OPTIMISATION AND UTILISATION OF RESISTANT STARCH FOR VALUE ADDITION IN RICE PRODUCTS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Optimisation and utilisation of resistant starch for value addition in rice products"** is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title of any other University or Society.

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Certified that this thesis, entitled **"Optimisation and utilisation of resistant starch for value addition in rice products"** is a bonafide record of research work done independently by **Mrs. Lilia Baby** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Lilia Baby

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ABBREVIATIONS

BD	Bulk Density
NSP	Non Starch Polysaccharides
RDS	Rapidly Digestible Starch
RS	Resistant Starch
RSRF	Resistant Starch Supplemented Rice flour
RT	Room Temperature
SDS	Slowly Digestible Starch
WAI	Water Absorption Index
WSI	Water Solubility Index
cfu/g	Colony-forming units per gram
g	Gram
g/cc	Gram per cubic centimeter
kg	Kilogram
%	Per cent

Introduction

1. INTRODUCTION

"The doctor of the future will no longer treat the human frame with drugs, but rather will cure and prevent disease with nutrition" - Thomas Edison

Rice is the staple food of over half the world's population. It is an important source of starch and other dietary carbohydrates, which plays a major role in meeting the energy requirements.

Rice starch exhibits a number of unique characteristics and is a better substitute for corn starch in a number of food applications (Juliano, 1984). Major novel and unique characteristics associated with rice starch are hypoallergenicity, bland flavour, small granule (3-10 μm), white colour, greater acid resistance, greater freeze-thaw stability of pastes and a wide range of amylose/amylopectin ratio (Ashogbon and Akintayo, 2012).

Before the early 1980s, starch was assumed to be fully digestible in human intestine. Englyst *et al.* (1982) during their research on measurement of non-starch polysaccharides recognised the presence of a small fraction of starch that was resistant to hydrolysis by exhaustive enzymes like α-amylase and pullulanase *in vitro* and coined the term "resistant starch" for this fraction. This fact has led to the classification of starch into two groups: 'available' starch (digestible) and 'resistant starch' (indigestible).

Resistant starch (RS) is one of the most abundant dietary sources of non-digestible carbohydrates and has a number of physiological effects beneficial for health. Since, RS is resistant to human digestive enzymes, the non-digested starch in large intestine is fermented by colonic micro flora and produces short chain fatty acids (Slavin, 2004) especially butyrate that encourage the growth of beneficial bacteria, indicative of prebiotic functionality. In the small intestine, since RS is not digested, glucose absorption by the intestinal cells is minimal leading to reduced glycaemic response (Vonk *et al.*, 2000). This in turn will help to improve glucose regulation in diabetics and facilitates better weight control for the obese. Thus, increasing RS content in the diet has the potential to provide several significant health benefits.

Resistant starch assists in increasing dietary fibre consumption because it can be incorporated into foods without having an impact on the taste and texture of the foods. Resistant starch products satisfy better terms in most of the food processing criteria than conventional insoluble fibre. The unique characteristics of RS like fine particle size, white colour, bland flavour, low water holding capacity and high water binding capacity, high gelatinisation temperature and good extrusion qualities were identified as a valuable supplement in a wide range of functional foods (Nugent, 2005).

Resistant starch levels in foods are determined by the nature of the starch and the processing techniques applied in its production. Resistant starch occurs naturally in foods including raw potatoes and bananas, but cooking can destroy it. It can be produced by a combination of gelatinisation and retrogradation process. Careful control of the processing conditions like moisture, pH, temperature, duration of heating, repeated heating-cooling cycles were found to have direct influence on the RS content of food (Fuentes-Zaragoza *et al.*, 2010). Apart from these factors Fuentes-Zaragoza *et al.* (2011) indicated the influence of processing, pressure, freezing, autoclaving as well as storage conditions on the formation of RS.

Physical and chemical modifications are commonly used to produce starches with special properties. Although, chemically modified starches are available for industrial purposes, the food industries prefer starches that have not been chemically altered. Therefore, physically modified starch, by use of moisture, heat, shear or radiation has gained wider acceptance (Adebowale *et al.*, 2005). Hydrothermal treatments are physical modifications that change the physicochemical properties of starch, without destroying its granule structure. Heat moisture conditioning, a process which involves the heating of starch at elevated temperature in controlled amount of steam has been used for modifying the properties of native starches. It involves incubation of starch granules in varying moisture levels for a certain period at different temperatures (Kweon *et al.*, 2000).

Since, rice forms a major portion of our diet, it is becoming more of a concern for people with diabetes due to its higher percentage of digestible starch causing a relatively higher glycaemic response (Shu *et al.*, 2009). Goni and Valentin-Gamazo (2003) recommended the increased consumption of low glycaemic foods rich in resistant starch, non-starch polysaccharides and oligosaccharides for diabetic patients.

In a rapidly changing world, with altered food habits and stressful life styles, consumers are demanding nutraceutical foods that contain basic nutritional properties with additional health benefits. Since, starchy foods are main source of energy in our diet, healthier starchy foods providing beneficial functionalities are essential to sustain good health. Therefore, it is of interest to study the RS content in differently processed rice starch and also the effect of RS incorporation into various rice products. Hence, the present study entitled "Optimisation and utilisation of resistant starch for value addition in rice products" was undertaken with the following objectives.

1. To standardise processing treatments for optimising resistant starch (RS) formation in rice starch and to evaluate the changes in rice starch properties.
2. To standardise rice flour supplemented with RS and to evaluate its quality attributes during storage.
3. To study the nutritional and organoleptic qualities of RS supplemented rice products and its effect on postprandial glycaemic response.

Review of literature

2. REVIEW OF LITERATURE

"*Leave your drugs in the chemist's pot if you can cure the patient with food*" the age old quote by Hippocrates is certainly the tenet of today. With the growing interest in self-care and integrative medicine coupled with our healthy embracing baby boomer population, recognition of the link between diet and health has been stronger. As a result, the market for foods that promote health beyond providing basic nutrition is flourishing. According to Osawa (1998), the primary function of food is to provide essential nutrients, the secondary function is to satisfy sensory attributes and the tertiary function is to prevent diseases at the molecular level. Viewing such an importance linked with food, the concept of functional food has been visualised.

The literature connected to the study entitled "Optimisation and utilisation of resistant starch for value addition in rice products" is presented under the following heads.

2.1 Importance and classification of starch
2.2 Definition and types of resistant starch
2.3 Food sources of resistant starch
2.4 Factors influencing formation of resistant starch
2.5 Physiological effects of resistant starch
2.6 Use of resistant starch in food industry

2.1. Importance and classification of starch

In plants, starch occurs as granules, storing the carbohydrates in an insoluble and tightly packed manner (Imberty *et al.* 1991). Sajilata *et al.* (2006) reported that starch occurs as granules in the chloroplast of green leaves and the amyloplast of seeds, pulses, and tubers.

Chemically, starches are polysaccharides composed of α-D-glucopyranosyl units linked together with α-D-(1–4) and/or α-D-(1–6) linkages (Zobel, 1988; Haralampu, 2000 and Sharma *et al.*, 2008). According to the authors, starch occurs in plant tissues as granules with a diameter in the range of one and 100 μm. British Nutrition Foundation (BNF, 1990) reported that starch is composed of two molecular types, amylose, the straight chain polyglucan comprised of approximately 1000 α-D-(1–4) linked glucose; and amylopectin, the branched polyglucan, comprised of approximately 4000 glucose units with branches occurring as α-D-(1–6) linkages.

Starch is the most abundant storage polysaccharide in plants and is the major dietary source of carbohydrates (Bjorck *et al.*, 1994; Skrabanja *et al.*, 1999; Sajilata *et al.*, 2006; Ratnayake and Jackson, 2008).

X-ray diffraction studies identified three forms of starch, *i.e.*, type A, type B and type C (Topping and Clifton, 2001). Type A starch common in cereals has amylopectin chain lengths of 23 to 29 glucose units formed as a double helix with amylose moieties packed inside. Type B found in raw potato and green banana starch consists of amylopectin of chain lengths of 30 to 44 glucose units with water interspersed. Type C found in legumes appears to be a mixture of both A and B forms. The authors also reported a new form of starch, *i.e.*, type V, which is produced when raw starch is heated with limited amount of water in which starch becomes associated with lipids.

Berry (1986) and Sajilata *et al.* (2006) classified starches as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) on the basis of the behaviour when incubated with enzymes. Rapidly digestible starch (RDS) consists mainly of amorphous and dispersed starch. It is quickly hydrolysed in the small intestine to glucose molecules within 20 minutes. Slowly digestible starch (SDS) is hydrolysed more slowly but digestion is still complete. It is hydrolysed to glucose within 20 to 110 minutes. Resistant starch (RS) is not hydrolysed even after 120 minutes of incubation. For nutritional purposes, Englyst *et al.* (1992) classified food starches as glycaemic starch and resistant starch. Glycaemic starches are those that are hydrolysed to glucose by enzymes in the digestive tract and can be further categorised as either rapidly digestible starch (RDS) or slowly digestible starch (SDS). Resistant starch is not hydrolysed by enzymes.

2.2. Definition and types of resistant starch

Before the early 1980s, starch was assumed to be fully digestible in human intestine. Englyst *et al.* (1982) recognised the presence of a small fraction of starch that was resistant to hydrolysis by enzymes like α-amylase and pullulanase *in vitro*.

The name "resistant starch" was coined by Berry (1986) to describe the incompletely digested starch in foods that had been cooked and then cooled. Resistant starch is the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals (Sievert *et al.*, 1991; Asp, 1992; Asp and Bjorck, 1992; Englyst *et al.*, 1992; Englyst *et al.*, 1996 and Perera *et al.*, 2010).

Englyst *et al.* (1992) classified starches on the basis of their digestibility into five groups namely RDS, RS1, RS2, $RS3_a$ and $RS3_b$. Readily digestible starch (RDS) are completely digested in small intestine, RS1 is physically inaccessible starch and are partially digested in small intestine, RS2 is resistant starch granules which have little digestion, $RS3_a$ is retrograded starch and are partially digested in small intestine and $RS3_b$ is retrograded starch, not digested in small intestine.

Depending on the resistance of starch to enzymes, Haralampu (2000), Nugent (2005), Sajilata *et al.* (2006) and Lunn and Buttriss (2007) subdivided resistant fractions into four: RS1, RS2, RS3, and RS4 which are also called as type I, II, III, and IV starches respectively. Ratnayake and Jackson (2008) and Sanz *et al.* (2009) classified RS in foods as RS1, RS2, RS3 and RS4. According to the authors, RS1 is physically inaccessible starch; RS2 is found in raw starch granules; RS3 is present in retrograded starch and RS4 is the starch that is chemically modified to obtain resistance to enzymatic digestion.

According to Bird *et al.* (2000) RS1 is the starch granules that are physically inaccessible to the digestive enzymes, as these are enclosed in the intact cell walls. According to Sajilata *et al.* (2006) RS1 is heat stable in normal cooking operations. Sharma *et al.* (2008) indicated that RS2 is the raw, ungelatinised native starch molecule present in granular form. The authors also indicated that in raw starch granules, starch is tightly packed in a radial pattern and therefore relatively dehydrated which limits the accessibility of enzymes.

According to Asp and Bjorck (1992) RS3 represents the most resistant fraction in the heat-processed foods and is mainly the retrograded amylose formed during cooling of gelatinised starch. Cummings *et al.* (1996), Haralampu (2000) and Nugent (2005) indicated RS4 as the chemically modified form, which cannot be broken down due to formation of new glycosidic linkages by substitution reactions. Bird *et al.* (2000) noticed RS4 type of resistant starch as esterified, cross-bonded starch.

Mermelstein (2009) indicated a fifth type of soluble polysaccharide called "resistant maltodextrins" namely Nutriose® and Fibresol®2 derived from processed starch. Fuentes-Zaragoza *et al.* (2010) classified RS into five categories: RS1-RS5. According to the authors, RS1 is the starch that is physically inaccessible to digestion, RS2 is raw or ungelatinised starch, RS3 is retrograded starch, RS4 is chemically modified starch and RS5 is an amylose-lipid complex in starch. A description of different types of RS and their sources reported by the authors are given in Table 1.

Table 1. Types of Resistant Starch (RS)

Types of RS	Description	Source
RS1	Inaccessible starch trapped in a digestion resistant matrix	Partially milled or whole grains, legumes, seeds or pasta
RS2	Raw or ungelatinised starch with a tightly packed crystalline Structure	Raw potatoes, green bananas, and genetically modified high-amylose corn
RS3	Retrograded starch from recrystallization of amylose after cooking and cooling	Bread and/or starch products cooked and cooled
RS4	Chemically modified starch that creates novel bonds between chains by cross-linking	Added to beverages, bread, cakes and porridges
RS5	Amylose-lipid complex retrograded with free fatty acids	Bread and other starch food products

2.3. Food sources of resistant starch

Whole grains are rich sources of fermentable carbohydrates including dietary fibre, resistant starch and oligosaccharides (Slavin, 2004). Lunn and Buttriss (2007) indicated that fibre provided by the whole grain includes a resistant starch component and varying amounts of soluble and fermentable fibres.

Resistant starch is naturally found in cereal grains, seeds and in heated starch or starch containing foods (Charalampopoulos *et al.*, 2002). Tharanathan and Mahadevamma (2003) noticed RS in legumes and indicated that in legumes, starch gets partially modified into resistant starch during processing. The authors also noticed lower digestibility of legume starch when compared to cereal starch due to the high amylose content. Rochfortt and Panozzo (2007) also noticed high RS in pulses which are useful in retaining their functionality even after cooking.

Bednar *et al.* (2001) pointed out higher RS concentrations in legumes due to the relationship between starch and protein, which is more resistant to hydrolysis. Giczewska and Borowska (2003) indicated very high diversity of resistant starch content in legumes which vary from a few per cent to about 80 per cent.

Yue and Waring (1998_a) noticed 0.5 to three per cent RS in native food sources, like peas, bananas and processed cereal foods like bread, pasta and breakfast cereals.

Bednar *et al.* (2001) indicated that starch present in spaghetti was more slowly digested due to the presence of densely packed starch. Nugent (2005) and Sajilata, *et al.* (2006) reported the occurrance of RS1 in cell or tissue structures of partly milled grains, seeds, and vegetables. The authors also indicated the occurrence of RS2 in raw starch granules like potato, green banana, high amylose corn and RS3 in cooked and cooled potatoes, bread, cornflakes and food products developed with repeated moist heat treatment. Nugent (2005) reported the presence of RS4 in breads and cakes in which modified starches have been used. Sharma *et al.* (2008) also indicated the presence of RS3 in cooked and cooled potatoes and breads.

Unripe banana was found to be rich in RS (Tribess *et al.*, 2009; Fuentes-Zaragoza *et al.*, 2010). However, Rodriguez-Ambriz *et al.* (2008) pointed out that although unripe banana is rich in indigestible carbohydrates, mainly RS and dietary fibre, cooking renders the native RS digestible.

2.4. Factors influencing formation of resistant starch

Berry (1986) identified amylose content, processing temperature and water content as the three key factors which influence RS formation after heat processing. Sievert and Pomeranz (1989) indicated starch/water ratio, autoclaving temperature and number of autoclaving- cooling cycles as the factors affecting RS formation in amylomaize VII starch. Apart from the starch type, process conditions, starch gelatinisation and the presence of other components like protein also influence the amount and the quality of RS formation (Eerlingen and Delcour, 1995).

In order to obtain optimum RS yields during retrogradation, Garcia - Alonso *et al.* (1999), indicated that starch gels should be cooled prior to freezing, followed by thawing at room temperature and drying at 60°C.

According to Thompson (2000_a) physical or chemical treatments given to starch also alter the RS levels in a food or ingredient. The author indicated processing conditions as the factors affecting the resistant starch content by influencing its gelatinisation and retrogradation. Giczewska and Borowska (2003) indicated that hydrothermal processing, either increase or decrease the fraction of RS, depending on the parameters of processing and varieties.

The physical form of grains or seeds in which starch is located, size and type of starch granules, associations between starch and other dietary components and processing, especially cooking and cooling were found to be the factors that determine whether starch is resistant to digestion (Bjorck *et al.*, 1994; Slavin, 2004).

Resistant starch formation depended on amylose/amylopectin ratio, starch-protein interaction, amylose-lipid complex and rate of starch retrogradation (Englyst *et al.*, 1992; Bravo *et al.*, 1998 and Mahadevamma and Tharanathan, 2004).

Nugent (2005) indicated that the physico-chemical properties of foods can directly affect the amount of RS present. Sajilata *et al.* (2006) stated several factors influencing the formation of RS which included inherent properties of starch, granular structure, amylose:amylopectin ratio, retrogradation of amylose, amylose chain length, heat and moisture, interaction of starch with other components like protein, dietary fibre and enzyme inhibitors and thermal processing.

Sharma *et al.* (2008) indicated that the structure of starch granule, physical characteristics of food, presence of other nutrients and antinutrients, amylose/amylopectin ratio and retrogradation of amylose as the factors affecting the digestibility of starch.

Careful control of the processing conditions like the moisture content, pH, temperature, duration of heating, repeated heating-cooling cycles were found to have direct influence on the RS content of food (Fuentes-Zaragoza *et al.*, 2010). Apart from these factors, Fuentes-Zaragoza *et al.* (2011) indicated processing pressure, freezing, autoclaving as well as storage conditions as the factors affecting the formation of some RS types especially RS3.

The manufacture of resistant starch usually involves repolymerisation (Yue and Waring, 1998_b), chemical modification (Wolf *et al.*, 1999), heating and retrogradation (Schmiedel *et al.*, 2000), extrusion cooking (Gebhardt *et al.*, 2001), and partial acid hydrolysis and hydrothermal treatments (Brumovsky and Thompson, 2001). According to Augustin *et al.* (2008) physically functional RS ingredient can be made by the application of physical processes to starch suspension.

Kim *et al.* (2006_a) indicated amylose content, volume of water used for cooking and cooling after cooking as the factors influencing the extent of RS formation.

Sievert and Pomeranz (1989) opined that higher amylose content lowers the digestibility of starch due to positive correlation between amylose content and yield of RS. Brumovsky and Thompson (2001) noticed an increase in RS levels in high-amylose starch. Bednar *et al.* (2001) indicated low digestibility of amylomaize due to high content of amylose. Tharanathan and Mahadevamma

(2003) also reported high amylose content in legumes leading to a higher RS content and low digestibility. The authors also indicated lower rate of digestibility in high amylose cereal starch. According to Vaidya and Sheth (2011) the nature of starch in terms of amylose and amylopectin is an important factor for starch digestibility and in turn affect RS formation.

Ring *et al.* (1988) indicated rapid retrogradation of amylose in cooked peas and the resulting material was found to be highly resistant to amylolysis. Mikulikova *et al.* (2008) reported that compared to cereal and pseudocereal starches, legume starches had high amylose levels leading to higher RS content. Vaidya and Sheth (2011) observed the effect of amylose on RS of cereal products and found a strong association of amylose and RS in maize products and weak positive association in wheat, rice and pearl millet products.

Fredriksson *et al.* (1998) studied the physico-chemical properties of starch in wheat, rye, high-amylose and normal-amylose barley and waxy maize and found that high-amylose barley retrograded to a greater extent than the other cereal starches. Tharanathan and Mahadevamma (2003) noticed quick retrogradation in cooked legumes leading to low digestion due to significant amount of type 3 RS (RS3). Vaidya and Sheth (2011) indicated the positive effect of starch- protein interaction on RS formation and indicated more retention of RS in wheat upon steaming due to high protein in wheat compared to rice.

An increase in RS levels by heat-moisture treatment was noticed by Lorenz and Kulp (1982), Hoover and Vasanthan (1994) and Kawabata *et al.* (1994) by transformation of single-chain amylose into its double helical crystals, perfection of existing crystals and increased interaction between amylose and amylopectin.

According to Sievert and Pomeranz (1989), conditions of heat- moisture treatment during starch gealtinisation is an important factor in determining the association of amylose and yield of RS upon cooling. Han and Lim (2009) noticed significantly higher RS content in japonica brown rice prior to cooking, as the moisture content of soaked rice reached 20 per cent, compared to rice containing 30 per cent moisture. Chung *et al.* (2009_a) reported an increase in RS content in cereals and pulses after heat-moisture treatment.

Brumovsky *et al.* (2009) applied heat-moisture treatments to potato, cassava, wheat and corn starches at 80, 100 and 120°C for 40 to 60 minutes at 30 per cent moisture and revealed an increase in RS content with the highest RS in corn starch treated at 120°C for 60 minutes.

Chung *et al.* (2010) noticed higher amount of RS in annealed and heat-moisture treated pulse starches which were subsequently gelatinised when compared to ordinary gelatinised native starches. However, Juansang *et al.* (2012) noticed low RS content in heat-moisture treated canna starch when compared to native starch.

Okoye (1992) noticed the influence of heating of starch in the absence of water on RS formation. Sang and Seib (2006) observed resistant fraction which survives cooking in high amylose corn starch by heat-moisture treatment and phosphorylation.

Berry (1986) observed appreciable amounts of RS in heat-processed foods. The author reported that RS levels of wheat starch reached up to 15 per cent, from the initial value of one per cent, after five repeated cycles of autoclaving at 134°C with excess water and subsequent cooling. In single autoclaving and cooling cycle the RS content was found to be nine per cent.

Bjorck *et al.* (1987) indicated that during autoclaving of wheat starch suspension, up to eight per cent starch was rendered resistant to enzymes. Sievert and Pomeranz (1989) also noticed the influence of number of autoclaving-cooling cycles in the formation of RS in amylomaize VII starch. The authors also indicated the pronounced effect of number of cycles on RS formation and indicated an increase of more than 40 per cent RS with an increase in the number of cycles up to 20.

Mangala *et al.* (1999) reported more RS content in ragi and cereal flour after autoclaving, which increased markedly during repeated autoclaving- cooling cycles due to an increase in the net crystallinity of RS by cooling in between successive autoclaving cycles. Hickman *et al.* (2009) noticed higher RS levels in wheat and corn starch autoclaved thrice at 121°C for 15 minutes and cooled within 15minutes, when compared to wheat and corn starch cooked at 100°C for 10 minutes. Kim and Kwak (2009) noticed a linear increase in RS content of corn starch with an increase in the number of autoclaving and cooling cycles at an autoclaving temperature of 120°C. The authors also noticed more pronounced effect with number of autoclaving and cooling cycles when compared to that of temperature. Dundar and Gocmen (2013) indicated the beneficial impact of high autoclaving temperature of 145°C and long storing time on RS formation in high amylose corn starch.

Slade and Levine (1991) reported that by applying temperature cycles it is possible to modify the resistant starch content of foods during storage.

Kim *et al.* (2006_a) indicated that cooling of cooked rice after autoclaving increased RS formation by promoting retrogradation of starch polymers. Ha *et al.* (2012) indicated high RS content in retrograded rice produced by repeated heating and cooling cycles than common rice. Sha *et al.* (2012) studied the impact of temperature on RS4 in rice starch and noticed dynamic change in the RS content. Among the temperature varying from 30°C to 200°C used for the study, the authors observed greatest increase in RS4 content in samples heated to 120°C.

Pillai and Thampi (2013) observed significantly high RS content in roasted beaten rice than the uncooked beaten rice and concluded that roasting alters the amount of resistant starch in food products.

Tovar and Melito (1996) noticed 19 to 31 per cent RS on dry matter basis in isolates from steam-heated legumes when compared to raw seeds. The authors also indicated more resistant starch in conventionally and high-pressure steamed beans when compared to raw pulses and suggested retrogradation as the major mechanism to reduce digestibility of starch.

Parboiling is another method of increasing RS production (Eggum *et al.*, 1993). According to Mangala *et al.* (1999) during parboiling of paddy, more than 90 percent of rice endosperm rich in starch becomes gelatinised and a part of it reassociates as RS upon cooling. Marsono and Topping (1999) also noticed higher RS in parboiled rice than raw rice.

Low-temperature and long-time baked product contained significantly higher amounts of RS than product baked under ordinary conditions (Liljeberg *et al.*, 1996). Sajilata *et al.* (2006) also indicated higher RS in baked products. Yadav (2011) noticed high RS content in bread baked at 120°C for long time when compared to bread baked at higher temperature of 200°C and 150°C for short periods.

Chen *et al.* (2010) observed higher levels of RS in deep fried and roasted foods when compared to braised foods. Vaidya and Sheth (2011) and Yadav (2011) also noticed a decrease in RS content of cereals, cereal products, potatoes and sweet potatoes during frying.

Faraj *et al.* (2004) studied the effect of extrusion cooking in hull-less barley flours and found a decrease in RS3 content of the native flours by extrusion cooking. On the contrary, Chanvrier *et al.* (2007) observed a slight increase in resistant starch content in wheat and maize starch (from 1.5% to 2.1%) after extrusion. Vaidya and Sheth (2011) also noticed less retention of RS in extruded cereal products.

