protein deficient, the consequence of deficiency gets carried forward to the old age too with geriatric issues. Though in initial stages the protein status of an individual is monitored, it is not very seriously considered in later stages of life. So, all age groups must be equally analysed, predominantly the young adult as they contribute majorly for the economic stability of the country because most of them fall into working and labour groups. If they are nutritionally affected, the economy of the country will be hampered.

1.1 Protein

Proteins play a vital role in the body as they are very important for carrying out major bodily functions such as ;(Chatterjee *et al.,* 2012)

- The basic component of muscles (for muscle build up), blood (as a constituent of haemoglobin) and skin (as they help in repairing the damaged and dead cells).

-acting as an alternative source of energy by gluconeogenesis, when there is insufficient carbohydrate intake.

-maintaining the acid- base balance in the body by neutralising the protons (H^+) ions released when they is high acid secretion in the body or else it will lead to acidosis.

-maintaining the fluid balance by holding fluids intact in the cells, when there is abnormalities in the protein synthesis it leads to oedema.

-acting as the first line of defence against infection by producing antibodies which are basically giant protein molecules.

-acting as a major regulator of the bodily functions, as majority of the hormones and enzymes are basically composed of proteins.

In case of balanced diet, 10-12% of energy contribution is by the proteins, which may be from cereals, pulses, milk and its products; animal products (egg, meat, fish, poultry etc.). The term "quality protein" plays a key role in knowing whether the food consumed is providing enough amounts of required amino acids to the body. Usually animal protein foods fall into this category as they provide all the necessary essential amino acids. Hence such foods are said to provide complete protein whereas the plant sources lack one more essential amino acid. Important sources of plant protein are cereals and pulses, but they lack in lysine and sulphur containing amino acids (cysteine, methionine) respectively (Subbulakshmi *et al.,* 2017). These amino acids are stored in the storage protein subunits namely albumin, globulins, prolamines and glutelins which undergo composition changes on storage or processing of the plant protein. Thus, they need to be combined with each other to provide amino acid composition that equalises animal protein, so that the above mentioned functions can be performed by protein in the body.

1.2 Plant protein

Usually the plant protein is termed to be inferior when compared to animal protein because they contain anti-nutrients which interfere with the nutrient availability. The anti-nutrients commonly present in cereals and pulses are oligosaccharides (raffinose,

starchyose), trypsin inhibitors, alpha-amylase inhibitors, lectins, phytates, tannins, saponins, polyphenols, oxalates etc.(Vasconcelos *et al*., 2010) So by the processing methods such as milling, soaking, fermentation, germination; these anti nutrient factors can be eliminated and the nutritional quality of the food can be increased. Based on the nature of the ant nutrient, the accurate processing methods are selected for best results.

1.3 Cereals

Cereals hold the lion's share in the Indian diet, despite the diet being calorie deficient. The major cereals used in Indian diet (about 80%) (Shakuntala *et al.,* 2000) especially in the Southern region is rice which is the staple food. Rice (*Oryza sativa*) was originated in south-east Asia and is usually grown in tropical countries with abundant rain and sunshine; it grows in diverse climatic conditions based on which varieties of rice are grown. The Indian varieties include there are nearly 1200 varieties of rice suitable for different ecosystem as released by ICAR-NRRI, Cuttack. Though rice has protein content of 5-7% which is less comparing to other cereal, it has high lysine content (3-4%) which is 50% more than wheat, so comparing to other cereals rice has better protein quality. Rice is available in the form of whole grain, flour, rice flakes, puffed rice, flaked rice, and by-products (bran oil) in Indian markets.

1.4 Pulses

Pulses are the sole protein source in case of vegan diet and are major protein constituent of vegetarian diets. The pulse which is not extensively used in whole of the country but is abundantly used in southern Indian especially in the coastal regions is cowpea *(Vigna catjang)*, which is available in brown and white versions. Cowpea is suitable to be grown in warm and tropical climates as it is tolerant to dry conditions. The most commonly used is the white cowpea which is generally used as a whole and used as a vegetable in tender form. (Shakuntala *et al.,* 2000) Comparing to other pulses the yield is low and it is difficult to digest in unprocessed form. It has high protein content of 16-30 %(OECD *et al.,* 2019).

1.5 Cereal –Pulse Combination

According to studies the best composition of cereal –pulse ratio earlier was 80:20 to improve the lysine intake as cereals are deficient in it. But current studies have improvised the ratio to 60:40 so that the amino acid requirements are met well.

(Swaminathan *et al* 2012). The 60:40 ratios is well balanced as it would rectify the sulphur containing amino acid levels of pulses and lysine levels of cereals which are the limiting amino acids in the respective foods groups. The earlier composition was cereal dominant whereas the current ratio is balanced with both cereals and pulses. Along with this if the cereals and pulses are processed to remove the anti-nutrients which interfere the nutrient availability, the protein quality of the meal would be upheld.

1.6 Processing Methods

The processing methods which can be generally carried out at household level are soaking, germination, natural fermentation; cooking, dry roasting etc. the studies claim those different processing methods decreases levels of different anti-nutrient. (For example: soaking decreases the level of phytic acid and raffinose (Akinyele *et al., 1989*)). Based on the anti-nutrient to be decreased the processing method should be used and for better results combination of processing methods would be best. For instance soaking of pulses prior to cooking would give better results than unsoaked pulses as it would even decrease the cooking time.

1.7 Desserts

The major taste contributed by desserts is sweetness which enhances the taste of bland ingredients in turn improving their palatability and increasing their shelf life by trapping moisture in case of sugar concentrated desserts (Fellows *et al*., 2000). The moisture of the ingredients is evaporated through dry roasting and it is combined with sugar to trap remaining moisture. In case of ladoo the use of sugar/jiggery increases the sugar concentration which in turn increases the shelf life of the food product by decreasing the microbial load(Yuvarani *et al*.,2016) when stored in suitable conditions at room temperature either by packaging in suitable packaging materials or in air tight containers. The shelf life of ladoos that are dry in composition is more compared to the moist ladoos such as that made out of using watery sugar syrup with lower concentrations. On an average the shelf life of ladoos are approximately 3weeks when stored at room temperature (Pandey *et al*., 2019).

1.8 Bagasse packaging

Bagasse is the waste product of the sugarcane plant obtained after sugar extraction. Earlier bagasse was used solely as biofuel but now it is used to manufacture packaging

and storage containers, plates, and bowls etc. as a substitute for the usage of wood pulp. Apart from this, it is biodegradable in nature which makes it a sensible choice over the plastic containers for packaging of sweets and confectionaries (*et.al.,* Xiong, 2017).

1.9 Nutritional labelling

Nutritional labelling comprises of the nutritional composition of the food which consists of two components namely the nutrient declaration that is the composition of macronutrients and the supplementary nutritional information that comprises of the specified micronutrients in the food. The benefit of nutritional labelling is that it helps the consumer to make healthier food choices.(*et.al.,* Narain,2016).

1.10 Budgeting of the product

The budgeting of the developed food product is a valuable measure to monitor the economic expenditure and at the same time rectify the methods that are expensive to stabilise the cost of the product and improve consumer acceptability.(*et al.,* Shekar, 2019)

The taste of cowpea in the form of ladoo would be more acceptable when compared to cowpea in the savoury form. In case of savoury dishes, cowpea generally has lower shelf life due to high perishability and bland taste. By the incorporation with rice the protein quality aims to have improvement.

Therefore, considering these aspects, the present research work has been taken up with following objectives;

- To develop ladoo with the locally available staple ingredients such as cowpea, rice and improve their acceptability by presenting them in the form of ladoo.
- To enhance the protein quality of the ladoo by simple processing methods such as fermentation and germination that can be used at the household level and can be carried out with low capital cost..

- To formulate cost effective protein-rich ladoo using the least number of ingredients and analyse the nutritional composition as well as the shelf life at different storage conditions.

CHAPTER 2

REVIEW OF LITERATURE

A product can be developed only after knowing the need for the development, in this case it is the protein deficiency in the Indian meal which due to poverty that is aiding in malnutrition. So it is very important to make use of the locally available, economically feasible and staple ingredients. In the present context, cowpea and rice are the locally available ingredients which are not expensive and are affordable by all classes of the society and are nutritious as well. After selection of ingredient the nutritional composition must be studied through previous researches and the possible techniques must be employed to improve the nutrient content, so that maximum nutrient is available to the body. The techniques chosen must be simple with no requirement of complicated equipment, so that it can be used at home level (in this case, soaking, natural fermentation and germination). The usage of local ingredient, easy processing techniques, storage and packaging technique through evidence based research work, will stabilise the cost of the product. The next step is utilising the data provided by the research articles which will act as a framework for the formulation of the product. The collected reviews are presented as follows;

2.1 Protein intake pattern
2.2 Nutritional composition
2.3 Processing method
2.4 Germination
2.5 Fermentation
2.6 Amino acid profile
2.7 Product development
2.8 Packaging of product
2.9 Shelf life study

2.0 REVIEW OF LITERATURE

2.1 PROTEIN INTAKE PATTERN

Sumedha Minocha *et al.*, (2017) analysed the protein intake pattern in India correlating with the agricultural yields. The study claimed that the Indian diet was cereal dominant and despite having adequate protein intake there was deficiency in the quality protein which ranged from 4% to 26% comprising of various age groups. Hence the study advised to improve the protein quality by increasing the pulse intake as it would be beneficial to all the groups of the society because milk ,being protein rich is affordable only by middle to higher class population. As majority of the Indian population fall into lower class, they suffer from poverty which in turn paves way for malnutrition. Hence the study concluded that the use of effective agricultural practices was useful for high-quality yield and better health status.

Sumathi Swaminathan *et al.*, (2012) conducted a survey through questionnaire method utilising the primary data from National Nutrition Monitoring Bureau (NNMB) to analyse the protein intake pattern in India. The study was done among the tribal community, rural and urban population. The outcome of the study depicted a drop in the protein and energy intake in the rural as well as urban setup whereas in case of tribal community it remained unchanged. The protein source in case of rural and tribal community was majorly from cereal and little from pulses, the urban population had their proteins from milk and its products along with plant protein. The population subjected to cereal rich diet were found to be adequate in protein content but had low Amino Acid Score (AAS) lacking in lysine. Hence the study claimed that the combination of legumes with cereals at the ratio of 80:20 or 60:40 to uplift the protein quality. The ratio of 60:40 was recommended over 80:20 as the latter being cereal dominant would fail to rectify lysine inadequacy.

K. Sheela *et al.*, (1999) evaluated the nutritional status in Karnataka, where the food intake pattern of both urban and rural population was analysed. The study revealed that irrespective of the dwelling scenario both the population had similar eating pattern which comprised of cereals and millets at larger proportion and the consumption of milk, meat, vegetables and fats were scarce. The study claimed that intake pattern depended on economic status and majority of the population fell into lower or middle-

class groups whose diet had 50% of cereals and rest 50% of other food groups (pulses/milk/animal products). The study highlighted that the energy intake was gender discriminated where men had adequate energy intake but women had energy deficiencies but both the sexes had protein inadequacy. Hence the study stated that the improvement of protein status through protein-rich diet was important, as the other micronutrient deficiencies were corrected through supplementation provided by nutrition programs.

2.2 NUTRITIONAL COMPOSITION

Onder Kabas *et al.*, (2007) examined the physical and nutritional characteristics of cowpea with an aim, to know the harvesting and processing technique to be applied on it, along with the knowledge about the nutritive composition. Through random sampling method 100 seeds out of 30 kg sample were selected and were subjected to physical analysis (linear dimension, sphericity, density, volume, porosity) and it was found that all the dimensions fell into acceptable range and same processing or harvesting methods can be applied to all seeds. The nutritional analysis revealed that the cowpea seeds had 90.58% of organic matter out of which maximum portion was proteins (20.31%) whereas rest was constituted of ash, nitrogen with small amounts of minerals. Therefore, the study claimed cowpea was rich in protein.

Alessandra Rangel *et al.*,(2004) conducted the biological evaluation of the cowpea protein on rats by feeding them with diet rich in protein isolates (casein/cowpea protein isolate(CPI)/vicilin).The isolates, CPI and vicilin were quite similar in amino acid profile with the latter being deficient in methionine and tryptophan whereas the former in cysteine and methionine. The in-vivo studies showed that casein rich diet had excess weight gain whereas CPI rich diet caused moderate weight gain that was beneficial with no toxicity. So, the study asserted that CPI was economic and effective (as anti-nutritional factors can be removed by heat treatment) to treat protein- energy malnutrition (PEM).

2.3 PROCESSING METHODS

A.C. Laurena *et al.*, (1987) assessed the effect of wet heat methods (boiling/pressurised boiling/pressurised steaming) and dry heat methods

(roasting/microwave heating) on the polyphenol content and protein digestibility of cowpea. On analysis it was found that both the methods are effective in polyphenol reduction. The study declared that the wet heat methods were better than dry heat methods as they cause effective digestibility of proteins.

I.O.Akinyele *et al.*, (1989) estimated to what extent does traditional method of processing cowpea affects its nutritional and anti-nutritional properties. The cowpea was processed into *ewa* (boiled cowpea), *akara* (dehulled, fried cowpea paste), *ghegiri* (soup) and *moin-moin* (dehulled, steamed cowpea paste). The samples were subjected to proximate analysis and it was seen that approximately 82% of makeable decrease in anti-nutritional factors was seen in all the processing methods. Hence the study claimed that traditional methods (soaking/cooking/dehulling) caused notable reduction in anti-nutrient contents.

M.A. Akpapunam *et al.*, (1985) subjected the cowpeas to germination, fermentation and cooking at different time intervals of 24, 36, 48 and 72 hours. The result proved that cooking only improved the protein digestibility whereas germination and fermentation improved the nutritional status by diminishing the anti-nutritional factors. The maximum benefit was seen in germination of 24 hours and fermentation of 36 hours. Hence the study depicted that fermentation or germination combined with cooking is highly beneficial.

S.S. Ibrahim *et al.*, (2002) estimated about the effects of soaking, germination, pressure cooking and fermentation on the anti-nutritional factors of cowpea with respect to unprocessed ones. The findings depicted that soaking for 16 hours removed maximum amount of anti-nutrients and pressure cooking these seeds were easier and quick. The germination accompanied by soaking in 0.03% of sodium bi-carbonate for 16 hours reduced the anti-nutrients; fermentation however increased the tannin content. Therefore ,the study concluded that the methods such as soaking and germination decreased the anti-nutritional content of cowpea.

2.4 GERMINATION

A.C. Uwaegbute *et al.*, (2000) investigated effect of the germination time on the sensory and chemical characteristics of the cowpea seeds. The cowpea seeds were

soaked for 12 hours and were germinated for 24, 48, 72, 96 hours. On proximate analysis of the samples it was seen that, nutrient composition increased on increase in germination time but on sensory evaluation it was found that though 96 hour germination had best results, spoilage set up by 24hours. Hence the study asserted 24 hours of germination to the best method for cowpea.

Yu Haey Kuo *et al.*, (2004) checked about the effect of different germination methods (dark/light) on the protein profile of various legumes (peas, lentils, beans) which were soaked in 0.07% sodium hypochlorite(30 minutes) washed and then soaked in water(5 ½ hours) and then germinated (dark/ light). The results confirmed that light germination was preferred over dark germination as the former increased the free amino acid content in the all the samples(except for peas which required alteration in time) whereas the latter was preferred to increase non protein amino acids. Hence, the study declared that based on amino acid required the light or dark method of germination can germination technique can be selected.

2.5 FERMENTATION

Ulf Svanberg *et al.*, (1997) established a theoretical discussion on the process of fermentation and the benefits which enhance the nutritional status of the foods. First area of focus was on fermenting the weaning foods with the sustained remark of improving the protein digestibility and preventing flatulence in the infants. The same theory was implied for adults as well, with additional benefits of increased micronutrient level and this was possible only due to reduction in the anti-nutritional factors such as tannins, phytates. Hence, the study depicted that fermentation enhanced the nutritional status of food.

K.H. Steinkraus *et al.*, (1997) gave theoretical information about the classification of the fermented foods based on the process of fermentation taken place into lactic acid fermentation, acetic acid fermentation, alcoholic fermentation, alkaline fermentation, salt concentrated fermentation. The article highlighted the benefits of fermented foods and techniques to assess optimum pH required for the process to occur in order to prevent spoilage especially due to mycotoxins. The study stated that proper fermentation can be achieved by optimum pH maintenance as it stops microbial spoilage..

J.K. Chavan *et al.*, (1989) studied on the optimum conditions which would enhance the nutritive value of cereals which are subjected to fermentation process. The study depicted that the pH range for optimum levels of fermentation activity is 3.6 to 4.1 as below it are signs of spoilage; similarly the Titrable acidity of 0.84 to 1.46 % is safe. The study depicted that process of fermentation increased the level of storage protein fraction, namely albumins and globulins.

L.A. Shekib *et al.*, (1994) estimated the changes undergone by cereals (rice/wheat) and pulses (lentil/ chickpea) when subjected to fermentation by soaking in 3% sodium chloride and 6% glucose for 36 hours. The results claimed that there was increase in protein content of both cereals and pulses as fermentation causes hydrolysis of storage proteins. Hence, the study claimed that fermentation is the inexpensive method to yield quality proteins.

N.E. Yousif *et al.*, (2003) assessed about the effect of natural fermentation on the protein digestibility of rice at in vitro conditions where it was subjected to different time intervals(0, 4, 8, 12, 16, 20, 24, 28, 32, 36 hours). The results claimed that the protein underwent changes only after 24 hours of fermentation and in case of protein fraction, the albumin and globulin content increased, improving the lysine and the sulphur containing amino acid content. The study declared that on considering the protein fraction, 36 hours of fermentation yielded the best result.

H.N. Said *et al.*, (2015) examined the effect of natural fermentation on the sensory and physiochemical property of rice which was soaked in 5% sodium chloride for 3, 12, 24, 36 hours. On analysis it was found out that lactic acid bacteria was dominant which caused the fermentation that in turn decreased the pH and Titrable acidity. The drop in pH was beneficial up to 36 hours of fermentation; however excess fermentation beyond 36 hours caused loss of starch, lipid and amylose content even thorough the protein was increased. Hence, the study claimed that the optimum time for fermentation was 36 hours without nutrient loss.

2.6 AMINO ACID PROFILE

Sirelkhatim Balla Elhardallou *et al.*, (2015) studied about the protein profile of the cowpea focusing on the amino acid content. The objective was to identify the contents

of all the essential as well as limiting amino acids. The protein profile of whole cowpea flour (WCF), dehulled defatted cowpea flour (DDCF), cowpea isolate of isoelectric method (CPII) and cowpea isolate of micellization method (CPIM) was analysed and it was found that CPIM had high protein content. CPIM and CPII had high amounts of essential and non-essential amino acids, with high amounts of lysine compared to WCF and DDCF. The limiting amino acids in case of DDCF were methionine, cysteine, threonine and in case of WCF were methionine, cysteine. Therefore, the study concluded that cereal-pulse combination rectified the limiting amino acid and improved protein quality of the product.

