DEVELOPMENT AND QUALITY EVALUATION OF GLUTEN FREE PASTA

By

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KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR – 679 573, MALAPPURAM

KERALA, INDIA

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PROJECT REPORT

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TAVANUR – 679 573, MALAPPURAM

KERALA

2015

DECLARATION

We hereby declare that this project report entitled **"Development and quality evaluation of GFP "** is a bonafide record of research work done by me during the course of research and that the project report has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Place: Tavanur

Date: 18-02-15

CERTIFICATE

Certified that this project report entitled **"Development and quality evaluation of GFP"** is a bonafide record of research work done jointly by Ajna Alavudeen, Akshara Prakash, Rahul, K and Remyasree, P.T under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to them.

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DEDICATED TO OUR

PROFESSION OF

FOOD ENGINEERING

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SYMBOLS AND ABBREVIATIONS

$	dollar
%	percentage
°C	degree centigrade
a_w	water activity
i.e	that is
AOAC	Association of official Analytical Chemists
BD	Bulk Density
CD	Celiac Disease
CFU	Colony Forming Units
CII	Confederation of Indian Industry
cm	centimetre
DDI	Distilled De-ionized Water
et al.	and others
g	gram
g/cm^3	gram per centimeter cube
g/g	gram per gram
g/ml	gram per millimeter
GDP	Gross Domestic Product
GF	Gluten Free
GFP	Gluten Free Pasta
H	hour
HTST	High Temperature Short Time
JSF	Jackfruit Seed flour

Kg	kilogram
KVK	Krishi Vigyan Kendra
m	metre
M.C	moisture content
min	minute
ml	milliliter
mm	millimeter
MoFPI	Ministry of Food Processing Industries
MPN	Most Probable Number
N	newton
N-sec	newton-second
NaCl	Sodium chloride
OCT	Optimum Cooking Time
PaMPF	Pasta from mild parboiled rice
PaSPF	Pasta from severe parboiled rice
PCA	Plate Count Agar
ppm	parts per million
rpm	Rotation per minute
RTE	Ready to Eat
T	treatment
w/v	weight by volume
w/w	weight by weight
µm	micro metre

Introduction

CHAPTER I

INTRODUCTION

The food processing sector is the largest industry in India and ranks 5th in terms of production (MoFPI, 2011), consumption, export and expected growth. Availability of raw materials, changing lifestyles and appropriate fiscal policies has given a considerable push to the industry's growth. The food sector contributes to about 28% of India's GDP. This sector serves as a vital link between the agriculture and industrial segments of the economy. Adequate focus on this sector could greatly alleviate our concerns on food security and food inflation. Strengthening this link is of critical importance to reduce wastage of agricultural raw materials, improve the value of agricultural produce by increasing shelf-life as well as by fortifying the nutritive capacity of the food products ensuring remunerative prices to farmers as well as affordable prices to consumers.

The Confederation of Indian Industry (CII) has estimated that the foods processing sectors has the potential of attracting US $ 33 billion of investment in 10 years and generate employment of 9 million person – days (MoFPI, 2011). Processing sector involves primary, secondary and tertiary processing. The primary processing relates to conversion of raw agricultural produce into a commodity that is fit for human consumption. It involves steps such as cleaning, grading, sorting, packing etc. Secondary and tertiary processing industries usually deal with higher levels of processing where new or modified food products are manufactured for example pasta and cookies are the outcome of these sectors.

Wheat is the staple food of millions of people in India as well as abroad. Only rice challenges wheat for the title of most important and popular food grain in the world. Whole wheat flour is used for making traditional products like chapattis and puri. Pasta products, bread and all yeast raised products, biscuits, cookies, crackers, noodles, and vermicelli are also made from wheat flour. These entire food products having a rubbery protenacious material called gluten. As lifestyle continues to become more hectic, an increased number of people are turning to ready-to-eat snack foods like gluten containing pasta, bread, oats etc. why because its taste, chewy texture, rising property, all these properties are given by 'gluten'. The word gluten comes from Latin word, "glue". Gluten is a basically a protein found in foods such as wheat, barley and rye. Gluten is the main structure-forming protein in flour, and is responsible for the elastic characteristics of dough. The protein components of gluten, gliadin and glutenin are storage proteins in wheat. Gluten is a proteinaceous material that can be separated from flour when the starch and other minor components of the flour are removed by washing out under running water. The resulting gluten contains approximately 65% water. On a dry matter basis, gluten contains 75-86% protein, the remainder being carbohydrate and lipid, which are held strongly within the gluten-protein matrix (Bloksma and Bushuk, 1998).

Gluten contains the protein fractions glutenin and gliadin. The former is a rough, rubbery mass when fully hydrated, while gliadin produces a viscous, fluid mass on hydration. Gluten, therefore, exhibits cohesive, elastic and viscous properties that combine the extremes of the two components. The gluten matrix is a major determinant of the properties of dough (extensibility, resistance to stretch, mixing tolerance, gas holding ability). Moreover, gluten properties useful in bakery products are viscoelastic properties for dough strength, film forming ability for gas and moisture retention, thermosetting properties for structural rigidity, water absorption and retention capacity for product softness, and natural flavor enclosing the starch granules and fibre fragments. Gluten exposure may create the conditions for human diseases, the best known of which is celiac disease.

Celiac disease (CD) is a permanent intolerance to gluten. Those who suffer from celiac disease manifest anomalies of the intestinal mucosa with partial or total atrophy of the villi following the consumption of food containing gluten (Hurst, 1991). Therefore this pathology is often correlated with a mal-absorption of several important nutrients, including iron, folic acid, calcium, and vitamins (Feighery, 1999). The only way to overcome the celiac disease is to consume only gluten free products.

The measure of celiac disease is increasing and seriously affecting the health of individuals. With the increasing awareness there exists a great scope for gluten free products. The gluten-free (GF) products market represents one of the most prosperous markets in the field of food and beverages in the immediate future. The retail market for gluten-free foods and beverages is exploding as a result of multiple triggers. The first giant marketer to create a gluten-free product was Anheuser-Busch, which debuted a gluten-free beer, Redbridge in 2006. In recent years there has been an increasing interest on GF foodstuffs. As it was recently pointed out by the report of Markets and Markets research service, the GF products market is experiencing a double-digit growth. The global GF product market is projected to reach a value of $ 6,206.2 million, growing at a compounded annual growth rate of 10.2 % by 2018. This means that GF product market represents one of the most prosperous markets in the field of food and beverages in the near future.

Gluten-free (GF) foodstuffs are typically based on rice and maize have a comparatively low content of poor-quality proteins, and are low in fiber, calcium, and iron. GF products also have a high fat and caloric content, to compensate for decreased sensorial acceptability (Thompson, 2009). Gluten-free products like pasta and cookies markets have increased in recent years. Pasta is also known as Noodles or Chowmein or Vermicelli or Macroni. These are all generic names of the same item. Pasta comes in different sizes and shapes like strings, Alphabets, Macroni, Fancy shapes like Shells, Wheels, Twists, Butterflies or Ribbons. Each pasta is complimented with the variety of sauces, vegetables, olive oil, meat to add to the taste and eating methods of the consumer. It is accompanied with Assorted Bread. Since the nutritional value of pasta is very high, it has become very popular for the young or old alike.

Replacement of the gluten network to produce GF products is a major technological challenge, gluten being the essential structure- building protein. Thus, substances that imitate the viscoelastic properties of gluten are always required in GF products (Mariotti *et al*., 2009).Starch retrogradation is useful to give rigidity to cooked pasta, and to reduce both the stickiness of the pasta surface and the loss of soluble materials into the cooking water. In order to have a good amount of retrograded starch in the products, it is necessary to induce starch disorganization by heat treatments carried out under specific moisture conditions, followed by cooling phases during which part of the starch, mainly amylose, can create a three-dimensional network by strongly linking short starch chains by junction zones (Mestres *et al.*, 1988).

Nowadays, the most used ingredients in gluten free pasta (GFP) production are rice and corn flours (Arendt et al., 2008), flours from pseudo cereals (Caperuto *et al*., 2001; Chillo *et al*., 2008), starches of different origin (Huang *et al*., 2001), dairy products and vegetable proteins (Wang *et al*., 1999). In some cases, also low amounts of emulsifiers (Charutigon *et al*., 2008; Chillo *et al*., 2008) and hydrocolloids (Singh *et al*., 2004) are added.

Now the production and consumption of pasta through extrusion cooking has notably increasing worldwide. Extrusion is a multi-variable unit operation comprising of mixing, shearing, cooking, puffing and drying in one energy efficient rapid continuous process. It has an important role in the food industry as an efficient manufacturing process. This technology has many distinct advantages like versatility, low cost, better product quality and no process effluents (Fellows, 2000). This processing technology is widely used to restructure starch and protein based materials thus allowing the manufacturing of a variety of textured convenience foods. Extrusion cooking is a high-temperature short-time (HTST) processing technology in which raw materials are exposed to a combination of moisture, pressure, temperature, and mechanical shear resulting in stable products (Dias *et al*., 2009).