Veena *et al.* (1995) indicated high amount of RS in legumes subjected to fermentation, germination and roasting. However, the authors noticed a decreased RS content in precooked samples.

Tribess *et al.* (2009) noticed significant variation in RS content of green banana with the method of drying employed during starch processing. Chen *et al.* (2010) noticed retention of large amount of RS in roughly processed foods.

Bravo *et al.* (1998) observed increased RS in cooked legumes during storage at 4°C for 24 hours. Farhat *et al.* (2000) reported that storage temperature is one of the important factors affecting retrogradation of starch. Mitra *et al.* (2007) indicated more retrogradation at lower temperature and observed 30 per cent retrogradation at 8°C, 25 per cent at room temperature and 20 per cent at 50°C. Blazek and Copeland (2010) also indicated increased retrogradation and formation of RS during storage of starch gels at lower temperature. Yadav (2011) also noticed an increase in RS content of bread and tubers during storage.

Kavita *et al.* (1998) observed an increase in RS contents of freshly gelatinised samples of corn, wheat, ragi, rice and potato flours on low temperature storage for 24 and 48 hours. The authors also indicated an increase in RS of cooked rice, potato, bengalgram and greengram during storage.

Carreira *et al.* (2004) evaluated the influence of low temperature storage on RS formation in beans, chick-pea, pasta and corn meal and indicated significant increase in the RS content of all the foods after 30 days of storage.

In a study conducted by Frei *et al.* (2003), six indigenous Philippine rice cultivars were cooked and stored in a refrigerator for 24 hours at a temperature of 4°C. The study revealed that storing rice in the refrigerator increased the RS formation. Park *et al.* (2009) noticed a decrease in the digestibility of starch during storage of gelatinised starch at the cycled temperatures of 4°C and 30°C when compared to the conventional storage at a constant low temperature.

Faraj *et al.* (2004) noticed a slight increase in RS3 when barley flour was stored at 4°C for 24 hour before oven drying. Kim *et al.* (2006_b) also observed an increase in RS level with increase in the storage time of extruded pastry wheat flour at 4°C for 7 to 14 days.

Marsono and Topping (1993) found significantly increased RS contents in rice-cooker and microwave cooked rice when they were stored at 0-4°C, compared with freshly cooked rice. Mitsuda (1993) found more retrogradation and consequently increased RS formation in rice stored at -20°C when compared to rice stored in a refrigerator at 4°C.

Kavita *et al.* (1998) observed a decrease in RS content of bengal gram, field bean, cow pea, and green gram during germination. Bravo *et al.* (1998) also indicated a decrease in RS content in freshly cooked moth bean, horse gram and black gram during sprouting.

Resistant starch formation was studied in two fermented products, namely *idli* and *dhokla* by Kavita *et al.* (1998) and found that fermentation decreased the RS contents in both the products. Elkhalifa *et al.* (2004) reported a decrease in RS content of sorghum flour during fermentation. Sajilata *et al.* (2006) also noticed a decrease in RS content during fermentation.

2.5. Physiological effects of resistant starch

Nugent (2005) indicated that resistant starch is one of the most abundant dietary sources of non-digestible carbohydrates and has a number of physiological effects beneficial for health. Sajilata *et al.* (2006) reported that RS received much attention due to its potential health benefits and functional properties. Koksel *et al.* (2007) indicated the health benefits of heat- moisture treated starch due to their decreased digestibility. Buttriss and Stokes (2008) pointed out that the physiological properties and the potential health benefits of RS varied widely depending on differences in the source, type and dose of resistant starch consumed. Perera *et al.* (2010) reported that resistant starch improves glycaemic and insulinaemic responses and exhibits special functions in the management of metabolic disorders like diabetes and hyperlipidemia and also in the prevention of cardiovascular and colonic diseases. Alexander (2012) indicated RS as a type of dietary fibre essential for prevention and treatment of obesity and type two diabetes mellitus due to its slow release of glucose post prandially, low energy density and colonic health benefits from fermentation in the colon.

Brown *et al.* (1996) stated that RS acts as a substrate for the growth of probiotic microorganisms and therefore is called a "prebiotic". The authors also suggested RS for use in probiotic compositions to promote the growth of beneficial microorganisms such as *bifido-bacterium.* Brown *et al.* (1997) noticed proliferation of *bifidobacteria* in the intestinal tract of pigs consuming high amylose starch which contained RS and indicated its potential benefit in acting as a prebiotic in humans. Brown *et al.* (1998) noticed that RS added yoghurt maintained the viable counts of *bifidobacteria* over several weeks, which was lacking in yoghurt prepared without RS. Silvi *et al.* (1999) studied the effect of resistant starch on human gut and observed that resistant starch modifies the human gut microflora by stimulating lactic acid bacteria. Sajilata *et al.* (2006) reported that since RS almost entirely passes the small intestine, it behaves as a substrate for the growth of probiotic microorganisms.

Asp and Bjorck (1992) stated that in small intestine RS is fermented by the micro flora of the large intestine and indicated high yield of butyric acid from unabsorbed starch, which inhibits the malignant transformation of large intestinal epithelial cells. Robertson *et al.* (2000) opined the health effect of RS due to its fermentation by the colonic microorganisms. Significant changes in faecal pH, bulking as well as greater production of SCFA in the caecum of rats fed with RS preparations have been noticed by Ferguson *et al.* (2000) and Tharanathan and Mahadevamma (2003), which are associated with the decreased incidence of colon cancer.

Champ *et al.* (2003) demonstrated the role of resistant starch in the stimulation of bacteria able to produce butyric acid. Topping *et al.* (2003) reported that RS promotes large-bowel health by preventing inflammatory bowel diseases and colorectal cancer. The authors also indicated that fermentation of complex carbohydrates (RS) by the large-bowel microflora produced metabolic products, especially short-chain fatty acids which promote normal colonic function. The undigested carbohydrate (RS) that reaches the colon is fermented by the intestinal microflora to short-chain fatty acids (SCFA) (Slavin, 2004) which is related to a decreased risk of colon cancer.

Dronamraju *et al.* (2007) reported the positive effect of RS on the control and prevention of colon cancer. Liu and Xu (2008) indicated the usefulness of RS as a preventive agent in individuals who are at high risk for developing colon cancer. Sharma *et al.* (2008) also reported that fermentation of resistant starch increases short-chain fatty acids in the colon and also produce high levels of butyric acid. The authors also indicated that butyrate is one of the main energy substrates for large intestinal epithelial cells and have an inhibitory effect on the growth and proliferation of tumor cells; hence RS fractions are useful for preventing colonic cancer.

Birkett *et al.* (1996) observed that RS significantly attenuates the accumulation of potentially harmful byproducts of protein fermentation in the human colon. A study conducted by Muir *et al.* (2004) noticed greater faecal output, lower faecal pH, higher faecal concentration and daily excretion of acetate and a higher faecal ratio of butyrate to total short-chain fatty acids and lower concentrations of total phenols and ammonia by feeding diet containing wheat fibre and RS.

Study conducted in pigs by Govers *et al.* (1999) observed improved conditions in the distal colonic regions which help to decrease the incidence of tumour by the combined consumption of RS and insoluble non-starch polysaccharides. Le-Leu *et al.* (2002) also noticed a significant increase in faecal bulk, SCFA and butyrate levels and lowered faecal pH in the faeces of rats leading to protection against colon cancer by the combined consumption of RS with bran. Fuentes-Zaragoza *et al.* (2011) reported the beneficial effects of RS in preventing constipation, increasing faecal bulk, decreasing production of mutagenic compounds and lowering the colonic pH and ammonia levels.

Consumption of natural resistant starch by humans is beneficial to glycaemic response in diabetic subjects (Giacco *et al.*, 1998 and Vonk *et al.*, 2000). Increased insulin sensitivity in healthy individuals with the use of RS was noticed by Robertson *et al.* (2003). Zhang *et al.* (2007) and Johnson *et al.* (2010) indicated increased insulin sensitivity in individuals with type II diabetes and insulin resistant individuals respectively.

In the small intestine, RS is slowly absorbed resulting in decreased postprandial glucose and insulin responses (Haralampu, 2000). Ou *et al.* (2001) indicated three mechanisms of RS influencing post prandial glucose level. One is by inhibiting alpha amylase from digesting starch into glucose, second is by increasing the viscosity of chyme in the small intestine which slows the rate of glucose uptake and third is by binding glucose which prevents its diffusion into the mucosal cells. Nugent (2005) opined that RS rich foods release glucose slowly leading to a lowered insulin response. The author also indicated significant reduction in postprandial insulinaemia and a small decrease in postprandial glycaemia by the consumption of RS containing foods. Foods containing RS moderate the rate of digestion and the slow digestion of RS has implications for its use in controlled glucose release application (Sajilata *et al.*, 2006).

Raben *et al.* (1994) and Reader *et al.* (1997) noticed reduction in post-prandial glycaemia and insulinaemia due to the metabolism of RS after five to seven hours of consuming food. *In vivo* studies conducted by Higgins (2004) indicated lower glucose and insulin responses within two to eight hours of consuming foods rich in RS.

Hoebler *et al.* (1999) and Jenkins *et al.* (2002) indicated reduced glycaemic response due to lack of available digestible starch in individuals consuming foods enriched with RS. The physiological effect of RS was found to be due to lowering the content of digestible starch with the replacement of RS (Nugent, 2005).

The influence of the physico-chemical composition of starchy foods in postprandial glucose and insulin responses was indicated by Heijnen *et al.* (1995). Diet rich in RS was associated with a reduced risk of diabetes (Pawlak *et al.*, 2004; So *et al.*, 2007). On the basis of animal studies conducted by the authors it was seen that high RS consumption improved insulin sensitivity via changes in ectopic fat storage. A nutrition intervention study by Johnson *et al.* (2010) in 20 insulin resistant subjects also revealed improved insulin sensitivity by the consumption of RS.

In a study conducted by Raben *et al.* (1994) among 10 healthy adult men proved the ability of meals containing high levels of RS in lowering the postprandial concentration of blood glucose levels. In a study conducted by Behall and Howe (1995) among ideal and overweight hyperinsulinaemic and non insulinaemic adults indicated decrease in glucose and insulin responses in both normal and hyperinsulinaemic subjects when amylose starch was given. Lintas *et al.* (1995_a and1995_b) reported an improved glucose response in volunteers with type 2 diabetes following the consumption of diets rich in natural RS from durum wheat spaghetti, pearled barley or unripe bananas and a worsened glycaemic response following the consumption of ripened bananas. In a feeding trial conducted on rats, Xue *et al.* (1996) observed low blood glucose levels after giving retrograded high amylose barley containing 18 per cent RS, when compared to a diet containing starch from waxy barley.

Reader *et al.* (1997) reported a decrease in postprandial blood glucose in persons suffering from type II diabetes mellitus by the consumption of food bar containing commercial RS3 ingredient. Achour *et al.* (1997) studied the effect of a meal containing 50g of RS3 in comparison with 50g fully digestible corn starch and indicated a decrease in blood glucose during absorptive state in subjects fed with retrograded amylose meal. Animal studies conducted by Murray *et al.* (1998) also indicated a reduction in the postprandial area under the curve for glucose and insulin by the intake of commercial RS3. Hoebler *et al.* (1999) in a study conducted among eight healthy subjects indicated that breakfast meals based on bread prepared by substituting high amylose maize starch for a part of wheat flour had a low glycaemic index compared to bread rich in amylose and spaghetti. In subjects fed with bread containing increased levels of RS2, Behall and Hallfrisch (2002) noticed low blood glucose responses. The authors suggested that more than 50 per cent amylose is needed for a significant change in postprandial glucose.

Reader *et al.* (2002) studied the effect of RS bar, traditional bar and candy bar on insulin and glucose response of type II diabetes mellitus subjects and indicated 50 per cent decrease in the glucose area among subjects who consumed RS bar.

Robertson *et al.* (2003) examined the effect of RS consumption on insulin sensitivity and indicated that high RS diet had a significant effect in reducing blood glucose levels. Robertson *et al.* (2005) also noticed increased insulin sensitivity due to high RS supplementation. The authors opined that high doses of RS2 and long term consumption are essential to enhance insulin sensitivity.

Behall and Scholfield (2005) noticed low glucose response in subjects fed with high amylose chips and muffins. The authors also noticed higher glucose and insulin response in hyperinsulinaemic subjects compared to normal individuals. Behall *et al.* (2006) studied the effect of muffins containing low, medium and high RS on post-prandial glucose response and indicated that high RS treatment decreased glucose area under the curve compared to low RS treatment.

Mitra *et al.* (2007) also indicated a decrease in fasting blood glucose in type II diabetes mellitus subjects by giving 150g of rice containing 8-10 per cent RS for 12 weeks. In a study conducted by Kendall *et al.* (2010), a declining trend in glucose in subjects consuming cereal bars and beverage containing varying levels of RS3 was observed. The authors also indicated that higher doses of RS3 are needed to produce significant decline in postprandial glucose. Johnson *et al.* (2010) noticed an improvement in insulin sensitivity by giving 40g of 60 per cent RS2 for 12 weeks in Type 2 diabetic subjects.

Hasjim *et al.* (2010) noticed reduced glucose and insulin area under the curve in adults when meal consisting of RS bread was given compared to white bread. Li *et al.* (2010) compared the postprandial glucose response in healthy subjects by giving RS2 rice, white rice and glucose and indicated the significant effect of RS2 rice and white rice in lowering glucose when compared to glucose load.

Alexander (2012) studied the effect of RS derived from corn by giving corn porridges containing 3.1 per cent, 8.4 per cent and 28.9 per cent RS to overweight and obese subjects and indicated that RS substitution improved acute and peak postprandial glucose response and observed significantly lower mean plasma glucose in subjects who consumed 28.9 per cent RS. Thus, the author noticed an improvement in acute and peak postprandial glucose response by RS supplementation. Kwak *et al.* (2012) noticed significant decrease in postprandial glucose concentrations in diabetic and pre-diabetic subjects by consuming a dose of six gram RS daily for four weeks.

Kimura (2013) analysed the effects of resistant starch on postprandial glycaemic response in obese animals and indicated the usefulness of RS in controlling glucose concentrations. Chiu and Stewart (2013) studied the effect of high and low RS rice on glycaemic response among healthy adults and the effect of RS was found to be evident with long term intake.

Behall *et al.* (1989) did not notice any significant response among healthy subjects who consumed a diet containing 70 per cent high amylose and 70 per cent high amylopectin separately for five weeks. Kim *et al.* (2003) also did not observe any improvement in blood glucose or insulin concentrations in streptozotocin-induced diabetic rats when RS rich diet was fed.

Nugent (2005) pointed out that there is a lack of consensus regarding the precise effects of RS on insulin and glucose responses. According to the author though various studies have reported an improvement in these measures following the consumption of RS rich test-meal, few studies did not show any effect or the effect was found to be physiologically irrelevant.

Yamada *et al.* (2005) reported that a single ingestion of bread containing 6 g RS significantly inhibited postprandial glucose and insulin responses in subjects with fasting blood glucose level of above 110 mg/dL. However, the authors did not notice such effects among subjects with fasting blood glucose level below 110 mg/dl. Shimada *et al.* (2008) indicated reduced levels of glucose-dependent insulinotropic polypeptide m-RNA in normal and type 2 dibetes rats fed with RS. A clinical trial conducted by Bodinham *et al.* (2010) also did not notice significant effect on the appetite and postprandial glycaemic response in healthy adults by supplementation 48g of RS.

Mathe *et al.* (1993) indicated the beneficial effect of RS in lowering plasma cholesterol levels in genetically obese and lean rats. Younes *et al.* (1995) also indicated the effectiveness of RS in lowering plasma cholesterol and triglyceride levels when compared to drugs.

Animal experiments conducted by Han *et al.* (2003) indicated the effect of RS from bean starch in reducing serum cholesterol. Kim *et al.* (2003) studied the effect of RS from corn or rice in reducing cholesterol levels in diabetic rats and indicated its significant effect in lowering plasma total cholesterol. Martinez-Flores *et al.* (2004) also reported the effect of diets containing cassava starch blended with RS or oat fibre in lowering total cholesterol levels in the serum and liver when compared to the diet of cassava starch without added fibre. Nugent (2005) reported the effect of RS in lowering lipid metabolism and noticed a decrease in total lipids, total cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), triglycerides and triglyceride-rich lipoproteins. Hypocholesterolemic effect of RS was demonstrated by Sajilata *et al.* (2006) in experiments conducted using RS diet containing 25 per cent potato. Mitra *et al.* (2007) indicated a decrease in total cholesterol and LDL cholesterol when 150g of RS3 rice containing 8-10 per cent RS was given daily to DM2 subjects for 12 weeks. Ha *et al.* (2012) reported the effect of the retrograded rice in lowering plasma cholesterol, liver cholesterol and triacylglycerol contents in adipose tissue when compared to those in the common rice group.

Higgins *et al.* (2004) noticed increased mobilisation and use of fat stores by consuming a diet rich in RS. The authors also indicated the significant effect of RS in increasing postprandial lipid oxidation and thus reducing the fat accumulation by replacing total dietary carbohydrate with RS.

Nugent (2005) indicated that RS-rich foods lead to a muted generation of hunger signals and reported the role of RS rich foods in the treatment of obesity and weight management. The effect of retrograded rice powder which had higher RS levels in lowering body weight gain was indicated by Ha *et al.* (2012). Kimura (2013) also reported the beneficial effect of RS for dietetic treatment of obesity.

Use of resistant starch in the diet as a bioactive functional food component to increase gut hormones and thus reducing energy intake was indicated by Keenan *et al.* (2006). Resistant starch consumption to reduce adiposity and weight gain in obesity-prone and obesity-resistant rats, due to a reduction in energy intake and changes in gut hormones was indicated by Belobrajdic *et al.* (2012).

In a study conducted by Raben *et al.* (1994) among healthy adults of ideal body weight indicated that fully digestible starch supplementation increased satiation upto six hours postprandial compared to RS supplementation. Willis *et al.* (2009) noticed fullness even after three hours of feeding muffin containing RS. Bodinham *et al.* (2010) noticed low food intake after RS supplementation in healthy adults over the entire 24 hour period. Anderson *et al.* (2010) indicated a decrease in post meal intake after RS supplementation in tomato soup. Amount of RS in soup treatment had correlated with reduced food intake at 120 minutes.

Kendall *et al.* (2010) observed opposite results in appetite ratings of subjects fed with 25g RS in cereal bar and found a decreasing trend in average satiety during the entire two hour post meal time period.

2.6. Use of resistant starch in food industry

Resistant starch has a great interest to product developers and nutritionists due to its potential physiological benefits and the unique functional properties (Yue and Waring, 1998_b and Baixauli *et al.*, 2008). Niba (2002) also indicated the physiological benefits of resistant starch and its potential in functional-food product development. Fuentes-Zaragoza *et al.* (2010) reported the importance of RS in improving the physical functionality, processing stability and nutritional functionality of the food.

Baghurst *et al.* (1996) indicated the importance of RS as a functional ingredient due to its low water-holding capacity. Fausto *et al.* (1997) indicated the usefulness of RS in various food products due to its desirable physiochemical properties like swelling, viscosity, gel formation and water-binding capacity. According to Yue and Waring (1998_b), the low water-holding capacity of RS make it a functional ingredient in the preparation of moisture-free food products and in improving the texture of the final product. The unique characteristics of RS, like fine particle size, white colour, bland flavour, low water holding capacity and high water binding capacity, high gelatinisation temperature and good extrusion qualities were identified as a valuable supplement in a wide range of functional foods (Nugent, 2005). Nobakhti *et al.* (2009) indicated that low water-holding capacity and high water binding capacity of RS, leads to a decrease in water activity and were found to be useful in increasing the shelf life of products.

The unique potential of RS as an ingredient to improve oral tactile perception, taste, palatability, colour and texture was indicated by Brown (2004). Buttriss and Stokes (2008) indicated the usefulness of RS in retaining the texture and taste of the product. The authors also indicated improvement in sensory properties in RS incorporated food products and their better acceptability when compared to traditional fibres, like brans and gums. Sharma *et al.* (2008) indicated the usefulness of RS as a nutritional ingredient in the development of foods which are appetizing and consumer friendly. Baixauli *et al.* (2008_a) studied the instrumental textural characteristics of muffins with added resistant starch and noticed a softer texture with elastic and cohesive nature, reflecting a more tender structure. The authors also studied the effect of progressively replacing wheat flour with resistant starch (RS) on muffins and found that volume, height, number and area of gas cells in muffins decreased significantly when the RS was incorporated at 15 per cent and above.

Tharanathan and Mahadevamma (2003) also indicated that resistant starch is useful in fortifying fibre apart from imparting special characteristics not otherwise attainable in high-fibre foods. In a study conducted by Sozer *et al.* (2007) it was seen that RS incorporated spaghetti was less sticky with minimum cooking loss when compared to spaghetti enriched with bran. Gelencser *et al.* (2008) indicated the usefulness of RS in food industry to enhance the dietary fibre content in cereal based products such as pasta or bread. Seremesic *et al.* (2013) studied the influence of RS3 and RS4 on the rheological and textural properties of cookie dough and indicated that increased concentrations of RS in formulation not only produced softer doughs but also increased fibre content.

Baghurst *et al.* (1996) indicated the usefulness of RS to reduce the negative effect of incorporating dietary fibre like dark color, reduced loaf volume, poor mouth feel and masking of flavour in various products. Yue and Waring (1998_a) and Sajilata *et al.* (2006) also indicated the usefulness of RS in minimising the negative effect of adding fibre to cereal formulations.

Po *et al.* (1994) and Myung- Hee-Kim *et al.* (2001) developed excellent quality sponge cakes by replacing 30 per cent of flour with four cycled autoclaved-cooled RS3 corn starch, cross- linked maize starch (RS4) and annealed and cross-linked RS4 maize starch (ARS4). The authors also developed yellow layer cake by replacing flour with four cycled autoclaved -cooled starch at 12.5 per cent level.

Sajilata *et al.* (2006) incorporated RS in batter of cakes, muffins and brownies and observed a favourable tenderness to the crumb after baking. The authors also indicated better expansion, mouth feel, colour, and flavour in baked products after the incorporation of RS. According to Sharma *et al.* (2008) incorporation of resistant starch in baked products, pasta products and beverages imparts improved textural properties due to its low water holding capacity, small particle size and bland flavour. In a study conducted by Korus *et al.* (2009) which focused on partial replacement of corn starch in gluten-free breads with tapioca and corn resistant starch it was seen that RS incorporated bread had soft crumb with increased total dietary fibre. Study conducted by Sankhon *et al.* (2013) reported that replacement of wheat flour with Parkia flour (African locust bean) at

5 to 15 per cent level in bread manufacture resulted in high loaf volume with good overall acceptability due to its RS content.

Pereira (2007), Homayouni *et al.* (2008) and Nobakhti *et al.* (2009) indicated the usefulness of different types of RS in low-moisture products like pasta, bread, biscuit, cake, muffins and breakfast cereals. The authors also explained the use of RS in moderate moisture products such as ice cream, milk desserts, cheese and even in high moisture ones like yoghurt and fermented yoghurt drink.

Addition of RS was found to be useful in cheese preparation without affecting the textural properties (Noronha *et al.*, 2007). The authors also indicated the possibilities of reducing the fat content of cheese by replacing with commercially available RS.

Sajilata *et al.* (2006) indicated the usefulness of RS as an ingredient to improve crispness in foods where high heat is applied during processing. In a study conducted by the authors, greater crispness was noticed in waffle where RS was incorporated when compared to traditional fibre incorporated waffle. Mahadevamma and Tharanathan (2007) also observed improved eating qualities especially crispness in RS incorporated products. Fuentes-Zaragoza *et al.* (2010) indicated improved crispness and expansion along with better mouth feel, colour and flavour in RS incorporated products when compared to traditional insoluble fibres.

Mahadevamma and Tharanathan (2007) reported the effectiveness of RS to reduce the oil absorption in fried products. Sanz *et al.* (2008) indicated the usefulness of type 3 resistant starch (RS3) as a barrier against fat absorption and in preventing moisture loss during frying in battered food. Sanz *et al.* (2010) also indicated the cooking stability of type 3 RS in fried battered products.

Yue and Waring (1998_a) developed dried pasta products containing up to 15 per cent resistant starch with little or no effect on dough rheology during extrusion. Tharanathan and Mahadevamma (2003) also indicated that flour can be replaced with RS on a 1-for -1 basis without significantly affecting dough handling or rheology. The quantity of RS used to replace flour depended on the starch being used, the application, the desired level, and the desired structure and function required in the product (Brown, 2004). The author indicated that in breads and rolls, which generally have a bland flavor, the maximum flour replacement is typically 10 to 20 per cent without noticeably changing the texture. For chemically leavened products, higher flour replacement levels are desired and in sweet products flour replacement levels can be as high as 75 per cent.

Sajilata and Singhal (2005) indicated the use of cross-linked starches based on maize, tapioca and potato of RS4 type in formulations needing pulpy texture, smoothness and flowability. Homayouni *et al.* (2014) indicated the usefulness of RS even in microencapsulation of probiotics.