I. Marta Evans *et al.,* (1974) analysed the protein content of cowpea and aimed at identifying the relation between total sulphur content with the limiting amino acid. The protein analysis depicted that most of the sulphur was present in the form of sulpho-amino acid with slight variation in total sulphur content. The negative co-relation claimed that the decrease in sulphur amino acid content was due to increase in albumin ratio. The study concluded that the drop in sulpho-amino acids content was mainly due to increase in albumin which acts as storage protein

R. Bressani *et al.,* (1961) did comparative study on the essential amino acid content of different pulses with an aim of identifying the abundant and limiting amino acids. The legume seeds used for study were black beans, red beans, rice beans and cowpea. It was discovered that cysteine and methionine were the limiting amino acid in all the legumes, taking the FAO protein reference as standard. The second limiting amino acid was leucine but the levels were acceptable comparing to cysteine and methionine. The protein content was found to be high in cowpea whereas with respect to essential amino acid both cowpea and black beans had high amount of tryptophan and all the legumes were high in lysine. Therefore through the study it was estimated that cowpea was a good source protein with respect to essential amino acids.

Ilka Maria Vasconcelos *et al.,* (2010) assessed about the variation in different cowpea varieties with respect to its amino acid content, anti-nutritional factors and seed protein content. It was evaluated that the optimum pH for seed solubility to extract protein isolate was 9 and on conducting SDS-PAGE it was found out that the predominant protein fractions were globulins followed by albumins. On analysing it was found out that globulin lacked threonine, prolamines lacked leucine and lysine, and glutelins

lacked lysine. The amino acid profile stated that all the varieties lacked the sulphur containing amino acids. Hence the study proved that no single protein fraction was equipped with all amino acids and combination of all yields somewhat efficient protein profile. The study stated that the anti-nutritional factors (trypsin and protease) could be diminished by infestation methods.

I.R. Agustin *et al.*, (2020) estimated about the effect of protein concentration on the solubility of protein in the cowpeas. The solubility range of the cowpea proteins were between the acidic pH of 4.0-5.0. The study claimed that cowpea protein were rich in albumin (containing cysteine, methionine, lysine) and globulin. The protein content was high in flour than in protein isolate.

R. Lasytity *et al.*, (1984) evaluated the composition of albumin, a protein fraction of cowpea legume. The study depicted that albumin consisted of cysteine and methionine but high contents of lysine was detected than the sulphur containing amino acids. Hence it concluded that cysteine and methionine content of pulse depended on albumin content.

K.M.G. Frota *et al.*, (2017) evaluated the protein profile of cowpea flour (WCF) and cowpea isolate (CPI). The results claimed that both the samples lacked in cysteine and methionine whereas the CPI lacked in even tryptophan. CPI was rich in all the other essential amino acids than WCF. Though it lacked tryptophan, WCF was preferred over CPI because even though the amino acid contents were high in CPI, it lacked tryptophan in addition which decreased its protein quality.

A.M. Hamad *et al.*, (1979) estimated about the protein quality concerning with available lysine of the cereals (rice, wheat, barley, oats) which are subjected to fermentation and germination process. The results showed that the lysine availability was high in fermented rice whereas in case of other cereals lysine was high in germinated samples. Hence it was proved that the best method for rice to increase lysine content was fermentation.

P. Tongual *et al.*, (1979) investigated the nutritional changes in the rice chip and meal subjected to lactic acid fermentation. After fermentation the samples were subjected to estimation of pH, Titrable acidity and proximate analysis. The results showed that the

pH and Titrable acidity were in acceptable range along with an increase in riboflavin content and slight increase in lysine content (though it remained as limiting amino acid).

2.7 PRODUCT DEVELOPMENT

S. Yuvarani *et al.*, (2016) develop the cereal pulse based multigrain ladoo and analysed for its nutritive value through AOAC methods. The preparation method was standardised with different variations and the standardised ladoo was subjected to sensory evaluation as well as proximate analysis. The antioxidant content was measured which claims that the cooking methods enhanced the vitamin C content.

2.8PACKAGING OF THE PRODUCT

W. Xiong *et al.*, (2017) made a review study on the bagasse composition which revealed that bagasse acts as an eco-friendly component and is of low cost. Apart from this the study revealed about the composition of natural fibre present in it, the processing method employed etc. which on the whole proves that it is the better choice over plastic.

2.9 SHELFLIFE STUDY

Laxmi Pandey *et al.*, (2019) analysed about the mineral composition of multigrain ladoo and how it underwent changes on subjecting to storage for 90days. The sensory characteristics and mineral content was estimated on day 1of ladoo preparation. The quality of ladoo on storage was estimated by fat acidity and peroxide values which would give clear picture on the rancidity of the ladoo. A decrease in peroxide value was seen by 90^{th} day claiming the ladoos to last for 90 days.

CHAPTER 3

MATERIALS AND METHODOLOGY

After analysing about the findings and conclusions so far with the help of the literature review, the method for formulating a product is set up. The section involves every minute detail about the raw materials used, the processing methods employed, the analysis method used. On the whole this section, gives information about all the analysis done on the product during the time of its study. The major areas of concern are proximate analysis, physio-chemical parameters, rheological parameters and microbial analysis. Apart from this, shelf life study, consumer acceptance and nutritional labelling also are an integral part. All these factors will contribute to the quality standards of the product to be developed. The methods employed for the food product analysis are the standardised procedures of from AOAC, BIS, and FSSAI which are modified as per the food product. The materials and methods section gives detailed information regarding the method, equipment and chemicals used.

3.0 MATERIALS AND METHODOLOGY

3.1 MATERIALS

3.1.1 Ingredients

The raw ingredients required for ladoo preparation (rice, cowpea, jaggery and ghee) were purchased from the local wholesale retailer in Mangalore. The baking soda, salt, sugar and citric acid used for fermentation and germination methods were of food grade purchased from the same whole sale retailer as well.

Table 1: *Ingredients Used For Ladoo Preparation.*

INGREDIENTS	COMPOSITION OF COWPEA-RICE LADOO (100Grams)
Rice, raw, milled *(Oryza sativa)*	30g
Cowpea, white (Vigna catjang)	30g
Jaggery, cane (Saccharum officinarum)	30g
Ghee (cow)	10ml
Cardamom	1g

Plate 1:
Ingredients Used For Ladoo Preparation.

3.1.2 Equipment and glassware:

- Equipment : Analytical Balance (Essae Model No FB-600), Hot Air Oven (Labotech Universal Hot Air Oven-Model No BDI50), Muffle Furnace (Rotek Muffle Furnace), Desiccator, pH Meter(EI Deluxe pH Meter Model 101), Spectrophotometer (Double Beam Spectrophotometer Model No 2375), Centrifuge (Remi Model No R-8C), Water bath (Labotech Serological Water bath Model No 57),
- Glassware And Other: Petri plates (80x17), Crucibles (Infusil), Burette (Borosilicate Glass3.3), Conical Flasks (Boro 3.3), Standard Flask (Borosilicate 3.3), Beaker (Boro Silicate 3.3), Micro Pipettes (Today Tech), Test Tubes (Boro Silicate 3.3), Cuvettes (Glass Square Cuvette, Light Path 10mm), Glass tube, Spatula.

3.1.3 Chemicals used:

All the chemical reagents used were of high grade manufactured from SRL, Merck, Himedia, Medilise, Loba Chemie , Spectrum, Indus, Avra, Oxford and Fisher.

- ✓ **Sisco Research Laboratories Private Limited, New Mumbai** : Di-ethyl ether(60-29-7), Anthrone pure (90-44-8), Di-nitro salicylic acid (64-17-5), Potassium sulphate (72-21-1),Potassium permanganate (73-53-9), Ethylene di-amide tetra acetic acid(87-61-7), Beta carotene (73-57-2), Acetone (67-64-1).
- ✓ **Medilise Chemicals, Kerala** : Hydrochloric acid(CAT No MH401), Sulphuric acid(CAT No MS1332), Sodium hydroxide pellets(CAT No MS336), Sodium potassium tartrate(CAT No MS1357), Copper sulphate(CAT No MC466), Glacial acetic acid (CAT No MA211),Chloroform (CAT No MC437), Oxalic acid (CAT No MO950), Ammonium ferrous sulphate (CAT No MA285), Ammonia (CAT No MS411).
- ✓ **Himedia Laboratories Private Limited, Mumbai**: Folin and Ciocalteau's phenol reagent, HI-LR (CAS No 0010-82-2), Bovine serum albumin (CAS No 9048-46-8).
- ✓ **Loba Chemie**: Sodium thiosulphate –extra pure (CAS No 7631-90-5).

- ✓ **Merck Life Science Private Limited, Mumbai :** Silica gel(DH7D671004), Dextrose Anhydrous Purified (DD9D690950),Potassium di-hydrogen phosphate(B.No DF9D691379), Di-Potassium phosphate anhydrous(B.No DH9D692054), Sodium carbonate anhydrous(B.No DE9D691277), potassium chromate (CAS 7789-00-6), sodium chloride (CAS 7647-14-5), silver nitrate (CAS 7761-88-8), ascorbic acid(CAS 50-81-7), iron chloride III (CAS 7705-08-0), ammonium chloride (CAS 12125-02-9).
- ✓ **Spectrum Reagent and Chemicals Private Limited, Cochin**: Iodine solution (Code I0105).
- ✓ **Changshu Hong sheng Fine Chemicals Co. Limited, China:** Ethanol (18-06-30)**.**
- ✓ **Indus Labs, Hassan:** Distilled water(CAS No 7732-18-5).
- ✓ **Oxford Lab Fine Chemicals, Mumbai** : Cholesterol extra pure (45-03-1)
- ✓ **Avra Synthesis Private Limited, Telangana**: Hexane petroleum fraction (CAS No110-54-3).
- ✓ **Fisher Scientific Chemicals, Mumbai:** Thiourea (CAS No 62-56-6), Diethyldithiocarbamate (CAS No 28-34-3), Trichloroacetic acid (CAS No 76-03-9).
- ✓ **Central Drug House Private Limited, New Delhi**: Calcium carbonate(CAS No 471-34-1), Erichrome black T (CAS No 60-00-4).
- ✓ **Qualigens Fine Chemicals, Mumbai:** Phenolphthalein indicator (77-09-8).

3.2 METHODOLOGY

3.2.1Quality assessment of ingredients:

All the raw materials (rice, cowpea, jaggery and ghee) were subjected to adulteration tests.(FSSAI)

Table 2: *Adulteration Tests for Quality Analysis*

INGREDIENT	ADULTERANT TESTED	TEST
Rice	Extraneous matter (dust, stone, straw, insect,	To 2-3 g of sample were placed in a

	hair)	petridish and were visually examined.
	Boric acid	To 1g of sample, 1ml of water and few drops of concentrated hydrochloric acid (HCl) were added. Mixed well and turmeric paper strip was dipped.
Cowpea	Extraneous matter (dust, stone, straw, insect, hair)	To 2-3 g of sample were placed in a petridish and were visually examined.
Jaggery	Washing soda	To 1g of melted sample, few drops of concentrated hydrochloric acid (HCl) were added. Mixed well
	Chalk powder	To 1g of melted sample, few drops of concentrated hydrochloric acid (HCl) were added. Mixed well
	Metanil yellow colour	To 1g of melted sample, 3ml of alcohol and few drops of concentrated hydrochloric acid (HCl) were added. Mixed well
Ghee	Coal tar dyes	To 1ml of melted sample, 5ml of

		concentrated sulphuric acid (H_2SO_4) was added in a test tube, shaken well.
	Vanaspati/ margarine	To 1ml of melted sample 1ml of concentrated hydrochloric acid (HCl) and a pinch of sugar was added . Mixed well
	Starch	To 1ml of melted sample, 0.1N iodine solution was added and mixed well.

3.2.2 Standardisation of the processing methods

Fermentation

The raw rice and cowpea seeds (about 20g) were subjected to fermentation by soaking in a bowl with tap water (1:2 ratio) for 36 hours at room temperature with lid closed.

3.2.2(b) Germination

The raw rice and cowpea seeds (about 20g) were subjected to germination by soaking in a bowl with tap water (1:2 ratio) for 12 hours, then the water was drained and the samples were tied in a cloth and were kept for germination at room temperature for 12 hours.

3.2.3 Processing of samples:

3.2.3 (a) Fermentation of rice

The rice samples were subjected to fermentation by three variations namely, variation 1 (rice +tap water; 1:4 ratio)(Tongual,1979), variation2 (rice +5%NaCl or common

salt;1:2 ratio)(Yousif, 2003) and variation 3(rice+3% salt+6% sugar, 1:3 ratio)(Zamora, 2000) for 36 hours at room temperature.

3.2.3 (b) Germination of cowpea

The cowpea samples were subjected to germination by three variations of soaking namely; variation 1(cowpea+ tap water for 12 hours with 1:10 ratio) (Uwaegbute,2000), variation 2(cowpea+0.03% baking soda for 16 hours with 1:10 ratio)(Ibrahim,2000) and variation 3(cowpea+0.07%citric acid for 30 minutes, rinse and soak in water for 5.5hours with 1:5 ratio)(Yu Haey Kuo,2004) and were germinated for 24 hours in room temperature.

Plate 2:
Variations Used For Fermentation Of Rice

Plate 3:
Variations Used For Germination Of Cowpea

3.2.4 Sensory evaluation of processed sample

The sensory evaluation for the processing methods was carried out using 9 point hedonic scale rating (Peryam,1957)

3.2.5 Processing of bulk sample

Rice

About 2.5kg of rice was soaked in 5 litres of filtered (RO) water containing 250grams of common salt (food grade) for 36 hours in a closed container to ferment naturally at room temperature. After 36 hours the water was drained and the samples were sundried for 5 hours and were milled to obtain flour. The flour was stored in polythene bags at 4°C until used.

3.2.5(b) Cowpea

About 2kg of cowpea seeds were soaked in 20 litres of filtered (RO) water containing 6 grams of baking soda(food grade) for 16 hours, after soaking the water was drained and

the seeds were tied in cloth and kept in airtight container for 24 hours to germinate. The seeds after germination were sundried for 4 hours followed by 5 hours of shade drying and were milled into flour. The flour was stored in polythene bags at room temperature in airtight container until use.

Plate 4:
Fermentation Of Rice Sample

Plate 5:
Germination Of Cowpea Sample

3.2.6 Accuracy of processing methods

3.2.6 (a) pH and Titrable acidity

The pH and Titrable acidity of water used before and after fermentation was analysed using digital pH meter(Garner,2010) and the acid base titration respectively.5ml of rice water was titrated against 0.1N sodium hydroxide solution using phenolphthalein indicator and the end point was derived on the appearance of pale pink colour. The acidity percentage was calculated.(Ghosh,2011).

Plate 6:
Digital pH Meter Used For Detection Of pH

3.2.6 (b) Percentage germination rate (%GR)

It is calculated by the formula;

$$\%GR = \frac{\text{number of seeds germinated}}{\text{Total number of seeds subjected to germination}} \times 100$$

The %GR of all the germinated samples is calculated to obtain the yield of the product (Shaieney, 2015).

3.2.7 Preparation of rice and cowpea flour blends

3.2.7 (a) Estimation of cowpea-rice blend with high protein content

The rice flour (fermented and non-fermented) and cowpea flour (germinated and non-germinated) were mixed in different proportions of 60:40 and 80:20 (Swaminathan, 2012) to analyse the protein content.

Table 3: *Flour Samples Used For Protein Analysis*

SET 1		
Variations	**Composition**	
Variation I	Unfermented rice flour (RF)	
Variation II	Fermented rice flour(Frf)	
Variation III	Non – germinated cowpea flour(CF)	
Variation IV	Germinated cowpea flour(Gcf)	
SET 2		
Variations	**Composition**	**Proportions**
Control	Unfermented rice flour (RF)+non-germinated cowpea flour(CF)	60:40
Variation 1	Unfermented rice flour (RF)+germinated cowpea flour(Gcf)	60:40
Variation 2	fermented rice flour (Frf)+non-germinated cowpea flour(CF)	60:40
Variation 3	fermented rice flour (Frf)+germinated cowpea flour(Gcf)	60:40
SET 3		
Variations	**Composition**	**Proportions**
Variation A	fermented rice flour (fRF)+germinated cowpea flour(gCF)	50:50
Variation B	fermented rice flour (fRF)+germinated cowpea flour(gCF)	60:40
Variation C	fermented rice flour (fRF)+germinated cowpea flour(gCF)	70:30

Variation D	fermented rice flour (fRF)+germinated cowpea flour(gCF)	80:20
Variation E	fermented rice flour (fRF)+germinated cowpea flour(gCF)	90:10

3.2.7(b) Estimation of protein (Lowry's method)

1 gram of powdered sample was mixed with 4ml of potassium phosphate buffer (pH 7.4) and was centrifuged at 5000rpm for 15 minutes. The supernatant(1 ml) was used for protein estimation. The standard sample was prepared using bovine serum albumin of 200µg/mL concentration, with aliquots of 0.2-1.0ml which were made up to 1.0ml with distilled water.5ml of alkaline copper sulphate solution was added and the test tubes were allowed to stand for 10 minutes, later 0.5ml of Folin Ciocalteau reagent(FCR) was added and all the test tubes were incubated in dark for 30 minutes. The absorbance was read at 660nm and the concentration was determined using spectrophotometer.

Plate 7:
Centrifuge Used For Centrifugation Of Flour Blends

Plate 8:

Flour Blends Before And After Centrifugation

(Homogenous layer)

(Two layers with the supernatant containing protein)

3.2.8 Preparation of ladoo

Plate 9:

Preparation Of The Cowpea-Rice Ladoo

STEP1: Heat pan, add ghee

STEP 2:Add the rice+cowpea flour

STEP 3:Mix well and roast on low flame

STEP 4:Raw flavor disappears and colour changes

STEP 5:Roast till colour turns brown

STEP 6: Add jaggery powder, cardamon

STEP 7:Mix well and cook until it starts combining

STEP 8: Transfer to a plate to cool

STEP 9: Shape into ladoos

The standardised proportion of raw ingredients as depicted in *Table 1* was used for preparation of ladoo. The pan was heated to which ghee was added followed by the rice and cowpea flour blend which was roasted on low flame until the raw flavour was eliminated and the mixture started to ooze ghee. At this time, the powdered jiggery, cardamom was added and mixed well. The mixture was allowed to cool and was shaped into ladoos and cooled again.

3.2.9 Sensory evaluation using preference test

The two samples with highest amount of protein as per Lowry's method was subjected to preference test with random sampling using the sample size of 100.(Choi,2002)

3.3 PROXIMATE ANALYSIS

Proximate analysis of the product was done employing the methods described by AOAC 2000(estimation of moisture, total ash, crude protein, crude fibre, crude fat) Hofreiter,1962(estimation of carbohydrates).

Note:

For the purpose of proximate analysis, the cowpea-rice ladoo samples were powdered and store in air tight container till further use. The methods used for proximate analysis were modified as with respect to the food product as mentioned in the FSSAI, lab-manual 3.The tests were performed in triplicates and the mean was taken.

3.3.1 Determination of moisture by air-oven method(AOAC,2000)

5g of the powdered ladoo sample was taken in a sterile glass petriplate whose weight was noted earlier. The triplicates of sample were dried in the hot air oven at 125^0 C for four hours. The samples were cooled in the desiccator for 30 minutes and the weight was noted(C_1).The samples were placed in the oven for one more hour at 125^0 C. The samples were cooled for 30 minutes in the desiccator and the weight was taken(C_2).The average of both the weights were taken and was substituted in the formula to obtain the percentage of moisture. Moisture (%) can be calculated as;

$$\frac{\textbf{(weight of petridish+sample prior drying)(g) - (weight of petridish+sample prior drying)(g)}}{\textbf{Weight of the food sample (g)}} \textbf{ x100}$$

$$\frac{\textbf{(B-C)}}{\textbf{A}} \textbf{ x 100}$$

Plate 10:
Hot Air Oven Used For Moisture Estimation

Plate 11:
Muffle Furnace for Ash Estimation

Plate 12:
Desiccator Used To Cool the Ash Sample

3.3.2 Determination of total ash by general method(AOAC,2000)

3g of powdered sample was taken in a crucible whose weight was previously noted. The triplicates of sample were taken in crucible which was placed in a hot-air oven for 3-4 hours at 100^0 C. The crucibles were charred by heating on the direct flame of Bunsen burner till the contents turned black. The crucibles were transferred to the Muffle furnace that was pre-heated at 500^0 C and left for 6-8 hours. The crucibles were cooled in the desiccator for 30 minutes and weighed (B_1). The crucibles were again placed in the Muffle furnace for an hour, cooled in the desiccator for 30 minutes and weighed again (B_2). The average of the weights was taken and the total ash was calculated by substituting in the formula.