Extrusion has been used to process cereal legume blends for many years (Harper and Jansen, 1985). When heating and working during the extrusion process, the macromolecules in food ingredients lose their native, organized tertiary structure and form a viscous plasticized material. At the end of the die, fast vaporization of the water present in food takes place, leading to structural reorganization and producing a series of textured products. Among these, pasta is the product made by extrusion.

Gluten free foods have become a staple in nearly every household because gluten intolerance and celiac disease is increasingly common. People often have the wrong impression that gluten free foods do not taste good and continue to eat items that contains gluten, inspite of the health problems and symptoms. To overcome these challenges this study was carried out to develop a new gluten free pasta (GFP) from starch rich food products like rice, arrowroot, jackfruit seed and amaranthus grain.

Rice (*Oryza sativa L.*) is an important cereal in many Asian countries. India produces about 92 million tonnes of paddy rice annually. Rice is the staple food in India. In common man term it is rich source of carbohydrate and biggest source of energy for majority of Indian population. Rice flour is used to make various foods such as noodles and confectionary products, and also utilized as substitute for wheat (*Triticumaestivum L.*) flour. Several researchers found out that it is possible to prepare breads with acceptable qualities by partial substitution of wheat flour with rice flour (Nishita and Bean, 1979; Noomhorm and Bandola, 1994; Kadan *et al*., 2001; Veluppillai *et al*., 2010). Absence of gluten provides an additional advantage and makes rice particularly suitable as an alternative to wheat in bakery products especially suitable for persons suffering from physiological disorder of gluten sensitive enteropathy.

So rice flour is widely used as a raw material to prepare GF products for its bland taste, white color, high digestibility, and hypoallergenic properties (Rosell *et al*.,2008). However, in spite of its advantages, rice is low in protein and has relatively poor technological properties for interacting and developing a cohesive network. It is comprised of 77.5% carbohydrate also contains a range of important nutrients, including vitamin B, vitamin E, proteins and minerals especially potassium which helps the body to reduce toxins. A diet based white rice leaves people vulnerable to the neurological disease beriberi due to a deficiency of thiamine (vitamin B).

Root and tubers are the third important food crops of mankind after cereals and grain legumes. It constitutes either staple or subsidiary food for about one fifth of the world population. The Arrowroot (*Maranta spp.*) plants could be a suitable food source, because they have good yields of high quality rhizomes, among other agronomic advantages. Arrowroot is very low in calories; 100 fresh roots provide only 65 calories, less than that of potato, yam, cassava, etc. Its chief starch is amylopectin (80%) and amylose (20%). Its powder is fine, odorless, granular starch that has found utility in food industry as thickener and stabilizing agent.

It has relatively more protein than that of other tropical food sources like yam, potato, cassava, plantains, etc. Arrowroot too is free from gluten. Arrowroot roots indeed are good source of folates. Folate along with vitamin B-12 is one of the essential components take part in DNA synthesis and cell division. Good folate diet when given during preconception periods and during pregnancy helps prevent neural-tube defects and other congenital malformations in the offspring. Further, it contains moderate levels of some important minerals like copper, iron, manganese, phosphorous, magnesium, and zinc. In addition, it is an excellent source of potassium. Potassium is an important component of cell and body fluids that help regulate heart rate and blood pressure. Therefore arrowroot as a raw material for the preparation of gluten free pasta contributes to several nutritional benefits.

In India malnutrition is prevalent due to inadequate intake of protein. In view of this, efforts were made for identifying and evaluating underutilized non-conventional cheap protein sources like jackfruit seed as an alternative. Jackfruit (*Artocarpus heterophyllus Lam*.) is one of the most popular tropical fruits grown in India. A member of family *moraceae* is a popular fruit of tropics. 10-15% of the total fruit weight is considered as its seed weight and having high carbohydrate and protein contents, dietary fibre, vitamins, minerals and phytonutrients.The calorific value was 357.665 kcal/100g. It was also rich in potassium (6466 ppm), magnesium (4582 ppm) and sodium (8906 ppm) (Albi *et a*l.,2014). Jacalin and artocarpin are the two lecithin present in the seeds and these has possess antibacterial, antifungal and anticarcinogenic properties (Roy chowdhary *et al*.,2012).

Also Jackfruit seed contains lignans, isoflavones, saponins, all phytonutrients and their health benefits are wide-ranging from anticancer to antihypertensive, antiaging, antioxidant, antiulcer, and so on (Omale and Friday, 2010). Jackfruit seed flour has great potential in the food industry, especially as thickener and binding agent in various food systems (Ocloo *et al*., 2010). Jackfruit seed flour has good ability of the flour to bind water and lipid. The flour had good capacities for water absorption (25%) and oil absorption (17%). Therefore incorporation of jackfruit seed flour in the developed GFP could lead to supplementing nutritional qualities as well as textural benefits apart from utilizing this under-utilized seed which otherwise is discarded.

Grain amaranth (*Amaranthushypochondriacus*) belongs to the amaranth family *amaranthaceae*, sub-family *amaranthoideae*, genus *amaranthus*. Different species of the amaranth plant were grown and consumed both as green vegetable and as cereal grains. It produces good yields of grain that contains relatively high levels of proteins and starch and a considerable (higher than any of the cereals) content of lipids. The protein has high lysine content higher than those of the other cereal proteins which are deficient in this amino acid. Thus, from the point of view of the nutritional value, amaranth grain is more advantageous than the cereal grains. Macronutrients content in amaranth flour is similar to wheat, and 2-3 times higher than other GF sources (Calderon de la Barca *et al*., 2010). Proteins from amaranth have better amino acid nutritional balance than other vegetable proteins, including cereals, and the fiber and mineral content in amaranth is much higher than in other GF grains (Pedersen *et al*., 1990). The high lysine content of amaranth grain makes it particularly attractive for use as a blending food source to increase the biological value of processed foods (Pedersen *et al*., 1987).

Amaranth grain consists of approximately 5 to 9% oil which is generally higher than other cereals. The lipid fraction of amaranth grain is similar to other cereals, being approximately 77% unsaturated, with linoleic acid being the predominant fatty acid. The lipid fraction is unique however, due to the unusually high squalene content (5 to 8%) of the total oil fraction. Also present in the amaranth oil fractions were tocotrienols (forms of vitamin E) which are known to effect lower cholesterol levels in mammalian systems. In addition to the unique characteristics of the major components of proteins, carbohydrates, and lipids, amaranth grain also contains high levels of calcium, iron, and sodium when compared to cereal grains. Amaranth flour has already been used to enrich cereal-based foods, including GFP. The amaranth proteins have a good digestibility, a majority of proteins belonging to the group of water-soluble albumins and salt-soluble globulins (Gorinstein *et al*., 2002). Therefore fortification of pasta mix with amaranthus could also be a good proposition because weakness of the ingredient is the strength of the other.

Extrusion of a mixture, instead of only the cereal portion would ensure a nutritious pre-cooked blended product with the elimination of prolonged cooking (Harper, 1989). So a product enriched with these raw materials viz. rice, arrowroot, jackfruit seed and amaranth will ensure a food which is safe to consume, nutritious and ready to eat GFP with more or less, same textural characteristics as that of wheat based pasta.

With this back ground, a project entitled "**Development and quality evaluation of extruded GFP"** was under taken with the following objectives:

1. To standardize the composition of Gluten Free Pasta (GFP) from rice, arrow root, jackfruit seed and amaranth.
2. To determine the quality characteristics of the developed extruded GFP.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

This chapter reviews the research work done by other investigators on application of high pressure extrusion processing to formulate extruded products. The qualities of these products in terms of their physical, functional, textural and biochemical characteristics are also reviewed.

2.1 GF Foods

The incidence of celiac disease or other allergic reactions/intolerances to gluten is increasing, largely due to improved diagnostic procedures and changes in eating habits. The worldwide number of sufferers of celiac disease has been predicted to increase by a factor of ten over the next number of years, resulting in a growing market for gluten-free cereal-based products. As lifestyle continues to become more hectic, an increased number of people are turning to ready-to-eat snack foods.

Extrusion technology seems promising in creating new gluten-free products with higher nutritional values. Rising demands for GF products parallels the apparent or real increase in celiac disease, or other allergic reactions/intolerances to gluten consumption (Lazaridou *et al.*, 2007). Consequently, there is still a need to find substances that could improve the quality of this type of product. Replacement of the gluten network to produce GF products is a major technological challenge, gluten being the essential structure-building protein. Thus, substances that imitate the viscoelastic properties of gluten are always required in GF products (Mariotti *et al.*, 2011). Despite several GF products are nowadays available on the market, baked products from gluten-free ingredients are generally of poor physicochemical and sensory quality, and lack fibre, vitamins and nutrients, which results in a worsening effect on the already nutritionally unbalanced diet of CD sufferers (Thomson, 2009).