Resistant starch was found to be useful in lowering the calorie content of foods when it was used to replace flour or other rapidly digested carbohydrates (Behall and Howe (1996), Aust *et al.* (2001) and Leszczynski (2004). Rochfortt and Panozzo (2007) also indicated the lower calorific value of RS incorporated products when compared to fully digestible starch. According to the authors, the RS could be incorporated into a wide range of mainstream food products without affecting the processing properties or the overall appearance and taste of the product.

Resistant starch was used to provide fibre in some commercially available low-carbohydrate foods and was found to be useful for those following low-carbohydrate dieting regimens (Nugent, 2005). Seremesic *et al.* (2013) opined that the application of resistant starch in cookie formulations improved nutritional quality by increasing the fibre content and reducing the energy value.

Eerlingen *et al.* (1994) and Yue and Waring (1998_b) indicated that the addition of RS increased the overall quality and organoleptic properties of the baked and extruded products. According to Leszczynski (2004) resistant starch incorporated food preparations reduced the availability of some saccharides in foods, without any negative impact on the organoleptic properties of food products.

A comparative study conducted by Aravind *et al.* (2013), it was seen that pasta enriched with commercial resistant starches was useful in lowering the starch digestibility when compared to pasta made with 100 per cent durum wheat.

Materials and Methods

3. MATERIALS AND METHODS

The methods followed and the materials used in the study entitled "Optimisation and utilisation of resistant starch for value addition in rice products" are given under the following heads.

3.1. Collection of rice and preparation of rice flour

3.2. Isolation of starch

3.3. Processing of isolated starch

3.4. Estimation of resistant starch (RS) and selection of treatments for further processing

3.5. Estimation of chemical constituents of selected processed starch

3.5.1. Moisture

3.5.2. Protein

3.5.3. Total carbohydrate

3.5.4. Amylose

3.5.5. Amylopectin

3.5.6. Non starch polysaccharides

3.5.7. *In vitro* starch digestibility

3.6. Selection of rice starch with maximum RS and standardisation of resistant starch supplemented rice flour (RSRF)

3.6.1. Selection of rice starch

3.6.2. Preparation of rice flour

3.6.3. Standardisation of resistant starch supplemented rice flour (RSRF)

3.7. Evaluation of physical qualities of RSRF

3.7.1. Bulk density

3.7.2. Water absorption index (WAI)

3.7.3. Water solubility index (WSI)

3.8. Preparation of rice products with RSRF and evaluation of organoleptic qualities of products

3.8.1. Preparation of *puttu*

3.8.2. Preparation of *idiappam*

3.8.3. Organoleptic qualities of products

3.8.3.1. Selection of judges

3.8.3.2. Preparation of score card

3.8.3.3. Selection of most acceptable products

3.9. *In vitro* starch digestibility of rice products prepared with RSRF

3.10. Shelf life of selected RSRF

3.10.1. Physical qualities

3.10.2. Proximate composition

3.10.3. Microbial enumeration

3.10.4. Insect infestation

3.10.5. Organoleptic qualities of rice products

3.10.6. *In vitro* starch digestibility of rice products

3.11. Postprandial glycaemic response of selected RSRF products

3.12. Cost of the developed RSRF

3.13. Statistical analysis

3.1. Collection of rice and preparation of rice flour

Rice variety 'Uma' was procured from Regional Agricultural Research Station (RARS), Pattambi, Kerala Agricultural University. Out of the 200kg of rice procured, five kilogram of rice was parboiled by conventional method using hot soaking process suggested by Srilakshmi (2004). Rice was steeped in hot water for twelve hours and half the amount of steeped water was drained off. Soaked rice was then steamed in the remaining water for thirty minutes and dried in the sun for three hours. Rice (195 kg) was kept as such as raw rice for further studies. Both raw and parboiled rice samples were milled and the polished rice was powdered into coarse flour. The flour was stored at room temperature until use.

3.2. Isolation of starch

Starch was isolated from both raw as well as parboiled rice flour by alkali steeping method suggested by Yanez and Walker (1986). The flour was mixed with five volumes of 0.25 per cent sodium hydroxide (NaOH) and kept for twenty four hours in order to remove the proteins. The supernatant was discarded and tested for protein. The process was repeated until the supernatant became clear and gave a negative reaction to the biuret test for protein. The residue left behind was washed repeatedly with water till the alkali was completely removed and the status was tested with a pH paper. The water was drained and the slurry was ground in a mixer grinder for five minutes and strained through a muslin cloth. The residue left behind was allowed to settle. Supernatant was discarded and the residue was dried at 60°C in a hot air oven for twelve hours. The dried material was ground and sieved through a 60 mesh sieve to get a fine powder of starch and stored in air tight container (Plate 1).

3.3. Processing of isolated starch

Isolated starch was processed by autoclaving at 121°C as also at 141°C separately over the three experimental periods of 20, 40 and 60 minutes. While autoclaving, water was added at varying levels. The details of the temperature and time of autoclaving and the per cent of moisture added to rice starch are given in Table 2. Altogether, 50 treatments were tried in this experiment.

Raw rice starch and parboiled rice starch were labelled as T_1 and T_2. The rice starches (T_3 to T_{26}) after autoclaving at 121°C and 141°C for 20, 40 and 60 minutes at four moisture levels were cooled to room temperature and dried in a hot air oven at 60°C till the moisture content was reduced below 12 per cent. All the above processes were repeated with a cooling regime of -20°C overnight instead of room temperature (T_{27} to T_{50}). Two replications were maintained in this experiment.

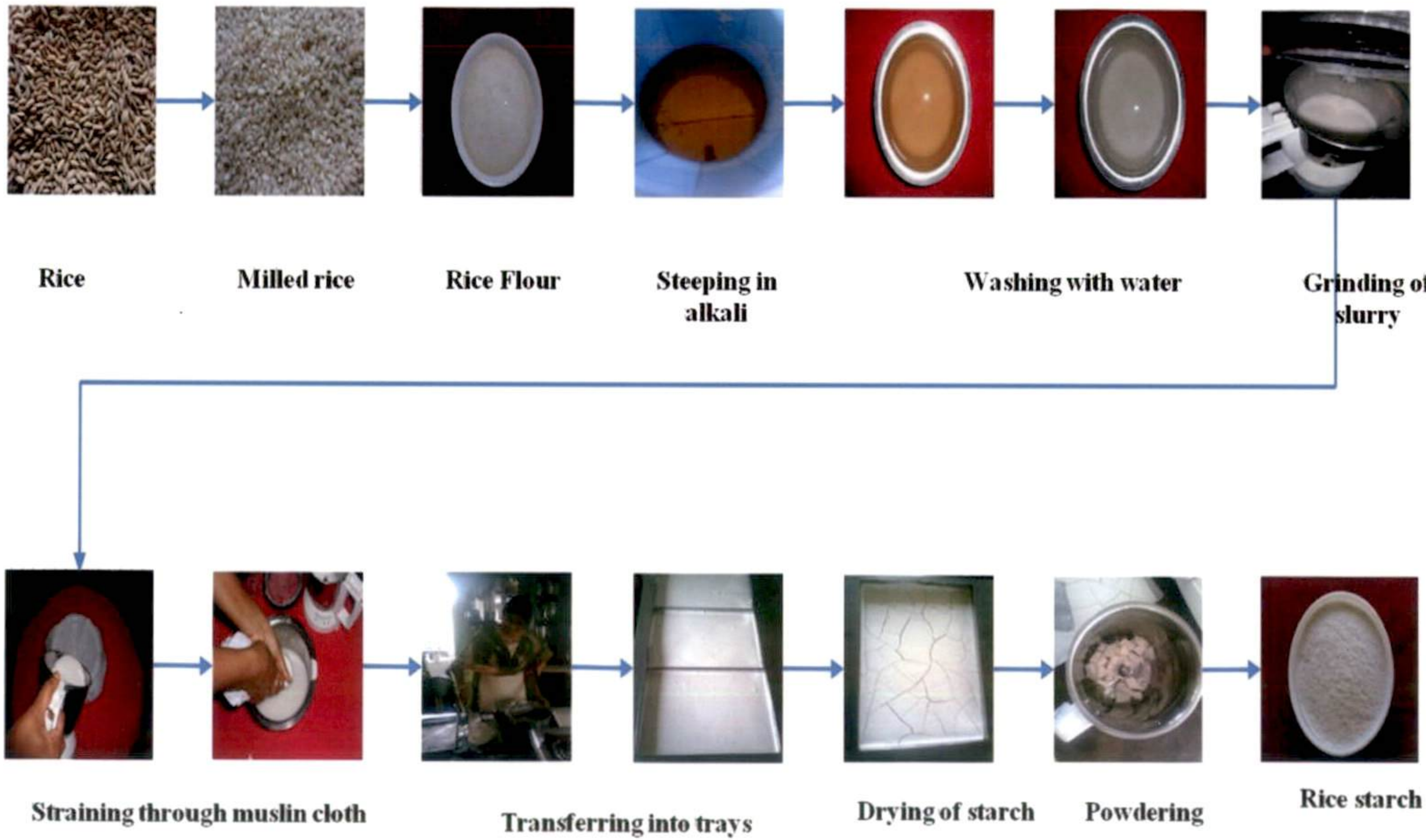


Plate 1. Isolation of starch

Table 2. Treatments for the processing of isolated starch

Treatment	Isolated starch	Autoclaving temperature	Time (Minutes)	Moisture (%)
T_1	Raw rice starch	-	-	-
T_2	Parboiled rice starch	-	-	-
T_3	Raw rice starch	121°C	20	0
T_4	Raw rice starch	121°C	40	0
T_5	Raw rice starch	121°C	60	0
T_6	Raw rice starch	141°C	20	0
T_7	Raw rice starch	141°C	40	0
T_8	Raw rice starch	141°C	60	0
T_9	Raw rice starch	121°C	20	10
T_{10}	Raw rice starch	121°C	40	10
T_{11}	Raw rice starch	121°C	60	10
T_{12}	Raw rice starch	141°C	20	10
T_{13}	Raw rice starch	141°C	40	10
T_{14}	Raw rice starch	141°C	60	10
T_{15}	Raw rice starch	121°C	20	20
T_{16}	Raw rice starch	121°C	40	20
T_{17}	Raw rice starch	121°C	60	20
T_{18}	Raw rice starch	141°C	20	20
T_{19}	Raw rice starch	141°C	40	20
T_{20}	Raw rice starch	141°C	60	20
T_{21}	Raw rice starch	121°C	20	40
T_{22}	Raw rice starch	121°C	40	40
T_{23}	Raw rice starch	121°C	60	40
T_{24}	Raw rice starch	141°C	20	40
T_{25}	Raw rice starch	141°C	40	40
T_{26}	Raw rice starch	141°C	60	40

Treatments T_3 to T_{26} after processing were cooled at room temperature

Treatments T_3 to T_{26} after processing were cooled at -20°C and labelled as T_{27} to T_{50}

3.4. Estimation of resistant starch (RS) and selection of treatments for further processing

3.4.1. Estimation of resistant starch (RS)

The RS content of all the starch samples (T_1 to T_{50}) were determined by the method suggested by Englyst *et al.* (1996_a) and Mc Cleary and Monaghan (2002) with slight modifications made by CTCRI, Trivandrum. For this, the total starch, Slowly Digestible Starch (SDS) and Rapidly Digestible Starch (RDS) content of the samples were determined.

Total starch was determined by the titrimetric method suggested by Moorthy and Padmaja (2002). One gram of sample was taken and 20 ml of 80 per cent ethanol was added and kept overnight and filtered through Whatman No.1 filter paper. Residue was transferred back into the conical flask using 20 ml 2N HCl and was then hydrolysed by leaving the flask on a hot plate at $100^{\circ}C$ for 30 minutes. The hydrolysates were cooled to room temperature and made up to 100 ml using distilled water. Pipetted out 10ml of one per cent potassium ferricyanide and five ml of 2.5N NaOH into Erlenmeyer flasks. The contents were mixed thoroughly and boiled. After boiling, the flame was lowered and one drop of methylene blue was added and titrated against the starch hydrolysate taken in a burette. End point was indicated by the rapid disappearance of the violet colour and appearance of golden yellow colour.

To determine the RDS and SDS, five grams of sample was mixed with 10 ml of HCl-KCl buffer (pH 1.5) and equilibrated at $37^{0}C$ for 10 minutes in a shaking water bath. Pepsin (SIGMA, USA) (0.4 ml) prepared by dissolving one gram pepsin in 10 ml HCl-KCl buffer solution was added to initiate proteolysis. Sample was incubated at $37^{\circ}C$ for one hour. Then, 40 ml of 0.2M sodium phosphate buffer (pH 6.9) containing 0.3M sodium chloride was added. After equilibrating for 10 minutes at $37^{\circ}C$, one ml of Panzynorm N (one tablet dissolved in 5.0 ml 0.2M sodium phosphate buffer of pH 6.9) was added and incubation was continued up to 120 minutes at 37°C in a shaking water bath. One ml of the supernatant was withdrawn from it at 20 minutes and at 120 minutes and was heated in a water bath separately at 100°C for five minutes in order to inactivate the enzymes. After cooling to room temperature, three ml of 0.2M sodium- acetate buffer (pH 4.8) was added to each sample and incubated at 60°C for 10 minutes. Dextrozyme GA (0.25 ml) (M/s Novo Industries, Denmark) was added and incubated at 60°C for 30 minutes. From this, 0.5 ml was taken and made upto 10 ml using distilled water. The made up solution (0.03 ml) was mixed with 2.97 ml glucose oxidase (EC 1.1.3.4) - peroxidase (EC 1.11.1.7) enzyme (M/s Beacon Diagnostics Pvt. Ltd. Gujarat, India). After 20 minutes, optical density was measured at 500 nm and glucose content was computed. From this, starch was determined by multiplying with a factor of 0.9. Similarly, five gram of the sample was subjected to the same procedure without adding pepsin, panzynorm and dextrozyme (control). Blank was prepared using 0.03 ml distilled water and 2.97 ml glucose oxidase (EC 1.1.3.4) - peroxidase (EC 1.11.1.7) enzyme. Starch computed at 20 minutes [Glucose expressed as (g/100g starch) x 0.9] was taken as the Rapidly Digestible Starch (RDS) and that measured at 120 minutes was taken as RDS + SDS (Slowly Digestible Starch). SDS was calculated using the equation: [Glucose value at (120 minutes - 20 minutes) x 0.9]. The starch remaining undigested after 120 minutes was taken as resistant starch (RS).

3.4.2. Selection of treatments for further processing

After determining the RS content of all the treatments (T_1 to T_{50}), five treatments which gave maximum RS content was selected for further processing. The selected starches were subjected to autoclaving and cooling cycles for two, three and four times to find out the effect of repeated autoclaving and cooling on RS formation in rice starch. Raw rice starch without any processing was taken as the control.

3.5. Estimation of chemical constituents of selected processed starch

Chemical constituents of selected five starches and control sample (raw rice starch) were estimated using standard procedures. Analysis was carried out in triplicate samples for the following constituents.

3.5.1. Moisture

Moisture content of rice starch was estimated using the method suggested by A.O.A.C. (1980). To determine the moisture content, five gram of starch was taken in a petridish and dried in a hot air oven at 60°C-70°C, cooled in a desiccator and weighed. The process of heating and cooling was repeated until a constant weight was achieved. The moisture content was calculated from the loss in weight during drying and expressed in g $100g^{-1}$ of the sample.

3.5.2. Protein

Protein content was estimated by the method of A.O.A.C (1980). Rice starch (0.5g) was digested with 10 ml Con. H_2SO_4 after adding 0.4 g of $CuSO_4$ and 3.5 g K_2SO_4 in a digestion flask until the colour of the sample was converted to green. After digestion, it was diluted with water and 25 ml of 40 per cent NaOH was pumped. The distillate was collected in two per cent boric acid containing mixed indicator and then titrated with 0.05N H_2SO_4. The nitrogen content obtained was multiplied with a factor of 6.25 to get the protein content and expressed in g $100g^{-1}$ of the sample.

3.5.3. Total carbohydrates

The total carbohydrate content was analysed colourimetrically using anthrone reagent (Sadasivam and Manickam, 1992). Rice starch (0.1g) was hydrolysed with five ml of 2.5N HCl and then cooled to room temperature. The residue was then neutralised with solid sodium carbonate until there was no effervescence. The volume was made up to 100 ml and centrifuged. The supernatant (0.1ml) was made up to one ml and then four ml anthrone reagent was added and heated for eight minutes, cooled rapidly and the intensity of green to dark green colour was read at 630 nm.

A standard graph was prepared using standard glucose at serial dilutions. From the standard graph, the amount of total carbohydrate present in the sample was estimated and expressed in percentage.

3.5.4. Amylose

Amylose content was determined by the method suggested by Sadasivam and Manickam (1992). To 100 mg of rice starch, one ml of distilled ethanol and 10 ml of 1N NaOH were added and kept overnight. The volume was made up to 100 ml. The extract (2.5ml) was taken and added 20 ml of distilled water and three drops of phenolphthalein. Then, 0.1N HCl was added drop by drop until the pink colour disappears. To this, one ml of iodine reagent was added and the volume was made up to 50 ml. The intensity of the colour developed was read at 590 nm. The amylose present in the sample was estimated from standard graph prepared using serial dilution of standard amylose solution and expressed in percentage.

3.5.5. Amylopectin

Amylopectin content was determined by the method suggested by Sadasivam and Manickam (1992). The amount of amylopectin was obtained by subtracting the amylose content from that of starch.

3.5.6. Non starch polysaccharides (NSP)

Non starch polysaccharide content was found out by the method suggested by Mangala *et al.* (1999). The amount of non-starch polysaccharide was obtained by subtracting the starch content from that of total carbohydrate.

3.5.7. *In vitro* starch digestibility

In vitro starch digestibility (RDS, SDS and RS) of selected five starches were estimated using the procedure suggested by Englyst *et al.* (1996_a) and Mc Cleary and Monaghan (2002) with slight modifications made by CTCRI, Trivandrum as detailed in 3.4.1

3.6. Selection of rice starch with maximum RS and standardisation of resistant starch supplemented rice flour (RSRF)

3.6.1. Selection of rice starch

Two rice starches with maximum RS content after repeated autoclaving and cooling cycles were selected to prepare resistant starch supplemented rice flour.

3.6.2. Preparation of rice flour

Raw rice was soaked in cold water for 4-6 hours, and water was drained. The soaked rice was spread over a clean cloth to remove excess water. Rice was powdered and sieved using a fine sieve, roasted for 10 minutes as suggested by Pasricha and Rebello (1989). Roasted rice flour was packed in air tight containers.

3.6.3. Standardisation of resistant starch supplemented rice flour (RSRF)

The two rice starches with maximum RS content were incorporated with the roasted raw rice flour so as to get 10, 15 and 20g RS/100g of rice flour. Roasted rice flour without RS incorporation was taken as the control. The quantities of rice flour and rice starch used to prepare RSRF are detailed in Table 3.

Table 3. Quantity of roasted rice flour and rice starch used to formulate RSRF

Specification / Formulation		Control	Resistant starch (g)		
			10	15	20
Roasted rice flour (g)		100	100	100	100
Rice starch (g)	No.1	----	10.85	16.28	21.70 g
	No.2	----	10.86	16.29	21.73

3.7. Evaluation of physical qualities of RSRF

The physical qualities of RSRF namely bulk density, water absorption index and water solubility index were assessed using standard procedures and compared with the qualities of roasted rice flour. All analysis was carried out in triplicate samples.

3.7.1. Bulk density

The bulk density was determined by the method described by Okaka and Potter (1977). Fifty gram sample was put into a 100 ml graduated cylinder. The cylinder was tapped 50 times and the bulk density was calculated as weight per unit volume of sample.

3.7.2. Water absorption index (WAI)

Water absorption index of flour was determined by the method of Anderson *et al.* (1969). The powdered flour (2.5g) was mixed with 30 ml distilled water using a glass rod and cooked at 90°C for 15 minutes in a water bath. The cooked paste was cooled to room temperature and transferred to centrifuge tubes and centrifuged for 10 minutes at 3000rpm. Water absorption index was calculated by the following expression:

$$WAI = \frac{\text{Weight of the sediment}}{\text{Weight of the sample}}$$

3.7.3. Water solubility index (WSI)

Water solubility index was determined by the method of Anderson *et al.* (1969). The powdered flour sample (2.5g) was mixed with 30 ml distilled water using a glass rod and cooked at 90°C for 15 minutes in a water bath. The cooked paste was cooled to room temperature and transferred to centrifuge tubes and centrifuged for 10 minutes at 3000rpm. Water solubility index was calculated by the following expression:

$$WSI = \frac{\text{Weight of the dissolved solids in supernatant}}{\text{Weight of the sample}} \times 100$$

3.8. Preparation of rice products with RSRF and evaluation of organoleptic qualities of products

Two most popularly used products namely *puttu* and *idiappam* were prepared using roasted rice flour and with resistant starch supplemented rice flour (RSRF)

(Plates 2-3). They were prepared using standard procedures as detailed in 3.8.1 and 3.8.2. *Puttu* and *idiappam* made of roasted rice flour were taken as the control products.

3.8.1. Preparation of *puttu*

Ingredients

Roasted rice flour - 50g

Coconut (scraped)- 5g

Salt - to taste

Water- As required

Procedure

The roasted rice flour was moistened by sprinkling salt water and lumps formed were crushed. Moistened flour and scraped coconut were filled in *puttu* maker in alternate layers and steamed for seven minutes (Pasricha and Rebello, 1989).

3.8.2. Preparation of *idiappam*

Ingredients

Roasted rice flour - 50g

Coconut (scraped) - 5g

Hot water- As required

Salt - to taste

Procedure

The roasted rice flour was mixed well with required quantity of boiled water and salt so as to make a soft dough. The dough was pressed through "*sevainazhi*" on to the steamer after putting scraped coconut and steamed for five minutes (Pasricha and Rebello, 2011).

Puttu and *idiappam* prepared with resistant starch supplemented rice flour (RSRF) were prepared in the same way as the control products. The quantity of

Rice Flour + 10g RS ($T_{31\,(1)}$)

Rice Flour + 15g RS ($T_{31\,(2)}$)

Rice Flour + 20g RS ($T_{31\,(3)}$)

Rice Flour + 10g RS ($T_{14\,(1)}$)

Rice Flour + 15g RS ($T_{14\,(2)}$)

Rice Flour + 20g RS ($T_{14\,(3)}$)

Rice Flour (Control)

Plate 2. *Puttu* prepared with resistant starch supplemented rice flour

Rice Flour + 10g RS ($T_{31\,(1)}$)

Rice Flour + 15g RS ($T_{31\,(2)}$)

Rice Flour + 20g RS ($T_{31\,(3)}$)

Rice Flour + 10g RS ($T_{14\,(1)}$)

Rice Flour + 15g RS ($T_{14\,(2)}$)

Rice Flour + 20g RS ($T_{14\,(3)}$)

Rice Flour (Control)

Plate 3. ***Idiappam*** **prepared with resistant starch supplemented rice flour**

ingredients used to prepare *puttu* and *idiappam* are furnished in Tables 4 and 5 respectively.

Table 4. Proportion of ingredients used to prepare *puttu*

Ingredients		Control	RSRF Resistant starch (g)		
			10	15	20
Roasted rice flour (g)		50	50	50	50
Rice starch (g)	No.1	----	5.43	8.14	10.85
	No.2	----	5.43	8.15	10.87
Coconut (scraped) (g)		5	5	5	5
Salt		to taste	to taste	to taste	to taste
Water (ml)		40	45	48	50

Table 5. Proportion of ingredients used to prepare *idiappam*

Ingredients		Control	RSRF Resistant starch (g)		
			10	15	20
Roasted rice flour (g)		50	50	50	50
Rice starch (g)	No.1	----	5.43	8.14	10.85
	No.2	----	5.43	8.15	10.87
Coconut (scraped) (g)		5	5	5	5
Salt		to taste	to taste	to taste	to taste
Water (ml)		50	55	55	55

3.8.3. Organoleptic qualities of products

Organoleptic evaluation of rice products was carried out in the morning using score cards by the panel of 10 selected judges.

3.8.3.1. Selection of judges

A series of organoleptic trials were carried out using simple triangle test at laboratory level to select a panel of 10 trained judges between the age group of 18 to 35 years as suggested by Jellinek (1985).

3.8.3.2. Preparation of score card

The sensory evaluation of *puttu* and *idiappam* was carried out using score card method (Swaminathan, 1974). Score cards were prepared based on a nine point hedonic scale giving a score of nine for like extremely to a score of one for dislike extremely (Appendix I). The quality attributes namely appearance, colour, flavour, texture, taste and overall acceptability of the two products were evaluated using this score card.