Ash (%) can be calculated as;

$$\frac{\text{(weight of the crucible+sample after drying)(g) – (weight of empty crucible)(g)} \quad \text{x100}}{\text{Weight of the food sample (g)}}$$

$$\frac{\text{(B-C)}}{\text{A}} \quad \text{x 100}$$

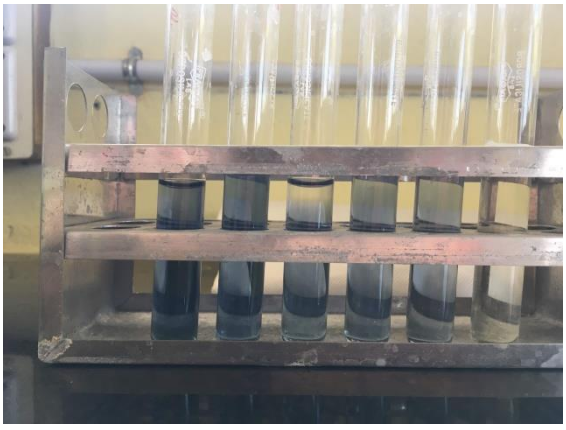
3.3.3 Determination of energy by bomb calorimeter method(AOAC,2000)

The food sample was outsourced to the Mangalore biotech laboratory for estimation as per AOAC 2000 method.

3.3.4 Determination of protein by Lowry's method

1 gram of powdered sample was mixed with 4ml of potassium phosphate buffer (pH 7.4) and was centrifuged at 5000rpm for 15 minutes. The supernatant(1 ml) was used for protein estimation. The standard sample was prepared using bovine serum albumin of 200µg/mL concentration, with aliquots of 0.2-1.0ml which were made up to 1.0ml with distilled water.5ml of alkaline copper sulphate solution was added and the test tubes were allowed to stand for 10 minutes, later 0.5ml of Folin Ciocalteau reagent(FCR) was added and all the test tubes were incubated in dark for 30 minutes. The absorbance was read at 660nm and the concentration was determined using spectrophotometer.

Plate 13:
Blue Coloured Complex In Lowry's Method



3.3.5 Determination of crude fat using AOAC method(AOAC,2000)

5-10 gms of food sample was weighed and transferred to a dry 250 ml Erlenmeyer flask. About 25-30ml of n- hexane solution was added and the contents of the flask were covered with aluminium foil to prevent the evaporation of hexane. The contents of

the flask were shaken every 30 minutes either by the shaker or manually and were left undisturbed for overnight. Next day the hexane liquid was poured out to a 100ml beaker whose weight was pre-determined and the hexane in the beaker was allowed to evaporate leaving behind the oil content of the food sample. This process was continued for 2 more days by freshly filling the flask containing the previously fat extracted sample with hexane and decanting, followed by evaporation of hexane. After 3 days the fat present in the beaker was weighed and the total fat content of the food was calculated using the formula.

Crude fat (%) can be calculated as:

$$\frac{\textbf{(weight of beaker with leftover fat)(g) - (weight of empty beaker) (g)}}{\textbf{weight of the food sample (g)}} \textbf{ x100}$$

$$\frac{\textbf{(B-C)}}{\textbf{A}} \textbf{ x 100}$$

Plate 14:
Decanting For Fat Estimation

3.3.6 Determination of total carbohydrates by colorimetric method (Hofreiter, 1962)

100mg of food sample was taken in boiling tube and was kept in boiling water bath with 5ml of 2.5N hydrochloric acid for hydrolysis for 3 hours. It was cooled and neutralised using 5g of sodium carbonate till effervescence ceased. The contents were made up to 100ml and centrifuged. 1ml of it was used for test sample in triplicates and 1ml was of distilled water was taken as blank.0.2 to 1ml of standard carbohydrate solution was taken which was made up to 1ml with distilled water.to all the test tubes,

4.0ml of anthrone reagent solution was added and was kept in the water bath for 8 minutes, cooled and the absorbance at 630nmwas read using the spectrophotometer.

Plate 15:
Spectrophotometer for Colorimetric Estimation

3.3.7 Determination of fibre (AOAC,2000)

2gms of food sample was weighed in a 500ml beaker to which 200ml of 0.255N of sulphuric acid solution was added and boiled for 30 minutes, It was filtered using a muslin cloth and washed with 200ml of boiling water, till all the acid was washed off. The filtrate was transferred to the same beaker to which 200ml of 0.313N of sodium hydroxide solution was added and boiled for 30 minutes. The contents were filtered in a filter paper washed with water and were dried for 2minutes in hot air oven for 250^0C and the dried sample was scraped off to the weighed crucible and was dried again for 100^0c for 4 hours. It was cooled and weighed . the contents were placed in the pre-heated Muffle furnace at 600^0C for 30 minutes, cooled and weighed. The crude fibre content was calculated by substituting in the formula.

Crude fibre(%) is calculated as follows;

$$\frac{\textbf{Weight of the crucible before ashing - Weight of the crucible before ashing}}{\textbf{Weight of the food sample(g)}}$$

3.4 PHYSIOCHEMICAL AND RHEOLOGICAL ANALYSIS

Food substances are differing in physical structure and chemical composition because it consists by complex groups. From these substances matrix was formed and it helps to determine the digestibility of the nutrient and the extent of nutrient release, in the digestive tract of an individual. The rheological property of food means relationship between structure and flow of food along with correlation of physical parameters with sensory evaluation(Abu-Jdayil, 2002).

3.4.2 Determination of pH using pH meter (Sharma, 2006)

The pH of the ladoo is determined by mixing 10grams of ladoo with 10ml of dis tilled water (whose pH is predetermined and set to 7). The mixture was made into slurry and the pH was determined by dipping the electrode into the ladoo slurry (which was calibrated earlier) and noting down the reading. The readings were taken in triplicates and the mean was derived.

3.4.3 Determination of acidity by titration method(Rangana, 2010)

5ml of food sample (5 grams of ladoo powder was mixed with 5ml of distilled water, whose pH is predetermined and set to 7) was titrated against 0.1N sodium hydroxide solution using phenolphthalein indicator and the end point was derived on the appearance of pale pink colour. The acidity percentage was calculated using the formula.

Calculation:

$$\text{Total Acidity} = \frac{56.1 \times (\text{volume of NaOH} \times \text{normality of NaOH})}{\text{Weight of food sample (g)}}$$

3.4.4 Determination of reducing sugar by DNSA method(Rangana, 2010)

1 gram of food sample was taken in a test tube which was added with 1 ml of distilled water and centrifuged for 10 minutes at 3000rpm. 1ml of supernatant was collected and used as test sample. 0.2-1ml of standard glucose solution was taken and was made up to 1ml with distilled water. The blank was prepared by adding 1ml of distilled water. All the test tubes were added with 3ml of di-nitro salicylic acid (DNSA)solution. The test tubes were covered with aluminium foil and were boiled for 5minutes in the water bath. The solutions were cooled and added with 1 ml of sodium potassium tartrate. It was calorimetrically read at 540nm.

3.4.5 Determination of bulk density by tapped method (Okaka and Potter, 1979)

The bulk density was determined by taking 50grams of the cowpea-rice ladoo powder into a 100 ml measuring cylinder and tapped the sample to a constant volume and the bulk density (gcm-3) was calculated using the formula.

Calculation:

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{weight of the food sample (g)}}{\text{Sample volume (cm}^3\text{)}}$$

3.5 SHELF-LIFE STUDY OF THE PRODUCT

3.5.1 Packaging of the food product

The ladoo prepared were packaged in a 750ml capacity biodegradable container manufactured Ecoware brand under the USDA standards. The food samples were stored in two variations; the first set was cling wrapped and was packed whereas second one did not consist of cling wrap. Both the sets were studied for time period of 30 days.

Plate 16:

Packaging of Ladoo in Bagasse Container

Plate 17:

Variations Used In Packaging Of Cowpea-Rice Ladoo

3.5.2 Microbial analysis of the food product

The food sample was outsourced for microbial analysis of yeast and mold, *Eschericia coli, Staphylococcus aureus* and total plate count by the Mangalore biotech laboratory under the FSSAI standard methods

3.5.3 Sensory evaluation of the food product

The food product was subjected to sensory evaluation using 9-point hedonic scale rating on the overall acceptability for the time period of 30 days with 5 sessions of analysis on day 1,7,14,21 and 28.(Choi, 2002)

3.5.4 Qualitative evaluation of the food product

The qualitative evaluation of the product was done using free fatty acid analysis and peroxide value analysis(AOAC,2000)

3.5.4(a) Estimation of free fatty acid of the food product

5 grams of food sample was taken to which 5ml of distilled water was added followed by 2 drops of 0.1N phenolphthalein indication. The contents were titrated against 0.1N sodium hydroxide solution until permanent pale pink colour appeared. The FFA percentage was calculated. The acceptable range was above 8%.

3.5.4(b) Estimation of peroxide value of the food product

5gms of sample was taken to which 30 ml of solvent (acetic acid and chloroform in 3:2 ratios) was added followed by 0.5ml of potassium iodide solution. The contents were placed in dark for 5 minutes and titrated against 0.01mol/L of sodium thiosulphate solution and the POV was calculated. The acceptable range was below 30 mEq/kg.

3.6 NUTRITIONAL LABELLING OF THE PRODUCT

The nutritional labelling of the food product was done using the USDFA standard method for which the following micro nutrients were estimated; iron, calcium, sodium, potassium, vitamin A, vitamin C and cholesterol as per AOAC methods.

Note :

For the estimation of iron, calcium, and potassium the ash solution of the food sample was used, whereas for the estimation of vitamin A, vitamin C, sodium and cholesterol the whole food sample was used.

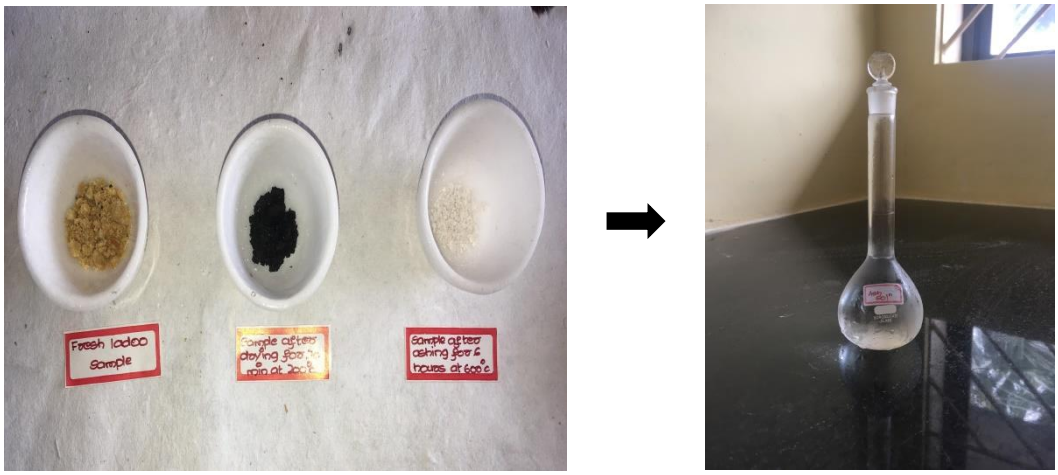
3.6.1 Preparation of ash solution for estimation of minerals(AOAC,2000)

5gms of powdered food sample was taken in a crucible which was weighed priorly. The crucible was placed in the hot air oven for 3-4 hours at 100^0C ,the crucible was charred on direct flame using Bunsen burner until the contents turned black. The charred crucible was placed in the Muffle furnace which was pre-heated to 500-600^0C for 6-8 hours. The ashed contents were cooled in the Muffle desiccator for 30 minutes. The crucible was placed in the furnace for one more hour and was cooled for 30 minutes in the desiccator. The cooled sample was dissolved with 5ml of concentrated hydrochloric

acid and a drop of water in the water bath, until the solution completely evaporated. The process was repeated with concentrated hydrochloric acid and water mixture of 4:1 ratio and then 1:4 ratio. The solution was boiled for 8 minutes and then was cooled. It was filtered through the Whatman's filter paper into 100ml standard flask which was made up to mark with distilled water. The aliquots were used for mineral analysis.

Plate 18:

Ash Solution Used For Micronutrient Analysis



3.6.2Estimation of iron by Wong's method(Wong, 1928)

1ml of the ash solution was used as the test sample to detect the iron content in the food sample. The standard iron solution was prepared using Mohr's salt of 100µg/ml concentration and 1to 5ml was used which was made up to 5ml using distilled water. The blank was prepared using 5ml of distilled water and 1ml of ash solution was made up to 5ml with distilled water. To all the test tubes, 1ml of 30% sulphuric acid and 1ml of 7% potassium per sulphate solution and 1ml of 40% potassium thiocyanate solution was added. The test tubes were kept at room temperature for 10 minutes and the absorbance was read at 490nm.

3.6.3 Estimation of calcium by EDTA method(AOAC, 2000)

The burette was rinsed and filled up to the mark with 0.1M EDTA solution. The standard calcium solution was taken for standardisation. 25ml of standard calcium solution was taken in the conical flask to which 10 ml of ammonium buffer and 20 ml of double distilled water was added followed by 2 drops of Erichrome Black T(EBT) indicator. The contents of Erlenmeyer's flask were titrated against the EDTA solution until the endpoint with blue coloured solution was obtained. For estimation of calcium in the food sample, 5ml of ash solution was taken in 250ml of standard flask and made

up to the mark with double distilled water. 50ml of the aliquot was taken to which 10ml of ammonium buffer and 2 drops of EBT indicator was added and titrated against EDTA solution until the endpoint was reached. Hence, the calcium content was calculated.

Plate 19:
Endpoint Of EDTA Titration

Plate 20:
Endpoint Of Mohr's Titration

3.6.4 Estimation of sodium by Mohr's titration method(Sezey, 2019)

The burette was rinsed and filled with 0.1N silver nitrate solution. The standardisation was carried out with standard sodium chloride solution . In a 250 ml beaker,10gms of food sample was added with 190ml of boiling double distilled water and was stirred. It was filtered and 50ml was taken in the Erlenmeyer's flask to which 1ml of 5% potassium chromate indicator solution was added and was titrated against the silver nitrate solution until it turned red-brown colour which was the endpoint. Through calculation , the sodium content was calculated.

3.6.5 Estimation of potassium by colorimetric method(AOAC, 2000)

The food sample for estimation of potassium by colorimetric method was outsourced to the Mangalore biotech laboratory for estimation as per AOAC 2000 method.

3.6.6 Estimation of vitamin A by colorimetric method(AOAC, 2000)

2.5 gms of food sample was dissolved in 15ml of 3:7 acetone-hexane mixture for overnight and was filtered and made up to 50ml with hexane which was used as test sample. 0.2 to 1.0 ml aliquots of standard beta carotene solution with concentration of 100µg/ml was used as standard solution which were made up to 10ml with petroleum ether. The blank was prepared with 0.3ml of acetone and 9.7ml of petroleum ether solution. All the test tubes were incubated for 15 minutes and the absorbance was read at 490nm.

3.6.7 Estimation of vitamin C by colorimetric method(AOAC, 2000)

1 gram of food sample was added with 10ml of 5%trichloroacetic acid solution and was centrifuged at 2000rpm for10 minutes. 1ml of the supernatant was taken as the test sample in triplicates, make it up to 3ml using 5%TCA solution. 0.5 to 2.5ml of standard solutions was pipetted out using standard ascorbic acid solution of 100µg/ml concentration which was made up to 3ml using 5%TCA solution. The blank was prepared using 3ml of 5% TCA solution. To all the test tubes,1 ml of DTC reagent was added and was incubated at 60^0C in the water bath for 1 hour and then cooled immediately for 15 minutes in ice. To all the test tubes 5ml of 9N sulphuric acid was added and shaken well. The test tubes were kept at room temperature for 20 minutes and then the absorbance was read at 540nm.

3.6.8 Estimation of cholesterol by Zak's method (Zak,1977)

To 1 gram of food sample, 4.9ml of ferric chloride precipitating agent was added and centrifuged for 15 minutes at 1500rpm.2.5ml of the supernatant was taken in triplicates, which was made up to 5 ml using ferric chloride diluting agent.0.5 to 2.5ml of standard cholesterol solution if concentration 100µg/ml was taken as standards and was made up to 5ml using ferric chloride diluting agent.5ml of ferric chloride diluting agent was taken as blank. To all the test tubes, 4ml of concentrated sulphuric acid was added and was placed in water bath for 30 minutes. It was cooled and the absorbance was read at 560nm.

3.7 BUDGETING OF THE FOOD PRODUCT

The budgeting of the food product was done by costing the amount of raw materials, the packaging materials, fuel usage with the yield of the product(Shekar,2019).

3.8 STATISTICAL ANALYSIS

All data are analysed using spss version 17.0 for windows (SPSS INC., CHICAGO, IL, USA) with a one-way analysis of variance (ANOVA). The post hoc analysis was used to analyse the differences between the individual means at a 5 percent significance level. The result of sensory evaluation (consumer acceptance) was analysed using repeated measures analysis of variance to test the differences between the mean sensory and hedonic ratings.

CHAPTER 4

RESULTS AND DISCUSSION

The methodology used for analysis of the cowpea rice ladoo, is tested and the outcome of the experiments is depicted in the section of results. In case of cowpea rice ladoo, protein analysis laid the basis for selection of proportion to be 50:50 and 60:40. Based on the result derived, the outcome is selected and further subjected to further analysis. The data obtained by the section of result and discussion gave a clear picture on the authenticity of the product along with the accuracy of the experiments undertaken. The results depicted that the macro-nutrients were dominant in the product based on proximate analysis, the physio-chemical parameters, rheological parameters which explained the physical components of the developed product, the microbial analysis was a predicting factor of product hygiene and keeping quality of the product. On the whole this section, illustrates the outcome of the developed ladoo.

4.0 RESULTS AND DISCUSSION

4.1QUALITY ASSESSMENT OF THE INGREDIENTS THROUGH ADULTERATION TEST

The raw materials were checked for adulterants in order to make sure that the ingredients used for the preparation of ladoo are of good quality.

Table 4: *Adulteration Test Depicting Purity of Raw Ingredients*

INGREDIENTS	ADULTERANT TESTED	OBSERVATION	RESULT
Rice	Extraneous matter (dust, stone, straw, insect, hair) Boric acid	No extraneous matters were observered. The color of the turmeric paper dipped in the solution remained unchanged	The rice sample was not adulterated.
Cowpea	Extraneous matter (dust, stone, straw, insect, hair)	No extraneous matters were observered.	The cow pea sample was not adulterated.
Jaggery	Washing soda Chalk powder Metanil yellow color	No effervescence was observed. No effervescence was observed. The solution did not turned blood red in color	The jaggery sample was not adulterated.