2.2Raw Materials

2.2.1 Rice (*Oryza sativa*)

Rice flour is widely used as a raw material to prepare GF products for its bland taste, white color, high digestibility, and hypoallergenic properties (Rosell and Marco, 2008). However, in spite of its advantages, rice is low in protein and has relatively poor technological properties for interacting and developing a cohesive network. Up to now, GFP made from rice flour has usually been prepared in one of two ways (Pagani,1986). In the first approach, native rice flour is treated with steam and extruded at high temperatures (more than 100° C) for promoting starch gelatinization directly inside the extruder-cooker. The second approach focuses on the use of pre-gelatinized flours, in which starch is already mostly gelatinized; the pre-treated flour can be formed into pasta by the continuous extrusion press commonly used in durum wheat semolina pasta making. In this regard, annealing and heat-moisture treatments have been proposed for rice flour and or cereal starch to induce new physiochemical properties. Because it is easy to use, pre-gelatinized flour is the most commonly used in industrial GFP production. Even if the effects of pre-gelatinization on starch from different sources (cassava, corn, rice, etc.) have been extensively investigated (Anastasiades *et al*., 2002; Lai and Cheng, 2004; Nakorn *et al*., 2009) there is not much information about the relationship between the induced starch properties and rheological properties of pre-gelatinized flour or its suitability for pasta-making or its cooking behavior. Recently, the use of flour from parboiled rice as a raw material for pasta products was proposed, by obtaining GFP with a good cooking behavior (Marti *et al*., 2010) due to the particular starch arrangements in the product (Marti *et al.*, 2011). Rice-based products are low in allergens and fat, easily digestible, and suited for gluten-free diets (Rosell and Marco, 2008). Pasta with appropriate cooking behaviour can be obtained from rice flour either by adding additives, or by applying appropriate heating or cooling treatments during pasta processing (Pagani, 1986).

A new method for making rice flour involving a dry milling technique of dried rice grain after soaking for at least 6 hr. was developed to substitute wheat flour (Song & Shin, 2007). Unlike a wet milling technique, wet rice grains after soaking for 6 hr. are dried under gelatinization temperature of rice starch and then the dried grains are milled, using a pin mill with a built-in standard sieve. Although many scientists have reported that wet milling is better than dry milling in producing rice flour (Kumar, Malleshi, and Bhattacharya, 2008; Sharma *et al*., 2008), the new developed rice flour also has some good processing qualities that allows for the production of rice flour as a substitute for wheat flour (Shin *et al*., 2010).

When rice flour is used as the only ingredient for pasta production, it requires additives or particular processing techniques to modify in a suitable way the properties of macromolecular components (starch and proteins) relevant to the structure of the final product. Either gelatinization of the rice flour or steaming of the pasta may improve the textural properties of the final product (Pagani,1986; Lai, 2001) and a process was developed for rice-based pasta, in which extrusion-cooking of the starting flour was followed by conventional pasta-making processes (Marti *et al*., 2010).

Colonna and Buleon (1992) studied the pasta from mild parboiled rice (PaMPF) and pasta from severe parboiled rice (PaSPF) showed the lowest values for starch enzymatic susceptibility, suggesting that the pasta-making of parboiled rice flours promoted a further relevant rearrangement in starch macromolecules that was effective in lowering cooking losses.

Marti *et al*. (2013) conduct a study and found that the cooking quality of GFP made from rice flours was greatly affected by the thermal treatments of the raw material. Regardless of extrusion conditions, severe parboiling process on paddy rice promoted new and effective starch networks in flour (highlighted by peculiar hydration and pasting properties), making rice suitable for GFP making.

Table 2.1 Composition of Rice Flour

Indices	**Values (g)**
Carbohydrate	81.1
Protein	6
Fat	1.3
Fibre	0.8
Moisture	10.8

Source: Marti et al. (2010)

2.2.2 Arrowroot (*Maranta arundinacea*)

Arrowroot is a starch obtained from the rhizomes (root stock) of several tropical plants, traditionally *Maranta arundinacea*. Arrowroot powder is a white, flavorless powder used to thicken sauces, soups, and other foods. Arrowroot powder is comprised of starches extracted from various tropical tubers, such as the arrowroot plant and cassava. Some lower quality arrowroot powder blends may also contain potato starch, so be sure to read ingredient lists carefully.

Arrowroot powder is twice the thickening power of wheat flour and, because it contains no protein, is gluten free. Unlike corn starch, arrowroot powder creates a perfectly clear gel and does not break down when combined with acidic ingredients, like fruit juice. Arrowroot also stands up to freezing, whereas mixtures thickened with corn starch tend to break down after freezing and thawing. Because arrowroot can stand up to acidic mixtures, it is excellent for thickening fruit gels and fruit sauces, like cranberry sauce, or acidic sauces like sweet and sour sauce. Arrowroot powder is excellent for making smooth homemade ice cream, as it interferes with the formation of large ice crystals and is not affected by freezing. Although it is good for ice cream, arrowroot powder is not suggested for use with other non-frozen dairy products, as it can produce a slimy or undesirable texture. An experiment conducted by M.D Erdman (1984), by comparing the characteristics of starch in arrow root plant.

Table 2.2 Proximate Analysis of Arrowroot Starch

Indices	**Values (g)**
Carbohydrate	85.3
Protein	0.7
Fat	0.4
Moisture	13.7

2.2.3 Jackfruit Seed (*Artocarpusheterophyllus Lam*.)

A study on the functional properties of raw and blended jackfruit seed flour for food application was carried out by chowdhury *et al*., (2012). The work to investigate the functional properties of raw and blended jackfruit seed flour with a view to provide useful information for its effective utilization along with wheat flour and finally the study reveals that jackfruit seed has a great potential in new food formulation along with wheat flour.

Abedin *et al.* (2012) studied on the nutritive compositions of jackfruit seeds, showed protein content was ranged from 13-18%. Crude fibre content of seed varied from 1.56-2.60%. Jackfruit is a good source of many mineral contents like N, P, K, Ca, Mg, S, Zn, Cu etc. Starch content in seed was found from 12.86 - 17.90%.

The seeds may be boiled, or roasted and eaten or boiled and preserved in syrup like chestnuts. Roasted, dried Seeds are ground to make flour which is blended with wheat flour for baking.

Table 2.3: Physicochemical Properties of JSF

Indices	**Values (%dry matter)**
Moisture	14.07±0.04
Crude fat	2.55±0.09
Protein	9.03±1.09
Total mineral matter	3.01±0.01
Carbohydrate	70.26±0.06
Energy (Kcal/100 g)	382.79±1.20

Source: Ocloo et al. (2010)

The starch content of the seed increased with maturity and different locations gave different seed contents (Rahman *et al*., 1999). Amylose content of jackfruit seed starch was 32% (Tulyathan *et al*., 2002). Jackfruit seed extract was found to inhibit the proteolytic activities of different animal pancreatic preparations effectively (Bhat and Pattabiraman, 1986). Tulyathan *et al*. (2002) reported on the good ability of the flour to bind water and lipid. The flour had good capacities for water absorption (25%) and oil absorption (17%) and the Brabender amylogram (6% concentration) of seed starch showed that its pasting temperature was 81°C; and its viscosity was moderate, remained constant during a heating cycle, and retrograded slightly on cooling.

Chowdhuri *et al*. (2012) conducted a study on functional properties of raw and blended jackfruit seed flour (JSF) for food applications and reveals that JSF has great potential in new food formulations along with wheat flour. The different functional behaviors of raw and blended JSF are influenced by milling operation, concentration of NaCl, effect of pH and on heating. As the flour and its blends have good water and oil absorption capacities up to 15%.

2.2.4 Amaranth *(Amaranthus hypochondriacus)*

Cabrera-Chavez *et al*. (2012) analysed the molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta. Incorporation of amaranth flour in rice pasta combined with extrusion-cooking improves the textural and nutritional quality of the final product. Addition of 25% amaranth flour significantly improves the nutritional characteristics of rice-based pasta without much dramatic worsening of cooking behavior. In this frame, introduction of the extrusion-cooking step prior to pasta-making is decisive, as pasta made from an extrusion-cooked mixture of rice flour and amaranth flour had the best textural and nutritional characteristics.

Nutrition value and use of grain amaranth were analyzed by Chavez et al., (2012) and found out that Grain amaranth has been tested and recognized by many authorities as a gluten-free foodstuff suitable for incorporation into the diet for celiac disease patients. On the other hand, lack of gluten is a limiting factor for application of grain amaranth into the composite flour for leavened products. Another fact limiting composite bread acceptance is a distinct aroma and flavor of amaranth, described as spicy, slightly pungent with bitter aftertaste. However, consumer's food acceptance not only depends on sensory, but also on non-sensory factors. The non-sensory factors include not only aspects such as price and convenience of preparation, but also the production methods, consumer's attitudes, awareness of health and the environmental, and product beliefs.

Table 2.4 Composition of Amaranth Flour

Indices	Values (g)
Starch	61.1
Protein	19.1
Lipid	9.7
Ash	3
Fibre	18.6

Source: Chavez et al. (2012)

2.3 Extrusion

Extrusion technology increases the level of dietary fibre in non-gluten-free ready to-eat (RTE) expanded snacks made from cereal and vegetable co-products (Stojceska *et al*., 2009; Stojceska *et al*., 2008).