3.8.3.3. Selection of most acceptable products

Organoleptic evaluation of *puttu* and *idiappam* made with RSRF and roasted rice flour (control) were carried out using score cards based on a nine point hedonic scale by the panel of ten judges. The quality attributes such as appearance, colour, flavour, texture, taste and overall acceptability of the products were evaluated. Two RSRF (one for each product) with highest mean score for all the organoleptic parameters were selected along with the control for further studies on storage stability and glycaemic response.

3.9. *In vitro* starch digestibility of rice products prepared with RSRF

In vitro starch digestibility of *puttu* and *idiappam* prepared with RSRF and control prepared exclusively with roasted rice flour with respect to RS, RDS and SDS were evaluated using the procedure as detailed in 3.4.1.

3.10. Shelf life of selected RSRF

Resistant starch supplemented rice flour (RSRF) with maximum acceptability for *puttu* and *idiappam* were packed in polythene bags of 200 gauge thickness and were stored for six months under ambient conditions. Roasted rice flour was taken as control.

The following evaluations were conducted in RSRF and roasted rice flour initially and after six months of storage. All the evaluations were carried out as a replicate trial in five replications.

3.10.1. Physical qualities

The physical qualities like bulk density, water absorption index and water solubility index of RSRF and raw rice flour were evaluated initially and after six months of storage as explained in 3.7.1, 3.7.2 and 3.7.3 respectively.

3.10.2. Proximate composition

Moisture, protein and total carbohydrate content of RSRF and raw rice flour were evaluated initially and after six months of storage as explained in 3.5.1, 3.5.2 and 3.5.3 respectively.

3.10.3. Microbial enumeration

Total microbial count of roasted rice flour and RSRF were assessed for bacteria, fungi and yeast initially and after six months of storage using serial dilution and plate count method as described by Agarwal and Hasija (1986). Ten gram of sample was added to 90 ml sterile water and agitated for 20 minutes. One ml of this solution was transferred to a test tube containing nine ml of sterile water to get 10^{-2} dilution. Similarly 10^{-3}, 10^{-4} and 10^{-5} dilutions were also prepared.

Enumeration of total microbial count was carried out using nutrient agar (NA) media for bacteria, potato dextrose agar (PDA) media for fungus and sabouraud's dextrose agar (SDA) media for yeast, which was obtained from Himedia Lab, Mumbai. The dilution used for assessing bacteria was 10^{-5} and for fungi and yeast 10^{-3}. One ml of each dilution was poured into separate sterilised petri plates followed by the addition of respective media. Sealed petri plates were incubated at $28^{\circ}C$. Enumeration of microbial count was done after 48 hours for bacteria and 72 hours for fungus and yeast.

3.10.4. Insect infestation

Presence of storage insects was assessed initially and after six months of storage by examining rice flour and RSRF under the microscope. Flour was sieved first with 60 BL sieve followed by 100 BL sieve and observed under microscope.

3.10.5. Organoleptic qualities of rice products

Organoleptic evaluation of *puttu* and *idiappam* with maximum acceptability and control prepared exclusively with roasted rice flour was carried out as per the procedure explained in 3.8.3 initially and after six months of storage.

3.10.6. *In vitro* starch digestibility of rice products

In vitro starch digestibility of *puttu* and *idiappam* prepared with maximum acceptable RSRF and control were assessed using the procedure explained in 3.4.1 initially and after six months of storage.

3.11. Postprandial glycaemic response of selected RSRF products

Postprandial glycaemic response of RS incorporated food products namely *puttu* and *idiappam* was evaluated by giving the products to five diabetic and five non diabetic subjects. This was compared by giving the two products prepared with non-supplemented rice flour. Both products were given to the subjects so as to get 50g of carbohydrate from the respective meal. The consent of all the subjects to participate in the study and to draw blood sample was taken.

Before giving the products, the fasting blood glucose level of the subjects was measured using glucometer manufactured by Nipro India Corporation Pvt. Ltd., Chennai. *Puttu* and *idiappam* prepared with and without RS supplemented flour was given as breakfast menu for the selected individuals during four separate days. The subjects were instructed to consume only the particular item given to them as breakfast on that particular day. The details of the products given to the subjects are given in Table 6. The blood glucose level of the subjects was measured after two hours of the meal. The experiment was conducted for a total of four days among each of the 10 selected subjects. The variation occurred in the blood glucose level of the subjects after giving the products was computed.

Table 6. Details of the products given to the subjects

Day	Product	Quantity of RS incorporation g/100g rice flour
1st	Control *puttu*	0
2nd	RSRF *puttu*	10
3rd	Control *idiappam*	0
4th	RSRF *idiappam*	15

3.12. Cost of the developed RSRF

Cost of the developed resistant starch supplemented *puttu* and *idiappam* flour was computed to assess the extent of expense incurred to prepare the flour. The cost was worked out on the basis of the cost of rice, processing charge of rice and cost of alkali. The cost of electricity was also taken into account. Details of computation are given in Appendix II.

3.13. Statistical analysis

The observations recorded were tabulated and the data were analysed statistically using Completely Randomised Design (CRD). Comparison of RS content was carried out using three and four factor ANOVA. Variations in chemical constituents between the treatments were statistically analysed by applying DMRT. The treatments were compared with control using 't' test. For organoleptic evaluation, Kendall's coefficient of concordance (W) was used to assess the degree of agreement among the judges. The effect of storage on various quality parameters was statistically analysed by paired t-test. Comparison of different parameters between RSRF and control samples during storage were statistically analysed using independent sample 't' test.

Results

4. RESULTS

The results pertaining to the study entitled "Optimisation and utilisation of resistant starch for value addition in rice products" are presented in this chapter under the following headings.

4.1. Resistant starch (RS) content of raw and parboiled rice starch

4.2. Effect of autoclaving and cooling on RS content in rice starch

- 4.2.1. Effect of autoclaving and cooling at room temperature on RS content
- 4.2.2. Effect of autoclaving and cooling at -20°C on RS content
- 4.2.3. Effect of cooling condition on RS content
- 4.2.4. Relative changes in RS content of rice starch due to autoclaving and cooling
- 4.2.5. Selection of processing conditions aimed to yield maximum RS content

4.3. Effect of repeated autoclaving and cooling on RS content and other chemical constituents of rice starch

- 4.3.1. Resistant starch
- 4.3.2. Rapidly digestible starch
- 4.3.3. Slowly digestible starch
- 4.3.4. Moisture
- 4.3.5. Protein
- 4.3.6. Total carbohydrate
- 4.3.7. Amylose
- 4.3.8. Amylopectin
- 4.3.9. Non starch polysaccharides

4.4. Quality evaluation of resistant starch supplemented rice flour (RSRF) and products

- 4.4.1. Physical qualities of RSRF
  - 4.4.1.1. Bulk density

4.1. Resistant starch (RS) content of raw and parboiled rice starch

In the present study, starch was isolated from rice variety Uma, procured from Regional Agricultural Research Station (RARS), Pattambi, Palakkad District, under Kerala Agricultural University. Out of the total 200kg of rice procured, five kilogram of rice was parboiled by conventional method using hot soaking process. Both raw and parboiled rice were powdered and starch was isolated. The quantity of starch obtained from raw and parboiled rice was 65.47 per cent and 66.6 per cent respectively.

The isolated raw and parboiled rice starches were analysed for RS content and the results are presented in Table 7.

Table 7. RS content of raw and parboiled rice starch

Treatments	**Resistant starch (%)**
Raw rice starch (**T_1**)	78.73
Parboiled rice starch (**T_2**)	76.29

The RS content of raw rice starch (T_1) was found to be 78.73 per cent compared to 76.29 per cent in parboiled rice starch.

4.2. Effect of autoclaving and cooling on RS content in rice starch

The effect of autoclaving and cooling on the RS content of raw rice starch is given in this section.

4.2.1. Effect of autoclaving and cooling at room temperature on RS content

The RS content of rice starch autoclaved at 121°C for 20 to 60 minutes and with zero, 10, 20 and 40 per cent moisture levels varied from 20.58 per cent (T_{23}) to 77.91 per cent (T_3). In the samples which were autoclaved at 141°C, the RS content varied from 42.17 per cent (T_{25}) to 79.61 per cent (T_{14}) (Table 8). The RS content of rice starch was found to be high in almost all samples which were autoclaved at 141°C (Fig.1a, 1b and 1c). In the samples which were autoclaved at 141°C for 20 minutes without any moisture (T_6) and with 20 per cent (T_{18}) moisture, a slight decrease in RS content was noticed when compared to the rice starch autoclaved at 121°C for 20 minutes without moisture (T_3) and with 20 per cent (T_{15}) moisture level.

Comparison of RS content using three factor ANOVA (temperature, time and moisture) (CD=6.31) under the same time of autoclaving at room temperature cooled condition indicated that at 121°C, a significantly higher yield of RS was obtained only in samples autoclaved for 20 minutes (T_{15}) with 20 per cent moisture. RS content was found to be significantly high when the samples were autoclaved at 141°C for 40 minutes with 10, 20 and 40 per cent moisture levels. Similarly, during 60 minutes of autoclaving, 141°C was favouring a significantly higher yield of RS content irrespective of moisture level.

A gradual decrease in RS content of samples was noticed with an increase in time of autoclaving both at 121°C and at 141°C without any moisture treatment. In the samples autoclaved at 121°C with 20 per cent and 40 per cent moisture levels also, the RS content gradually decreased with an increase in the time of autoclaving. With 10 per cent moisture level also, the RS content decreased from 74.29 per cent at 20 minutes autoclaving to 72.13 per cent at 60 minutes autoclaving. At 141°C, though a gradual decrease in the RS content occurred in the samples autoclaved without any moisture treatment, with 10 per cent and 20 per cent moisture levels, the RS content increased with increase in the time of autoclaving. The details are depicted in Fig. 2a and 2b.

Table 8. Effect of temperature, time and moisture on RS content of rice starch cooled at room temperature

Temperature (°C)	Time (Minutes)	Moisture (%)							
		0		10		20		40	
		Resistant starch (%)							
121	20	T_3	77.91	T_9	74.29	T_{15}	70.03	T_{21}	28.61
	40	T_4	67.36	T_{10}	63.96	T_{16}	48.76	T_{22}	25.3
	60	T_5	56.87	T_{11}	72.13	T_{17}	45.49	T_{23}	20.58
141	20	T_6	76.58	T_{12}	77.01	T_{18}	61.9	T_{24}	43.63
	40	T_7	70.59	T_{13}	78.41	T_{19}	63.9	T_{25}	42.17
	60	T_8	69.79	T_{14}	79.61	T_{20}	66.73	T_{26}	43.57

CD = 6.31

With respect to the effect of adding moisture on the RS content of the samples, it was seen that in samples autoclaved at 121°C for 20 as well as 40 minutes, the RS content gradually decreased with an increase in the moisture level from zero to 40 per cent (Fig. 3a and 3b). However, at 60 minutes of autoclaving, the RS content increased initially with 10 per cent moisture level and with 20 per cent and 40 per cent moisture levels it gradually decreased to 20.58 per cent (T_{23}). At 141°C also, an increase in RS content occurred with 10 per cent moisture level when they were autoclaved for 20, 40 and 60 minutes. With 20 per cent and 40 per cent moisture levels, the RS content gradually decreased in all samples when they were autoclaved for 20, 40 and 60 minutes.

Comparison of RS content using three factor ANOVA (temperature, time and moisture) (CD=6.31) during different moisture regimes under the same temperature and time of autoclaving at room temperature cooled samples

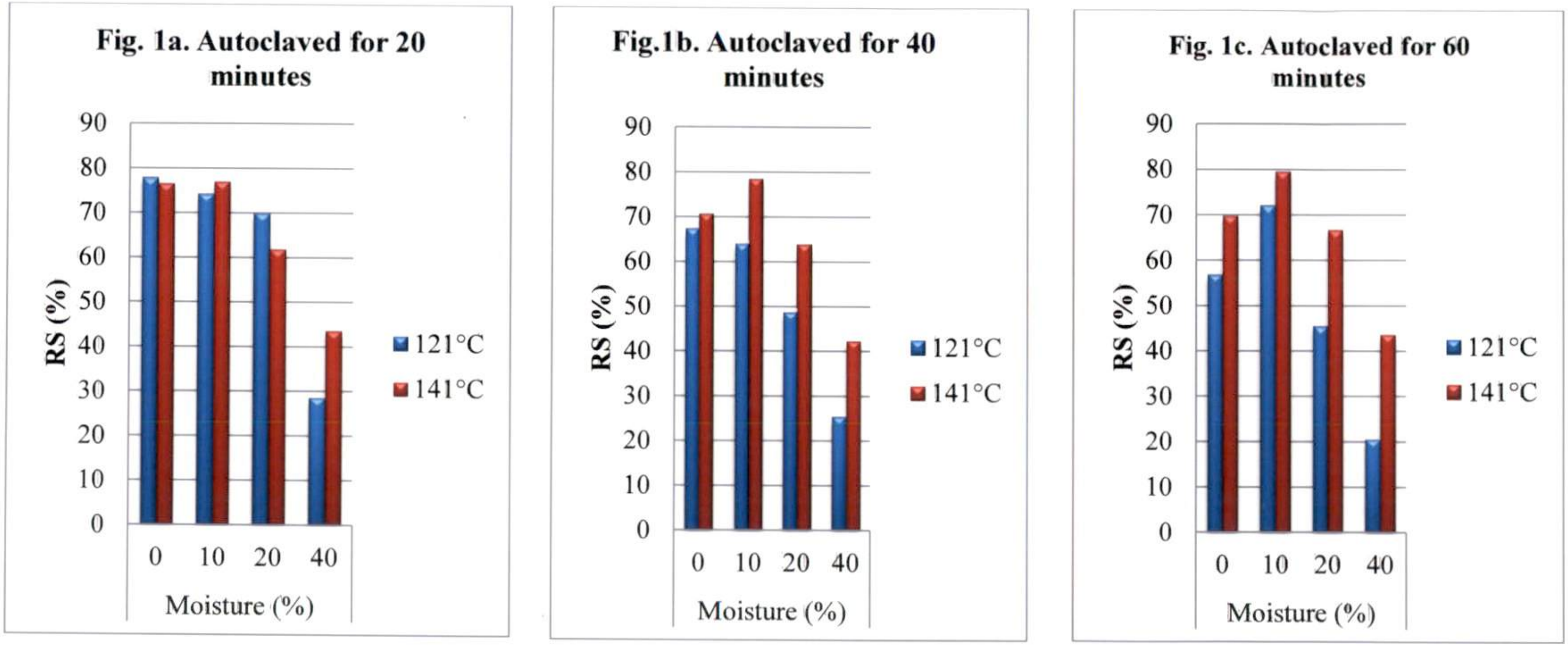


Fig.1. Effect of temperature on RS content of rice starch cooled at room temperature

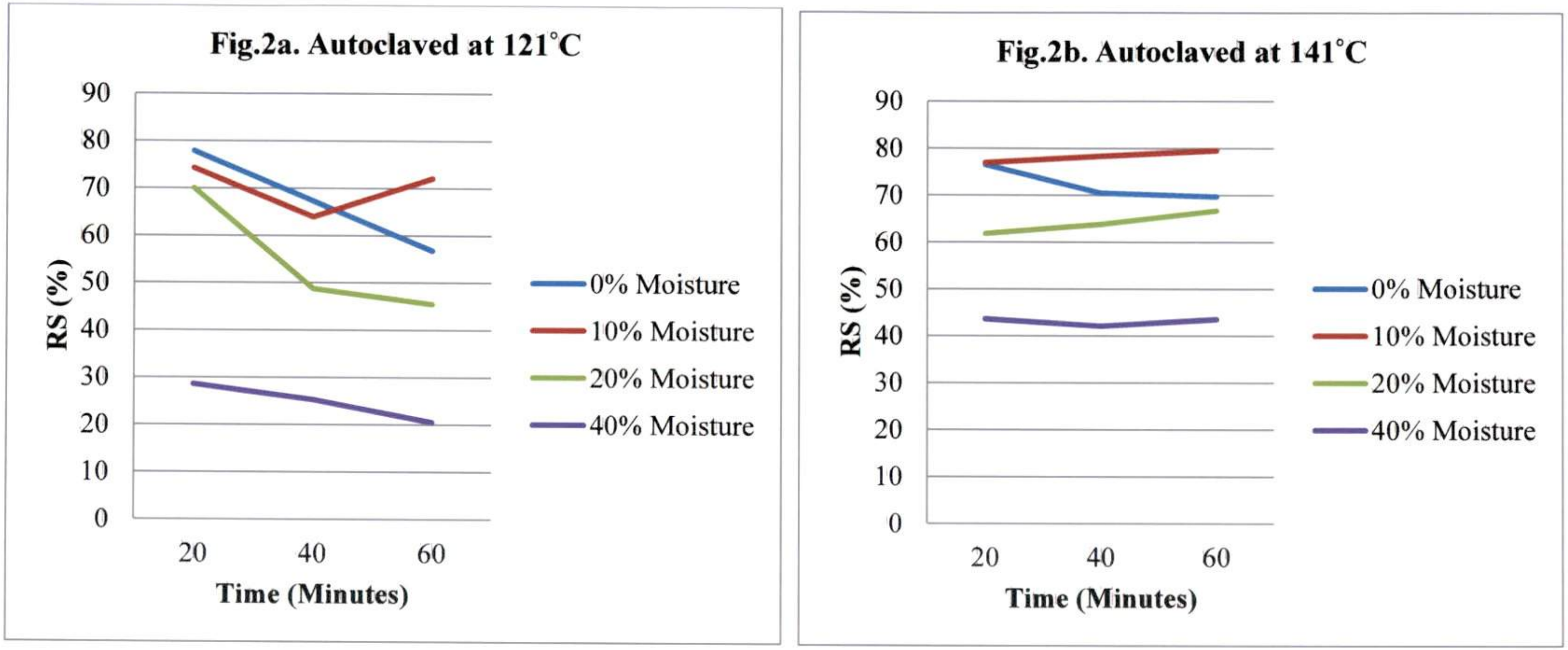


Fig.2. Effect of time on RS content of rice starch cooled at room temperature

indicated a significant decrease in RS content with 20 and 40 per cent moisture levels in samples autoclaved at 121°C for 20, 40 and 60 minutes and at 141°C for 20 and 40 minutes when compared to the samples autoclaved without any moisture. At 10 per cent moisture level a significant increase of RS was observed in samples autoclaved at 121°C for 60 minutes and at 141°C for 40 and 60 minutes when compared with the RS content of samples processed without moisture.

4.2.2. Effect of autoclaving and cooling at -20°C on RS content

The effect of temperature and time of autoclaving and the application of moisture on the resistant starch content of rice starch cooled at -20°C is given in Table 9.

The RS content varied from 16.93 per cent (T_{45}) to 80.6 per cent (T_{35}) in samples which were autoclaved at 121°C for 20 to 60 minutes and with zero, 10, 20 and 40 per cent moisture levels. In the samples which were autoclaved at 141°C, the RS content varied from 40.02 per cent (T_{50}) to 82.82 per cent (T_{31}). Here also, the RS content was found to be high in almost all samples which were autoclaved at 141°C. Rice starches which were autoclaved at 141°C for 60 minutes with 10 per cent (T_{38}) and with 40 per cent (T_{50}) moisture levels, a slight decrease in RS content was noticed when compared to the corresponding samples (T_{35} and T_{47}) autoclaved at 121°C (Fig. 4a, 4b and 4c).

Comparison of RS content on the basis of three factor ANOVA (temperature, time and moisture) (CD=5.75) during two temperatures under the same time of autoclaving and cooling at -20°C indicated that in samples autoclaved for 20 minutes, RS content increased significantly at 141°C when compared to 121°C with 10, 20 and 40 per cent moisture levels. Similar observation of significant increase in RS was noticed at 40 minutes of autoclaving without moisture and also with 10 and 40 per cent moisture levels. During 60 minutes of autoclaving, samples autoclaved at 121°C for 60 minutes with 10 and 40 per cent moisture had significantly high RS content.

Table 9. Effect of temperature, time and moisture on RS content of rice starch cooled at -20°C

Temperature (°C)	Time (Minutes)	Moisture (%)							
		0		10		20		40	
		Resistant starch (%)							
121	20	T_{27}	71.28	T_{33}	66.95	T_{39}	51.64	T_{45}	16.93
	40	T_{28}	65.72	T_{34}	68.17	T_{40}	53.88	T_{46}	19.7
	60	T_{29}	67.81	T_{35}	80.6	T_{41}	59.49	T_{47}	50.31
141	20	T_{30}	72.8	T_{36}	76.31	T_{42}	62.03	T_{48}	46.86
	40	T_{31}	82.82	T_{37}	82	T_{43}	56.44	T_{49}	62.28
	60	T_{32}	74.61	T_{38}	73.63	T_{44}	61.81	T_{50}	40.02

CD = 5.75

With respect to the effect of time on the RS content of the samples (Fig. 5a and 5b) cooled at -20°C, a gradual increase in RS content was noticed with an increase in time of autoclaving with three moisture levels at 121°C. In the samples which were autoclaved at 121°C without any moisture the RS content decreased from 71.28 per cent (T_{27}) at 20 minutes of autoclaving to 67.81 per cent (T_{29}) at 60 minutes of autoclaving. In samples autoclaved at 141°C the RS content showed an increase initially at 40 minutes of autoclaving with 10 and 40 per cent moisture levels and at 60 minutes it showed a decreasing trend.