Ghee	Coal tar dyes	On addition of concentrated sulphuric acid(H_2SO_4) the solution did not turn red in color	
	Vanaspati/ margarine	On addition of concentrated hydrochloric acid (HCl) the solution did not turn red or pink in color and the sample did not separate into two different layers.	The ghee sample was not adulterated.
	Starch	On addition of 0.1N iodine solution the solution did not turn blue or violet in color.	

Table 4 indicates the purity of raw materials used for preparation of the ladoo. It is evident from the table that the raw materials are of good quality and are free from adulteration.

4.2 STANDARDISATION OF THE PROCESSING METHODS

The processing methods were standardized in order to make sure that the raw materials (rice and cowpea) are subjected to the processing methods (fermentation and germination) gives acceptable outcomes in terms of sensory characteristics.

Table 5: *Processing Method Used On Sample*

PROCESSING METHOD	**OBSERVATION**	**INFERENCE**
FERMENTATION	• The fermented rice sample was acceptable in terms of sensory characteristics such as appearance, odor, and color. • The fermented cowpea sample was unacceptable in terms of sensory characteristics because it had foul smell, slimy appearance and brown faded color.	The rice samples were suitable for the process of fermentation.
GERMINATION	• The rice sample did not undergo germination. • The cowpea sample had undergone germination with acceptable sensory characteristics in terms of appearance, color ,	The cowpea samples were suitable for the process of germination.

Table 5 depicts the outcome of processing method on rice and cowpea. From the table it is portrayed that on the basis of sensory characteristics, through the 9-scale hedonic scale rating (n=10) the rice sample was subjected to fermentation and cowpea sample was subjected to germination.

4.2 (a) Processing of samples:

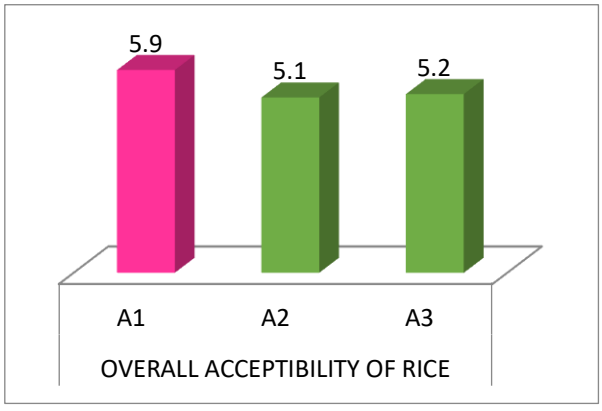
The samples were processed using different variations in order to select the best method for fermentation and germination.

- **Processing of rice**

Table 6: *Statistical Outcome of Fermentation of Rice (N=10)*

Particulars	Mean	Standard Deviation	ANOVA f value	p value	Inference
A1	5.90	1.912	0.574	0.570	There is no much significant difference between the samples.
A2	5.10	1.792			
A3	5.20	1.751			

Figure 1:
Different Methods of Rice Fermentation (N=10)



A1-fermentation of rice with 5% common salt (1:2 ratio)

A2-fermentation of rice with tap water(1:4 ratio)

A3- fermentation of rice with 6% sugar and 3% common salt (1:3 ratio)

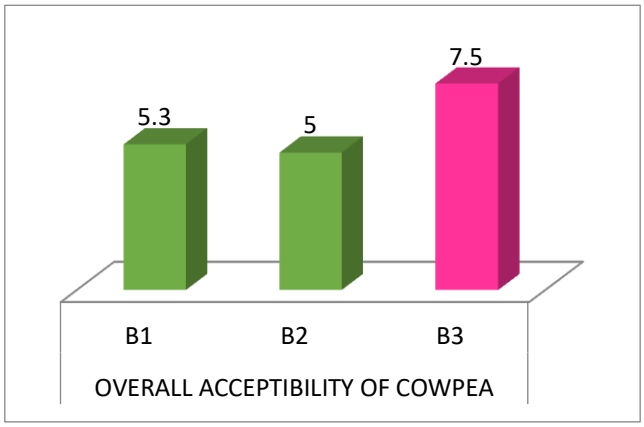
Table 6 illustrates the statistical analysis of overall acceptability of rice. Hence ,**Table 6** and **Figure 1** depicts that the rice samples do not have must significant difference because the f value is below 1 and thus there is no much difference between the three variations. Hence, the one with highest mean value of 5.9 the sample fermented with 5% common salt (1:2 ratios) was selected as the best.

- Processing of cowpea

Table 7: *Statistical Outcome of Germination of Cowpea (N=10)*

Particulars	Mean	Standard Deviation	ANOVA f value	p value	Inference
A1	5.30	0.949	14.540	0.000	There is high significant difference between the samples.
A2	5.00	1.491			
A3	7.50	0.850			

Figure 2:
Different Methods of Cowpea Germination (N=10)



B1- germination of cowpea with 0.03% baking soda (1:10 ratio)

B2-germination of cowpea with 0.07% citric acid(1:5 ratio)

B3-germination of cowpea with tap water (1:10 ratio)

Table 7 depicts the statistical analysis of overall acceptability of cowpea and the **Figure 2** illustrates that the cowpea samples have a high significant difference because the f value is above 1 and thus there is more difference between the three variations. Hence, the one with highest mean value 7.5, (B3-germination of cowpea with tap water (1:10 ratio)). The one germinated with tap water was preferred on the basis of overall acceptability when compared to the ones germinated with baking soda and citric acid

which did not have much variation but the ones germinated with tap water had considerable difference with other two variations.

Taking the above results into consideration, the bulk sample was prepared by following these methods of fermentation and germination.

4.2(b) Accuracy of processing methods

Titrable acidity and pH determines the acid concentration of the solution which is a remarkable factor to know whether the process of fermentation was accurate.

- ✓ In the case of fermented rice water, the pH of water was 6.39 before fermentation and the pH level dropped to 3.86 after fermentation. The Titrable acidity was found to be 0.42% after fermentation. The results were within the acceptable range which indicated that the fermentation was accurate.

- ✓ In case of germination, the percentage germination rate depicted that how many grains had successfully germinated and in case of cowpea the percentage germination rate was 81.3% as 4065 grains were germinated out of 5000 grains. The result was within the acceptable range.

Table 8: *Methods to Assess the Accuracy of Processing On Flour*

Processing methods	**Particulars**	**Normal range**	**Results**
Fermentation	pH	3.6-4.2	3.86
	Titrable acidity	0.3-0.6%	0.42%
Germination	Percentage germination rate	73-90%	81.3%

Table 8 depicts the accuracy of the processing method and from the table it is clear that the fermentation and germination process were conducted accurately.

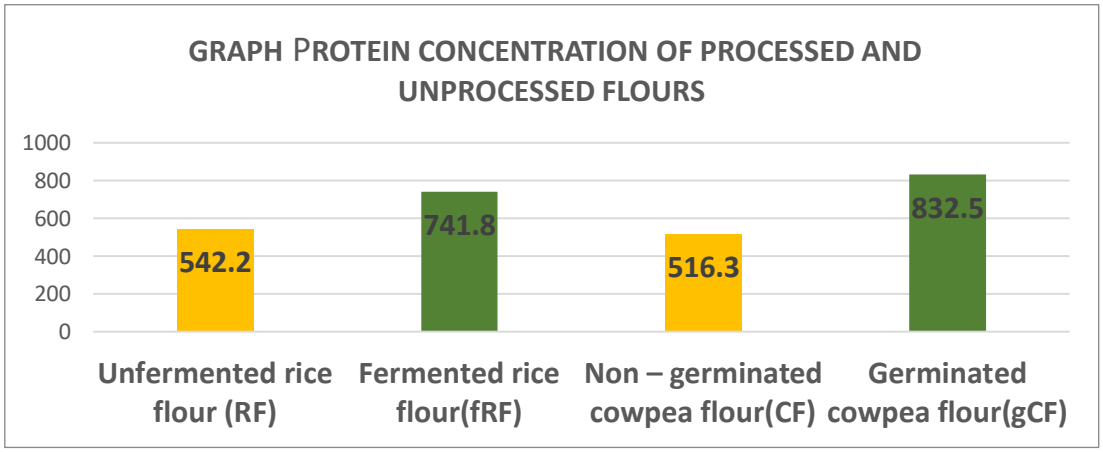
4.3 PROTEIN ESTIMATION OF DIFFERENT RICE AND COWPEA FLOUR BLENDS BY LOWRY'S METHOD

The concentration of protein was determined using spectrophotometric method and different flour blends were used.

Table 9: *Protein Concentration of Processed and Unprocessed Flours*

Variations	Composition	Concentration of protein per gram of sample (µg/mL)
Variation Ⅰ	Unfermented rice flour (RF)	542.2
Variation Ⅱ	Fermented rice flour(fRF)	741.8
Variation Ⅲ	Non – germinated cowpea flour(CF)	516.3
Variation Ⅳ	Germinated cowpea flour(gCF)	832.5

Figure 3:
Protein Concentration of Processed and Unprocessed Flours



From **Table 9** it is analyzed that there was change in the protein content after processing. **Figure 3** illustrates that the fermented rice blend and the germinated

cowpea blend had more protein content compared to the unprocessed ones. Hence it was found that the processing methods increased protein content.

Table 10: *Protein Concentration of Flour Blends with 60:40 Proportions*

Variations	Composition	Proportions	Concentration of protein per gram of sample (µg/mL)
Control	Unfermented rice flour (RF)+non-germinated cowpea flour(CF)	60:40	517.5
Variation 1	Unfermented rice flour (RF)+germinated cowpea flour(gCF)	60:40	693.2
Variation 2	fermented rice flour (fRF)+non-germinated cowpea flour(CF)	60:40	703.2
Variation 3	fermented rice flour (fRF)+germinated cowpea flour(gCF)	60:40	840.1

Table 10 depicts that the protein concentration of variation 3 and 4 was high compared to other variations, so the combination of fermented rice and germinated cowpea was used in different proportions to choose the two variations with highest protein content. **Figure 4** illustrates the protein concentration of the flour blends.

Figure 4:

Protein Composition of Different Flour Blends In 60:40 Proportions

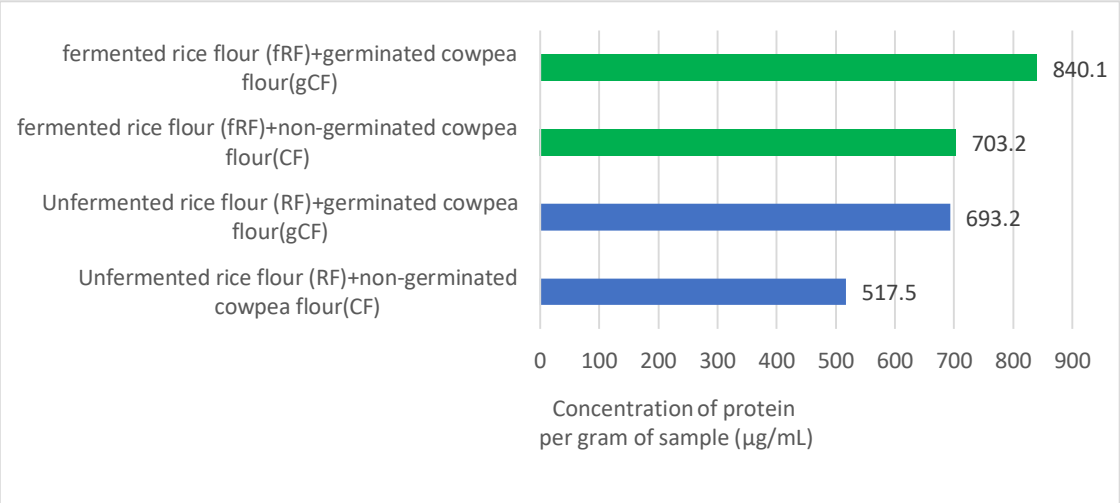
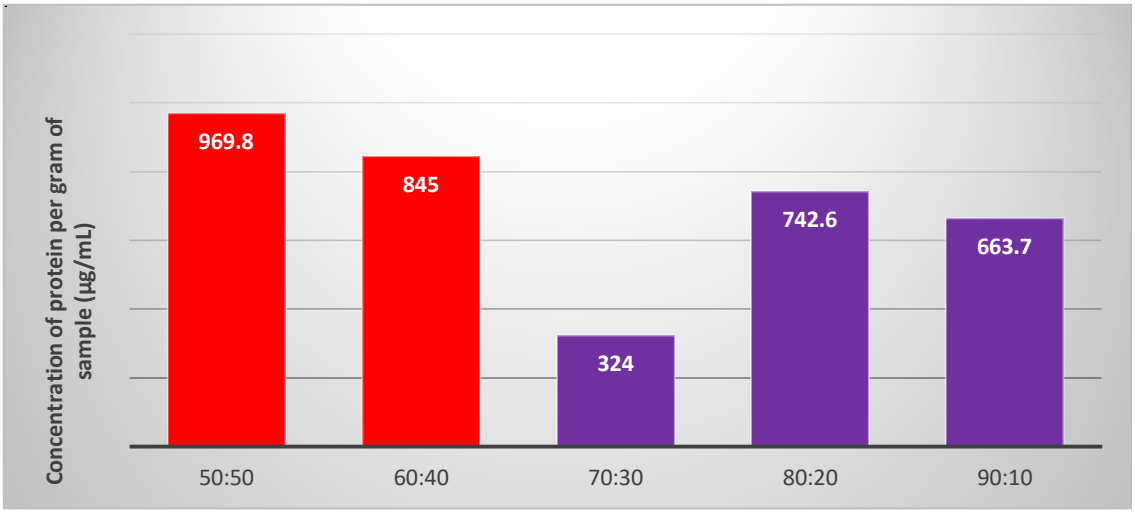


Table 11: *Protein Concentration of Processed Flour Blends*

Variations	Composition	Proportions	Concentration of protein per gram of sample (µg/mL)
Variation A	fermented rice flour (fRF)+germinated cowpea flour(gCF)	50:50	969.8
Variation B	fermented rice flour (fRF)+germinated cowpea flour(gCF)	60:40	845.0
Variation C	fermented rice flour (fRF)+germinated cowpea flour(gCF)	70:30	324.0
Variation D	fermented rice flour (fRF)+germinated cowpea flour(gCF)	80:20	742.6
Variation E	fermented rice flour (fRF)+germinated cowpea flour(gCF)	90:10	663.7

Table 11 shows that the rice and cowpea blend with 50:50 and 60:40 proportions had high protein content. Hence these proportions were used to prepare the ladoo for selection of the best one based on consumer preference. The **Figure 5** illustrates the protein concentration with 50:50 and 60:40 proportions having the high value.

Figure 5:
Protein Composition of Different Processed Flour Blends



4.4 SENSORY EVALUATION USING PREFERENCE TEST

Two variations of the fermented rice and germinated cowpea blends with high protein content (*50:50 and 60:40 named A55 and A64 respectively*) were used to prepare ladoos and were subjected to preference test by a sample size of 100. It was seen that 68 people out of 100 liked *A55* whereas remaining 32 liked *A64.*

Figure 6:
Preference Test of Cowpea -Rice Ladoo (N=100)

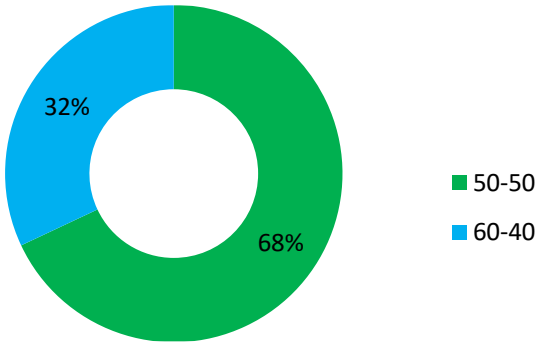


Figure 6 depicts the outcome of preference test**.** The sample size taken was 100 with the probability level as 0.05, so the critical reference value was 61. Therefore, 68 people chose *A55,* the final proportion of the ladoo had rice and cowpea in the ratio of 1:1

4.5 ASSESSMENT OF FINAL PRODUCT

The ladoo prepared with the flour blend of 50:50 was assessed by 50 people on its overall characteristics, and the results depicted that the consumers liked the product and would buy if introduced in the market and would recommend other people to buy it too. All of them felt that it would be liked by all the age groups and they were surprised to know the ladoo was made out of cowpea and preferred to eat cowpea in ladoo form rather than in the savory dishes and felt that in the form of ladoo the bland taste of cowpea improved and was palatable with accurate amount of sweetness. Over all the consumers found the cowpea ladoo to be a good product.

In case of purchase, most of them preferred to buy it once in 15 days and the sensory attribute that was liked the most was taste.

Figure 7:
Sensory Characteristics Of Cowpea Ladoo Liked Most By Consumers (N=50)

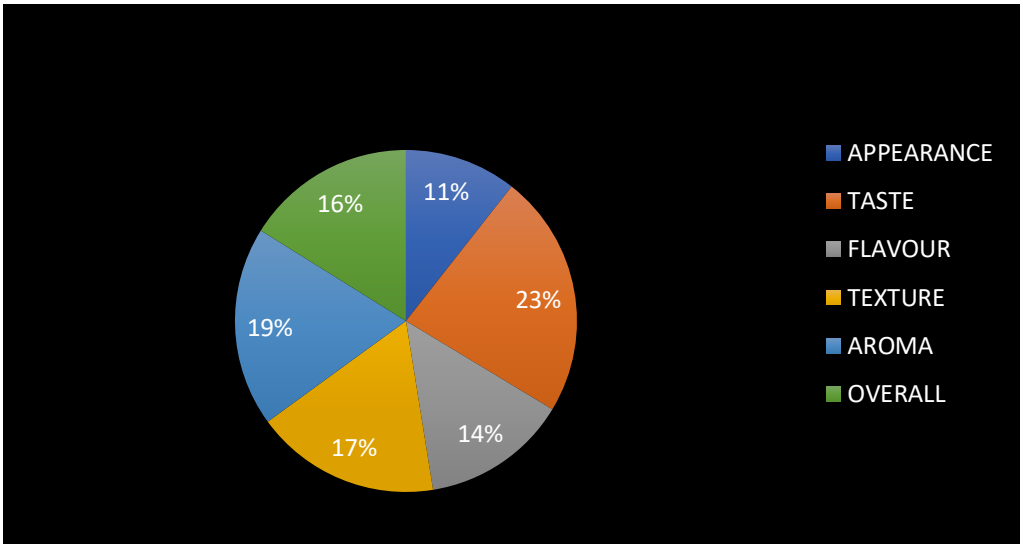


Figure 7 illustrates that the consumers liked all the characteristics of ladoo equally and were satisfied with the overall product.