ValentinaStojceska *et al*. (2010) performed extrusion technology by incorporating a number of different fruits and vegetables, such as apple, beetroot, carrot, cranberry and gluten-free teff flour cereal. The materials were added at the level of 30% into the gluten-free balanced formulation (control) made from rice flour, potato starch, corn starch, milk powder and soya flour. Different process conditions, such as water feed rate 12%, solid feed rate 15–25 kg/h, screw speed 200–350 rpm, barrel temperatures: 80°C at feed entry and 80–150°C at die exit were used. The increases of dietary fibre components depend on the types of samples used as a source and, in this study, a greater increase in dietary fibre content was observed with Teff samples, followed by apple, cranberry, carrot and beetroot. The formation of gluten-free expanded products with high dietary fibre levels can be achieved by controlling extrusion conditions, such as temperatures, solid feed rate and screw speed combinations and the selection of appropriate raw ingredients.

The screw has a number of sections, including a feed section to compress particles in to a homogenous mass, a kneading section to compress, mix and shear the plasticized food and in high shear screws, a cooking section (Leszek and Zuilichem, 2011). Pressure flow is caused by the build-up of pressure behind the die and by material movement between the screw and barrel. Slipping can be minimized by special groves on the inside of the barrel.

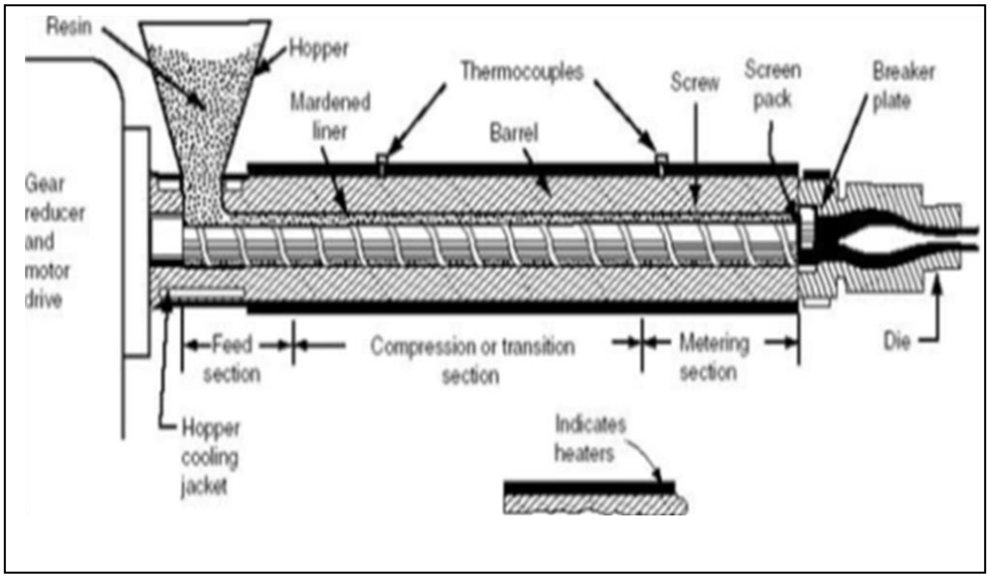

Figure. 2.1 Single Screw Extruder (Ref: Fellows, 2000)

Single screw extruders have lower capital and operating costs and require less skill to operate and maintain than twin screw machines (Fellows, 2000). Ever since extrusion involves simultaneous mixing, kneading and cooking, it causes a large number of complex changes to a food, including hydration of starches and proteins, homogenization, gelation, shearing, melting of fats, denaturation or re-orientation of proteins, plastification and expansion of the food structure. For many years the empirical knowledge of extruder operators outstripped scientific theory of the sequence and nature of these interactions and their effects. The two factors that most influence the nature of the extruded product are the rheological properties of the food and the operating conditions of the extruder. However, computer modeling of fluid flow behavior and heat transfer inside the extruder barrel has more recently led to a greater understanding of the operation of extruders (Harper, 1989).

Extrusion technology has gained in popularity due to following reasons (Fellows, 2000)

- Versatility - A very wide variety of products are possible by changing the ingredients, the operating conditions of the extruder and the shape of the dies. Many extruded foods cannot be easily produced by other methods.

- Reduced costs - Extrusion has lower processing costs and higher productivity than other cooking or forming processes. Some traditional processes, including manufacture of cornflakes and frankfurters, are more efficient and cheaper when replaced by extrusion.
- High production rates and automated production - Extruders operate continuously and have high throughputs. For example, production rates of up to 315 kg/h for snack foods, 1200 kg/h for low-density cereals and 9000 kg/h for dry expanded pet foods are possible.
- Product quality - Extrusion cooking involves high temperatures applied for a short time and limited heat treatment therefore retains many heat sensitive components.
- No process effluents - Extrusion is a low-moisture process that does not produce process effluents. This eliminates water treatment costs and does not create problems of environmental pollution.

Extrusion-cooking had no major effect on rice proteins, but decreased the solubility of amaranth proteins (mainly buffer-soluble albumins), confirming previous reports (Silva-Sanchez *et al.* 2004). Mariotti *et al*. (2011) have reported that urea-soluble proteins extracted from GF rice and maize pasta participate in the formation of disulfide-linked aggregates. Our data show that buffer-soluble proteins from amaranth may form disulphide bonds (mainly during extrusion-cooking) that maintain a protein network desirable in GF matrices.

Extrusion-cooking of flours or flour mixtures reportedly increases the optimum cooking time (OCT), and extrusion at high temperature creates in rice pasta a hydrophilic structure that absorbs high water amounts (Marti *et al*. 2010). However, the amount of water absorbed during cooking showed no significant differences among the various samples considered here, regardless of their formulation and processing conditions (not shown).

Cabrera-Chavez *et al*. (2012) conducted a study on the molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta. The high firmness of rice-only pasta is due to the elevated content of starch in rice flour, and to starch retrogradation in the extrusion-cooking process. The addition of amaranth flour increases the amount of proteins and fiber, that act synergistically in decreasing the extent of retrograded starch. Antagonistic and synergistic relationships have been reported between fiber and other food components (mainly starch and protein) and have been related to restricted water movement during the cooking of pasta products (Brennan, Kuri, & Tudorica, 2004). Thus, the decrease in firmness of fiber enriched pasta may be associated with a decrease in starch swelling and gelatinization (Brennan & Tudorica, 2008).

2.4 Physical Properties

2.4.1 Water Activity (a_w)

Linko *et al.* (1982) studied the extrusion cooking and bioconversions of cereals and reported that at typical high temperatures and pressure prevailing in HTST extrusion cooking an water activity (a_w) approximately 1 may be reached even at well below 20% moisture, explaining the high degree of cooking obtainable during extrusion cooking of cereal based materials at very low moisture levels.

Onwulata *et al*. (2006) studied on the physical properties of extruded products as affected by cheese whey and reported that evaporation of water is a main cause of product expansion. Product expansion increased directly with decrease moisture at high shear, for whey substituted products.

Pilli *et al.* (2011) studied on the starch-lipid complexes in model system and real food produced using extrusion-cooking technology i.e., (rice starch added with oleic acid) and real food (rice starch added with pistachio nut flour). Both formulas were extruded at the same processing conditions (temperature profiles, screw speed and water feed content). The formation of starch-lipid complexes in real food is strongly dependent on water feed content. In fact, at barrel temperature of 128°C, the highest melting enthalpy of real food (6.7 joule/gram) was obtained only at 21% of water feed content whereas in the model system it was obtained both at 16 and 21%. These results point out the importance to consider all components present in the extruded food in order to study biopolymers modifications that occur during processing. Industrial relevance of this was the additions of lipids alter the physical and chemical properties of starchy foods.

2.4.2 Bulk density

This was determined by taking the flour sample in a measuring cylinder filled up to a certain mark the initial volume and initial weight of the sample were recorded then the flour samples were given equal tapping and final volume was recorded, the bulk density of the sample was calculated from these data. The method adopted was slightly modified from the method of Narayana and Narasingarao (2011).

2.5 Functional properties

2.5.1 Water Absorption Capacity

Water absorption capacity was evaluated following abbey and ibeh method with minor modification.1gm of flour was dissolved in 25 ml of distilled water and vortexed thoroughly and centrifuged at 2500 g for 10 min. the residue obtained up on decanting the soluble fraction was weighed. The water absorption capacity was expressed as ml of water absorbed by 1 g of flour or in % (v/w).

2.5.2 Oil Absorption Capacity

Oil absorption capacity determined using 1 g of flour and 10 ml of refined vegetable oil the method of Beuchat was employed for the oil absorption capacity determination. The sample was then allowed to stand at room temperature for 30 min followed by centrifugation at 5000gm for 30 min the volume of the supernatant was noted in a 10 ml measuring cylinder. It was expressed as ml of oil absorbed by 1 g of flour or in % value.