As the moisture level increased, the RS content also increased initially with 10 per cent moisture levels both at 40 minutes and 60 minutes of autoclaving at 121°C. At 141°C also with 10 per cent moisture level an increase in the RS content was noticed when rice starch was autoclaved for 20 minutes. When the moisture content increased to 20 per cent and 40 per cent, the RS content

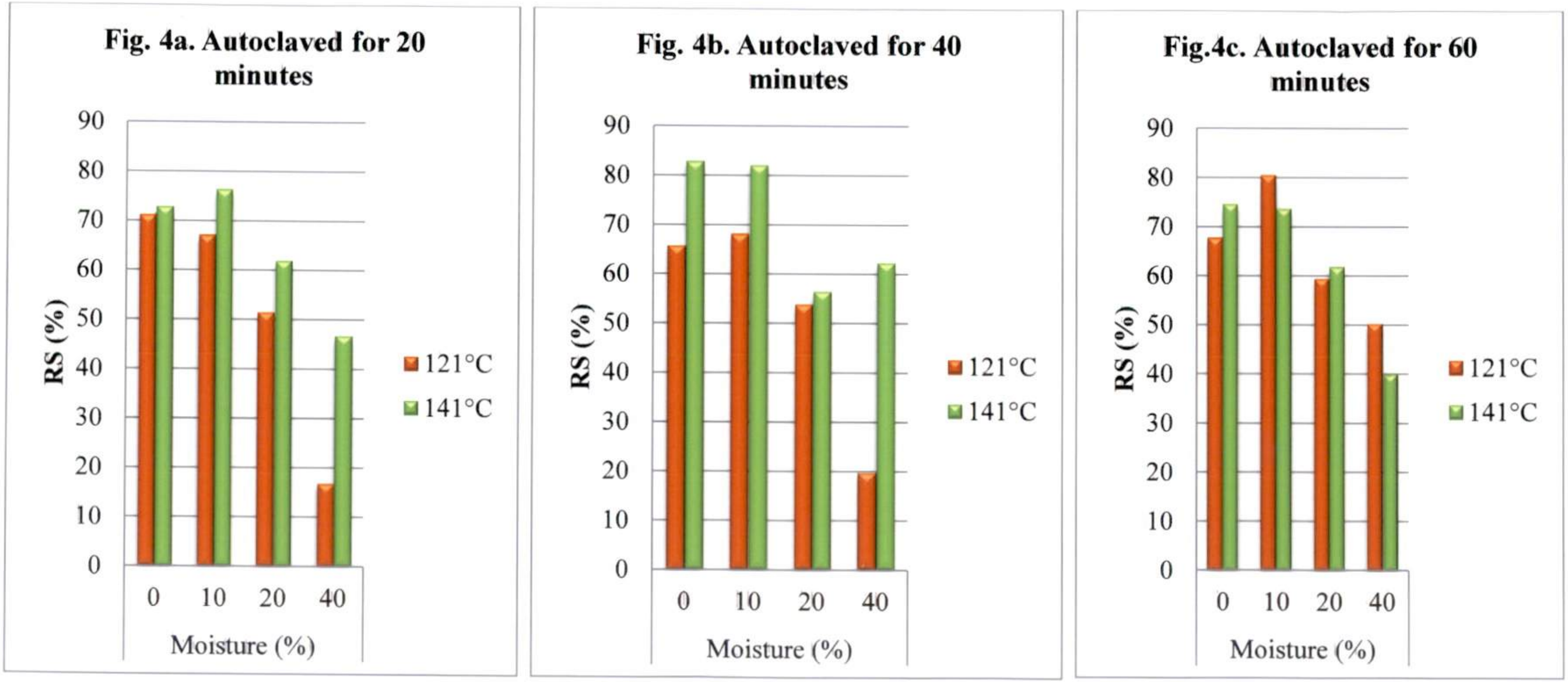
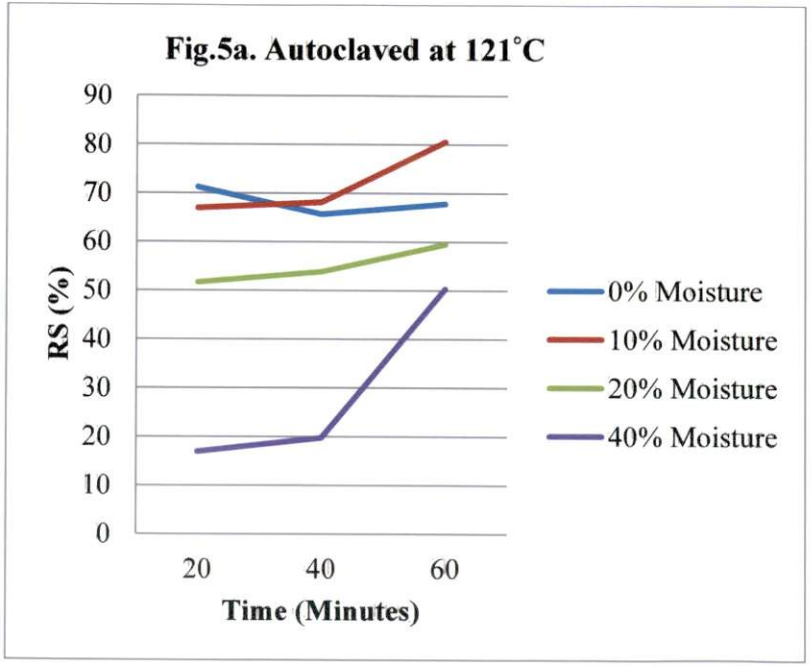


Fig.4. Effect of temperature on RS content of rice starch cooled at -20°C



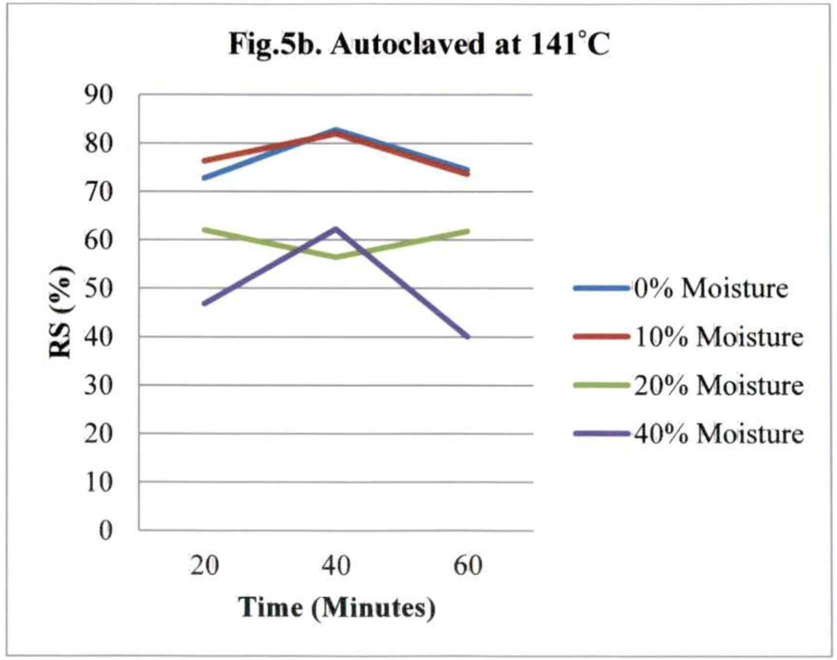


Fig.5. Effect of time on RS content of rice starch cooled at -20°C

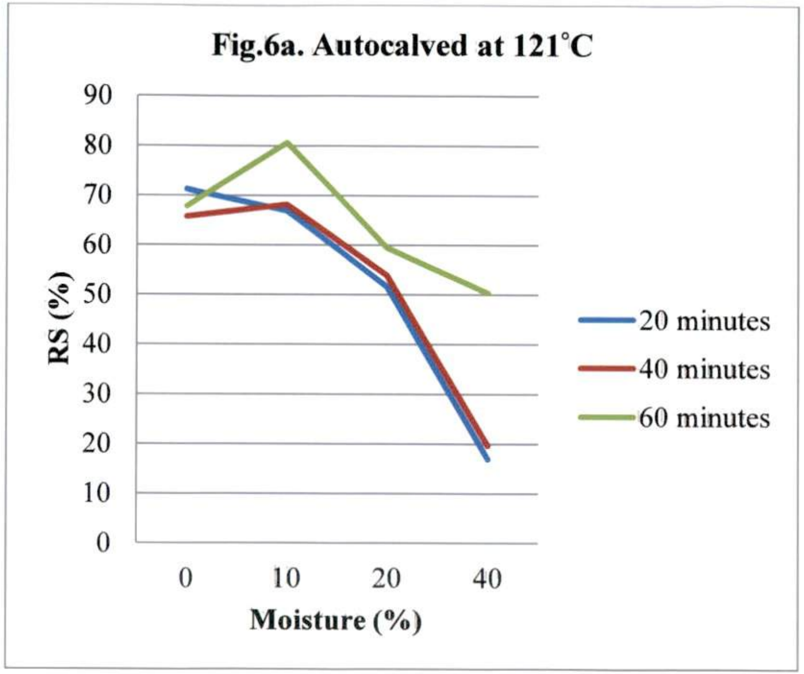
decreased in samples autoclaved both at 121°C and 141°C for 20, 40 as well as 60 minutes (Fig. 6a and 6b).

Comparison of RS content using three factor ANOVA (temperature, time and moisture) (CD= 5.75) during different moisture regimes under the same temperature and time of autoclaving in -20°C cooled samples showed a significant decrease in RS content in all samples autoclaved with 20 and 40 per cent moisture levels at 121°C and 141°C for 20, 40 and 60 minutes when compared to the samples processed without adding any moisture. When the autoclaving time was 60 minutes, at 121°C a significantly higher yield of RS was noticed with 10 per cent moisture level also (T_{35}) when compared to the samples autoclaved without moisture (T_{29}).

4.2.3. Effect of cooling condition on RS content

The effect of cooling conditions on RS content of rice starch is given in Table 10. Comparison of RS content using four factor ANOVA (temperature, time, moisture and cooling condition) during different moisture regimes under the same temperature and time of autoclaving indicated significantly high RS in the samples which were cooled at room temperature after autoclaving at 121°C for 20 minutes at four different moisture levels. However, at 60 minutes of autoclaving at 121°C, RS content was found to be significantly high in all samples which were cooled at -20°C (Fig. 7a, 7b and 7c).

At 141°C of autoclaving for 20 minutes a slight increase in the RS content was noticed in samples cooled at room temperature without adding any moisture and with 10 per cent moisture level (Fig. 8a, 8b and 8c). With 20 per cent and 40 per cent moisture levels, the RS content was found to be high in samples cooled at -20°C. All these variations were found to be statistically insignificant.



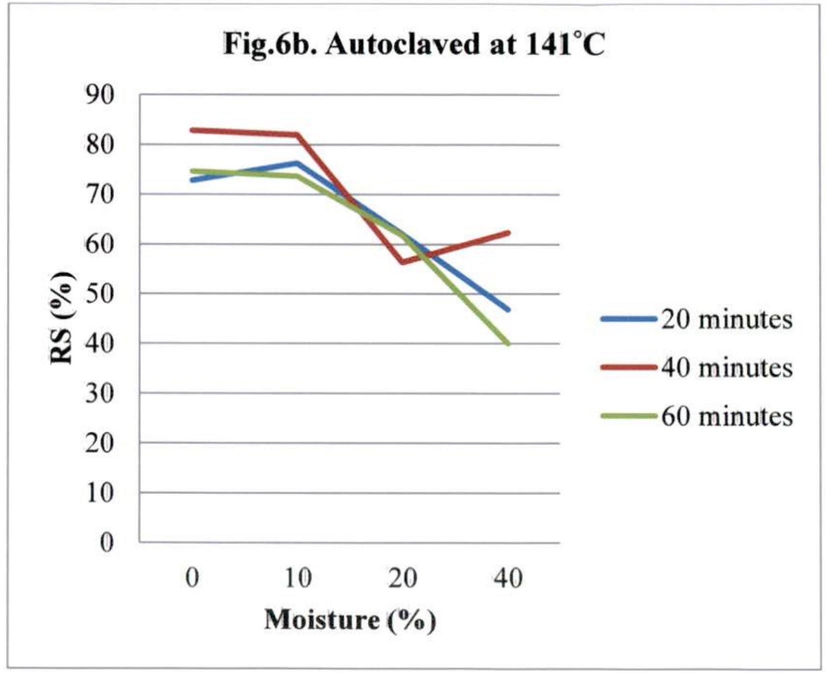


Fig.6. Effect of moisture on RS content of rice starch cooled at -20°C

Table 10. Effect of cooling conditions on RS content of rice starch

Temperature (°C)	Time (Minutes)	Moisture (%)							
		0		10		20		40	
		-20°C	**RT**	**-20°C**	**RT**	**-20°C**	**RT**	**-20°C**	**RT**
121	20	71.28	77.91	66.95	74.29	51.64	70.03	16.93	28.61
	40	65.72	67.36	68.17	63.96	53.88	48.76	19.7	25.3
	60	67.81	56.87	80.6	72.13	59.49	45.49	50.31	20.58
141	20	72.8	76.58	76.31	77.01	62.03	61.9	46.86	43.63
	40	82.82	70.59	82	78.41	56.44	63.9	62.28	42.17
	60	74.61	69.79	73.63	79.61	61.81	66.73	40.02	43.57

RT- Room temperature

CD = 6.07

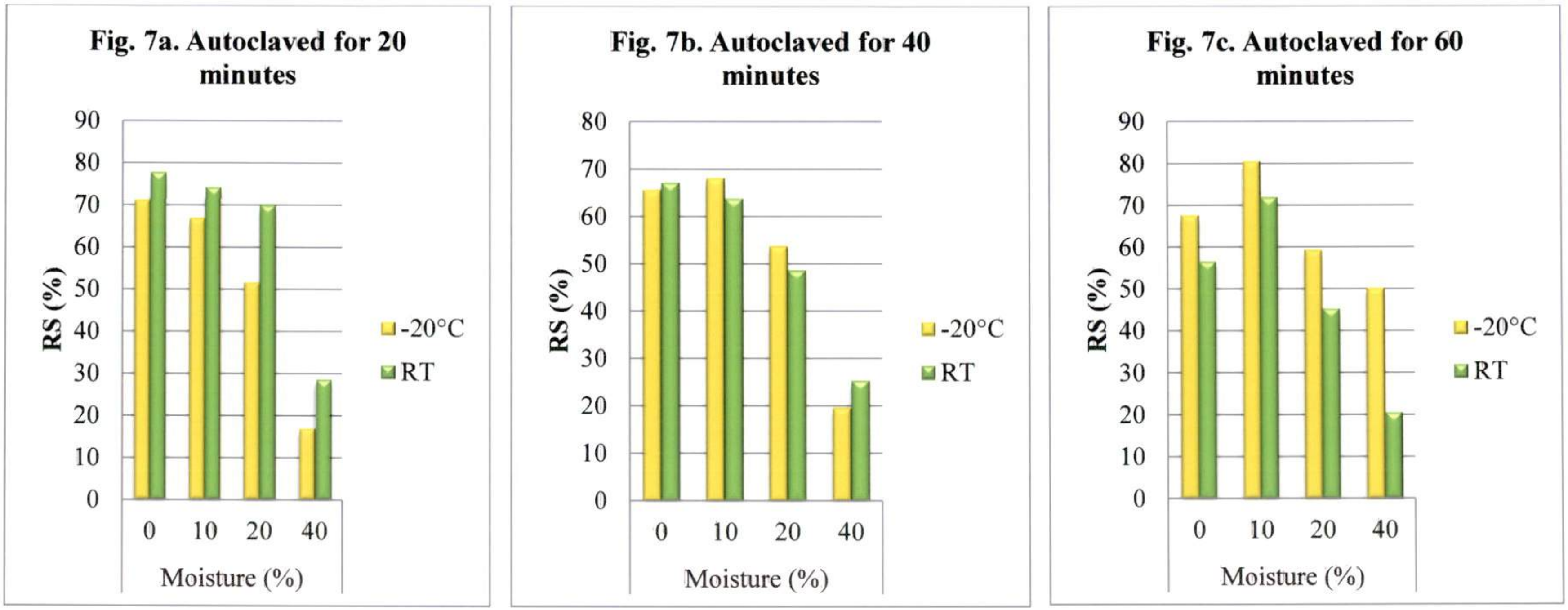


Fig.7. Effect of cooling conditions on RS content of rice starch autoclaved at 121°C

In samples autoclaved at 141°C for 40 minutes the RS content was found to be high in samples cooled at -20°C with 10 and 40 per cent moisture as well as without moisture. The increase noticed without moisture and with 40 per cent moisture level was found to be statistically significant.

At 60 minutes of autoclaving at 141°C, the RS was found to be high in all samples which were cooled at room temperature except in samples autoclaved without any moisture. However the increase noticed was statistically insignificant.

4.2.4. Relative changes in RS content of rice starch due to autoclaving and cooling

The relative changes occurred in the RS content of rice starch due to autoclaving and cooling at room temperature and at -20°C when compared to the RS content of raw rice starch are given in Table 11 and 12 respectively.

Table 11. Relative changes in RS content of rice starch during processing (cooled at room temperature)

Temperature (°C)	Time (Minutes)	Relative changes in RS content* (%)			
		Moisture (%)			
		0	10	20	40
121	20	-1.04	-5.63	-11.05	-63.66
	40	-14.4	-18.8	-38.06	-67.86
	60	-27.8	-8.38	-42.22	-73.85
141	20	-2.73	-2.19	-21.38	-44.58
	40	-10.3	-0.4	-18.84	-46.44
	60	-11.4	+1.1	-15.24	-44.65

*Resistant starch content in raw rice starch was taken as the base (78.73%)

A slight increase in RS content when compared to raw rice starch occurred only in samples autoclaved at141°C for 60 minutes with 10 per cent moisture. In all other processing conditions, the RS content decreased when the samples were cooled at room temperature (Table 11).

Maximum relative decrease was noticed in samples autoclaved at 121°C for 60 minutes with 40 per cent moisture (73.85%). Relative decrease in RS content over raw rice starch varied from 0.4 per cent to 73.85 per cent.

Table 12. Relative changes in RS content of rice starch during processing (cooled at -20°C)

Temperature (°C)	Time (Minutes)	Relative changes in RS content* (%)			
		Moisture (%)			
		0	10	20	40
121	20	-9.46	-15	-34.41	-78.49
	40	-16.5	-13.4	-31.56	-74.97
	60	-13.9	+2.4	-24.44	-36.1
141	20	-7.54	-3.08	-21.21	-40.48
	40	+5.2	+4.2	-28.31	-20.89
	60	-5.23	-6.48	-21.49	-49.16

*Resistant starch content in raw rice starch was taken as the base (78.73 %)

From Table 12, it is clear that in samples cooled at -20°C after autoclaving of starch at 121°C and 141°C for 20 to 60 minutes and with 10 to 40 per cent moisture and without moisture, a decrease in RS content occurred when compared to the RS content of rice starch without processing. Maximun decrease was found in samples autoclaved at 121°C for 20 minutes with 40 per cent moisture (78.49%).

A slight increase in RS content was found in samples autoclaved at 121°C for 60 minutes with 10 per cent moisture and in samples autoclaved at 141°C for 40 minutes without moisture as also with 10 per cent moisture. In samples cooled at -20°C the relative decrease in RS content varied from 3.08 per cent to 78.49 per cent when compared with the RS content of raw rice starch.

4.2.5. Selection of processing conditions aimed to yield maximum RS content

On the basis of the effects of temperature and time of autoclaving and moisture levels and cooling conditions on the RS content, five treatments with maximum RS content was selected for further studies. The details of the selected treatments are presented in Table 13. In these selected treatments, RS content varied from 78.41 to 82.82 per cent. The highest RS content was observed in rice starch autoclaved at 141°C for 40 minutes without moisture and cooled at -20°C. The lowest was noticed in rice starch autoclaved at 141°C for 40 minutes with 10 per cent moisture and cooled at room temperature.

Table 13. Summary of processing conditions aimed to yield maximum RS

Treatment	Temperature (°C)	Time (Minutes)	Moisture (%)	Mode of cooling	RS (g/100g starch)
T_{31}	141	40	0	-20°C	82.82
T_{37}	141	40	10	-20°C	82
T_{35}	121	60	10	-20°C	80.60
T_{14}	141	60	10	RT	79.61
T_{13}	141	40	10	RT	78.41

RT- Room temperature

On the basis of this, best processing conditions suitable for maximum RS yield from rice starch was found to be autoclaving at 141°C for 40 minutes (Table 14). Better retention of RS content was seen in samples cooled at -20°C, though

RS can very well be realised even at room temperature cooling. Maximum moisture level suitable was found to be 10 per cent. Autoclaving of samples at 141°C for 40 minutes and at 121°C for 60 minutes yielded maximum RS content.

Table 14. Processing conditions suitable to yield maximum RS from rice starch

Temperature of autoclaving (°C)	Time of autoclaving (Minutes)	Maximum moisture level (%)	Mode of cooling
141	40	10	-20°C
121	60	10	
141	60	10	RT

4.3. Effect of repeated autoclaving and cooling on RS content and other chemical constituents of rice starch

Five rice starches with high resistant starch content selected from the previous experiment were subjected to repeated autoclaving and cooling for two, three and four times. The effect of repeated autoclaving and cooling on the RS content and other chemical constituents of rice starch are presented in this section.The rice starch without any autoclaving and cooling was taken as the control.

Variations in chemical constituents between the treatments were statistically analysed by applying DMRT. The effects of various treatments were compared with the corresponding control values using 't' test.

4.3.1. Resistant Starch (RS)

The effect of repeated autoclaving and cooling on the resistant starch (RS) content of rice starch are presented in Table 15.

The resistant starch content of rice starch subjected to two autoclaving and cooling cycles varied from 78.6 per cent (T_{14}) to 82.17 per cent (T_{31}). In the samples which were autoclaved and cooled three and four times, the content varied from 80.03 per cent (T_{13}) to 82.65 per cent (T_{35}) and from 83.47 per cent (T_{35}) to 92.12 per cent (T_{31}) respectively.

The yield of RS due to repeated autoclaving and cooling at varying temperature and time with and without moisture was realised to the maximum extent in the case of starch samples autoclaved at 141°C for 40 minutes without moisture and cooled at -20°C (T_{31}), the process being repeated two/four times. When the output of RS was observed after two autoclavings and coolings, the yield realised from T_{13} (80.8%) and T_{37} (80.77%) were found to be on par with T_{31} (82.17%). Contrary to the situation under two and four autoclavings, T_{35} yielded the maximum RS under three autoclavings and the treatment except T_{13} were on par. When the autocalvings were repeated four times, T_{14} (92%) yielded as much RS as that from T_{31} (92.12%).

The RS content of all the samples which were autoclaved and cooled three and four times with and without moisture were found to be significantly higher than the samples which were not subjected to any processing treatment (control) in which the RS content was found to be 78.73 per cent.

An increase in the RS content was noticed with an increase in the number of autoclaving and cooling in all the samples irrespective of the temperature and time of autoclaving, moisture content and cooling conditions.

Table 15. Effect of repeated autoclaving and cooling on RS (%) content of rice starch

Treatments	Processing conditions				RS (%)		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Number of autoclaving and cooling		
					Two	Three	Four
T_{31}	141	40	0	-20°C	82.17^{a}	82.27^{ab}	92.12^{a}
T_{37}	141	40	10	-20°C	80.77^{ab}	81.02^{ab}	87.24^{b}
T_{35}	121	60	10	-20°C	78.62^{b}	82.65^{a}	83.47^{c}
T_{14}	141	60	10	RT	78.6^{b}	81.97^{ab}	92^{a}
T_{13}	141	40	10	RT	80.8^{ab}	80.03^{b}	85.44^{bc}
Control *vs* Treatments^{+}					NS	S	S

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=78.73 per cent, $^{+}$Based on t-test
RT- Room temperature
NS -Not significant
S- Significant

4.3.2. Rapidly Digestible Starch (RDS)

Rapidly digestible starch content (Table 16) of samples subjected to two and three autoclaving and cooling cycles varied from 7.21 per cent (T_{37}) to 15.8 per cent (T_{31}) and 6 per cent (T_{31}) to 15.1 per cent (T_{37}) respectively. In rice starch which was autoclaved twice, RDS content was found to be high in the samples autoclaved at 141°C for 40 minutes without any moisture and cooled at -20°C. In samples autoclaved thrice, maximum was seen in rice starch which was autoclaved at 141°C for 40 minutes with 10 per cent moisture and cooled at -20°C. In rice starch which was subjected to autoclaving and cooling for four times, the RDS varied from 4.42 to 14.8 per cent with maximum content in the sample which was autoclaved at 121°C for 60 minutes with 10 per cent moisture and cooled at -20°C.

Among the different treatments tried with repeated autoclaving, maximum RDS (15.8%) was found in samples autoclaved at 141°C for 40 minutes without any moisture and cooled at -20°C twice followed by T_{37} with 15.1 per cent RDS in samples autoclaved at 141°C for 40 minutes with 10 per cent moisture and cooled at -20°C thrice.

Thus, a uniform higher output was noticed in T_{35} with repeated autoclaving and cooling cycles. Under a restricted autoclaving of upto two times maximum yield of RDS was recorded under T_{31} (15.8%) and the yield of T_{13} (11.2%) and T_{35} (10.2%) were on par with T_{31}. As the autoclaving was incremented by one, maximum yield resulted under T_{37} (15.1%) and all the treatments except T_{31} were on par. When the autoclaving was further augmented by one, the yield was found to be maximum from T_{35} (14.8%) and was found to be superior to all other treatments. In general, a steep decrease in the yield of RDS was noticed under four autoclavings in relation to the RDS content of control (14.04 %) in the different processing treatments under consideration except in T_{35}.

Table 16. Effect of repeated autoclaving and cooling on the RDS (%) content of rice starch

Treatments	Processing conditions				RDS (%)		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Number of autoclaving and cooling		
					Two	Three	Four
T_{31}	141	40	0	-20°C	15.8^{a}	6^{b}	4.42^{d}
T_{37}	141	40	10	-20°C	7.21^{b}	15.1^{a}	8.61^{c}
T_{35}	121	60	10	-20°C	10.2^{ab}	12.9^{a}	14.8^{a}
T_{14}	141	60	10	RT	7.57^{b}	9.64^{ab}	7.05^{c}
T_{13}	141	40	10	RT	11.2^{ab}	10.5^{ab}	11.7^{b}
Control *vs* Treatments^{+}					NS	NS	S

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=14.04 per cent, $^{+}$Based on t-test
RT- Room temperature
NS -Not significant
S- Significant

In T_{31}, the RDS content gradually decreased with an increase in the number of autoclaving and cooling cycles

4.3.3. Slowly Digestible Starch (SDS)

The changes noticed in the SDS content of rice starch with an increase in the number of autoclaving and cooling cycles are given in Table 17. As revealed in the table, maximum SDS (13.8%) was observed in rice starch which was subjected to two autoclavings at 141°C for 60 minutes with 10 per cent moisture and cooled at room temperature (T_{14}). In samples which were subjected to three autoclaving and cooling cycles, the SDS content varied from 3.92 per cent (T_{37}) to 11.73 per cent (T_{31}). In four times autoclaved and cooled samples, highest SDS was found in T_{37} (4.15%) and the lowest in T_{14} (0.95%).

When the ouput of SDS was observed at two autoclaving and cooling cycles, the maximum yield realised from T_{14} (13.8%) was found to be on par with all other processing treatments except T_{31} in which the SDS content was found to be very low (2.04%). Under three autoclavings, maximum SDS content was noticed in T_{31} (11.73%). The SDS content of T_{14} (8.39%) and T_{13} (9.46%) were on par with T_{31}. In four autoclaving and cooling cycles, maximum SDS was found in T_{37} (4.15%) and all the other treatments were found to be on par. The yield of SDS under four autoclaving and cooling was found to be significantly low when compared to the SDS content of 7.23 per cent noticed in control irrespective of the temperature, time, moisture and cooling applied to rice starch. In two and three autoclaved samples the variation noticed in the SDS content from the control was found to be statistically insignificant.