Figure 8:
Frequency of Purchasing the Ladoo

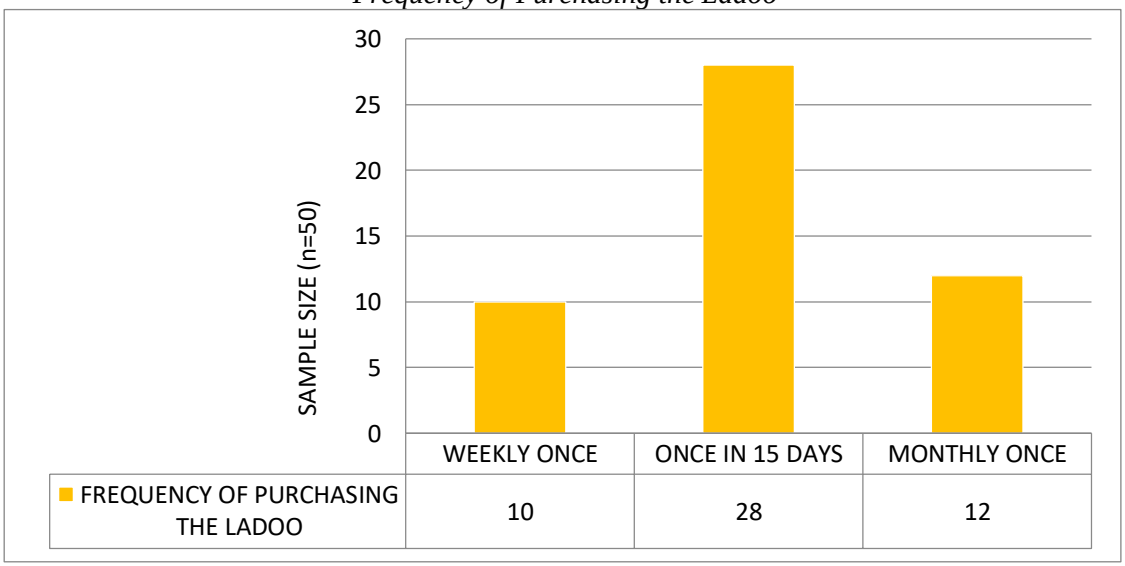


Figure 8, picturizes that most of the consumers preferred to purchase the ladoo once in 15 days. However a small portion (n=10) opted for weekly purchase too and a few of the population (n=12) opted monthly purchase.

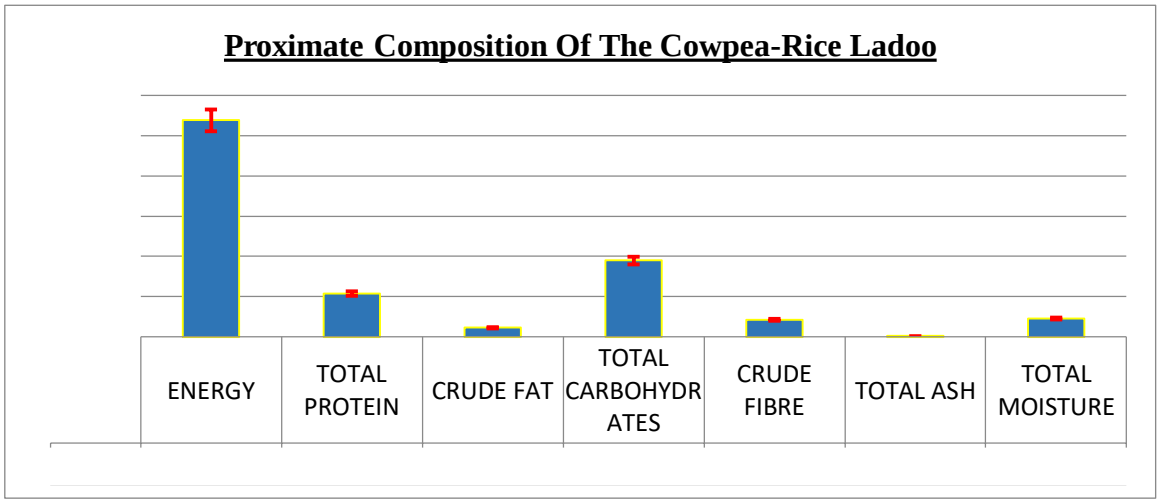
4.6 PROXIMATE ANALYSIS

The proximate composition of ladoo per 100grams is illustrated in the **Figure 9** and from the **Table 12** it is clear that the ladoo comprises of crude protein, total energy, total carbohydrates, crude fat, crude fiber, total ash and total moisture in the descending order of their values.

Table 12: *Proximate Composition of the Cowpea-Rice Ladoo*

PARTICULARS	COMPOSITION (100grams)	STANDARD DEVIATION	PERCENTAGE
ENERGY	**537.76 kcals**	**± 0.49**	**57%**
TOTAL PROTEIN	**10.80 grams**	**± 0.03**	**13%**
CRUDE FAT	**2.29 grams**	**± 0.01**	**2%**
TOTAL CARBOHYDRATES	**109.03 grams**	**±0.18**	**16%**
CRUDE FIBRE	**4.209 grams**	**± 0.02**	**5%**
TOTAL ASH	**0.211 grams**	**± 0.01**	**1%**
TOTAL MOISTURE	**4.523 grams**	**±0.02**	**6%**

Figure 9:
Proximate Composition of the Cowpea-Rice Ladoo



- Energy is the calorific composition of the food which determines the nutrient intake and in case of cowpea ladoo the energy provided is **537.76** kcals/100grams of sample. Each ladoo comprises of **57%** of calories.

- Total carbohydrates are the total count of simple and complex sugars in the food sample and the cowpea ladoo consisted of **109.03**g/100grams of sample. Each ladoo comprises of **16%** of carbohydrates.

- The crude protein is the amount of protein present in the protein present in the ladoo and it mainly depends on the nitrogen content of the food. The cowpea ladoo has a crude protein value of **10.80** g/100 grams of sample. Each ladoo comprises of **16%** of protein claiming it to be a protein- rich product.

- Crude fiber consists of non-digestible components which have low energy value but provide bulk in the diet and cowpea ladoo has **4.209** g/100grams of sample. Each ladoo comprises of **5%** of fiber.

- Crude fat is the complex mixture of the fat- soluble components present in sample and in cowpea ladoo the crude fat composition is **2.29** g/100grams of sample. Each ladoo comprises of **2%** of fat.

- Total moisture is the water content present in the food sample which may be naturally present or manually added and cowpea ladoo had the total moisture of **4.523** g/100 grams of sample. Each ladoo comprises of **6%** of moisture.

- Total ash is the residue obtained after incineration of the food sample and the ash content of cowpea ladoo was **0.211**g/100grams of sample. Each ladoo comprises of **1%** of ash.

4.7 PHYSIOCHEMICAL AND RHEOLOGICAL ANALYSIS

Table 13: *Physiochemical Data of Cowpea-Rice Ladoo*

PARTICULARS	RESULTS	STANDARD DEVIATION
pH	**6.17**	**±0.01**
TOTAL ACIDITY	**13.53%**	**± 0.01**
BULK DENSITY	**70.1 g/cm^3**	**± 0.01**
REDUCING SUGAR	**5.84 g/100g**	**±0.02**

Table 13 depicts the physiochemical and rheological properties of the ladoo which acts as a basis for the physical and chemical attributes, to be acceptable. The pH of the ladoo

being **6.17** was within the acceptable range as it was neutral. The acidity was **13.53%** which was within the acceptable range of 9.05-15%. The bulk density of **70.1 g/cm^3** for 50 gm of sample claimed it to be moderately bulk in density. The reducing sugar was **5.84 g/100g** predominantly due to maltose.

4.8 SHELFLIFE STUDY OF THE PRODUCT

- *PACKAGING:* The ladoo packaged in the biodegradable bagasse container with cling wrap was fresh and had not lost its minimal moisture but the one which was not wrapped with cling film lost its moisture content and was dry in nature.

- *SHELF-LIFE:* The ladoos packed with cling wrap film on it had a shelf life of 21 days (WEEK 3).On the sensory analysis based on ANOVA and Benofferi p test the values were acceptable for week 3 within limits whereas the product underwent microbial spoilage in week4 so shelf life till week 3(21 days) was termed to be safe for consumption from the date of manufacture.

Table 14: *Statistical Analysis of Shelf Life Study Based On Sensory Characteristics*

Parameter		N	Mean	Std. Deviation	Repeated Measures ANOVA F Value	P
APPEARANCE	Week 1	20	8.40	0.68	164.049	0.000
	Week 2	20	7.80	0.62		
	Week 3	20	6.70	0.47		
	Week 4	20	4.50	0.61		
COLOUR	Week 1	20	8.25	0.91	89.936	0.000
	Week 2	20	7.80	0.62		
	Week 3	20	6.80	0.70		
	Week 4	20	4.70	0.73		
TEXTURE	Week 1	20	8.25	0.85	117.162	0.000
	Week 2	20	7.80	0.62		
	Week 3	20	6.75	0.64		
	Week 4	20	4.65	0.67		
TASTE	Week 1	20	8.35	0.67	159.302	0.000
	Week 2	20	7.95	0.51		
	Week 3	20	6.70	0.57		
	Week 4	20	4.55	0.69		

Parameter		N	Mean	Std. Deviation	Repeated measures ANOVA F value	P
AROMA	Week 1	20	8.30	0.80	159.537	0.000
	Week 2	20	7.90	0.55		
	Week 3	20	6.50	0.51		
	Week 4	20	4.55	0.69		
OVERALL ACCEPTIBILITY	Week 1	20	8.45	0.60	198.221	0.000
	Week 2	20	7.85	0.59		
	Week 3	20	6.70	0.47		
	Week 4	20	4.50	0.61		

Table 14 depicts that the sensory characteristics depleted after each week and was unacceptable by the week 4. On analyzing the ANOVA result for each characteristic it was seen that the overall acceptability was as high as 198.221 which was due to drastic variations in the sensory characteristics. The Benofferi p value was 0.000 for all parameters, claiming the data to be significant as it was below 0.02.

- The parameter color had drastic change over the period of 4 weeks with the highest F value of 164.049.
- The parameter aroma also had noticeable depletion with every passing week and the F value was found to 159.537 which were high.
- The parameter taste also has similar F value of 159.302 claiming evident change in taste every passing week.
- There was no much difference in texture; hence the F value was around 117.162.
- There was least variation in colour of the ladoo with every consecutive week. So the F value for parameter colour was less within the range of 89.936.

Hence, the statistical analysis proved the deterioration of ladoo by week 4 with the noticeable change in aroma, appearance, taste and least variation in color and texture of the ladoo.

Table 15: *Post Hoc Analysis of Shelf Life Study Based On Sensory Characteristics*

Parameter: COLOUR

	Paired Differences			Bonferroni test p value	
	Mean	Std. Deviation	change (%)		
Week 1 - Week 2	.450	1.099	5.45	.497	NS
Week 1 - Week 3	1.450	1.234	17.58	.000	HS
Week 1 - Week 4	3.550	1.099	43.03	.000	HS
Week 2 - Week 3	1.000	.918	12.82	.001	HS
Week 2 - Week 4	3.100	.788	39.74	.000	HS
Week 3 - Week 4	2.100	1.119	30.88	.000	HS

Parameter: TEXTURE

	Paired Differences			Bonferroni test p value	
	Mean	Std. Deviation	change (%)		
Week 1 - Week 2	.450	.945	5.45	.278	NS
Week 1 - Week 3	1.500	1.000	18.18	.000	HS
Week 1 - Week 4	3.600	1.095	43.64	.000	HS
Week 2 - Week 3	1.050	.826	13.46	.000	HS
Week 2 - Week 4	3.150	.813	40.38	.000	HS
Week 3 - Week 4	2.100	.912	31.11	.000	HS

Parameter: AROMA

	Paired Differences			Bonferroni test p value	
	Mean	Std. Deviation	change (%)		
Week 1 - Week 2	.400	.995	4.82	.528	NS
Week 1 - Week 3	1.800	.894	21.69	.000	HS
Week 1 - Week 4	3.750	.851	45.18	.000	HS
Week 2 - Week 3	1.400	.681	17.72	.000	HS
Week 2 - Week 4	3.350	.813	42.41	.000	HS
Week 3 - Week 4	1.950	.826	30.00	.000	HS

Parameter: TASTE

	Paired Differences				
	Mean	Std. Deviation	change (%)	Bonferroni test p value	
Week 1 - Week 2	.400	.821	4.79	.253	NS
Week 1 - Week 3	1.650	.988	19.76	.000	HS
Week 1 - Week 4	3.800	.834	45.51	.000	HS
Week 2 - Week 3	1.250	.716	15.72	.000	HS
Week 2 - Week 4	3.400	.821	42.77	.000	HS
Week 3 - Week 4	2.150	.933	32.09	.000	HS

Parameter: OVERALL ACCEPTIBILITY

	Paired Differences				
	Mean	Std. Deviation	change (%)	Bonferroni test p value	
Week 1 - Week 2	.600	.821	7.10	.024	sig
Week 1 - Week 3	1.750	.639	20.71	.000	HS
Week 1 - Week 4	3.950	.826	46.75	.000	HS
Week 2 - Week 3	1.150	.745	14.65	.000	HS
Week 2 - Week 4	3.350	.933	42.68	.000	HS
Week 3 - Week 4	2.200	.696	32.84	.000	HS

Table 15 illustrates the post hoc analysis of the ladoo which determined the change that occurred by comparing two consecutive weeks and in all the cases, there was no significant difference between week1 and week 2 of all the parameters.

- Whereas the total acceptability between wee1 and week 2 had slight significant difference of 0.024 which was within the acceptable range. This data claimed that there was no much change in the ladoo from week1 to week 2 as the percentage of change was below 6-7%.
- The data of other weeks that is week 1 with week 3 and 4 claimed that there was high significant difference in all the attributes and the percentage of change was from 17-47% with high range width.

- The data of week 2 with comparison with week 3and 4 claimed to have high significant difference with the percentage of change from 12-43% which was a high range width.
- The data between week3 and 4 did not have much variation and ranged from 30-32% which proved that the depletion of attributes started by end of week3 and was not more in the week 4.
- Therefore from the post hoc analysis, it is clear that the onset of deterioration of ladoo was from the end of week 3 till the week 4.
- *MICROBIAL ANALYSIS*: the microbial analysis of the ladoo is depicted in in table which has a detailed note on the microbial load on the day of ladoo preparation (DAY 1) and on the day of spoilage (DAY 28). The tabular representation of the comparison in the microbial load is depicted in fig according to which the yeast and mold were the reason of spoilage hence storage in dry condition is suitable.

Table 16: *Microbial Analysis of Ladoo on Day1 and Day 28*

PARAMETERS	**MAXIMUM PERMISSIBLE LIMITS**	**TEST (DAY 1)**	**TEST (DAY 28)**
TOTAL PLATE COUNT	10^5 Cfu/g	$3.5x10^2$ Cfu/g	$3.6x10^2$ Cfu/g
Escherichia coli	Absent Cfu/25g	Absent	Absent
Staphylococcus aureus	10^4 Cfu/g	$<1x10^1$ Cfu/g	$<1x10^1$ Cfu/g
Yeast and molds	10^1 Cfu/g	$<1x10^1$ Cfu/g	$1x10^1$ Cfu/g

Table 16 shows that on day 1 the microbial count was within the permissible limit whereas on day 28 the yeast and mold had developed evidently more than the limit and the total plate count were higher than the acceptable range.

- The free fatty acid value from week 1 to week 4 was **13.53%, 10.95%, 8.19%** and **3.54%** respectively. The peroxide vale from week 1 to week 4 was **18 mEq/kg, 20 mEq/kg, 31.4 mEq/kg** and **39.8 mEq/kg** respectively.

4.9 NUTRITIONAL LABELLING

Table 17: *Micronutrient data of the ladoo*

Nutrients	Concentration per 100 gram of ladoo
IRON	421.5mg
CALCIUM	264.94mg
SODIUM	102.07mg
POTASSIUM	893.5mg
VITAMIN A	0.164mg
VITAMIN C	0.349mg
CHOLESTROL	3.711mg

Table 17 depicts the concentration of important micronutrients which are printed on the packaged product for nutritional information of cowpea-rice ladoo. From the data it is clear that the ladoo is low in cholesterol, potassium and sodium which are major factors of concern by the consumers. Whereas it is a fair source of vitamin A and C along with calcium and iron, hence these nutrients can be enriched. But as the ladoo is claimed to be protein rich, these nutrients can be provided to the body by other food sources

4.10BUDGETING OF THE PRODUCT

Table 18: *Budgeting of the ladoo*

INGREDIENTS	**QUANTITY**	**AMOUNT(Rs)**
Rice (sona masoori , loose)	600g	27
Cowpea (white, loose)	600g	54
Jaggery (loose)	600g	29
Ghee (packaged)	200ml	282
Cardamom powder (loose)	20g	40
Total	**2020g**	**432**
PACKAGING MATERIAL	10 no's	100
MISCELLANEOUS		20
GRAND TOTAL		***552***

From the total quantity of approximately two kilogram of raw ingredients, 80 ladoos were made of each weighing 25 grams. Each bagasse container of 750 ml capacity contained 8 ladoos with total quantity of 200grams per container. Hence, 10 such containers were used along with cling wrap and both costed 90 and 20 rupees respectively which were calculated collectively under packaging material. The miscellaneous included gas cost, labor and others such as printing labels etc. Hence, the cost of each packaged cowpea-rice ladoo was 55.2 rupees. **Table 18** depicts the overall budget of the cowpea –rice ladoo in terms of the quantity and the cost. However, the cost is affordable when compared to other sweets available in the market.

4.11 PAKAGED PRODUCT

The final packaged product consisted of the brand name "CORI MUNCHIES" and the logo representing the ladoo. The nutritional column consisted of the nutritional table with information on calories, net quantity, macro and micro nutrient profile (of per serving and whole package) along with the daily values as per 2000 calories.

Plate 21:
Product Name and Logo

Plate 22:

Nutritional Information on the Packaged Product

Nutrition Facts	
8 servings per container	
Serving size	**200 (25g)**
Amount Per Serving	
Calories	**130**
	% Daily Value*
Total Fat 0.5g	**1%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Total Carbohydrate 27g	**10%**
Dietary Fiber < 1g	**3%**
Total Sugars 1g	
Includes 0g Added Sugars	**0%**
Protein 3g	**6%**
Vitamin D 0mcg	0%
Calcium 0.858mg	0%
Iron 0.0189mg	0%
Potassium 10.48mg	0%
Vitamin A	0%
Vitamin C	0%

*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Plate 23:

The Final Packaged Product

CHAPTER 5

SUMMARY AND CONCLUSION

This section gives a clear picture on the overall findings done on the cowpea-rice ladoo and according to this the ladoo is high in energy, protein and carbohydrates. The physiochemical properties such as pH and total acidity were within the acceptable limits. The bulk density of the product claims to be moderately bulk. The micronutrients were found to be low in total composition. The shelf life was up to 3weeks and the budgeting of each pack of ladoo was economically feasible. Hence overall the ladoo was developed taking proximate, physiochemical, microbial aspects into consideration. Hence it is a healthier option as it is low in cholesterol, sodium and potassium.

5.0 SUMMARY AND CONCLUSION:

5.1 SUMMARY:

- Adulteration test claimed the raw ingredients used for the preparation of cowpea-rice ladoo to be free from any sort of contamination, which was a basic criteria to develop a high quality ladoo.
- Simple processing methods employed at household level such as natural fermentation, germination were easy to apply and at the same time cost effective which would stabilise the product cost.
- The processing methods natural fermentation and germination were highly effective in enhancing the nutrient availability of the rice and cowpea by eliminating the anti-nutrients which blocked nutrient absorption by the body.
- Selection of appropriate processing techniques for rice and cowpea would favour the acceptability and palatability of the final product and variant of a processing method best suitable for the rice or cowpea would improve its role in the final product.
- Accuracy of the processing methods such as pH and acidity determination for natural fermentation of rice being 3.86 and 0.42% which fell and total germination rate in case of cowpea being 81.3% which fell within the acceptable range, claimed the processing method employed to be accurate.
- For developing a protein rich product, the protein profile of unprocessed and processed rice and cowpea samples claimed the fermented rice and germinated cowpea sample to have 1.36times and 1.6 times more protein concentration from the unprocessed samples claiming that fermentation and germination increased the protein availability.
- The simple with fermented rice and germinated cowpea at the proportion of 60:40 had 1.31 times more protein than the combination with processed and unprocessed sample, claiming that when booth the flour blends are processed the protein availability is high.
- The proportion of 50:50 and 60:40 of fermented rice flour to germinated cowpea had protein concentration of 969.8 and 845 which was 1.78 times more protein than other combinations.
- On evaluating the consumers' preference, about 68% chose the cowpea-rice ladoo of 50:50 proportion, which laid the basis for formulating of the ladoo.