2.5.3 Swelling Power

The JSF has a swelling power of 5.264. The value was comparable with that reported by Ocloo *et al* (2010) that is 4.77. Swelling power is a measure of hydration capacity, because the determination is a weight measure of swollen starch granules and their occluded water. Food eating quality is often connected with retention of water in the swollen starch granules.

2.6 Texture Analysis

Agulera *et al*. (1984) studied on the air classification and extrusion of navy bean fractions and reported that the increase in hardness coincided with an increase in expansion of the extrudates. This property which is a physical quantification of the textural properties, was not significantly different for all extrudates under concern. They also reported that a similar trend for pure corn, where increases in extrusion temperature gave more expanded and softer products.

The high shear stress and temperature seem to favor the formation of a strengthened starchy network, involving the majority of starch macromolecules (as exhibited by its low cooking loss and pasting viscosity) with a positive effect on the texture of cooked pasta in terms of high consistency parameters. A similar behavior was also found by Wang *et al*. (1999) who investigated the suitability of pea flour for pasta-making using a twin-screw extruder: pasta obtained by extrusion-cooking exhibited superior firmness, flavor, and texture after cooking, compared to pasta-products prepared from the same flour using a conventional extruder.

Anton and Luciano (2007) studied the instrumental texture evaluation of extruded snack foods. Texture evaluation of extruded snacks is a complex subject, where the combination of the techniques involves sensory, instrumental and microstructure analysis. From a practical perspective, empirical methods are suggested as alternative to fundamental techniques, especially to food scientists and food manufacturers interested in predicting consumer perception of texture.

2.7 Biochemical Analysis

2.7.1 Analysis of Total Carbohydrates, Protein and Gluten

Dias *et al*. (2008) studied the development and assessment of acceptability and nutritional properties of extruded snacks from germ-free maize flour. The product presented a calorie reduction of up to 47.5% in comparison with products available on the market. The total fat of the extruded product was found to be less than 0.1 % as that compared to the snacks found in the market.

Dias *et al*. (2009) studied the increasing concerns about the health risks of saturated and trans-fatty acid consumption that led to the development of alternative agents for this use. It shows the use of rapeseed oil as a replacement for partially hydrogenated vegetable oil in snack flavouring. Products with several different rapeseed oil contents were designed, packed, and then stored for twenty weeks at room temperature. Fatty acids compositions, reactive substances, shear strength and sensory acceptability were assessed throughout storage time. Total replacement reduced saturated fat by 72.5% in relation to market available snacks. Trans fatty acids were initially absent in these products.

Ernesto *et al*. (2009) studied the effects of thermoplastic extrusion process parameters (raw material moisture content and temperature) and the addition of functional ingredients (lycopene and soy protein) on quality characteristics for extruded corn snacks with the objective of developing an easy-to-eat functional product.

2.8 Sensory Analysis

Jing *et al*. (1991) reported on the effects of extrusion conditions on sensory properties of corn meal extrudates and found that temperature and moisture had a significant effect on the flour aroma of extrudates. Increasing the temperature and decreasing the moisture resulted in a marked increase in toasted corn taste probably related to the chemical reactions in corn meal during extrusion cooking. Screw speed has less effect on toasted corn taste. The chemical reactions responsible for toasted corn aroma were probably also responsible for toasted flavour. The denseness of extrudates affected significantly the barrel temperature during extrusion and by the interaction between temperature and screw speed. At low temperature with decreasing screw speed, increases denseness of extrudates. Thus the crispness of the extrudates were related to the denseness of the extrudates.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

This chapter deals with the methodologies used to perform the preparation of samples, blending of prepared flours, extrusion of rice, arrow root, jackfruit seed and amaranth flour blended in different proportions. The chapter also describes the standardized methods to carry out the physical, textural and sensory tests to analyze the qualities of the developed GFP.

3.1 Raw Materials

Raw materials selected for the study were Rice, Arrowroot, Jackfruit seed and Amaranth. Rice and arrow root flour were procured from local markets and the amaranth grain were obtained from the KVK, Tavanur. Jackfruit seed is collected from farms and houses in Tavanur.

3.2 Preparation of Samples for Extrusion

Raw rice flour and arrowroot flour are procured from market. Freshly harvested and dried amaranth grain obtained from KVK, Tavanur was cleaned with air in order to remove the light particles such as chaff etc. Then the amaranth seed is milled in a food processor, to obtain the flour. Jackfruit seed was cleaned with water in order to remove the surface dirt and dried in a hot air oven at 55± 5°C until the moisture content of the cut pieces were brought down to 5%. The dried pieces were milled in a hammer mill (Plate 3.1) to obtain the jackfruit seed flour (JSF). The preparation of JSF is shown in figure 3.1.

Jackfruit seed
↓
Washing
↓
Cutting
↓
Drying (60°C for 24 h, untill M.C of 5%)
↓
Removing white outer layer
↓
Milling (hammer mill)
↓
JSF

Figure 3.1 Preparation of JSF

These raw materials were blended in two different combinations such as:

i. Rice, jackfruit seed and amaranth
ii. Rice, jackfruit seed and arrowroot

Plate 3.1 Hammer mill

The treatments showing the proportions of Rice, Jackfruit seed, Arrow Root and Amaranth were shown in Table 3.1. These proportions were arrived at after preliminary trials, experiments and screening products with less than normal quality.

Table 3.1 Treatments showing proportions used for the study.

Treatments	Rice Flour (%)	Jackfruit Seed flour (%)	Arrow Root flour (%)	Amaranth flour (%)
Control	100	0	0	0
T_1	30	20	50	0
T_2	50	30	20	0
T_3	70	20	0	10
T_4	40	30	25	5

3.3 Extrusion Process

The blends were extruded in a single screw extruder (La monferrina, Model Dolly, Italy) (Plate 3.2). The screw zone of the extruder can be divided into five sections. The first section allows the flour to get uniformly mixed inside the extruder. The second cooking section consists of a flat plate through which the ingredients get heated up to the required temperature. The third section consists of a rotating screw with pressure variance in which the particles of the blend are brought together. The fourth section contains an extruder die with the required size to enable puffing of the product and the fifth section is the cutting section where the expelled extrudates were sliced to specific length.

Plate 3.2 (a) Single screw extruder **(b) Screw of extruder**

3.4 Experimental Procedure

The blends of rice; jackfruit seed; amaranth and arrowroot in different combination as shown in Table 3.1 is mixed well and salt at the rate of 1.5 g for 200 g flour is added to the blend and mixed well. The flour was introduced into the inlet of the extruder, and calculated amount of water is sprayed at definite intervals to blend until a workable consistency is obtained. Mixing process in the extruder is carried out for 45 min. When the flour blend achieves optimum moisture content then the extrusion process is to become started. In general 20% water is added per treatment until a workable consistency is obtained. The flow chart for production of GFP is shown in figure 3.2. The GFP then obtained through various treatments were then packed in polypropylene (PP) cover and stored for conducting further analysis.

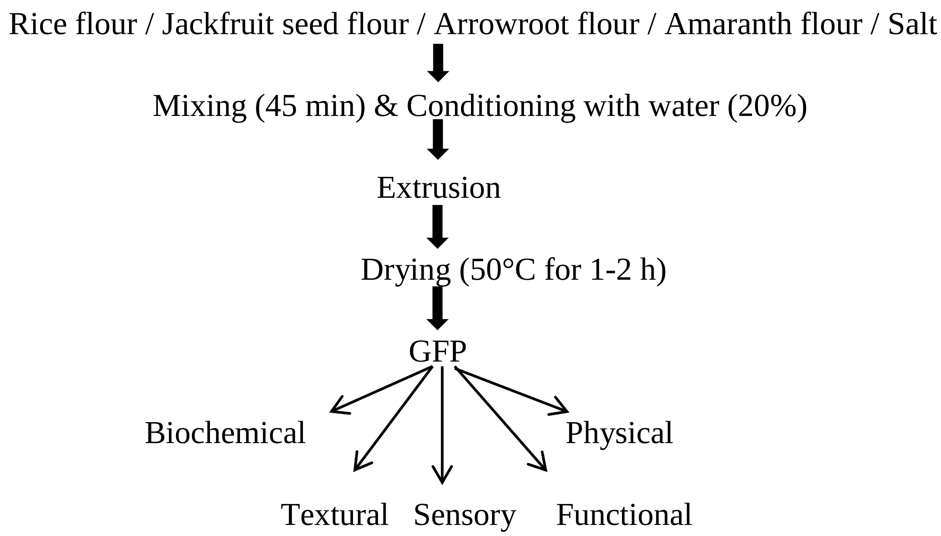

Figure 3.2: Flow Chart for Production GFP

3.5 Standardization of the Composition of GFP

The percentage composition of fractions in the developed GFP is standardized based on three parameters predominant in determining the quality characteristics. Such as textural characteristics, the main physical property i.e. bulk density and the sensory quality. All experiments were conducted in triplicates and average values are reported.