4.3.4. Moisture

The moisture content of rice starch subjected to repeated autoclaving and cooling cycles varied from 4.79 per cent (T_{14}) to 8.65 per cent (T_{35}) and from

Table 17. Effect of repeated autoclaving and cooling on the SDS (%) content of rice starch

Treatments	Processing conditions				SDS (%)		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Number of autoclaving and cooling		
					Two	Three	Four
T_{31}	141	40	0	-20°C	2.04^{b}	11.73^{a}	3.46^{a}
T_{37}	141	40	10	-20°C	12^{a}	3.92^{b}	4.15^{a}
T_{35}	121	60	10	-20°C	11.1^{a}	4.47^{b}	1.77^{a}
T_{14}	141	60	10	RT	13.8^{a}	8.39^{ab}	0.95^{a}
T_{13}	141	40	10	RT	7.97^{ab}	9.46^{ab}	2.84^{a}
Control *vs* Treatments^{+}					NS	NS	S

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=7.23 per cent, $^{+}$Based on t-test
RT- Room temperature
NS -Not significant
S- Significant

5.18 per cent (T_{14}) to 7.10 per cent (T_{31}) in two and three autoclaving and cooling cycles respectively. In the samples subjected to four autoclaving and cooling cycles, moisture content varied from 5.79 per cent in T_{37} to 7.1 per cent in T_{13} (Table 18). The lowest moisture content was observed in T_{14} which was subjected to autoclaving at 141°C for 60 minutes with 10 per cent moisture and cooled at room temperature for two and three times.

In the samples which were subjected to two and four autoclaving and cooling cycles, the moisture content varied significantly between the treatments. However, in the starch which was subjected to autoclaving three times, the moisture content of T_{35} and T_{13} were found to be on par.

The moisture content of all the samples subjected to repeated autoclaving and cooling cycles were found to be significantly low when compared with the moisture content of 10.66 per cent noticed in the unprocessed starch (control).

4.3.5. Protein

The protein content of rice starch subjected to two autoclavings varied from 0.09 per cent in T_{35} to 0.50 per cent in T_{31}. In the samples which were autoclaved three and four times, the protein content varied from 1.95 per cent (T_{31} and T_{14}) to 2.19 per cent (T_{13}) and from 1.75 per cent (T_{13}) to 2.19 per cent (T_{14}) respectively (Table 19).

In two autoclaved samples, the highest protein content noticed in T_{31} (0.50%) was found to be significantly higher than all the other treatments. Under three autoclavings, the maximum protein content was obtained in T_{13} (2.19%). The protein content of T_{37} and T_{35} (2.13%) were found to be on par with T_{13}. In four autoclaved samples also, the highest protein content observed in T_{14} (2.19%) was significantly higher than the protein content of rice starch subjected to all other four processing treatments.

Table 18. Effect of repeated autoclaving and cooling on moisture (%) content of rice starch

Treatments	Processing conditions				Moisture (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Two	Three	Four
T_{31}	141	40	0	-20°C	8.18^{c}	7.10^{a}	6.03^{c}
T_{37}	141	40	10	-20°C	8.51^{b}	6.96^{b}	5.79^{e}
T_{35}	121	60	10	-20°C	8.65^{a}	5.80^{c}	6.43^{b}
T_{14}	141	60	10	RT	4.79^{e}	5.18^{d}	5.86^{d}
T_{13}	141	40	10	RT	7.93^{d}	5.76^{c}	7.1^{a}
Control *vs* Treatments^{+}					S	S	S

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=10.66 per cent, $^{+}$Based on t-test
RT- Room temperature
S- Significant

Table 19. Effect of repeated autoclaving and cooling on protein (%) content of rice starch

Treatments	Processing conditions				Protein (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Two	Three	Four
T_{31}	141	40	0	-20°C	0.50^{a}	1.95^{b}	1.95^{b}
T_{37}	141	40	10	-20°C	0.12^{c}	2.13^{a}	2.04^{b}
T_{35}	121	60	10	-20°C	0.09^{c}	2.13^{a}	2.01^{b}
T_{14}	141	60	10	RT	0.15^{bc}	1.95^{b}	2.19^{a}
T_{13}	141	40	10	RT	0.26^{b}	2.19^{a}	1.75^{c}
Control *vs* Treatments^{+}					S	S	S

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=2.22 per cent, $^{+}$Based on t-test
RT- Room temperature
S- Significant

When the protein content of samples subjected to repeated autoclaving and cooling cycles was compared with the protein content of unprocessed starch, a significant decrease was noticed in processed samples irrespective of the time and temperature of autoclaving, moisture content and cooling.

4.3.6. Total carbohydrate

The details pertaining to the effect of repeated autoclaving and cooling on the total carbohydrate content of rice starch are presented in Table 20.

In rice starch which were autoclaved and then cooled twice, the total carbohydrate content varied from 94 per cent (T_{13}) to 97 per cent (T_{37}). In the samples which were autoclaved and cooled three and four times, the content varied from 95 per cent (T_{31} and T_{14}) to 97.5 per cent (T_{37}) and 95 per cent (T_{13}) to 97 per cent (T_{37}) respectively.

The total carbohydrate content of rice starch subjected to two, three and four autoclaving and cooling cycles was found to be high in T_{37} which was autoclaved at 141°C for 40 minutes with 10 per cent moisture and cooled at -20°C. The carbohydrate content noticed in T_{35} and T_{13} autoclaved three times were found to be on par with the maximum carbohydrate content noticed in T_{37}. Though, maximum carbohydrate content of 97 per cent was observed in T_{37} in the samples which were autoclaved two and four times, all the other four treatments were found to be on par with T_{37} only in samples subjected to four autoclaving and cooling cycles.

When the total carbohydrate content of starches subjected to various processing treatments were compared with the unprocessed starch, the content present in processed samples were found to be significantly high irrespective of the temperature, time and number of autoclaving, moisture and cooling conditions.

Table 20. Effect of repeated autoclaving and cooling on the total carbohydrate (%) content of rice starch

Treatments	Processing conditions				Total carbohydrate (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Two	Three	Four
T_{31}	141	40	0	-20°C	94.5^{b}	95^{b}	96.5^{a}
T_{37}	141	40	10	-20°C	97^{a}	97.5^{a}	97^{a}
T_{35}	121	60	10	-20°C	95^{b}	95.5^{ab}	96^{a}
T_{14}	141	60	10	RT	94.5^{b}	95^{b}	95.5^{a}
T_{13}	141	40	10	RT	94^{b}	96^{ab}	95^{a}
Control *vs* Treatments[+]					S	S	S

DMRT column wise comparison

Figures with even alphabets in the same column form one homogeneous group

Control=93 per cent, [+]Based on t-test

RT- Room temperature

S- Significant

4.3.7. Amylose

The amylose content of rice starch subjected to two autoclaving and cooling cycles varied from 28.6 per cent (T_{35}) to 34per cent (T_{37}). In samples which were autoclaved and cooled three and four times, the amylose content ranged from 31per cent (T_{14}) to 34.8 per cent (T_{31}) and 30.8 per cent (T_{13}) to 34.8 per cent (T_{37}) respectively (Table 21).

In samples subjected to two autoclavings, the maximum amylose content of 34 per cent was noticed in rice starch autoclaved at 141°C for 40 minutes with 10 per cent moisture and cooled at -20°C (T_{37}) and was found to be significantly higher than all the other treatments where as in the samples which were autoclaved and cooled three and four times no significant difference was noticed.

The variation observed in the amylose content of rice starches subjected to two, three and four autoclaving and cooling cycles was not significantly different from control. In the case of two autoclaving and cooling cycles, maximum output of amylose content was noticed in T_{37} and was significantly higher than all other treatments. When the number of cycles were further increased up to four times such a significant variation ceased to exist.

4.3.8. Amylopectin

In rice starch subjected to two autocalving and cooling cycles, the amylopectin content varied from 66 per cent (T_{37}) to 71.4 per cent (T_{35}). In the samples which were autoclaved and cooled three and four times, the content varied from 65.2 per cent (T_{31}) to 69 per cent (T_{14}) and 65.2 per cent (T_{37}) to 69.2 per cent (T_{13}) respectively (Table 22).

The maximum amylopectin content observed in two cycle process was in T_{35}. All the other treatments except T_{37} were on par with T_{35}. In the three cycle process, no significant difference among treatments was found. The same results hold good in the four cycle process also. The variation observed

Table 21. Effect of repeated autoclaving and cooling on the amylose (%) content of rice starch

Treatments	Processing conditions				Amylose (%)		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Number of autoclaving and cooling		
					Two	Three	Four
T_{31}	141	40	0	-20°C	31.4^b	34.8^a	33.4^a
T_{37}	141	40	10	-20°C	34^a	34.4^a	34.8^a
T_{35}	121	60	10	-20°C	28.6^b	31.8^a	32.6^a
T_{14}	141	60	10	RT	31^b	31^a	34.6^a
T_{13}	141	40	10	RT	33^b	33.2^a	30.8^a
Control *vs* Treatments$^+$					NS	NS	NS

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=35.6 per cent, $^+$Based on t-test
RT- Room temperature
NS -Not significant

Table 22. Effect of repeated autoclaving and cooling on the amylopectin (%) content of rice starch

Treatments	Processing conditions				Amylopectin (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Two	Three	Four
T_{31}	141	40	0	-20°C	68.6^{a}	65.2^{a}	66.6^{a}
T_{37}	141	40	10	-20°C	66^{b}	65.6^{a}	65.2^{a}
T_{35}	121	60	10	-20°C	71.4^{a}	68.2^{a}	67.4^{a}
T_{14}	141	60	10	RT	69^{a}	69^{a}	65.4^{a}
T_{13}	141	40	10	RT	67^{a}	66.8^{a}	69.2^{a}
Control *vs* Treatments^{+}					NS	NS	NS

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=64.4 per cent, $^{+}$Based on t-test
RT- Room temperature
NS -Not significant

due to processing on the amylopectin content of rice starch irrespective of conditions was found to be statistically insignificant when compared to unprocessed starch.

4.3.9. Non starch polysaccharides (NSP)

Non starch polysaccharide content of rice starch subjected to two autoclaving and cooling process varied from 6.26 per cent (T_{14}) to 11.3 per cent (T_{37}) (Table 23). In samples subjected to three autoclaving process, maximum NSP was found in T_{37} (11.4%) and the lowest was in T_{14} (7.62%). In the samples which were autoclaved and cooled four times, the content varied from 8.45 per cent in T_{13} to 11.3 per cent in T_{37}.

In all the three autoclavings, maximum was seen in the samples subjected to autoclaving at 141°C for 40 minutes with 10 per cent moisture and cooled at -20°C (T_{37}) and was found to be significantly high only in samples autoclaved twice. In four cycle process, all the other four treatments were found to be on par with T_{37}, while in rice starch subjected to three autoclaving, except T_{14} (7.62%) all the other three treatments were found to be on par.

There was no significant difference between the processed starch using different treatments and control irrespective of the number of cycles.

4.4. Quality evaluation of resistant starch supplemented rice flour (RSRF) and products

From the experiment conducted to study the effect of repeated autoclaving and cooling on RS formation, two treatments with highest RS content was selected for the standardisation of resistant starch supplemented rice flour (RSRF). It was seen that among the five different treatments experimented with varying temperature and time of autoclaving, moisture and cooling conditions at two, three and four autoclaving and cooling cycles, maximum RS (92.12%) was noticed in rice starch autoclaved at 141°C for 40 minutes without moisture and cooled at

Table 23. Effect of repeated autoclaving and cooling on the non starch polysaccharide (%) content of rice starch

Treatments	Processing conditions				Non starch polysaccharides (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling (-20°C /RT)	Two	Three	Four
T_{31}	141	40	0	-20°C	8.79[b]	9.29[ab]	9.54[a]
T_{37}	141	40	10	-20°C	11.3[a]	11.4[a]	11.3[a]
T_{35}	121	60	10	-20°C	8.87[b]	10.6[a]	10.3[a]
T_{14}	141	60	10	RT	6.26[c]	7.62[b]	9.79[a]
T_{13}	141	40	10	RT	9.13[b]	10.3[a]	8.45[a]
Control *vs* Treatments[+]					NS	NS	NS

DMRT column wise comparison
Figures with even alphabets in the same column form one homogeneous group
Control=8.13 per cent, [+]Based on t-test
RT- Room temperature
NS -Not significant

-20°C for four times (T_{31}) followed by T_{14} (92%) which was also autoclaved and cooled four times at141°C for 60 minutes with 10 per cent moisture and cooled at room temperature.

The selected two rice starches were incorporated with *puttu* and *idiappam* flour so as to get 10, 15 and 20g of RS per 100 g of rice flour. Roasted *puttu* and *idiappam* flour without RS incorporation was taken as the control. The physical qualities of resistant starch supplemented rice flour (RSRF) and control were carried out. Two popular roasted rice flour based products namely *puttu* and *idiappam* were prepared with the resistant starch supplemented rice flour and the control. The *in vitro* starch digestibility of products was also assessed. The results are given in this section from 4.4.1 to 4.4.3.

4.4.1. Physical qualities of resistant starch supplemented rice flour (RSRF)

4.4.1.1. Bulk density

The bulk density of RS supplemented *puttu* flour and *idiappam* flour varied from 0.77 to 0.79 g/cc and 0.76 to 0.78 g/cc respectively (Table 24). Both the flour without RS supplementation (control) had a bulk density of 0.79 g/cc.

The bulk density of RS supplemented *puttu* flour was found to be on par with the bulk density of *puttu* flour without RS supplementation (control). The bulk density of *idiappam* flour prepared with varying levels of RS supplementation was also found to be on par. However, the bulk density of $T_{14\,(1)}$ and $T_{14\,(2)}$ prepared so as to yield 10 and 15g RS respectively per 100g of rice flour varied significantly from the bulk density of *idiappam* flour prepared wihout any RS supplementation.

Table 24. Bulk density of RS supplemented rice flour

Treatments	Effective yield of RS (g/100g of rice flour)	Bulk density (g/cc)	
		Puttu flour	*Idiappam* flour
$T_{31\ (1)}$	10	0.77[a]	0.78[ab]
$T_{31\ (2)}$	15	0.79[a]	0.78[ab]
$T_{31\ (3)}$	20	0.78[a]	0.77[ab]
$T_{14\ (1)}$	10	0.78[a]	0.76[b]
$T_{14\ (2)}$	15	0.79[a]	0.76[b]
$T_{14\ (3)}$	20	0.79[a]	0.77[ab]
Control		0.79[a]	0.79[a]

Figures with even alphabets in the same column form one homogeneous group

4.4.1.2. Water absorption index (WAI)

Water absorption index of RS supplemented *puttu* flour varied from 3.73 ($T_{14(3)}$) to 4.45 ($T_{31(2)}$) (Table 25). The WAI of *puttu* flour without RS supplementation was found to be 5.03. In *idiappam* flour, the water absorption index of RS supplemented flour varied from 4.2 ($T_{14\ (3)}$) to 4.53 ($T_{31\ (3)}$). The *idiappam* flour without RS supplementation had a water absorption index of 4.72. The water absorption index of RS supplemented *puttu* and *idiappam* flour was found to be significantly lower than the flour without RS supplementation. The highest WAI noticed in $T_{31\ (2)}$ in the case of *puttu* flour and $T_{31\ (3)}$ in *idiappam* flour supplemented with RS so as to yield 15 and 20 g of RS per 100g rice flour was also found to be significantly different from all other treatments prepared with RS supplementation in their respective category.

4.4.1.3. Water solubility index (WSI)

Water solubility index of *puttu* flour supplemented with RS varied from 0.60 per cent to two per cent (Table 26). *Puttu* flour supplemented with RS so as to yield 10g of RS per 100g rice flour ($T_{31\,(1)}$) showed the highest WSI where as the flour supplemented with RS so as to yield 15g RS per 100g rice flour ($T_{31\,(2)}$) showed the lowest WSI. The WSI of $T_{31\,(3)}$ and $T_{14\,(1)}$ were on par with $T_{31\,(1)}$ as regards *puttu* flour.

Table 25. Water absorption index of RS supplemented rice flour

Treatments	Effective yield of RS (g/100g of rice flour)	Water absorption index (ratio)	
		Puttu **flour**	***Idiappam*** **flour**
$T_{31\,(1)}$	10	4.05^{c}	4.31^{cd}
$T_{31\,(2)}$	15	4.45^{b}	4.36^{c}
$T_{31\,(3)}$	20	3.97^{cd}	4.53^{b}
$T_{14\,(1)}$	10	4.2^{c}	4.39^{c}
$T_{14\,(2)}$	15	3.81^{de}	4.21^{d}
$T_{14\,(3)}$	20	3.73^{e}	4.2^{d}
Control		5.03^{a}	4.72^{a}

Figures with even alphabets in the same column form one homogeneous group

Table 26. Water solubility index of RS supplemented rice flour

Treatments	Effective yield of RS (g/100g of rice flour)	Water solubility index (%)	
		Puttu **flour**	***Idiappam*** **flour**
$T_{31\,(1)}$	10	2.00^{a}	1.09^{b}
$T_{31\,(2)}$	15	0.60^{c}	0.84^{b}
$T_{31\,(3)}$	20	1.46^{ab}	1.06^{b}
$T_{14\,(1)}$	10	1.37^{ab}	2.2^{a}
$T_{14\,(2)}$	15	1.24^{bc}	1.04^{b}
$T_{14\,(3)}$	20	0.65^{c}	1.01^{b}
Control		0.65^{c}	0.68^{b}

Figures with even alphabets in the same column form one homogeneous group

In case of *idiappam* flour significantly higher WSI was noticed in $T_{14(1)}$ when compared to other RS supplemented *idiappam* flours. Water solubility index of *idiappam* flour supplemented with RS ranged between 0.84 per cent ($T_{31(2)}$) to 2.2 per cent ($T_{14\ (1)}$) (Table 26). Lowest WSI was noticed in control which was found to be on par with all other treatments except $T_{14\ (1)}$.

4.4.2. Organoleptic evaluation of products prepared with RSRF

Puttu and *idiappam* prepared using resistant starch supplemented rice flour and rice flour without RS supplementation were evaluated organoleptically for different quality attributes like appearance, colour, flavour, texture, taste and overall acceptability using score card. The mean scores and rank scores obtained for various quality attributes of *puttu* and *idiappam* are presented in this section.

4.4.2.1. *Puttu*

The agreement among judges on evaluating the quality attributes like appearance, colour, flavour, texture, taste and overall acceptability of *puttu* was found to be statistically significant based on Kendall's (W) value.

As revealed in Table 27, the mean score for appearance of *puttu* prepared using RS supplemented flour varied from 7.8 to 8.5 with a mean rank score in between 2.65 and 5.1. The mean score of *puttu* prepared using rice flour without RS supplementation was 9 with a rank score of 6.7.

The mean score for colour varied from 7.7 ($T_{31\ (2)}$) to 8.4 ($T_{14\ (1)}$) with a rank score in between 2.35 and 4.95. When compared to the *puttu* prepared with RS supplemented flour, *puttu* prepared without supplementation obtained the highest score of 9 and a rank score of 6.7.

Table 27. Mean scores for organoleptic qualities of *puttu* prepared with RSRF

Treatments	Effective yield of RS (g/100g of rice flour)	Mean scores					
		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
$T_{31\ (1)}$	10	8.3 (4.25)	8.1 (4.3)	7.9 (3.85)	8.2 (4.45)	8.1 (4.4)	8.3 (4.95)
$T_{31\ (2)}$	15	7.8 (2.65)	7.7 (2.35)	7.7 (2.95)	8.1 (4.25)	7.9 (3.8)	7.8 (3.1)
$T_{31\ (3)}$	20	7.9 (2.8)	7.8 (2.85)	7.3 (1.75)	7.4 (2.55)	7.4 (2.3)	7.5 (2.3)
$T_{14\ (1)}$	10	8.5 (5.1)	8.4 (4.95)	8.4 (5.5)	8.1 (4.35)	8.4 (5.1)	8.4 (5.25)
$T_{14\ (2)}$	15	8.2 (3.7)	7.9 (3.55)	7.9 (3.75)	7.7 (3.05)	7.8 (3.35)	7.7 (3.1)
$T_{14\ (3)}$	20	7.9 (2.8)	7.9 (3.3)	7.8 (3.5)	7.6 (2.45)	7.4 (2.25)	7.6 (2.5)
Control		9.0 (6.7)	9.0 (6.7)	8.9 (6.7)	9.0 (6.9)	9.0 (6.8)	9.0 (6.8)
Kendall's (W) Value		0.528**	0.521**	0.643**	0.526**	0.601**	0.659**

For flavour and texture, the mean score of *puttu* prepared with RS supplemented rice flour varied from 7.3 to 8.4 and 7.4 to 8.2 respectively. The rank score varied from 1.75 to 5.5 for flavour and 2.55 to 4.45 for texture. For flavour, the highest mean and rank score were obtained for the *puttu* prepared with rice flour supplemented with RS from T_{14} so as to yield 10g RS per 100g rice flour. *Puttu* prepared with the same supplementation of 10g RS ($T_{31\ (1)}$) obtained the highest mean and rank scores for texture. When compared to these treatments, *puttu* prepared with rice flour without RS supplementation obtained the high mean score of 8.9 for flavour and 9 for texture with rank scores of 6.7 and 6.9 respectively.

For taste and overall acceptability also $T_{14\ (1)}$ obtained the highest mean scores of 8.4 with a rank score of 5.1 for taste and 5.25 for overall acceptability. $T_{31\ (3)}$ and $T_{14\ (3)}$ obtained the lowest mean scores of 7.4 for taste. The lowest score of 7.5 for overall acceptability was found to be in $T_{31\ (3)}$.

Thus, among the different RS supplemented *puttu*, $T_{14(1)}$ which was supplemented with RS prepared from rice starch autoclaved at 141°C for 60 minutes with 10 per cent moisture and cooled at room temperature with four autoclaving and cooling cycles and to yield 10g RS per 100g rice flour obtained the highest mean score for appearance, colour, flavour, taste and overall acceptability.

4.4.2.2. *Idiappam*

Based on Kendall's (W) value, significant agreement was observed among the judges in the evaluation of all the organoleptic qualities of *idiappam*.

The mean score for appearance of *idiappam* prepared using RS supplemented flour varied from 7.7 ($T_{31\ (3)}$) to 8.7 ($T_{31\ (2)}$) with a rank score in the range of 1.80

to 5.30 (Table 28). Mean score for *idiappam* prepared without RS supplementation was found to be 8.9 with a rank score of 6.35.

The mean score for colour varied from 7.5 ($T_{31\ (3)}$) to 8.4 ($T_{31\ (2)}$ and $T_{14\ (1)}$) with a rank score in between 1.7 and 5. When compared to the *idiappam* prepared with RS supplemented flour, *idiappam* prepared without any supplementation obtained the highest score of 8.9 and a rank score of 6.6.

For flavour and texture, the mean score of *idiappam* prepared with RS supplemented rice flour varied from 7.4 to 8.2 and 7.3 to 8.4 respectively. The rank scores varied from 1.95 to 4.55 for flavour and 1.75 to 5.25 for texture. The highest mean score for flavour was obtained for *idiappam* prepared with rice flour supplemented with RS to yield 15g RS per 100g rice flour in $T_{31\ (2)}$ and also for *idiappam* prepared with rice flour supplemented with RS to yield 10g RS per 100g rice flour in $T_{14\ (1)}$. For texture, highest mean and rank scores were obtained for *idiappam* prepared with rice flour supplemented with RS to yield 15g RS per 100g rice flour in $T_{31(2)}$. When compared to these treatments, *idiappam* prepared with rice flour without RS supplementation obtained the highest mean score of 8.9 for both flavour and texture. The rank scores were found to be 6.85 and 6.8 respectively for flavour and texture.

For taste, highest mean score of 8.3 was obtained for *idiappam* prepared with rice flour supplemented with RS to yield 15g RS per 100g rice flour in $T_{31\ (2)}$ and also for *idiappam* prepared with rice flour supplemented with RS to yield 10g RS per 100g rice flour in $T_{14\ (1)}$. Mean rank scores were found to be 5 and 5.1 respectively for $T_{31\ (2)}$ and $T_{14\ (1)}$. *Idiappam* prepared with rice flour supplemented with RS to yield 20g RS per 100g rice flour in $T_{31\ (3)}$ obtained the lowest mean score of 7.3 and rank score of 1.85 for taste.

Table 28. Mean scores for organoleptic qualities of *idiappam* prepared with RSRF

Treatments	Effective yield of RS (g/100g of rice flour)	Mean scores					
		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
$T_{31\ (1)}$	10	8.2 (3.60)	8.2 (3.9)	7.9 (3.45)	7.8 (3.1)	7.9 (3.5)	8 (3.5)
$T_{31\ (2)}$	15	8.7 (5.30)	8.4 (4.9)	8.2 (4.55)	8.4 (5.25)	8.3 (5.0)	8.4 (5.25)
$T_{31\ (3)}$	20	7.7 (1.80)	7.5 (1.7)	7.7 (2.7)	7.4 (2.05)	7.3 (1.85)	7.5 (2.0)
$T_{14\ (1)}$	10	8.5 (4.95)	8.4 (5.0)	8.2 (4.35)	8.2 (4.85)	8.3 (5.1)	8.3 (4.75)
$T_{14\ (2)}$	15	8.1 (3.25)	8.2 (4.0)	8.1 (4.15)	8.1 (4.2)	7.9 (3.7)	8 (3.85)
$T_{14\ (3)}$	20	8 (2.75)	7.7 (1.9)	7.4 (1.95)	7.3 (1.75)	7.4 (2.05)	7.5 (1.95)
Control		8.9 (6.35)	8.9 (6.6)	8.9 (6.85)	8.9 (6.8)	9 (6.8)	8.9 (6.7)
Kendall's (W) Value		0.599**	0.702**	0.57**	0.743**	0.721**	0.702**

The mean score for overall acceptability varied from 7.5 ($T_{31\ (3)}$ and $T_{14\ (3)}$) to 8.4 ($T_{31(2)}$) with rank score in beteen 1.95 and 5.25. When compared to the *idiappam* prepared with RS supplemented flour, *idiappam* prepared without any supplementation obtained the highest score of 8.9 and a rank score of 6.7.