- The proximate analysis claimed the ladoo formulated to be rich in energy (537.76kcals) and protein (10.80g) along with carbohydrates (109.03g) per 100gms of sample.
- The physiochemical property consisted of the pH being **6.17** was within the acceptable range as it was neutral. The acidity was **13.53%** which was within the acceptable range of 9.05-15%. The bulk density of **70.1 g/cm^3** for 50 gm of sample claimed it to be moderately bulk in density. The reducing sugar was **5.84 g/100g** predominantly due to maltose.
- The novel technique of packaging the ladoo in the bagasse container made out of sugar cane pulp was an initiative to minimise the use of non –biodegradable packaging materials like plastic and each container costed 9.45 rupees which was affordable.
- The ladoo packaged with cling wrap on the container stayed fresh when compared to the ones without cling wrap and this was mainly due to loss of moisture. Hence the ladoo was packed in bagasse container with a cling wrap later.
- The shelf life study claimed that the ladoo was acceptable for 3 weeks (28 days) from the date of manufacture as the ladoo underwent spoilage on the fourth week. The hedonic scale rating dropped almost to the half on week 4 when compared to week 1.
- The microbial analysis on day 1 claimed the product to be hygienic as all the microbial count was within the permissible range. However on day 28 it was seen that there is rise in yeast and mold concentration, which in turn was the reason for spoilage. It can be rectified by storing the ladoos in cool temperature.
- The free fatty acid value and peroxide vale depleted every preceding week and was unacceptable by week 4, indicating spoilage of the ladoo.
- The nutritional labelling claimed that the ladoo is low in cholesterol, potassium and sodium which are major factors of concern by the consumers. Whereas it is a fair source of vitamin A and C along with calcium and iron, hence these nutrients can be enriched.
- The budgeting of the ladoo with a net weight of 200gms containing 8 ladoos of 25 grams each was 55.2 rupees which was much lower than any other sort of pulse based ladoo available in the market.

- Further improvements need to employed in storing the ladoos at room temperature for more than 21 days and the nutrients that are low in the ladoo can be fortified.

5.2 CONCLUSION:

Protein is very important part of the diet for all age groups; the ones who are at risk of protein deficiency and go unnoticed are the young adults. A protein rich ladoo was formulated by using least number of ingredients which are locally available and are staple foods of a particular region such as cowpea and rice. As they are locally available the cost of the final product can be economically feasible and affordable by all groups of the society. The protein availability was increased by simple processing methods and by introducing the raw ingredients in a new like ladoo form different from traditional cooking methods such as curry, sabzi attracts the individuals to consume it and the ladoo was long lasting it can be easily incorporated in daily eating pattern. The ladoos were the rich source of energy, carbohydrates and protein. It was lacking in micronutrient which can availed from other food sources. It is a healthier option as it is low in cholesterol, sodium and potassium. By incorporating the cowpea rich ladoo in the diet, the protein that is been lacking in the diet can be partially compensated and it is a healthier and safer option than supplements.

5.3 RECOMMENDATIONS

- The anti-nutrients profiling before and after processing will be a sustainable evidence to prove the absorption of protein present in the ladoo.
- The amino acid profiling of the cowpea and rice through chromatographic techniques will be more precise in supporting the claim of the ladoo being a complete protein source.
- Enriching the ladoo with nutrients that are less in quantity such as calcium, iron, vitamin A and C will improve its nutritional status.
- Novel techniques for ladoo preparation to make it sterile, in order to hike its shelf-life period.
- Variation in the cooking and processing method to prevent the nutrient loss of the final product- the ladoo.

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APPENDIX

APPENDIX I

PREPARATION OF REAGENTS

1. **Processing of samples**

- *5% Salt solution*

5gms of iodised salt was weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the salt dissolved and was made up to the mark.

- *3% Salt and 6% Sugar solution*

3gms of iodised salt and 6 gms of crystal sugar were weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the salt and sugar dissolved and was made up to the mark.

- *0.03% baking soda solution*

0.03 gms of food grade baking soda(E500) was weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the baking soda crystals dissolved completely and was made up to the mark.

- *0.07% citric acid solution*

0.07 gms of food grade citric acid crystals weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the crystals dissolved and was made up to the mark.

2. **Titrable acidity of fermented rice water**

- *0.1N sodium hydroxide solution*

4gms of sodium hydroxide pellets were weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the pellets dissolved completely and was made up to the mark.

3. **Protein estimation by Lowry's method**

- *Standard protein solution*

20mg of bovine serum albumin was weighed and transferred to a 100 ml standard flask. It was made up to mark with distilled water. The concentration of the standard protein solution was 200 μg/ml.

- *Alkaline copper sulphate solution*

The alkaline copper sulphate was prepared by mixing 50 ml of solution A and 1ml of freshly prepared solution B.

- *0.1 N sodium hydroxide solution:*

4gms of sodium hydroxide pellets were weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the pellets dissolved completely and was made up to the mark.

- *1% sodium potassium tartrate solution:*

1 gram of sodium potassium tartrate crystals were weighed and were transferred to 100ml standard flask. It was made up to the mark by distilled water. It is freshly prepared.

SOLUTION A - *2% sodium carbonate solution:*

2 grams of sodium carbonate was weighed and was transferred to 100 ml standard flask. It was made up to mark with 0.1N sodium hydroxide solution.

SOLUTION B -*0.5% copper sulphate solution:*

0.5grams of anhydrous copper sulphate crystals were weighed and transferred to 100ml standard flask. It was made up to mark with 1% sodium potassium tartrate solution.

- *Folin Ciocalteau Reagent (FCR)*

50ml of Folin Ciocalteau Reagent was prepared by taking 25 ml of Folin Ciocalteau Reagent solution in 50ml measuring cylinder and making it up to 25ml by distilled water.

- *Potassium phosphate buffer (pH 7.4)*

3.03gms of di-potassium hydrogen phosphate crystals and 1.035gms of potassium di-hydrogen phosphate crystals were weighed separately and were transferred to a 250ml standard flask. It was made up to the mark with distilled water.

The pH was adjusted by dipping the electrode of pH meter in the buffer solution and adjusting the pH with 0.1N sodium hydroxide (if less than 7.4) or with 0.1M hydrochloric acid (if pH more than 7.4)

4. Proximate analysis of the food sample

(a) Protein estimation by Lowry's method

- *Standard protein solution*

20mg of bovine serum albumin was weighed and transferred to a 100 ml standard flask. It was made up to mark with distilled water. The concentration of the standard protein solution was 200 μg/ml.

- *Alkaline copper sulphate solution*

The alkaline copper sulphate was prepared by mixing 50 ml of solution A and 1ml of freshly prepared solution B.

- *0.1 N sodium hydroxide solution:*

4gms of sodium hydroxide pellets were weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the pellets dissolved completely and was made up to the mark.

- *1% sodium potassium tartrate solution:*

1 gram of sodium potassium tartrate crystals were weighed and were transferred to 100ml standard flask. It was made up to the mark by distilled water. It is freshly prepared.

SOLUTION A - *2% sodium carbonate solution:*

2 grams of sodium carbonate was weighed and was transferred to 100 ml standard flask. It was made up to mark with 0.1N sodium hydroxide solution.

SOLUTION B -*0.5% copper sulphate solution*:

0.5grams of anhydrous copper sulphate crystals were weighed and transferred to 100ml standard flask. It was made up to mark with 1% sodium potassium tartrate solution.

- *Folin Ciocalteau Reagent (FCR)*

50ml of Folin Ciocalteau Reagent was prepared by taking 25 ml of Folin Ciocalteau Reagent solution in 50ml measuring cylinder and making it up to 25ml by distilled water.

- *Potassium phosphate buffer (pH 7.4)*

3.03gms of di-potassium hydrogen phosphate crystals and 1.035gms of potassium di-hydrogen phosphate crystals were weighed separately and were transferred to a 250ml standard flask. It was made up to the mark with distilled water.

The pH was adjusted by dipping the electrode of pH meter in the buffer solution and adjusting the pH with 0.1N sodium hydroxide (if less than 7.4) or with 0.1M hydrochloric acid (if pH more than 7.4)

(b) Carbohydrate estimation by colorimetric method

- *2.5N hydrochloric acid*

91.2ml of concentrated hydrochloric acid was measured in the volumetric cylinder and transferred to a 1000ml standard flask using a funnel and was made up to the mark with distilled water.

- *Standard glucose solution*

100mg of anhydrous dextrose was weighed and transferred into 100 ml standard flask and was made up to mark with distilled water. The concentration of stock solution was 1mg/ml. to prepare standard glucose solution, 10ml of the stock solution was taken in 100ml standard flask and was made up to mark with distilled water. The concentration of standard glucose solution was 100µg/ml.

- *Anthrone reagent*

0.2gms of anthrone was weighed in the analytical balance and was transferred to 100ml standard flask using the funnel. It was dissolved using ice cold concentrated sulphuric acid and was made up to the mark. (Note: The solution must be freshly prepared just before use).

(c) Fibre estimation by AOAC method

- *0.255N sulphuric acid solution*

7ml of concentrated sulphuric acid was measured in a volumetric cylinder and was transferred into 1000ml standard flask using funnel, made up to mark with distilled water.

- *0.313N sodium hydroxide solution*

12.38 Gms of sodium hydroxide pellets were weighed using analytical balance and transferred to a 1000ml standard flask and was added with distilled water until the pellets dissolved. It was made up to mark with distilled water.

5. Physiological and rheological analysis

(a) Reducing sugar estimation

- *Standard glucose solution*

100mg of anhydrous dextrose was weighed and transferred into 100 ml standard flask and was made up to mark with distilled water. The concentration of stock solution was 1mg/ml. to prepare standard glucose solution, 10ml of the stock solution was taken in 100ml standard flask and was made up to mark with distilled water. The concentration of standard glucose solution was 100µg/ml.

- *Sodium potassium tartrate solution*

30 gms of sodium potassium tartrate crystals were weighed and were transferred to 100ml beaker. It was dissolved in 50ml of distilled water. It is freshly prepared.

- *2M sodium hydroxide solution*

0.16 gms of sodium hydroxide pellets were weighed in the analytical balance and transferred to a 100ml beaker. It was dissolved in 20ml of distilled water.

- *3,5-DNSA solution*

1 gram of di-nitro-salicylic acid was weighed and taken in a 100ml beaker to which 20ml of 2M sodium hydroxide solution was added.

- *DNSA reagent*

50ml of sodium potassium tartrate solution and 20ml of 3,5-DNSA solution was freshly prepared and transferred to a 100ml standard flask, mixed well. The solution was made up to mark with distilled water.

(b) Total acidity

- *0.1N sodium hydroxide solution*

4gms of sodium hydroxide pellets were weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the pellets dissolved completely and was made up to the mark.

6. Shelf life study of the food product

(a)Estimation free fatty acid of the food product

- *0.1N sodium hydroxide solution*

4gms of sodium hydroxide pellets were weighed in the analytical balance and transferred to a 100ml standard flask using a funnel, it was mixed with distilled water and shaken well till the pellets dissolved completely and was made up to the mark.

- *0.1N phenolphthalein indicator*

0.5g of phenolphthalein was taken in a beaker to which 100ml of 50% of ethyl alcohol solution(50ml of ethyl alcohol was dissolved in 50ml of distilled water) was added and mixed well. The solution was transferred to a stopper bottle.

(b)Estimation of peroxide value of the food product

- *Acetic acid-chloroform solvent*

60ml of glacial acetic acid was weighed in a measuring cylinder and transferred to a stopper bottle, to which 40ml of chloroform was measured in the cylindrical flask and added. The stopper bottle was kept closed until use.

- *0.1N sodium thiosulphate solution*

24.8gms of sodium thiosulphate were weighed in the analytical balance and transferred to a 1000ml standard flask using a funnel, it was mixed with distilled water and shaken well till the crystals dissolved completely and was made up to the mark.

7. Nutritional labelling of the product

(a) Estimation of iron by Wong's method

- *30% sulphuric acid solution*

30ml of concentrated sulphuric acid was taken in a 100ml standard flask which was made up to mark with double distilled water.

- *7% potassium per sulphate solution*

7gms of potassium per sulphate crystals were weighed in the analytical balance and were transferred to 100ml standard flask using funnel which was made up to mark with double distilled water.

- *40% potassium thiocyanate solution*

40gms of potassium thiocyanate was weighed in the analytical balance and were transferred to 100ml standard flask using funnel which was made up to mark with double distilled water.

- *Concentrated potassium permanganate solution*

10gms of potassium permanganate was weighed in the analytical balance and transferred through a funnel to the 100ml standard flask and was made up to mark with double distilled water.

- *Standard iron solution*

0.702gms of crystalline ferrous ammonium sulphate (Mohr's salt) was weighed in the analytical balance and dissolved in 100 ml of double distilled water and 50ml of concentrated sulphuric acid and was warmed slightly.2-3 drops of concentrated potassium permanganate solution was added until the solution turned permanent pink colour. The solution was transferred to 1000ml standard flask and made up to with double distilled water. The concentration of the solution was 1mg/ml. 10 ml of stock solution was taken in a 100ml standard flask which was made up to the mark with double distilled water. The concentration of standard iron solution was 100µg/ml.

(b)Estimation of calcium by EDTA method

- *0.1M EDTA solution*

3.72gms of disodium EDTA dihydrate was weighed in the analytical balance and transferred to 1000ml plastic bottle . 100ml of double distilled water was added an

stirred well until the contents dissolved. 700ml of double distilled water was added and mixed well, it was made up to and was kept for a day before using.

- *EBT indicator*

0.2gm of Erichrome black T was weighed in the analytical balance and was transferred to a 100ml beaker, 20ml of absolute alcohol was added and dissolved well. It was stored in the dropper bottle.

- *Standard calcium solution*

1gm of calcium carbonate was weighed in the analytical balance and was dried in the hot air oven for 2 hours at 80^0C and then cooled in the desiccator for 1 hour. 0.5 GMS of the cooled calcium carbonate was weighed using the analytical balance and transferred to a 250ml standard flask and was made up to mark with double distilled water. 25ml of this solution was taken and added to a 250ml standard flask and 5ml of concentrated hydrochloric acid was added and left for 3-4 minutes for the carbon-dioxide to evolve. The contents were transferred to 500ml standard flask and were made up to mark with double distilled water.

- *8.5M ammonium buffer*

16.9gms of ammonium chloride was dissolved in 143ml of concentrated ammonium solution which was transferred to 250ml standard flask and was made up to mark with double distilled water.

(c)Estimation of sodium using Mohr's titration method

- *0.1N silver nitrate solution*

1.698gms of silver nitrate was weighed in the analytical balance and transferred to 100ml standard flask using the funnel it was made up to mark using double distilled water.

- *5% potassium chromate solution*

5 gms of potassium chromate was weighed in the analytical balance and was transferred to the 100ml standard flask using funnel it was made up to the mark using double distilled water.

- *Standard sodium chloride solution*

0.585gms of sodium chloride was weighed using the analytical balance and was transferred to the 100ml standard flask using funnel it was made up to the mark using double distilled water.

(e)Estimation of vitamin A by colorimetric method

- *Acetone-hexane mixture*

30ml of acetone was measured using the measuring cylinder and transferred to a stopper bottle. To the same bottle 70ml of hexane was measured using the measuring cylinder and added. The bottle was kept in dark until used.

- *Standard beta carotene solution*

The stock solution was prepared by adding 100mg of standard beta carotene was measured using the analytical balance which was transferred to 100ml standard flask and was made up to using petroleum ether. The concentration was 1mg/ml.10ml of the stock solution was taken in a 100ml standard flask and was made up to mark using petroleum ether. The concentration of standard beta carotene solution was 100µg/ml.

(f)Estimation of vitamin C by colorimetric method

- *5% trichloroacetic acid solution*

5gms of trichloroacetic acid crystals were weighed using the analytical balance and were transferred to the 100ml standard flask using funnel it was made up to the mark using distilled water.

- *9N sulphuric acid solution*

24.97ml of concentrated sulphuric acid was measured using the measuring cylinder and was transferred to 100ml standard flask using funnel. It was made up to mark using distilled water.

- *DTC reagent*

0.4gms of tiourea,0.05gms of copper sulphate crystals and 3.0gms of 2, 4 Dinitrophenyl hydrazine were weighed separately and were transferred to 100ml standard flask using funnel. The contents were dissolved and made up to mark using 9N sulphuric acid.

- *5% oxalic acid*

5gms of oxalic acid crystals were weighed using the analytical balance and were transferred to the 100ml standard flask using funnel it was made up to the mark using distilled water.

- *10% acetic acid solution*

10ml of glacial acetic acid was measured using the measuring cylinder and was transferred to 100ml standard flask using funnel. It was made up to mark using distilled water.

- *5% oxalic acid in 10% acetic acid solution*

100ml of 5% oxalic acid and 50ml 10% acetic acid solution were taken in a stopper bottle and mixed well.

- *Standard ascorbic acid solution*

100mg of ascorbic acid was weighed and transferred into 100 ml standard flask and was made up to mark with distilled water. The concentration of stock solution was 1mg/ml. to prepare standard glucose solution, 10ml of the stock solution was taken in 100ml standard flask and was made up to mark with distilled water. The concentration of standard glucose solution was 100µg/ml.

(g)Estimation of cholesterol by Zak's method

- *Stock ferric chloride*

840 mg of pure dry ferric chloride was weighed using the analytical balance and dissolved in 100 ml of glacial acetic acid.

- *Ferric chloride precipitating solution*

10 ml of stock ferric chloride reagent was taken in 100 ml of standard flask and made up to the mark with pure glacial acetic acid and kept in dark.

- *Ferric chloride diluting reagent*

8.5 ml of stock ferric chloride was diluted to100 ml with pure glacial acetic acid and kept in dark.

- *Standard cholesterol solution*

100 mg of cholesterol was dissolved in 100 ml of glacial acetic acid. The concentration the stock solution was 1mg/ml. 10 ml of stock was dissolved in 0.85 ml of stock ferric chloride reagent and made up to 100 ml with glacial acetic acid. The concentration of working standard is 100 µg/ml.

APPENDIX 2

QUESTIONNAIRES AND FORMS USED FOR PRODUCT ANALYSIS

FORM 1:

Sensory Evaluation For Selection Of Processing Method (9-point hedonic scale rating)

Name:_____ Age: _____ Date:_____

You are receiving a set of samples consisting of rice which is coded in series. Kindly taste each of from the set and drink water provided before tasting the next sample. Evaluate them by numbering on the basis of attributes given below.