3.5.1 Bulk Density

Bulk density is the measure of expansion that has occurred as a result of extrusion. The density was obtained by taking the weight of the 2 cm samples that filled a specific volume (Jhoe *et al*., 2009) and calculated using the formula:

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

3.5.2 Texture Analysis

Instrumental analysis of texture in foods provides fast and relatively inexpensive access on product characteristics and consumer acceptance. For the experiment, all extruded samples were air dried in an oven at 60ºC for three hours or until moisture content of 6% was reached which is ideal operating condition for the texture analyzer.

Each dried sample with three replications was kept in PP cover for evaluation. The texture analyzer (TA. XT plus texture analyser, Stable micro systems Ltd.) (Plate 3.3) was operated with a 100 kg load cell and a sharp blade probe (55 mm wide, 40 mm high and 9 mm thick) to find the peak force of extrudates in terms of firmness and shearing force of extrudates that were 4-5 cm long (Jisha *et al*., 2010). A force time curve was recorded and analyzed.

Plate 3.3 Experimental set up of texture analyzer

3.5.3 Sensory Evaluation

Sensory evaluation is the scientific discipline used to evoke measures to analyse and interpret reactions to those characteristics of food as they are perceived by the senses of sight, smell, taste, touch and hearing. Sensory attribute of quality, guide the consumer in his selection of foods, also for determining the conformity of a food with established government or trade standard and food grade. As the final criterion of food quality is human evaluation, the value of objective measurements must be evaluated by their correlation with sensory measurements. A successful implementation of sensory evaluation programme requires major components like proper laboratory facilities, sensory panels and rigorous training programme (Reece, 1979). For the pasta product with different compositions the sensory evaluation was carried out by a heterogeneous population consisting of twelve people with different age group and sex. They ranked the samples according to overall flavour, taste, texture and acceptability of the prepared pasta. Cooked GFP delicacy obtained using standard ingredients in equal proportion were served to the sensory panel. The specimen score card is shown in figure 3.3.

SENSORY EVALUATION CARD

Name of the panelist: Date:

TREATMENT NO	**COLOUR**	**TASTE**	**TEXTURE**	**OVER ALL ACCEPTABILITY**

5-Like Very much

4-Like

3-Neither like nor dislike

2-Dislike

1-Dislike very much **Signature of the panelist**

Figure: 3.3 Score card for sensory evaluation

3.6 Physical Properties

After standardization of the mix based on the characteristics described in section 3.5.1, 3.5.2 and 3.5.3, the product with standardized mix was taken for further analysis for characterization. The physical property such as water activity (a_w), functional properties such as water absorption capacity, oil absorption capacity and swelling power; proximate analysis such as moisture content, crude protein, crude fibre, carbohydrate and gluten and the microbial analysis of the GFP were determined.

3.6.1 Water activity

Water activity (a_w) is a measurement of the energy status of water in a system. The concept of water activity is of particular importance in determining product quality and safety. It predicts safety and stability with respect to microbial growth, chemical and biochemical reaction rates and physical properties (Anonymous, 2004). The water activity of the developed extrudates was measured using a standard water activity meter. The powdered extrudates were placed in a prepared sample cup by making sure the cup is entirely within the chamber (Plate 3.4). Then load the sample into the instrument, the readings were noted.

Plate 3.4 water activity meter

3.7 Functional Properties

3.7.1 Water Absorption Capacity

In order to determine water absorption capacity, ground extrudates (1g) was weighed into 25 ml graduated conical centrifuge tubes and about 10 ml of water added. The suspensions were allowed to stand at room temperature (30 ± 2°C) for 1 hr. The suspension was then centrifuged at 200 x g (2000 rpm) for 30 min. The volume of water on the sediment was measured and the water absorbed expressed as per cent water absorption based on the original sample weight (Albi and Jayamuthunagai, 2014).

3.7.2 Oil Absorption Capacity

Albi Abraham and J. Jayamuthunagai (2014) determine the method of oil absorption capacity One gram sample was weighed into 25 ml graduated conical centrifuged tubes and about 10 ml of refined vegetable oil (density, 0.91 g/ml) was added. The suspension was centrifuge at 200 x g (2000 rpm) for 30 minute. The volume of oil on the sediment was measured and the oil absorbed was expressed as percent oil absorption based on the original sample weight.

3.7.3 Swelling Power

This was determined by the method suggested by Albi Abraham, and J. Jayamuthunagai, (2014) with slight modification for small samples. One gram of the sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80°C for 30 min. The mixture was continually shaken during the heating period. After heating, the suspension was centrifuged at 1000 x g for 15 min. The supernatant was decanted and the weight of the paste was noted. The swelling power was calculated using the equation:

$$\text{Swelling power (g/g)} = \frac{(w2-w3)\times 1}{w1}$$

W_1: weight of the sample

W_2: weight of the centrifuge tube with sample

W_3: weight of the centrifuge tube with swollen material

3.8 Proximate Analysis

3.8.1 Estimation of Crude Protein

Protein is the most essential nutrient present in many food crops. The major element present in the protein is nitrogen, which generally constitutes 16% of the total make up.

Determination of the nitrogen content is the easiest way to compute the crude proteins using Kjeldahl digestion tubes on Kjeldahl apparatus (Plate 3.5). The crude protein content was obtained by multiplying the total nitrogen content by a factor of 6.25 and estimated according to the procedure given by the (AOAC official methods of analysis 2008, Moorthy *et al.*, 2002).

$$\text{Protein (\%)} = \frac{(\text{Titre value} - \text{Blank volume}) \times \text{Normality of Hcl} \times 14.007 \times 6.25}{\text{Weight of the sample}} \times 100$$

Plate 3.5 Kjeldhals apparatus

3.8.2 Estimation of Crude Fibre

Crude fiber consists of cellulose, variable proportion of hemicellulose and highly variable proportion of lignin along with some minerals. Estimation is based on treating the moisture and fat free sample successively with dilute alkali. During these steps, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The reagents were: 0.255N (±0.005) H_2SO_4 - Mix 6.79 ml of H_2SO_4 in water and make up to 1 litre. (1.25%), 0.313N (±0.005) NaOH -Dissolve 12.5 g NaOH in water and made up to 1 litre (1.25%). The loss in weight gives crude fibre content.

2 g of the dried sample was ground and boiled with 200 ml of H_2SO_4 for 30 minutes. The mass was then filtered through muslin cloth and washed with boiling water until washings are no longer acidic. This was then boiled with 200 ml NaOH for 30 minutes. Filter through muslin cloth again and washed with 25 ml of boiling 1.25% H_2SO_4, 350 ml portion of water and 26 ml alcohol. Remove the residue. Transfer to ashing dish (W_1). Dry the residue for 2 hour at

130±2°C. Cool the dish in the desiccator and weigh (W_2). Ignite for 30 minutes at 600±15°C. Cool in a desiccator and reweigh (W_3).

$$\% \text{ Crude Fibre} = \frac{(w2-w1)-(w3-w1)}{\text{weight of the sample}}$$

3.8.3 Estimation of Carbohydrate

Phenol–Sulfuric Acid Method

The estimation of carbohydrate was carried out as suggested by DuBois *et al*., 1956. A 2 ml aliquot of a carbohydrate solution is mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 ml of concentrated sulfuric acid was added rapidly to the mixture. After allowing the test tubes (Plate 3.6) to stand for 10 min, they were vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development .Then, light absorption at 490 nm was recorded on a spectrophotometer. Reference solutions were prepared in identical manner as above, except that the 2 ml aliquot of carbohydrate is replaced by DDI water. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurements.

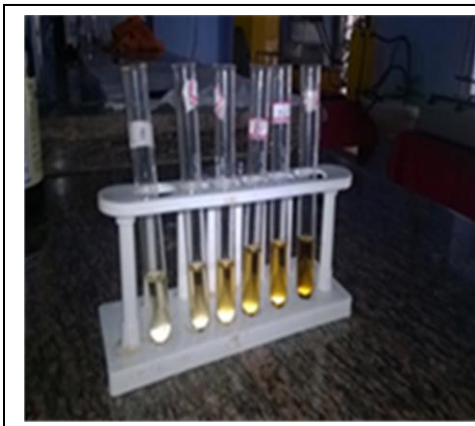

Plate 3.6: Carbohydrate Analysis

3.9 Gluten content

Gluten is made up of gliadin and glutenin. 25g of flour sample was weighed and put in a china dish. Water was added from a burette. Measure the volume of water need to make soft dough. Dough was kept in a beaker filled with water for half an hour. Thin dough with water was made by keeping on removing starch till clear water comes out. Iodine test was carried out to conform that the dough is free of starch. Extracted gluten was dried at at 130±1˚C for 2 hours and weighed.

3.10 Microbial analysis

Microbiological analysis of prepared samples included determination of total viable count and total coliform count. Ten grams of samples were homogenized with 90 ml sterile peptone water to obtain a 10^{-1} dilution. Further tenfold serial dilution was made using the same diluents till a dilution of 10^{-6} was obtained. Aliquot of (0.1ml) suitable dilution was spread plated in triplicates onto prepared, sterile and dried petridishes of suitable media for the enumeration of different organisms. The total number of viable microbes per gram of GFP was obtained by multiplying the number of colony forming units (CFU) on the plate with respective dilution factor and then was converted into logarithmic form. Plate count Agar (PCA) was used for total viable count enumeration. Experiments were conducted in triplicate. Total coliform was enumerated by Most Probable Number (MPN) method.