Thus, among the different RS supplemented *idiappam*, $T_{31(2)}$ which was supplemented with RS prepared from rice starch autoclaved at 141°C for 40 minutes without moisture and cooled at -20°C with four autoclaving and cooling cycles and to yield 15g RS per 100g rice flour obtained the highest mean score for all the five quality attributes as well as for overall acceptability.

4.4.3. *In vitro* starch digestibility of products prepared with RSRF

In vitro digestibility of starch in *puttu* and *idiappam* prepared with RSRF were assessed with respect to RS, RDS and SDS and compared with the products prepared without RS incorporation.

4.4.3.1. Resistant starch (RS)

The resistant starch content of *puttu* prepared with rice flour supplemented with RS at varying levels varied from 11.66 per cent to 20.77 per cent (Table 29). The highest RS content (20.77%) was noticed in *puttu* prepared with rice flour supplemented with RS to yield 10g RS per 100g rice flour in $T_{14\ (1)}$ and was found to be significantly higher than all other treatments. *Puttu* prepared with rice flour supplemented with RS to yield 20g RS per 100g rice flour in $T_{14\ (3)}$ had the lowest RS content of 11.66 per cent. The RS content of *puttu* prepared without supplementation was found to be 8.59 per cent.

Table 29. Resistant Starch (RS) content of *puttu* and *idiappam* prepared with RSRF

Treatments	Effective yield of RS (g/100g of rice flour)	RS (%)	
		Puttu	*Idiappam*
$T_{31\,(1)}$	10	18.4[b]	19.31[b]
$T_{31\,(2)}$	15	12.65[c]	17.09[d]
$T_{31\,(3)}$	20	11.82[c]	16.95[d]
$T_{14\,(1)}$	10	20.77[a]	21.2[a]
$T_{14\,(2)}$	15	16.95[b]	18.24[c]
$T_{14\,(3)}$	20	11.66[c]	16.80[d]
Control		8.59[d]	16.73[d]

Figures with even alphabets in the same column form one homogeneous group

The resistant starch content of *idiappam* prepared with RS supplemented flour varied from 16.80 per cent to 21.2 per cent (Table 29). The RS content was found to be maximum in *idiappam* prepared with 10g RS per 100g rice flour in $T_{14(1)}$ and was found to be significantly higher than all other treatments. Minimum RS content of 16.80 per cent was noticed in *idiappam* prepared with rice flour supplemented with RS to yield 20g RS per 100g rice flour in $T_{14\,(3)}$ and was found to be on par with $T_{31\,(2)}$ and $T_{31\,(3)}$. *Idiappam* prepared without supplementation had 16.73 per cent RS and was also found to be on par with the RS content of $T_{31(2)}$, $T_{31\,(3)}$ and $T_{14\,(3)}$.

4.4.3.2. Rapidly digestible starch (RDS)

The rapidly digestible starch content of *puttu* prepared with RS supplemented flour varied from 50.09 per cent to 77.26 per cent (Table 30). The highest RDS content was noticed in *puttu* in which 20g RS per 100g rice flour was incorporated ($T_{31(3)}$). The *puttu* prepared with a supplementation of RS to yield 10g RS per 100g rice flour in $T_{31\,(1)}$ had the lowest RDS content (50.09%). *Puttu* prepared exclusively with rice flour had the RDS content of 83 per cent and was found to be significantly higher than all other treatments.

Table 30. Rapidly Digestible Starch (RDS) content of *puttu* and *idiappam* prepared with RSRF

Treatments	Effective yield of RS (g/100g of rice flour)	RDS (%)	
		Puttu	***Idiappam***
$T_{31\ (1)}$	10	50.09^{g}	68.49^{a}
$T_{31\ (2)}$	15	72.70^{d}	57.26^{e}
$T_{31\ (3)}$	20	77.26^{b}	55.00^{f}
$T_{14\ (1)}$	10	63.89^{f}	60.11^{d}
$T_{14\ (2)}$	15	71.06^{e}	66.77^{b}
$T_{14\ (3)}$	20	76.12^{c}	65.71^{c}
Control		83^{a}	66.68^{b}

Figures with even alphabets in the same column form one homogeneous group

The RDS content of *idiappam* prepared with rice flour supplemented with RS at varying levels ranged between 55 per cent and 68.49 per cent (Table 30). *Idiappam* prepared with rice flour supplemented with RS to yield 10g RS per 100g of flour in $T_{31\ (1)}$ had the highest RDS content of 68.49 per cent and was significantly higher than all other treatments. The lowest RDS content of 55 per cent was observed in *idiappam* prepared with rice flour supplemented with RS to yield 20g RS per 100g flour in $T_{31\ (3)}$. The RDS content of *idiappam* prepared without RS was found to be 66.68 per cent and was found to be on par with $T_{14\ (2)}$.

4.4.3.3. Slowly digestible starch (SDS)

Slowly digestible starch content of *puttu* prepared with rice flour supplemented with RS at varying levels ranged between 10.92 per cent and 31.51 per cent (Table 31). The highest SDS content was found in *puttu* prepared with rice flour supplemented with RS to yield 10g RS per 100g flour in $T_{31\ (1)}$ and was found to be significantly higher than all other treatments. Lowest SDS content was noticed in *puttu* prepared with rice flour supplemented with RS to yield 20g RS per 100g flour in $T_{31\ (3)}$. The SDS content of *puttu* prepared without RS was found to be 8.42 per cent.

Table 31. Slowly Digestible Starch (SDS) content of *puttu* and *idiappam* prepared with RSRF

Treatments	Effective yield of RS (g/100g of rice flour)	SDS (%)	
		Puttu	*Idiappam*
$T_{31\ (1)}$	10	31.51[a]	12.19[f]
$T_{31\ (2)}$	15	14.65[b]	25.65[b]
$T_{31\ (3)}$	20	10.92[d]	28.05[a]
$T_{14\ (1)}$	10	15.34[b]	18.69[c]
$T_{14\ (2)}$	15	11.99[c]	14.99[e]
$T_{14\ (3)}$	20	12.22[c]	17.49[d]
Control		8.42[e]	16.59[d]

Figures with even alphabets in the same column form one homogeneous group

Slowly digestible starch content of *idiappam* prepared with RS supplemented flour ranged from 12.19 per cent in $T_{31\ (1)}$ to 28.05 per cent in $T_{31\ (3)}$ (Table 31). Highest SDS content was observed in *idiappam* prepared with rice flour supplemented with RS to yield 20 g RS per 100g flour. The lowest SDS content (12.19%) was observed in *idiappam* prepared with rice flour supplemented with RS to yield 10g RS per 100g flour in $T_{31(1)}$. Slowly digestible starch content of *idiappam* prepared without RS was found to be 16.59 per cent and was found to be on par with $T_{14\ (3)}$.

4.5. Storage stability of resistant starch supplemented rice flour (RSRF)

On the basis of the mean organoleptic scores obtained for *puttu* and *idiappam* prepared with RSRF, one treatment from each product with highest mean organoleptic scores were selected for storage study. For *puttu* $T_{14(1)}$ and for *idiappam* $T_{31(2)}$ obtained the highest mean scores for various quality attributes as well as for overall acceptability. These two RSRF were packed in polythene bags of 200 gauge thickness and stored for six months under ambient conditions. Roasted rice flour for each product without RS supplementation was taken as the control. All these were analysed for physical qualities, proximate composition, *in vitro* starch digestibility, microbial count and insect infestation before and after storage. *Puttu* and *idiappam* were also prepared with the flour initially and after storing the flour for six months and evaluated the organoleptic qualities. The results are presented in this section.

4.5.1. Physical qualities of RSRF during storage

4.5.1.1. *Puttu* flour

The results pertaining to the physical qualities like bulk density, water absorption index and water solubility index of RS suppelemented *puttu* flour before and after storage are given in Table 32 and in Figure 9a, 9b and 9c.

Initially, the resistant starch supplemented *puttu* flour had a bulk densiy of 0.78g/cc which decreased to 0.77g/cc on storage. In *puttu* flour without RS supplementation the initial bulk density of 0.79g/cc decreased to 0.78g/cc. In both, the decrease was statistically insignificant.Variation noticed in the bulk density of *puttu* flour with and without RS supplementation was also found to be statistically insignificant before as well as after storage.

The water absorption index of *puttu* flour with and without RS supplementation was found to be 4.2 and 5.03 initially, which decreased to 4.16 and 4.46 respectively at the end of storage. The decrease was found to be statistically significant only in *puttu* flour without RS supplementation. The variation observed in the water absorption index of RS supplemented *puttu* flour when compared to the flour without RS supplementation was found to be statistically significant before as well as after storage.

Table 32. Physical qualities of RS supplemented *puttu* flour ($T_{14\,(1)}$)

Physical qualities	RSRF			Control			RSRF *vs* Control	
	Initial (1)	Final (2)	't' value	Initial (3)	Final (4)	't' value	't' value	
							(1) *vs* (3)	(2) *vs* (4)
Bulk density (g/cc)	0.78	0.77	1.74 NS	0.79	0.78	1.51 NS	1.99NS	2.13NS
Water absorption index (ratio)	4.2	4.16	0.83 NS	5.03	4.46	6.33*	9.31**	20.84**
Water solubility index (%)	1.37	0.98	2.19 NS	0.65	0.64	0.50 NS	5.22**	2.98*

Table 33. Physical qualities of RS supplemented *idiappam* flour ($T_{31\,(2)}$)

Physical qualities	RSRF			Control			RSRF *vs* Control	
	Initial (1)	Final (2)	't' value	Initial (3)	Final (4)	't' value	't' value	
							(1) *vs* (3)	(2) *vs* (4)
Bulk density (g/cc)	0.78	0.76	3.99 NS	0.79	0.77	1.72 NS	1.41NS	2.11NS
Water absorption index (ratio)	4.36	4.36	0.048 NS	4.72	4.68	0.392 NS	3.46*	26.79**
Water solubility index (%)	0.84	0.78	2.28 NS	0.68	0.67	1.51 NS	9.39**	2.72 NS

NS – Not significant
* - Significant at 5% level
**- Significant at 1% level

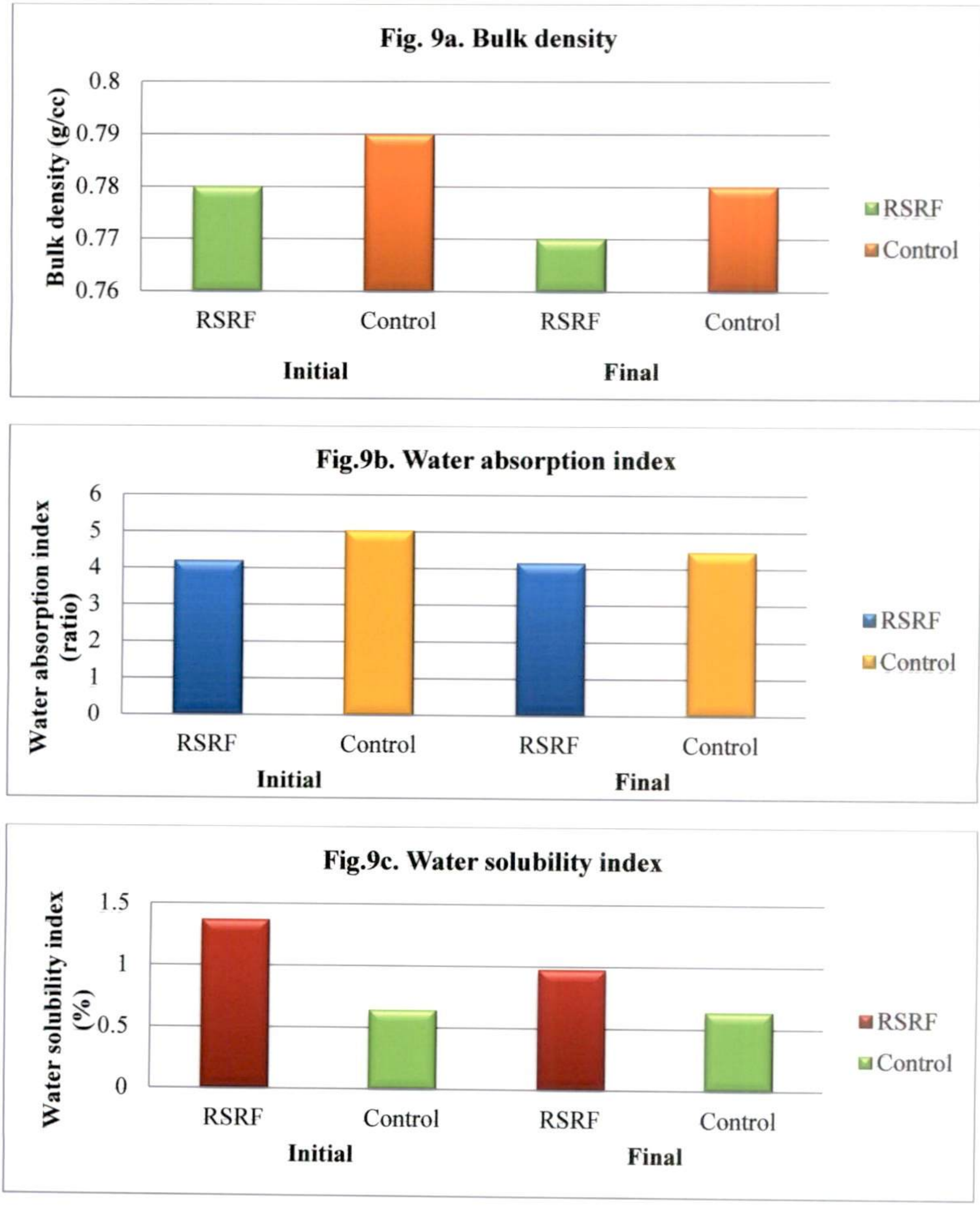


Fig. 9. Physical qualities of RS supplemented *puttu* flour during storage

RSRF- Resistant starch supplemented rice flour

The water solubility index of RS supplemented *puttu* flour was found to be 1.37 per cent before storage which decreased to 0.98 per cent at the end of storage. In *puttu* flour without RS supplementation, water solubility index was found to be 0.65 per cent which showed a slight decrease (0.64%) at the end of storage. The water solubility index of RS supplemented *puttu* flour was found to be significantly high when compared to *puttu* flour without RS supplementation before as well as after storage.

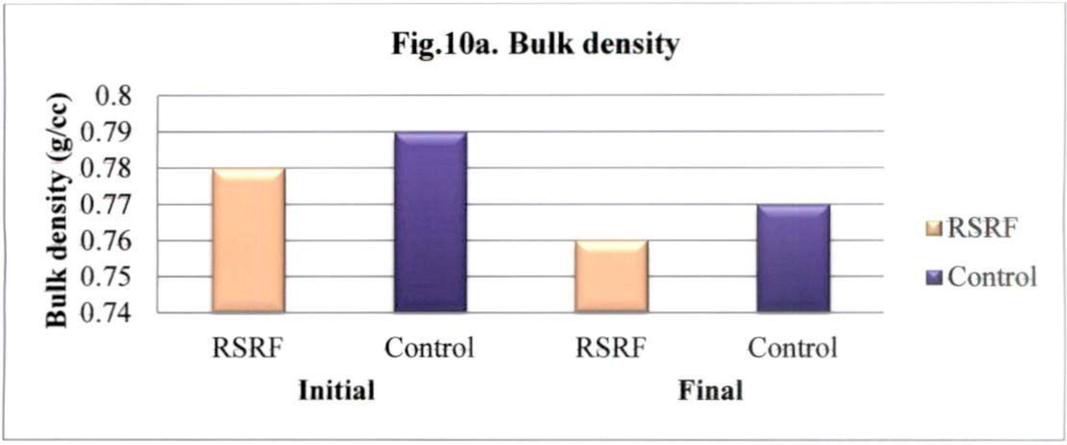
4.5.1.2. *Idiappam* flour

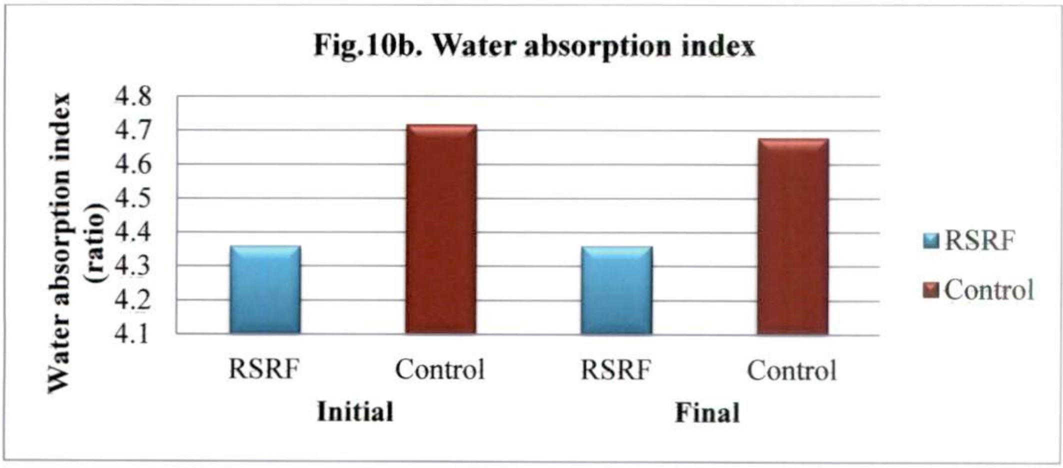
The changes noticed in the physical qualities of RS supplemented *idiappam* flour are given in Table 33 and in Figure 10a, 10b and 10c.

The bulk density of *idiappam* flour with and without RS supplementation was found to be 0.78 and 0.79g/cc initially which decreased to 0.76 and 0.77g/cc at the end of storage. Variation noticed in the bulk density of *idiappam* flour with and without RS supplementation was found to be statistically insignificant before and after storage.

Initially, the RS supplemented *idiappam* flour had a water absorption index of 4.36 which did not change during storage. In *idiappam* flour without RS supplementation the initial water absorption index of 4.72 decreased to 4.68 at the end of storage. The variation observed in the water absorption index of RS supplemented *idiappam* flour when compared to the *idiappam* flour without RS supplementation was found to be statistically significant before as well as after storage.

Initially, the water solubility index of *idiappam* flour with and without RS supplementation was found to be 0.84 and 0.68 per cent respectively. At the end of storage, the WSI of RS supplemented *idiappam* flour decreased to 0.78 per cent while in flour without supplementation it decreased to 0.67 per cent. The variation





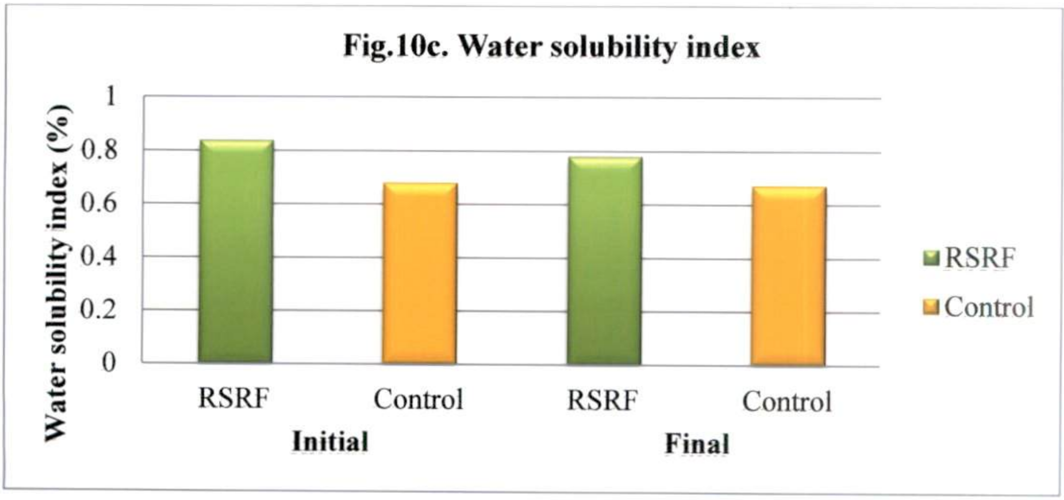


Fig.10. Physical qualities of RS supplemented *idiappam* flour during storage

RSRF- Resistant starch supplemented rice flour observed in the water solubility index of RS supplemented *idiappam* flour when compared to the *idiappam* flour without RS supplementation was found to be statistically significant before storage.

The changes observed in all the three physical qualities at the end of storage were found to be statistically insignificant in the flours with and without RS supplementation.

4.5.2. Proximate composition of RSRF during storage

4.5.2.1. *Puttu* flour

The results pertaining to the moisture, protein and total carbohydrate content of RS supplemented *puttu* flour before and after storage are given in Table 34.

Moisture content of RS supplemented *puttu* flour was found to be 5.43 per cent which increased to 6.57 per cent at the end of storage. In *puttu* flour without RS supplementation the initial moisture content of 5.40 per cent increased to 6.06 per cent at the end of storage. The increase observed in the moisture content of both flours during storage was found to be statistically significant. However, the variation observed in the moisture content of RS supplemented flour and the flour without RS supplementation was found to be statistically significant only during the later part of storage.

The protein content of RS supplemented *puttu* flour was found to be 7.47 per cent which decreased to 5.80 per cent on storage. In *puttu* flour without RS supplementation the initial protein content of 7.67 per cent decreased to 4.49 per cent during storage. The decrease noticed in the protein content of flour during storage was found to be statistically significant.

Table 34. Proximate composition of RS supplemented *Puttu* flour ($T_{14\ (1)}$)

Proximate composition	RSRF			Control			RSRF *vs* Control	
	Initial (1)	Final (2)	't' value	Initial (3)	Final (4)	't' value	't' value (1) *vs* (3)	't' value (2) *vs* (4)
Moisture (g/100g)	5.43	6.57	6.49*	5.40	6.06	10.53**	0.162^{NS}	7.38**
Protein (g/100g)	7.47	5.80	32.90**	7.67	4.49	54.50**	4.95**	15.91**
Total carbohydrate (g/100g)	80.33	79	0.203^{NS}	73.33	72.33	1.73^{NS}	7.42**	1.06^{NS}

Table 35. Proximate composition of RS supplemented *Idiappam* flour ($T_{31\ (2)}$)

Proximate composition	RSRF			Control			RSRF *vs* Control	
	Initial (1)	Final (2)	't' value	Initial (3)	Final (4)	't' value	't' value (1) *vs* (3)	't' value (2) *vs* (4)
Moisture (g/100g)	5.73	6.73	111.92**	5.38	6.82	36.34**	4.98**	1.32^{NS}
Protein (g/100g)	6.15	5.78	4.91*	7.09	5.78	12.99**	16**	0.00^{NS}
Total carbohydrate (g/100g)	87.67	86.33	1.0^{NS}	81	80.67	0.09^{NS}	3.78*	1.45^{NS}

NS – Not significant

* - Significant at 5% level

**- Significant at 1% level

The variation noticed between the protein content of *puttu* flour supplemented with RS when compared to the non supplemented flour was found to be statistically significant before as well as after storage.

Resistant starch supplemented *puttu* flour had 80.33 per cent carbohydrate initially which decreased to 79 per cent at the end of storage. In *putttu* flour without RS supplementation the total carbohydrate content of 73.33 per cent before storage decreased to 72.33 per cent at the end of storage. The decrease observed in the total carbohydrate content of *puttu* flour with and without RS supplementation during storage was found to be statistically insignificant.

The decrease noticed between the total carbohydrate content of RSRF *puttu* flour when compared to the flour without supplementation was statistically significant only during the initial period of storage.

4.5.2.2. *Idiappam* flour

Initially, the moisture content was found to be 5.73 per cent in RS supplemented *idiappam* flour which increased to 6.73 per cent at the end of storage (Table 35). In *idiappam* flour without RS supplementation, the initial moisture content of 5.38 per cent increased to 6.82 per cent at the end of storage. The increase noticed in the moisture content of RSRF and rice flour during storage was found to be statistically significant.

The variation noticed between the moisture content of RS supplemented *idiappam* flour and control flour was statistically significant only during the initial period of storage.