ATTRIBUTES	RICE SAMPLES		
	A1	A2	A3
APPEARANCE			
COLOUR			
TEXTURE			
AROMA			
TASTE			
OVERALL ACCEPTIBILITY			
(9-Like Extremely,8-Like Very Much,7-Like Moderately,6-Like Slightly,5-Neither Like nor Dislike,4-Dislike Slightly,3-Dislike Moderately,2-Dislike Very Much,1-Dislike Extremely.)			
COMMENTS:			

FORM 2:

Sensory Evaluation For Selection Of Processing Method (9-point hedonic scale rating)

Name:_____ Age: _____ Date:_____

You are receiving a set of samples consisting of cowpea which is coded in series. Kindly taste each of from the set and drink water provided before tasting the next sample. Evaluate them by numbering on the basis of attributes given below.

ATTRIBUTES	COWPEA SAMPLES		
	B1	B2	B3
APPEARANCE			
COLOUR			
TEXTURE			
AROMA			
TASTE			
OVERALL ACCEPTIBILITY			
(9-Like Extremely,8-Like Very Much,7-Like Moderately,6-Like Slightly,5-Neither Like nor Dislike,4-Dislike Slightly,3-Dislike Moderately,2-Dislike Very Much,1-Dislike Extremely.)			
COMMENTS:			

FORM 3:

Paired preference test to choose the best ladoo

Name: _____ Date: _____

Rinse your mouth before starting the test.

Taste at least half of both the samples A55 and A64 given, you may drink water after tasting each sample. Re -tasting is not allowed.

Circle the best sample you liked out of the two on the basis of overall acceptability.

A55 A64

Answer these questions considering the ladoo you preferred;

1. Did you like the product?

☐ Yes ☐ No

2. What quality did you like the most?

☐ Appearance ☐ Taste ☐ Flavour ☐ Texture ☐ Aroma ☐ Overall

3. Would you like to buy this product if it is introduced in the market?

☐ Yes ☐ No

4. Would you recommend people to buy this product?

☐ Yes ☐ No

5. If the product is cost-effective then how frequently would you purchase this product?

☐ Weekly ☐ Once In 15 Days ☐ Monthly

6. If the size of the ladoo is increased, how many ladoos would you consume at one time?

☐ Yes ☐ No

7. Could you make out that the ladoo was make from cowpea and rice flour?

☐ Yes ☐ No

If yes, based on what did you recognise_____

☐ Yes ☐ No

8. Would you prefer eating cowpea in this ladoo form or in savoury dishes?

☐ Yes ☐ No

9. Did you feel that the bland taste of cowpea improved in the form of ladoo?

☐ Yes ☐ No

10. Do you think that this ladoo would be liked by all age group?

☐ Yes ☐ No

11. Would you like to alter the sweetness of the ladoo?

☐ Increase Sweetness ☐ Decrease Sweetness ☐ Keep It As It Is

12 on the whole what did you feel about the product?

☐Very Good ☐ Good ☐ Average ☐Bad ☐ Very Bad

FORM4:

Shelf life Study Of Ladoo Using 9 Point Hedonic Scale Rating

Taste the sample given and rate it from 9 to 1 based on your preference for the particular characteristics mentioned below;

9-Like Extremely
8-Like Very Much
7-Like Moderately
6-Like Slightly,
5-Neither Like nor Dislike
4-Dislike Slightly
3-Dislike Moderately
2-Dislike Very Much
1-Dislike Extremely

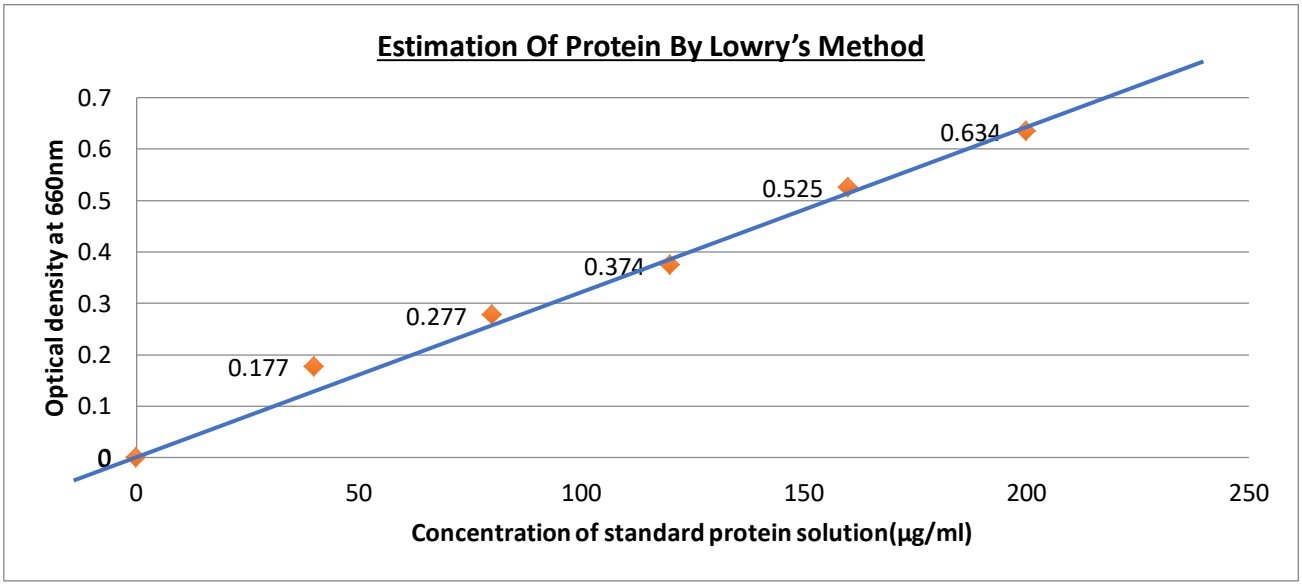
Sensory characteristics	Week1	Week2	Week3	Week4
Appearance				
Colour				
Texture				
Taste				
Aroma				
Overall acceptability				

APPENDIX 3

OPTICAL DENSITY OF STANDARD SOLUTION USED FOR COLORIMETRIC AND TITRIMETRIC ANALYSIS

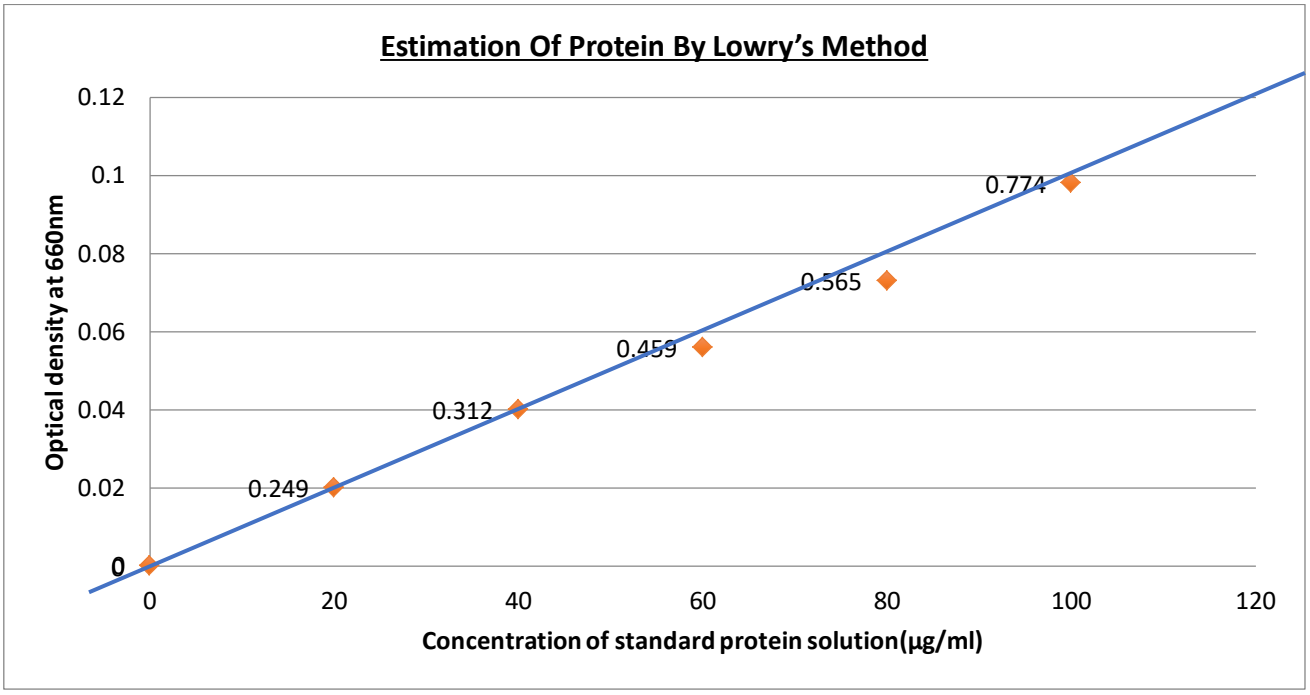
- **Estimation Of Protein By Lowry's Method (For flour samples):**

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard protein solution(ml)	0.2	0.4	0.6	0.8	1.0	-
Concentration of standard protein solution(µg/ml)	40	80	120	160	200	-
Volume of distilled water(ml)	0.8	0.6	0.4	0.2	-	1.0
Volume of copper sulphate solution(ml)	5.0	5.0	5.0	5.0	5.0	5.0
Volume of Folin-Ciocalteau Reagent (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Incubate in dark for 30 minutes						
Optical density of standard solution at 660nm	**0.177**	**0.277**	**0.374**	**0.525**	**0.634**	**0.000**



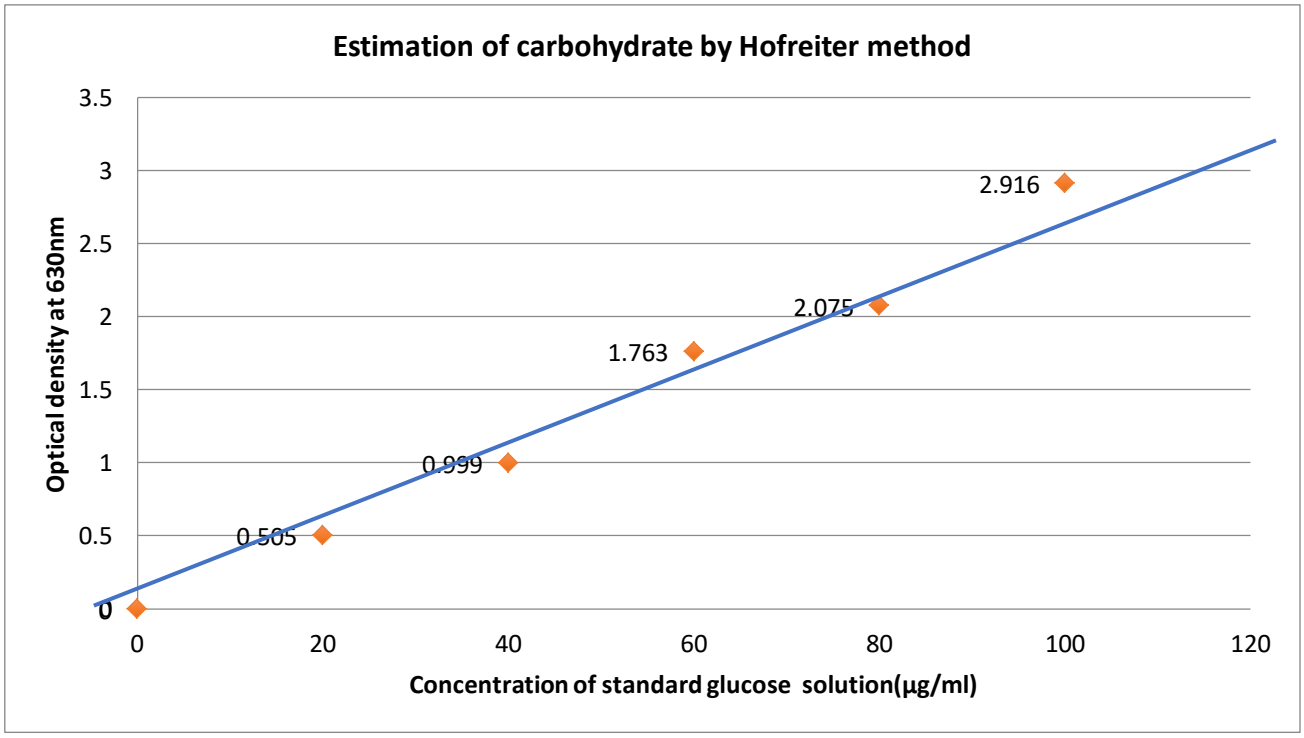
- **Estimation Of Protein By Lowry's Method (For food sample):**

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard protein solution(ml)	0.2	0.4	0.6	0.8	1.0	-
Concentration of standard protein solution(µg/ml)	40	80	120	160	200	-
Volume of distilled water(ml)	0.8	0.6	0.4	0.2	-	1.0
Volume of copper sulphate solution(ml)	5.0	5.0	5.0	5.0	5.0	5.0
Volume of Folin-Ciocalteau Reagent (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Incubate in dark for 30 minutes						
Optical density of standard solution at 660nm	**0.249**	**0.312**	**0.459**	**0.565**	**0.774**	**0.000**



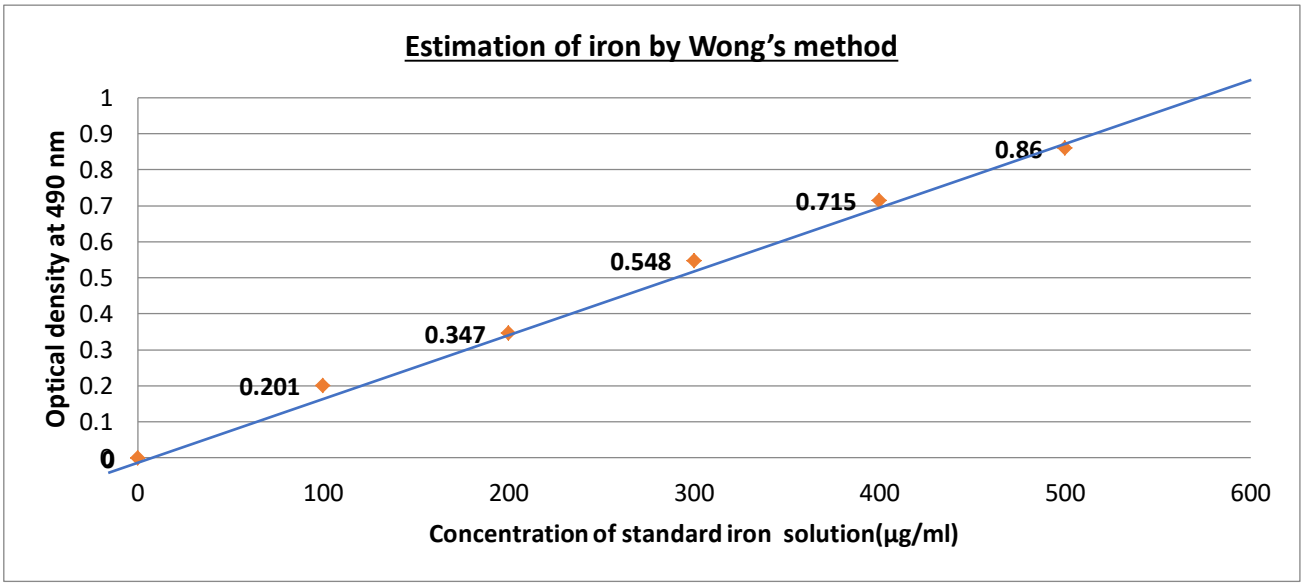
➢ **Estimation of carbohydrate by Hofreiter method**

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard glucose solution(ml)	0.2	0.4	0.6	0.8	1.0	-
Concentration of standard carbohydrate solution(µg/ml)	20	40	60	80	100	-
Volume of distilled water(ml)	0.8	0.6	0.4	0.2	-	1.0
Volume of Anthrone reagent solution(ml)	4.0	4.0	4.0	4.0	4.0	4.0
Incubate in water bath for 8 minutes						
Optical density of standard solution at 630nm	**0.505**	**0.999**	**1.763**	**2.075**	**2.916**	**0.000**



➢ **Estimation of iron by Wong's method**

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard iron solution(ml)	1.0	2.0	3.0	4.0	5.0	-
Concentration of standard carbohydrate solution(µg/ml)	100	200	300	400	500	-
Volume of distilled water(ml)	4.0	3.0	2.0	1.0	-	5.0
Volume of 30% sulphuric acid solution(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Volume of 7% potassium per sulphate solution(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Volume of 40% potassium thiocyanate solution(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Incubate at room temperature for 10 minutes						
Optical density of standard solution at 490nm	**0.201**	**0.347**	**0.548**	**0.715**	**0.86**	**0.000**



- Estimation of calcium by EDTA method

ESTIMATION OF CALCIUM			
Burette : 0.1M EDTA solution Conical flask: 10ml of diluted ash solution+4 ml of ammonium buffer + 8 ml of double distilled water + 2 drop of Erichrome Black T(EBT) indicator. End point: blue coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	33.0	33.0
2	0	33.1	33.1
3	0	33.0	33.0
			MEAN = **33.06 cm^3**

CALCULATION:

Amount Of Calcium

$$= \frac{(\text{molarity of EDTA} \times \text{volume of EDTA}) \times \text{molecular weight of calcium}}{\text{Volume of sample}} \times 100$$

Amount of calcium present in 10 of ash solution:

$$\frac{0.1 \times 33.06 \times 40.07}{10} \times 100 = \textbf{1324.71 mg}$$

As 5 gms of sample was present in 100ml of ash solution, 10 ml consists of 0.5 gms of food sample .therefore , 1 gram of sample contains;

1324.71 x 2 **=2649.42mgs of calcium**

- Estimation of sodium by Mohr's titration method

ESTIMATION OF SODIUM			
Burette : 0.1n silver nitrate solution Conical flask: 10ml filtered food sample+ 1ml of 5% potassium chromate indicator solution End point: red-brown coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	22.2	22.2
2	22.2	44.4	22.2
3	0	22.2	22.2
			MEAN = 22.2 cm^3

CALCULATION:

Chloride ion calculation =

$$\frac{\text{Volume of silver nitrate x normality of silver nitrate x molecular weight of chloride}}{\text{Volume of sample}} \times 100$$

$$= \frac{22.2 \times 0.1 \times 35.45}{10} \times 100 = \textbf{786.99 mg (1)}$$

Sodium chloride ion calculation =

$$\frac{\text{Volume of silver nitrate x normality of silver nitrate x molecular weight of sodium chloride}}{\text{Volume of sample}} \times 100$$

$$\frac{22.2 \times 0.1 \times 58.44}{10} \times 100 = \textbf{1297.36 mg (2)}$$

Sodium ion calculation = Sodium chloride ion calculation - Chloride ion calculation

=1297.36 - 786.99

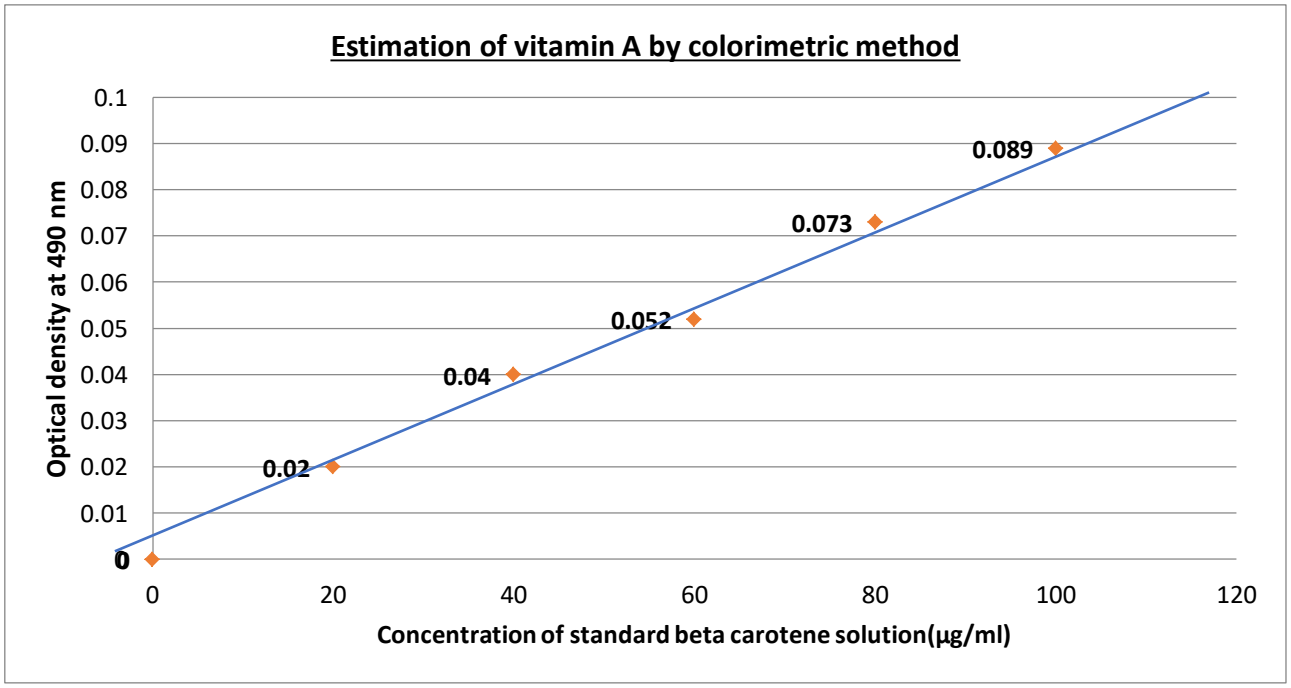
=**510.37 mg**.