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

This chapter deals with the results obtained and discussion of the experiments conducted on extruded gluten free pasta from rice, jackfruit seed, arrow root and amaranth blended in different proportions.

4.1 Standardization of the Composition of GFP

Standardization methods for the production of GFP are explained in the section 3.5.1, 3.5.2 and 3.5.3. Results obtained from the procedures are described below.

4.1.1 Bulk Density (BD)

The bulk density of the extrudates determined using the methodologies described in section 3.5.1 are given in table 4.1. Bulk density is depended upon the particle size of the samples. Bulk density is a measure of heaviness of a flour mix and also the measure of expansion during extrusion process. It is important for determining packaging requirements and material handling.

Table 4.1 BD of GFP

Treatments	BD (g/cm^3)
Control	0.366
T_1	0.293
T_2	0.428
T_3	0.303
T_4	0.393

Though higher bulk density indicates high mass per unit volume, it indicates more expansion during the extrusion process which is a desirable characteristics of the extruded product. More expansion leads to a better extruded pasta product after cooking since that could give better softness and chewiness in mouth. On the other hand a very high bulk density relates to difficulties in packaging and product damage during transport. Therefore a bulk density value of 0.393 g/cm^3 for treatment T_4 can be considered optimum.

4.1.2 Texture Analysis

The textural properties of the extrudates were determined by measuring the peak force and breaking force by the methodologies described as per section 3.5.2. Textural properties are discussed in terms of firmness, shearing force. The results for these textural properties are tabulated in Table 4.2.

Table 4.2 Textural Analysis of Treatments

Treatments	Firmness (N)	Shearing force (N-sec)
Control	28492.7	3581.9
T_1	22985.9	2778.3
T_2	20535.9	3430
T_3	23280.8	4954.8
T_4	366108	3581.9

From the results of the firmness it may be revealed that the firmness of treatment T_4 is the higher i.e. 36610.8 N than other treatments. This is a desirable quality parameters of pasta whereas the highest shearing force is noticed for the treatment T_3 i.e. 4954.8 N-Sec. This indicates that very high force is required to break the product even after the product is cooked. On the other hand shearing force obtained for the treatment T_4 and control are more or less, same and moderate which is considered better in terms of quality. Therefore the treatment T_4 may be considered superior with respect to the textural quality

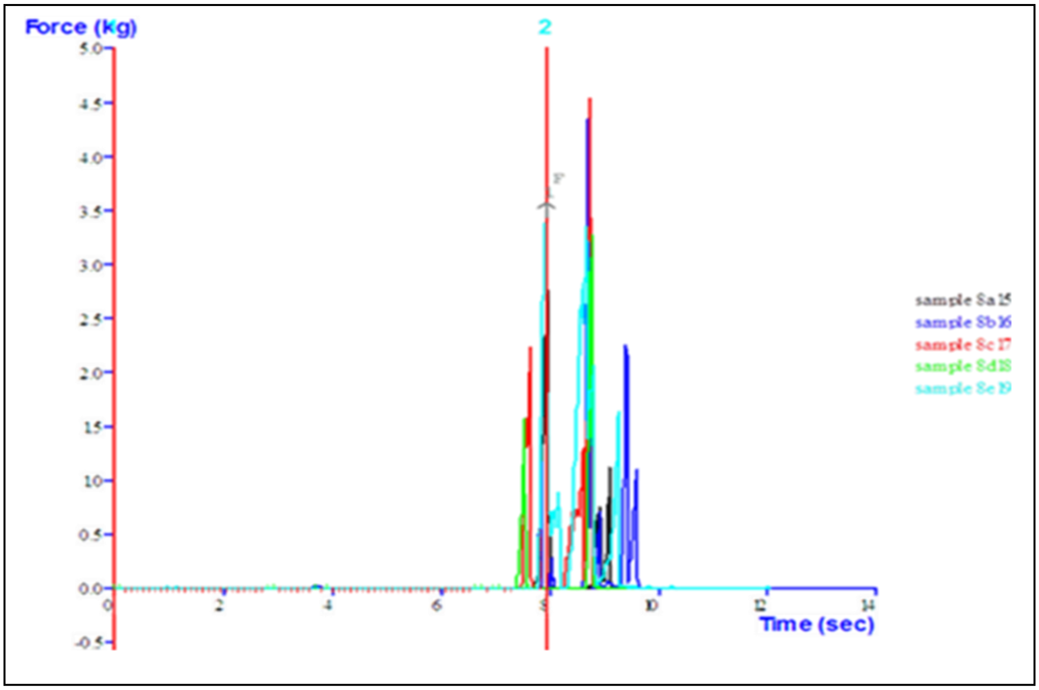

Figure.4.1 Texture Profile Analysis (TPA) of T_4

4.1.3 Sensory Evaluation

The success or failure of a new expanded food product is directly related to sensory attributes, where texture plays a major role (Iwe, 2000; Anton and Luciano, 2007). For better acceptability and sustained marketing of the optimised pasta, various sensory parameters such as appearance, colour, flavour, taste and overall acceptability were considered. The result of the sensory evaluation conducted using 12 member sensory panel is depicted in figure 4.2

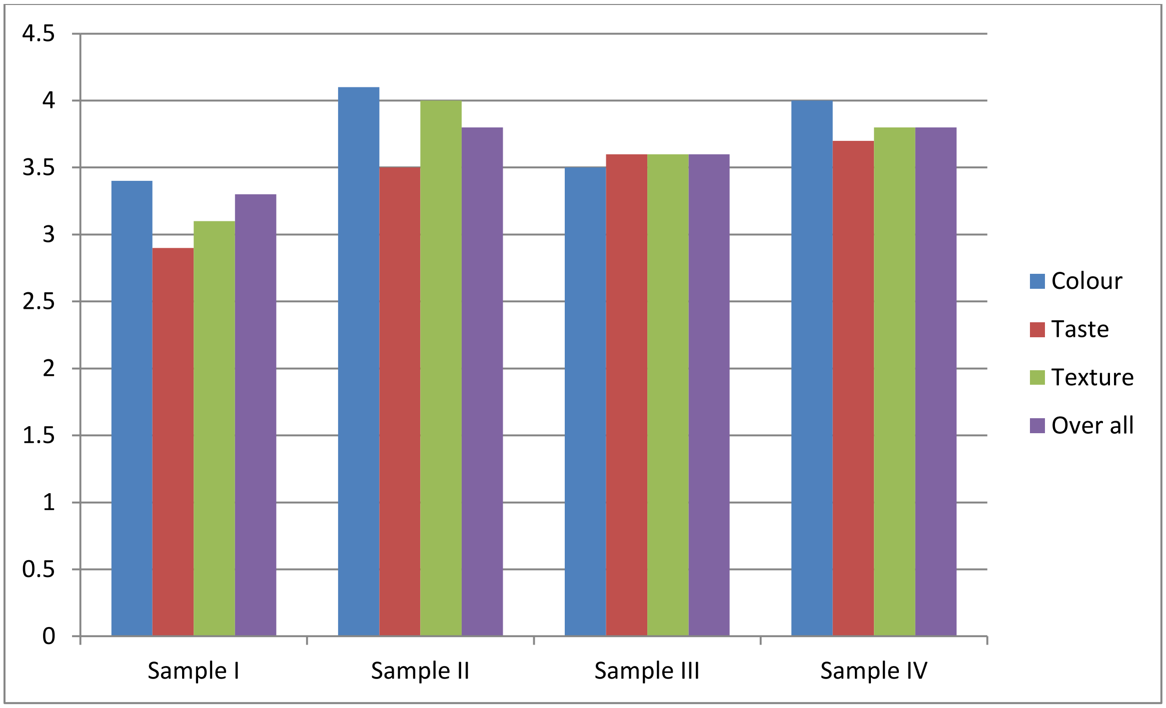

Figure 4.2 Sensory Score of Extrudates

From this graph, it may be concluded that the treatment T_4 had the maximum score for all the characters viz. appearance, flavour, taste, texture and overall acceptability. Therefore the treatment T_4 may be considered superior when compared to other treatments. The cooked GFP of treatment T_4 and control is shown in plate 4.1.

(a) Control

(b) Treatment T4

Plate 4.1 Cooked GFP Delicacy Using Control and Treatment T_4

Based on the results of studies on bulk density, textural properties and sensory evaluation the treatment T_4 with mix proportion of 40% rice, 30% jack fruit seed powder, 25% arrow root and 5% amaranth flour was found to be better when compared to other treatments and their properties is taken as standard proportion for further experiments and analysis.