As 10 gms of sample was present in 200ml of ash solution, 10 ml consists of 0.5 gms of food sample Hence , one gram of food sample has;

510.37 x 2 **= 1020.756 mg** of sodium.

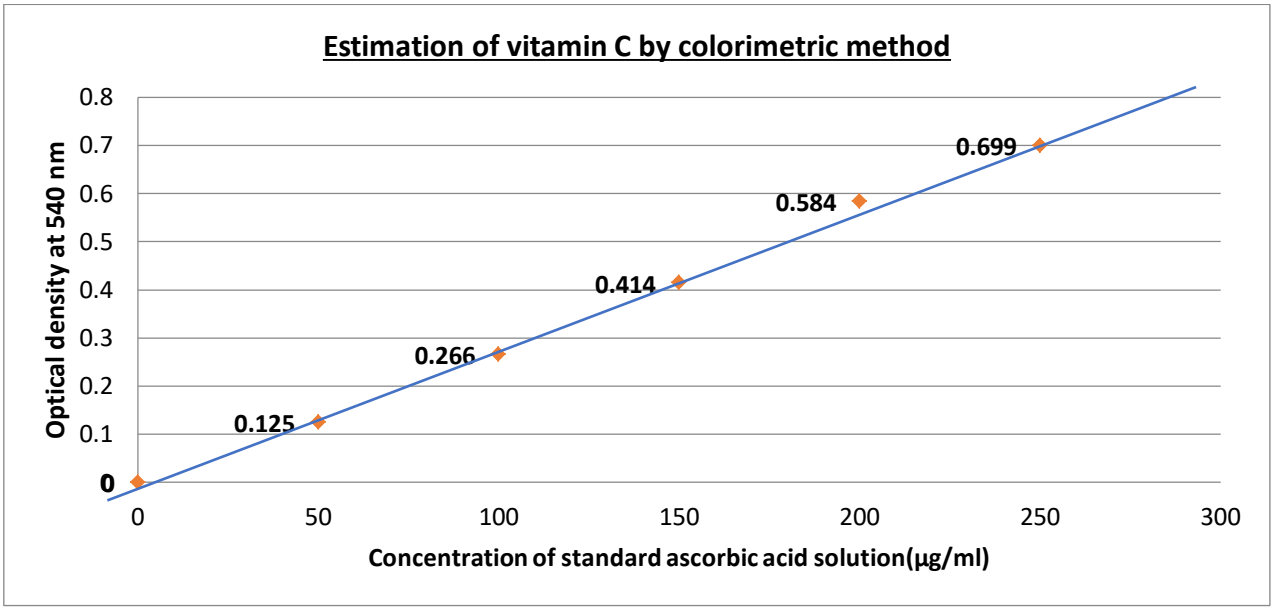
➢ Estimation of vitamin A by colorimetric method

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard beta carotene solution(ml)	0.2	0.4	0.6	0.8	1.0	-
Concentration of standard beta carotene solution(µg/ml)	20	40	60	80	100	-
Volume of petroleum ether(ml)	9.8	9.6	9.4	9.2	9.0	9.7
Volume of acetone solution(ml)	-	-	-	-	-	0.3
Incubate at room temperature for 15 minutes						
Optical density of standard solution at 490nm	**0.02**	**0.04**	**0.052**	**0.073**	**0.089**	**0.000**



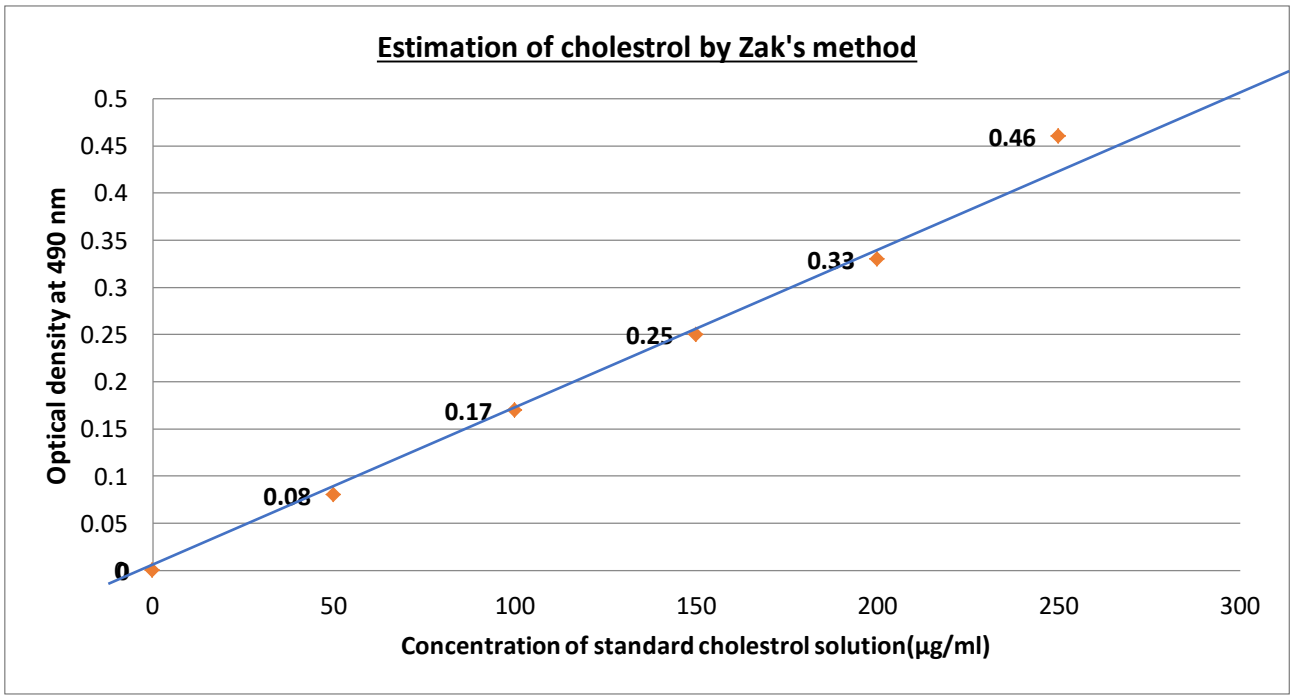
➢ Estimation of vitamin C by colorimetric method

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard ascorbic acid solution(ml)	0.5	1.0	1.5	2.0	2.5	-
Concentration of standard ascorbic acid solution(µg/ml)	50	100	150	200	250	-
Volume of 5%trichloroacetic acid solution(ml)	2.5	2.0	1.5	1.0	0.5	3.0
Volume of DTC reagent solution solution(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Incubate at 600c in water bath for 1 hour and cooled immediately for 15 minutes in ice.						
Volume of 9N sulphuric acid solution (ml)	5.0	5.0	5.0	5.0	5.0	5.0
Incubate at room temperature for 20 minutes						
Optical density of standard solution at 540nm	**0.125**	**0.266**	**0.414**	**0.584**	**0.699**	**0.000**



➢ Estimation of cholesterol by Zak's method

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard cholesterol solution(ml)	0.5	1.0	1.5	2.0	2.5	-
Concentration of standard cholesterol solution(µg/ml)	50	100	150	200	250	-
Volume of ferric chloride diluting agent solution(ml)	4.5	4.0	3.5	3.0	2.5	5.0
Volume of concentrated sulphuric acid solution(ml)	4.0	4.0	4.0	4.0	4.0	4.0
Volume of 7% potassium per sulphate solution(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Volume of 40% potassium thiocyanate solution(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Incubate in the water bath for 30 minutes						
Optical density of standard solution at 490nm	**0.08**	**0.17**	**0.25**	**0.33**	**0.46**	**0.00**

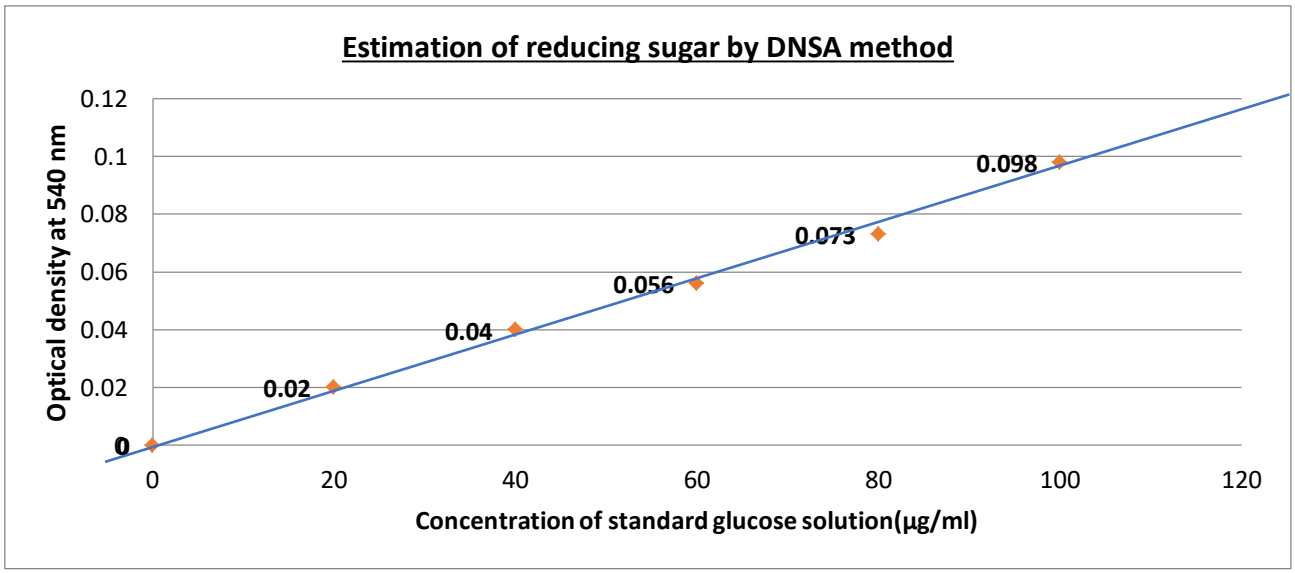


APPENDIX 4

PHYSIOCHEMICAL PROPERTY OF FOOD PRODUCT

- **Estimation of reducing sugar by DNSA method**

Aliquots of standard solution	S1	S2	S3	S4	S5	Blank
Volume of standard glucose solution(ml)	0.2	0.4	0.6	0.8	1.0	-
Concentration of standard carbohydrate solution(µg/ml)	20	40	60	80	100	-
Volume of distilled water(ml)	2.5	2.0	1.5	1.0	0.5	3.0
Volume of DNSA solution(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Incubate in water bath for 5 minutes						
Optical density of standard solution at 540nm	**0.02**	**0.04**	**0.056**	**0.073**	**0.098**	**0.00**



- **Estimation of acidity by titration method**

ESTIMATION OF TOTAL ACIDITY			
Burette : 0.1N sodium hydroxide solution Conical flask: 5 grams of food sample+ 5ml of distilled water + 2 drop of 0.1N phenolphthalein indication. End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	12	12
2	12	24.1	12.1
3	24.1	36.2	12.1
			MEAN = **12.06 cm^3**

CALCULATION:

$$\frac{56.1 \times \text{Volume of NaOH} \times \text{Normality of NaOH}}{\text{Weight of food sample}}$$

$$\frac{56.1 \times 12.06 \times 0.1}{5} = \mathbf{13.53\%}$$

APPENDIX 5

QUALITATIVE ANALYSIS OF THE PRODUCT

- **Estimation of free fatty acid value**

ESTIMATION OF FREE FATTY ACID VALUE (WEEK 1)			
Burette : 0.1N sodium hydroxide solution Conical flask: 5 grams of food sample+ 5ml of distilled water + 2 drop of 0.1N phenolphthalein indication. End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Burette reading (cm^3)
1	0	12	12
2	12	24.1	12.1
3	24.1	36.2	12.1
			MEAN = **12.06 cm^3**

CALCULATION:

$$\frac{56.1 \times \text{Volume of NaOH} \times \text{Normality of NaOH}}{\text{Weight of food sample}}$$

$$\frac{56.1 \times 12.06 \times 0.1}{5} = \mathbf{13.53\%}$$

ESTIMATION OF FREE FATTY ACID VALUE (WEEK 2)			
Burette : 0.1N sodium hydroxide solution Conical flask: 5 grams of food sample+ 5ml of distilled water + 2 drop of 0.1N phenolphthalein indication. End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Burette reading (cm^3)
1	0	9.8	9.8
2	9.8	19.5	9.7
3	19.5	29.5	9.8
			MEAN = **9.76 cm^3**

CALCULATION:

$$\frac{56.1 \times \text{Volume of NaOH} \times \text{Normality of NaOH}}{}$$

Weight of food sample

$$\frac{56.1 \times 9.76 \times 0.1}{5} = \mathbf{10.95\%}$$

ESTIMATION OF FREE FATTY ACID VALUE (WEEK 3)			
Burette : 0.1N sodium hydroxide solution Conical flask: 5 grams of food sample+ 5ml of distilled water + 2 drop of 0.1N phenolphthalein indication. End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Burette reading (cm^3)
1	0	7.3	7.3
2	7.3	14,6	7.3
3	14.6	21.9	7.3
			MEAN = **7.30 cm^3**

CALCULATION:

$$\frac{56.1 \times \text{Volume of NaOH} \times \text{Normality of NaOH}}{\text{Weight of food sample}}$$

$$\frac{56.1 \times 7.30 \times 0.1}{5} = \mathbf{8.19\%}$$

ESTIMATION OF FREE FATTY ACID VALUE(WEEK 4)			
Burette : 0.1N sodium hydroxide solution Conical flask: 5 grams of food sample+ 5ml of distilled water + 2 drop of 0.1N phenolphthalein indication. End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Burette reading (cm^3)
1	0	3.1	3.1
2	3.1	6.3	3.2
3	6.3	9.4	3.2
			MEAN = **3.16 cm^3**

CALCULATION:

$$\frac{56.1 \times \text{Volume of NaOH} \times \text{Normality of NaOH}}{\text{Weight of food sample}}$$

$$\frac{56.1 \times 3.16 \times 0.1}{} = \mathbf{3.54\%}$$

5

- **Estimation of peroxide value**

ESTIMATION OF PEROXIDE VALUE (BLANK)			
Burette : 0.01mol/L of sodium thiosulphate Conical flask: 5mk of distilled water + 30 ml of acetic acid-chloroform +0.5ml of potassium iodide solution End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	3.0	3.0
2	3.0	6.0	3.0
3	6.0	9.0	3.0
			MEAN = **3.0 cm^3**

ESTIMATION OF PEROXIDE VALUE (WEEK 1)			
Burette : 0.01mol/L of sodium thiosulphate Conical flask: 5gms of sample + 30 ml of acetic acid-chloroform +0.5ml of potassium iodide solution End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	2.1	2.1
2	2.1	4.2	2.1
3	4.2	6.3	2.1
			MEAN = **2.1 cm^3**

CALCULATION:

$$\frac{\text{(Blank titre value - test titre value) x molarity of sodium thiosulfate x 1000}}{\text{Weight of food sample}}$$

$$\frac{(3\text{-}2.1) \times 0.1 \times 1000}{5} = \textbf{18 mEq / Kg}$$

ESTIMATION OF PEROXIDE VALUE (WEEK 2)			
Burette : 0.01mol/L of sodium thiosulphate Conical flask: 5gms of sample + 30 ml of acetic acid-chloroform +0.5ml of potassium iodide solution End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	2.0	2.0
2	2.0	4.0	4.0
3	4.0	6.0	2.0
			MEAN = **2.0 cm^3**

CALCULATION:

$$\frac{\text{(Blank titre value - test titre value) x molarity of sodium thiosulfate x 1000}}{\text{Weight of food sample}}$$

$$\frac{\text{(3-2.0) x 0.1 x 1000}}{5} = \textbf{20 mEq / Kg}$$

ESTIMATION OF PEROXIDE VALUE (WEEK 3)			
Burette : 0.01mol/L of sodium thiosulphate Conical flask: 5gms of sample + 30 ml of acetic acid-chloroform +0.5ml of potassium iodide solution End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	1.4	1.4
2	1.4	2.9	1.5
3	2.9	4.3	1.4
			MEAN = **1.43 cm^3**

CALCULATION:

$$\frac{\text{(Blank titre value - test titre value) x molarity of sodium thiosulfate x 1000}}{\text{Weight of food sample}}$$

$$\frac{\text{(3-1.43) x 0.1 x 1000}}{5} = \textbf{31.4 mEq / Kg}$$

ESTIMATION OF PEROXIDE VALUE (WEEK 4)			
Burette : 0.01mol/L of sodium thiosulphate Conical flask: 5gms of sample + 30 ml of acetic acid-chloroform +0.5ml of potassium iodide solution End point: pale pink coloured solution			
Trial number	Initial burette reading (cm^3)	Final burette reading (cm^3)	Mean burette reading (cm^3)
1	0	1.0	1.0
2	1.0	2.1	1.1
3	2.1	3.1	1.0
			MEAN = **1.01 cm^3**

CALCULATION:

$$\frac{\text{(Blank titre value - test titre value) x molarity of sodium thiosulfate x 1000}}{\text{Weight of food sample}}$$

$$\frac{\text{(3-1.01) x 0.1 x 1000}}{5} = \textbf{39.8 mEq / Kg}$$