4.2 Physical Properties

4.2.1 Water Activity (a_w)

The water activities of the extrudates were determined by methodologies explained in section 3.6.1. Food stability usually decreases with increase in water activity. So this property was used as a critical control point to correlate whether the products made were in a safe level. Products with low aw, signify safe for consumption (Linko *et al*., 1982). The values of water activity exhibited by the extrudates also confirmed with the necessary moisture for starch gelatinization essential for efficient enzymatic hydrolysis. Water activity was found minimum value of 0.3 for the optimized product made out of standardized mix proportions containing high amount of carbohydrate. Also it can be concluded that the GFP made out of optimized mix proportion that is (40% rice, 30% Jackfruit seed, 25% arrowroot and 5% amaranthus) has high carbohydrate content which reduces water activity and thus reduce microbial spoilage.

4.3 Functional properties

4.3.1 Water Absorption Capacity

Water absorption capacity of the sample was calculated as per the methodologies explained under section 3.7.1. Water absorption capacity was found to be 2.4 ml/g for the standardized product (T_4). Protein and carbohydrate play an important role in binding water. Protein in jackfruit seed consist of more hydrophilic subunit structure which can bind more water. Also high content of carbohydrate (presence of hydrocolloid starch) could lead to water absorption .The result obtained shows that the flour has a good ability to bind water.

4.3.2 Oil Absorption Capacity

The oil absorption capacity is 1.97 ml/g. High oil absorption suggests the hydrophobic structure in the protein subunits. This is in agreement with the findings of Ocloo *et al*. (2010).

Oil absorption is an important property in food product development because it impacts flavor and mouth feel to foods.

4.3.3 Swelling Power

The standardized GFP was found to present swelling power of 3.64 g/g. Swelling power increases with increase in temperature and this is an indication of water absorption index of granules during heating. Swelling power is a measure of hydration capacity, being a weight measure of swollen starch granules and their occluded water. Food eating quality is often connected with retention of water in the swollen starch granules.

4.4 Proximate Analysis

4.4.1 Carbohydrate

The major component of the flour was carbohydrate. The carbohydrate content of the optimized sample was found to be 45 g. The high carbohydrate content gives better water and oil absorption capacity, textural and sensory quality apart from being an energy source.

4.4.2 Protein

Generally gluten containing pasta has commonly less amount of protein. The protein content of the GFP with standardized mix was found to be 2.16 g. Increased protein content produced a less expanded and more rigid network resulting in higher resistance to shear (Chaiyakul *et al*., 2009). This study also confirms that extrusion resulted in the regularity of nitrogen reduction by facilitating high process temperatures. Low amount of protein was due to the nitrogen losses in the course of extrusion by the formation of isopeptide bonds with simultaneous emission of ammonia (Kasprzak and Rzedzicki, 2008; Jhoe *et al*., 2009). The determination of protein is highly related to the determination of nitrogen amount. The protein content is higher in all composition except in rice flour. Rice flour has only 9.4 % protein content but this deficiency is cleared with protein rich jackfruit seed flour and amaranth flour.

4.4.3 Crude Fibre

The crude fibre determination of the extrudates was done with the methodologies explained under section 3.8.2. The per cent crude fibre of the flour was found to be 2 %.

4.4.4 Gluten Content

In order to verify the presence of Gluten, its content was estimated as per the procedure explained in section 3.9. It was found that the gluten was absent in the sample. This could be due to the fact that gluten is naturally absent in jackfruit seed flour, amaranth flour, rice flour and arrowroot flour.

4.5. Microbial Analysis

The microbial test conducted as described in section 3.10 reveals that the microbial load of the standardized sample was 1x 10^2 CFU/ml.

The concluded results of the physical, functional, proximate and microbial analysis of the standardized Gluten Free Pasta with 40% rice, 30% Jackfruit seed flour, 25% arrowroot and 5 % amaranth flour are shown in Table 4.3.

Table 4.3: Properties of Standardized GFP

Sl. No	Property	Value
1	Water Activity (a_w)	0.3
2	Water Absorption Capacity	2.4 ml/g
3	Oil Absorption Capacity	1.97 ml/g
4	Swelling Power	3.64 g/g
5	Carbohydrate	45 g
6	Protein	2.16 g
7	Crude Fibre	2%
8	Gluten Content	Absent
9	Microbial Load	$1x10^2$ CFU/ml

Summary and Conclusion

SUMMARY AND CONCLUSION

As the eating patterns are changing day by day, snack foods play very important role in the diet of the modern consumer. The conventional pasta available in market contain gluten which gives the product typical textural quality. But too much exposure to gluten may create the condition for human disease such as coeliac disease which is permanent to intolerance to gluten leading to mal-absorption of several nutrients. Therefore development of Gluten Free Pasta (GFP) product mimicking all the textural characteristics of gluten based pasta is a major challenge. Therefore this study was undertaken to develop a GFP product using ingredients rich in nutrients, available at low cost and will supplement necessary properties. The major objectives of the study were to standardizing the mix composition of the GFP produced from rice, Jackfruit seed flour, arrowroot flour and amaranth flour each one of which has a definite qualitative parameters such as nutritional fortification, textural qualities etc. In short the weakness of the one ingredient is the strength of the other. The second objective is to study the physical, functional, proximate and microbial characterization of the products.

The standardization of the mix blend was carried out with three sets of proportion of rice, jackfruit seed flour, arrowroot flour and amaranth flour taken with proportions of 30%, 20%, 50% and 0%; 50%, 30%, 20% and 0%; 70%, 20%, 0% and 10% and 40%, 30%, 25% and 5%, rice: jackfruit seed: arrowroot: amaranth respectively. Product prepared with 100% rice was taken as control. The blends were prepared and extruded in laboratory model single screw extruder having 5 sections namely mixing, cooking, pressurization, puffing and cutting. The product obtained was packed and analyzed.

The standardization of the mix proportion was carried out based on the bulk density, textural characteristics and sensory evaluation. After standardization, the characterization of the product with optimum blend proportions was carried out. From the result of the study water activity, bulk density was found that the product with blend percentage of 40% rice, 30% jackfruit seed flour, 25% arrowroot and 5% amaranth (treatment T_4) shows moderate bulk density value of 0.393 g/cm^3 which may be concluded optimum. The textural analysis shows that the T_4 shows the maximum firmness value of 36610.8 N when compared to other values. Also the moderate shearing force of 3581.1 N-Sec. This indicates a better textural quality when compared to the other treatments. The sensory panel adjudged that the delicacy prepared using

GFP with its proportions as that of T_4 as best in terms of appearance, flavor, taste, texture and overall acceptability. Based on these findings, T_4 with the proportions of (rice 40%, 30% jackfruit seed flour, 25% arrowroot flour and 5% amaranth) is taken as standard mix proportion.

The standardized product was then characterized. The water activity was found to be 0.3. The functional property such as water absorption capacity, oil absorption capacity and swelling power was found to be 2.4 ml/g, 1.97 ml/g and 3.65 g/g respectively. The proximate analysis showed that the carbohydrate, protein, crude fibre and gluten content of the optimized sample was found to be 45 g, 2.16 g, 2% and 0% respectively. It was found that the product contain no gluten. Microbial load of the GFP was found to be $1x10^2$ CFU/ml. Thus within the study a GFP from locally available materials, one of which such as jackfruit seed considered as waste was developed. The product developed was nutritionally rich, texturally superior and microbiologically safe for long term storage. The mix proportion was standardized and characterization of the product was also carried out.

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Abstract

DEVELOPMENT AND QUALITY EVALUATION OF GLUTEN FREE PASTA

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ABSTRACT

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ABSTRACT

The rising demand of gluten-free (GF) products for celiac people has led to important technological research on the replacement of the gluten matrix in the production of high quality gluten-free foods. A fair proportion of the gluten free products currently on the market are nutritionally inadequate. Lifelong adherence to a GF diet remains the cornerstone treatment for the disease. However, gluten is a major component of wheat and rye flours, and its replacement in bakery products remains a significant technological challenge. Considering the above cited facts a study was under taken to develop and evaluate the qualities of gluten free pasta (GFP)

The objectives of this study was to develop and analyze a GFP made with the combination of rice, jackfruit seed, arrow root and amaranth flour in different proportion by utilizing their physical, functional, nutritional properties and biochemical properties. The standardization of the mix proportion (rice - 40 %, jackfruit seed – 30%, arrowroot – 25% and amaranth 5%) was found out based on the results obtained from bulk density (0.393 g/cm^3), textural characteristics (Firmness - 36610.8 N and Shearing force – 3581.1 N-Sec) and sensory values. After standardization, the characterization of the product with optimum blend proportions was subjected to qualitative analysis such as physical property (water activity), functional properties (water absorption capacity, oil absorption capacity and swelling power), proximate analysis (carbohydrate, protein, crude fibre and gluten content) and microbial analysis were determined. The water activity of the stadandized GFP was found to be 0.3, water absorption capacity as 2.4 ml/g, oil absorption capacity as 1.97 ml/g, swelling power as 3.65 g/g, carbohydrate content was found to be 45 g, protein as 2.16 g, crude fibre as 2% and contain no gluten (0%). Microbial load was found to be $1x10^2$ CFU/ml. The study reveal that GFP obtained could contribute to nutritionally rich, texturally superior and microbiologically safe for long term storage for coeliac disease (CD) patients as well as non-coeliac patients.