The protein content of RS supplemented *idiappam* flour (Table 35) was found to be 6.15 per cent before storage which decreased to 5.78 per cent at the end of storage. In *idiappam* flour without RS supplementation, the initial protein content of 7.09 per cent decreased to 5.78 per cent at the end of storage. The decrease was found to be statistically significant in both types of flours.

The variation noticed between the protein content of RS supplemented *idiappam* flour and the *idiappam* flour without RS supplementation was found to be statistically significant only during the initial period of storage.

Initially, the RS supplemented *idiappam* flour had a total carbohydrate content of 87.67 per cent which decreased to 86.33 per cent during storage (Tabe 35). In *idiappam* flour without RS supplementation, the initial total carbohydrate content of 81 per cent decreased to 80.67 per cent at the end of storage. In both types of flour, the decrease was statistically insignificant.

The decrease observed between the total carbohydrate content of *idiappam* flour with and without RS supplementation was statistically significant only during the initial period of storage.

4.5.3. Total microflora of RSRF during storage

4.5.3.1. *Puttu* flour

The RS supplemented *puttu* flour was evaluated for bacteria, fungi and yeast initially as also after six months of storage and results pertaining to microbial enumeration are presented in Table 36.

Initially, the RS supplemented *puttu* flour had bacterial count of 2.33 x 10^3 cfu/g which increased to 5 x 10^3 cfu/g at the end of storage. In *puttu* flour without RS supplementation the initial bacterial count of 6.67 x 10^3 cfu/g increased to 9.33 x 10^3 cfu/g during storage.

Table 36. Total microflora of *puttu* flour

Total microflora	RSRF		Control	
	Initial	Final	Initial	Final
Bacteria (x 10^3 cfu/g)	2.33	5	6.67	9.33
Yeast (x 10^2 cfu/g)	ND	0.33	ND	0.67
Fungi (x 10^2 cfu/g)	ND	0.67	ND	0.67

Yeast count was not detected initially in *puttu* flour with and without RS supplementation. However, after storage yeast count of 0.33 x 10^2 cfu/g and 0.67 x 10^2 cfu/g were detected in *puttu* flour with and without RS supplementation.

The fungal count was also not detected in both flour initially. At the end of storage the fungal count of 0.67 x 10^2 cfu/g was noticed in both types of flour.

4.5.3.2. *Idiappam* flour

The results pertaining to microbial enumeration of *idiappam* flour are presented in Table 37. Initially, the RS supplemented *idiappam* flour had a bacterial count of 3x10^3 cfu/g which increased to 6.67 x 10^3 cfu/g during storage. In *idiappam* flour without RS supplementation the initial bacterial count of 1.67 x 10^3 cfu/g increased to 3.67 x 10^3 cfu/g at the end of storage.

Table 37. Total microflora of *idiappam* flour

Total microflora	RSRF		Control	
	Initial	Final	Initial	Final
Bacteria(x 10^3 cfu/g)	3	6.67	1.67	3.67
Yeast (x 10^2 cfu/g)	ND	0.33	ND	0.67
Fungi (x 10^2 cfu/g)	ND	0.33	ND	0.67

Initially, yeast and fungal count were not detected in *idiappam* flour prepared with and without RS supplementation. At the end of storage, yeast count of 0.33 x 10^2 cfu/g and 0.67 x 10^3 cfu/g and fungal count of 0.33 x 10^2 cfu/g and 0.67 x 10^2 cfu/g were detected in both types of flour.

4.5.4. Insect infestation of RS supplemented flour during storage

Insect infestation of *puttu* and *idiappam* flour supplemented with RS was evaluated initially and at the end of six months of storage and compared with rice flour without RS supplementation. Insect infestation was not detected in both types of flour during storage.

4.5.5. Organoleptic qualities of the products prepared with RSRF during storage

Puttu and idiappam were prepared using RS supplemented rice flour before and after storing the flour for six months and were evaluated organoleptically for different quality attributes. The mean scores obtained for various quality attributes of *puttu and idiappam* are presented in this section.

4.5.5.1. *Puttu*

As revealed in Table 38, the mean scores for the appearance of *puttu* prepared with and without RS supplementation were found to be 8.5 and 9 which decreased to 8.3 and 8.4 in *puttu* prepared with stored flour.

The mean score for colour of *puttu* prepared with RS supplementation was found to be 8.4 initially which decreased to 7.9 at the end of storage. In *puttu* prepared without RS supplementation, the mean score for colour was found to be 9 which showed a decrease (8.1) at the end of storage.

Table 38. Mean score for the organoleptic qualities of *puttu* prepared with RSRF ($T_{14\ (1)}$) during storage

Rice flour used	Appearance		Colour		Flavour		Texture		Taste		Overall acceptability	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
RSRF	8.5	8.3	8.4	7.9	8.4	7.7	8.1	8	8.4	7.8	8.4	8
Rice flour	9.0	8.4	9.0	8.1	8.9	7.6	9.0	7.9	9.0	7.6	9.0	7.9

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For flavour and texture, the initial mean scores of *puttu* prepared with RS supplemented flour were found to be 8.4 and 8.1 respectively. With stored flours, the mean scores decreased to 7.7 for flavour and 8 for texture. The mean score of flavour of *puttu* prepared with rice flour was 8.9 initially and 7.6 after storage. In the case of texture it was 9 and 7.9 respectively.

Initially, the mean score for taste of *puttu* prepared with RS supplemented flour was 8.4 and it decreased to 7.8 in product prepared with stored flour. In the case of *puttu* prepared using rice flour, initial mean score for taste was 9 and it decreased to 7.6 in *puttu* made with stored flour.

The overall acceptability score of *puttu* prepared with RS supplemented flour was found to be 8.4 initially which decreased to 8 in *puttu* prepared with stored flour. For *puttu* prepared using rice flour without supplementation the mean score was 9 initially which decreased to 7.9 during storage.

4.5.5.2. *Idiappam*

As revealed in Table 39, initially, mean score for appearance of *idiappam* prepared using rice flour with and without RS supplementation were 8.7 and 8.9 respectively. In *idiappam* prepared with stored flour the mean scores decreased to 8.1 and 8.6 respectively.

The initial mean score for colour of *idiappam* prepared with RS supplemented flour was 8.4 which decreased to 8.1 in the product prepared with stored flour. For *idiappam* prepared without RS supplementation, the initial mean score of 8.9 decreased to 8.2.

In *idiappam* prepared with RS supplemented flour, initial mean score for flavour was found to be 8.2 which decreased to 8.1 at the end of storage, where as

Table 39. Mean score for the organoleptic qualities of *idiappam* prepared with RSRF ($T_{31\ (2)}$) during storage

Rice flour used	Appearance		Colour		Flavour		Texture		Taste		Overall acceptability	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
RSRF	8.7	8.1	8.4	8.1	8.2	8.1	8.4	7.8	8.3	8.1	8.4	8.2
Rice flour	8.9	8.6	8.9	8.2	8.9	8.1	8.9	8.1	9	8.2	8.9	8.4

idiappam prepared without RS supplementation decreased to 8.1 from the initial score of 8.9.

For texture and taste, the mean scores of *idiappam* prepared with RS supplemented flour were found to be 8.4 and 8.3 initially which decreased to 7.8 and 8.1 in products prepared with stored flour. The mean score for texture and taste of *idiappam* prepared without any supplementation were found to be 8.9 and 9 respectively before storage which correspondingly decreased to 8.1 and 8.2 at the end of storage.

Initially, the mean scores for overall acceptability of *idiappam* prepared with and without RS supplementation were 8.4 and 8.9 respectively and the mean scores dereased to 8.2 and 8.4 in products prepared with stored flours.

4.5.6. *In vitro* starch digestibility of RSRF products during storage

The *in vitro* starch digestibility of *puttu* and *idiappam* prepared with RS supplemented rice flour was evaluated with respect to RS, RDS and SDS contents. This was compared with the *in vitro* starch digestibility of *puttu* and *idiappam* prepared with rice flour without RS supplementation. The results are given in Table 40 and 41 respectively. Both the products were prepared using rice flour before as also after storage for six months.

4.5.6.1. *Puttu*

Resistant starch content of *puttu*, prepared with RS supplemented rice flour, was found to be 20.77 per cent initially which increased to 22.30 per cent when it was prepared with stored flour. The RS content of *puttu* prepared using rice flour without RS supplementation was found to be 8.59 per cent when it was prepared with rice flour before as well as after storage. The changes observed in the RS

Table 40. *In vitro* starch digestibility of RS supplemented *puttu* ($T_{14\ (1)}$)

In vitro starch digestibility (%)	RS supplemented *puttu*			Control *puttu*			RSRF *vs* Control	
	Initial (1)	Final (2)	't' value	Initial (3)	Final (4)	't' value	't' value	
							(1) *vs* (3)	(2) *vs* (4)
Resistant starch	20.77	22.3	3.56 NS	8.59	8.59	0.008 NS	114.58**	18.90**
Rapidly Digestible Starch	63.89	63.97	0.242 NS	83	84	20.00*	81.56**	38.54**
Slowly Digestible Starch	15.34	13.73	63.80*	8.42	7.41	12.63*	43.31**	70.66**

Table 41. *In vitro* starch digestibility of RS supplemented *idiappam* ($T_{31\ (2)}$)

In vitro starch digestibility (%)	RS supplemented *idiappam*			Control *idiappam*			RSRF *vs* Control	
	Initial (1)	Final (2)	't' value	Initial (3)	Final (4)	't' value	't' value	
							(1) *vs* (3)	(2) *vs* (4)
Resistant starch	17.09	18.44	0.58 NS	16.73	16.73	0.03 NS	0.82 NS	0.70 NS
Rapidly Digestible Starch	57.26	58.56	4.3 NS	66.68	71.83	1.51 NS	2.72 NS	53.08**
Slowly Digestible Starch	25.65	23.00	26.4*	16.59	11.44	1.29 NS	2.31 NS	73.11**

NS – Not significant

* - Significant at 5% level

**- Significant at 1% level

content of *puttu* prepared with fresh flour and stored flour was found to be statistically insignificant both in RS supplemented and non supplemented flour.

The variation noticed between the resistant starch content of *puttu* prepared using flour with and without RS supplementation was found to be statistically significant during both periods of storage.

The RDS content of *puttu* prepared with RS supplemented flour was found to be 63.89 per cent (before storage) which increased slightly to 63.97 per cent at the end of storage. The increase was found to be statistically insignificant. The RDS content of *puttu* prepared with rice flour without RS supplementation was 83 per cent which increased to 84 per cent during storage. The increase observed was found to be statistically significant.

The RDS content of *puttu* prepared with rice flour without RS supplementation was found to be high when it was prepared with fresh as well as stored rice flour. The increase was found to be statistically significant.

Puttu prepared using RS supplemented rice flour had an SDS content of 15.34 per cent initially, which decreased to 13.73 per cent when *puttu* was prepared with stored rice flour. The SDS content of *puttu* prepared without RS supplemented flour decreased to 7.41 per cent from the initial SDS content 8.42 per cent when it was prepared with stored rice flour and the decrease was found to be statistically significant.

The variation noticed between the SDS content was found to be statistically significant when it was prepred with stored and fresh flour.

4.5.6.2. *Idiappam*

The *idiappam* prepared with RS supplemented flour had an RS content of 17.09 per cent initially which increased to 18.44 per cent when it was prepared with stored flour. *Idiappam* prepared using rice flour without any RS supplementation had 16.73 per cent RS irrespective of storage. The changes noticed were found to be statistically insignificant. Though, the RS content was found to be slightly high in *idiappam* prepared with RS supplemented flour before as well as after storage, the increase was found to be statistically insignificant.

The rapidly digestible starch content of *idiappam* prepared with RS supplemented flour was found to be 57.26 per cent which increased to 58.56 per cent when it was prepared using the stored flour. In *idiappam* prepared exclusively with rice flour, the RDS content increased to 71.83 per cent from the initial value of 66.68 per cent. Here also, the increase observed in the RDS content of *idiappam* prepared using stored rice flour was found to be statistically insignificant.

The variation noticed between the RDS content of *idiappam* prepared with the two types of flour was statistically insignificant during initial periods of storage. Whereas, the variation noticed between the RDS content of *idiappam* prepared with the two types of flour was found to be statistically significant after storage.

The slowly digestible starch content of *idiappam* prepared with RS supplemented flour was found to be 25.6 per cent before storage which decreased to 23 per cent at the end of storage. The decrease was found to be statistically significant. In *idiappam* prepared without RS supplementation, the SDS content decreased to 11.44 per cent from the initial value 16.59 per cent when it was prepared with stored rice flour.The decrease was statistically insignificant.

The variation observed between the SDS content of *idiappam* prepared with and without RS supplemented flour was also found to be statistically insignificant during initial periods of storage.Whereas, significant variation was observed at the end of storage period.

4.6. Postprandial glycaemic response of selected RSRF products

Postprandial glycaemic responses of the selected RSRF and control *puttu* and *idiappam* were assessed in 5 diabetic and 5 non diabetic individuals. The results of the postprandial glycaemic response after consuming the products are given in Table 42 and 43 respectively.

4.6.1. *Puttu*

In normal subjects the blood glucose concentration after consumption of RS supplemented *puttu* increased in the range 7-25mg/dl where as in the same subjects the range varied from 11-28mg/dl when *puttu* prepared without RS supplementation was given.

In diabetic subjects, the increase in blood glucose was in the range 17-57 mg/dl when RSRF *puttu* was given. However, the increase in blood glucose ranged from 28-92mg/dl when subjects consumed *puttu* without RS supplementation.

4.6.2. *Idiappam*

In normal subjects the blood glucose concentration after consumption of RS supplemented *idiappam* increased in the range 8-29mg/dl, whereas in the same subjects the range was from 12-45mg/dl when *idiappam* prepared without RS supplementation was given.

Table 42. Postprandial glycaemic response of *puttu* prepared with RS supplemented rice flour ($T_{14\ (1)}$)

Volunteers		RSRF *puttu*			Control *puttu*		
		Fasting blood sugar (mg/dl)	Postprandial blood sugar (mg/dl)	Increase in blood glucose concentration (mg/dl)	Fasting blood sugar (mg/dl)	Postprandial blood sugar (mg/dl)	Increase in blood glucose concentration (mg/dl)
Normal subjects	N1	92	101	9	94	116	22
	N2	92	117	25	94	105	11
	N3	97	104	7	99	127	28
	N4	102	125	23	98	119	21
	N5	88	108	20	90	104	14
NIDDM subjects	D1	108	125	17	117	145	28
	D2	105	155	50	106	198	92
	D3	115	172	57	109	157	48
	D4	110	154	44	109	147	38
	D5	112	148	36	111	152	41

Table 43. Postprandial glycaemic response of *idiappam* prepared with RS supplemented rice flour ($T_{31\ (2)}$)

Volunteers		RSRF *Idiappam*			Control *idiappam*		
		Fasting blood sugar (mg/dl)	Postprandial blood sugar (mg/dl)	Increase in blood glucose concentration (mg/dl)	Fasting blood sugar (mg/dl)	Postprandial blood sugar (mg/dl)	Increase in blood glucose concentration (mg/dl)
Normal subjects	N1	99	120	21	99	111	12
	N2	91	99	8	90	109	19
	N3	104	121	17	96	124	28
	N4	99	128	29	93	138	45
	N5	86	95	9	90	110	20
NIDDM subjects	D1	108	133	25	116	152	36
	D2	107	135	28	112	199	87
	D3	106	172	66	106	147	41
	D4	110	140	30	118	158	40
	D5	106	132	26	112	150	38

In diabetic subjects the increase in blood glucose was in the range of 25-66 mg/dl when RSRF *idiappam* was given. The increase in blood glucose varied from 36-87mg/dl when subjects consumed *idiappam* without RS supplementation.

4.7. Cost of the developed RSRF

The cost was worked out for the RS supplemented *puttu* and *idiappam* flour which were prepared by incorporating selected starch with the roasted rice flour. The cost of production of RS supplemented *puttu* and *idiappam* flour were worked out on the basis of the cost of rice, processing charge of rice, cost of alkali used for the production of starch and electricity charges. The cost of production of 1kg *puttu* flour supplemented with 10g resistant starch and 1kg *idiappam* flour supplemented with 15g resistant starch were found to be Rs.83.83 and Rs.92.65 respectively.

Discussion

5. DISCUSSION

The results of the study entitled "Optimisation and utilisation of resistant starch for value addition in rice products" are discussed in this chapter under the following headings.

5.1. Resistant starch in raw and parboiled rice starch
5.2. Effect of temperature, time, moisture and cooling conditions on RS content of rice starch
5.3. Effect of repeated autoclaving and cooling on *in vitro* digestibility of starch and proximate composition of rice starch
5.4. Quality characteristics of RSRF and RS supplemented rice products
5.5. Effect of storage on the quality aspects of RSRF and RS supplemented products
5.6. Glycaemic response of RSRF products

5.1. Resistant starch in raw and parboiled rice starch

In the study, starch was isolated from rice variety Uma with high amylose content of 26.4 per cent. Starch content of parboiled rice was found to be slightly higher (66.6%) than raw rice (65.47%). However, the yield of RS from raw rice (78.73%) was more when compared to parboiled rice (76.29%).

The relatively higher yield of starch in parboiled rice might be due to the lower solubilistion of starch of parboiled rice in water when compared to raw rice as suggested by Rao and Juliano (1970). Otegbayo *et al.* (2001) in a study conducted on the effect of parboiling on physico-chemical qualities of two rice varieties of Nigeria indicated increased carbohydrate content in parboiled rice when compared to non-parboiled samples. Kim *et al.* (2006_a) also noticed higher starch content in parboiled uncooked rice. The higher starch content of parboiled rice might also be due to the gelatinisation of starch during parboiling which makes the grain to expand filling up the surrounding air space as indicated by Otegbayo *et al.* (2001). Rao and Juliano (1970) observed that during parboiling reassociation of starch and increase in some carbohydrate components change the molecular size of rice.

The low RS noticed in the starch isolated from parboiled rice when compared to raw rice starch might be due to the changes in the starch molecules occurring during parboiling making it more digestible. Otegbayo *et al.* (2001) indicated solubilisation and leaching of amylose molecules into the surrounding water during soaking and subsequent steaming during parboiling and decrease in the RS content. Gunaratne *et al.* (2013) also noticed a decrease in the RS content by 50 per cent in certain rice varieties during parboiling. Contradictory to this, Kim *et al.* (2006_a) reported high RS as well as fast digestible starch in parboiled rice and noticed more proportion of RS in the total starch fraction of parboiled rice. The reversal phenomenon noticed might be due to the varying time of parboiling as also the size of the rice grain considered.

The increased RS content noticed in raw rice might be due to the low digestibility of raw rice starch when compared to parboiled rice as indicated by Chitra *et al.* (2010) which might be due to the native semicrystalline structure of the raw rice starch.

5.2. Effect of temperature, time, moisture and cooling conditions on RS content of rice starch

When the effect of temperature on RS content of raw rice starch was studied, it was seen that the autoclaved samples had comparatively low RS content both at 121°C and 141°C in association with the two cooling conditions when compared to the RS content of 78.73 per cent in raw rice starch. The percentage decrease varied from 10.92 to 68.46 per cent in starch which were autoclaved at 121°C and from 0.50 to 45.23 per cent at 141°C which were cooled at room temperature (Table 44). In the samples cooled at -20°C, the decrease varied from 8.66 to 63.19 per cent at 121°C and from 1.80 to 36.85 per cent at 141°C. Thus, in all samples the decrease in the RS content was found to be more in rice starch which were autoclaved at 121°C. Though, studies conducted by Sajilata *et al.* (2006) on the effect of repeated autoclaving and other processing and cooling methods indicated an increase in the RS content of foods, the decrease noticed in the present study might be due to the single step autoclaving and cooling given to the rice starch. A parallel study by Chen *et al.* (2010) indicated a decrease in RS during cooking of food.

However, with an increase in the temperature of autoclaving from 121°C to 141°C, the RS content increased to a greater extent in both the samples which were cooled at room temperature as well as at -20°C. The beneficial effect of high autoclaving temperature on RS formation was reported with respect to high amylose corn starch by Dundar and Gocmen (2013). Kim and Kwak (2009) and Brumovsky *et al.* (2009) also indicated a linear increase in the RS content with increase in the temperature of autoclaving as observed in the present study.

With an increase in the time of autoclaving, a decrease in the RS content occurred in rice starch especially in samples which were processed without moisture and cooled at room temperature. Here, the RS content decreased to 15.57 per cent when the samples were autoclaved for 60 minutes at 121°C and to 7.82 per cent at 40 minutes of autoclaving at 141°C (Table 45). However, in the same conditions of autoclaving a slight increase in RS occurred in samples cooled at -20°C. Here, the RS content increased by 3.18 per cent when the samples were autoclaved for 60 minutes at 121°C and to 13.76 per cent at 40 minutes of autoclaving at 141°C.

Table 44. Variation (%) in RS content due to temperature of autoclaving in raw rice starch

Cooling conditions / Temperature (°C)	Moisture (%)							
	0		10		20		40	
	RT	-20°C	RT	-20°C	RT	-20°C	RT	-20°C
121	-14.42	-13.28	-10.92	-8.66	-30.44	-30.14	-68.46	-63.19
141	-8.14	-2.52	-0.50	-1.80	-18.48	-23.68	-45.23	-36.85

RT- Room Temperature

In samples autoclaved with the addition of moisture, the RS content increased by 155.38 per cent when the samples were autoclaved for 60 minutes at 121°C and cooled at -20°C. The effect of increased time of autoclaving on RS formation was positive and telescoping (20→40, 40→60) especially in samples cooled at -20°C. As observed in the present study, Cheung and Chau (1998) and Brumovsky *et al.* (2009) also noticed an increase in the RS content of foods with an increase in cooking time. Milasinovic *et al.* (2010) and Song *et al.* (2012) observed increased RS in maize and rice starch respectively due to autoclaving for longer periods. The increase in the RS content due to prolonged heating might be due to more gelatinisation and the release of more amylose molecules and easy reassociation of polymer chains during cooling leading to better retrogradation of starch as indicated by Cheung and Chau (1998) and Song *et al.* (2012).

The effect of moisture application on RS formation indicated that with an increase in the moisture content from zero to 10 per cent the yield of RS increased both in room temperature and -20°C cooled samples. The RS content increased up to 8.33 per cent and 5.33 per cent respectively in room temperature cooled and -20°C cooled samples (Table 46). Li and Gao (2010) also noticed significant increase in the RS content of heat moisture treated mung bean starch when compared to native starch. Maruta *et al.* (1998) also noticed an increased yield of RS with the application of moisture and indicated that it is due to the rearrangement of molecules to the enzyme resistant structure during heat moisture treatment under non gelatinised condition.

However, with an increase in the per cent of moisture from 10 to 20 per cent and from 20 to 40 per cent the yield of RS decreased considerably in samples autoclaved both at 121°C and 141°C. Greater decrease occurred at the maximum moisture application of 40 per cent in which the RS decreased up to 54.66 per cent and 47.31 per cent respectively in room temperature and -20°C cooled samples. When the samples were treated with excess water, the water will act on the hydrogen bonds between the molecular chains and the chains become flexible for

Table 45. Variation (%) in RS content with increase in the time of autoclaving

Temperature (°C)	Increase in time (Minutes)	Moisture (%)							
		0		10		20		40	
		RT	-20°C	RT	-20°C	RT	-20°C	RT	-20°C
121	20➞40	-13.54	-7.80	-13.90	+1.82	-30.37	+4.34	-11.57	+16.36
	40➞60	-15.57	+3.18	+12.77	+18.23	-6.71	+10.41	-18.66	+155.38
141	20➞40	-7.82	+13.76	+1.82	+7.46	+3.23	-9.01	-3.35	+32.91
	40➞60	-1.13	-9.91	+1.53	-10.21	+4.43	+9.51	+3.32	-35.74

RT- Room Temperature

Table 46. Variation (%) in RS content with increase in moisture application

Temperature (°C)	0 % ➡ 10%		10 % ➡ 20%		20 % ➡ 40%	
	RT	**-20°C**	**RT**	**-20°C**	**RT**	**-20°C**
121	+4.07	+5.33	-21.91	-23.51	-54.66	-47.31
141	+8.33	+0.74	-18.08	-22.27	-32.81	-17.26

RT- Room Temperature

enzyme action as reported by Kurakake *et al.* (1997) and Li *et al.* (2011). This might also be due to increased digestibility of gelatinised starch as indicated by Okoye (1992). Studies conducted by Jacobs *et al.* (1997), Kweon *et al.* (2000), Gunaratne and Hoover (2002), Zavareze *et al.* (2010) and Juansang *et al.* (2012) also indicated a decrease in RS content in foods during heat moisture treatment. Contradictory to this, studies conducted by Luo *et al.* (2003) and Chung *et al.* (2009_a) indicated increase in RS with an increase in moisture application.

In the present study, increase in RS content occurred only at the minimum moisture application of 10 per cent. A moisture application beyond 10 per cent, the RS content decreased to a greater extent. Sievert and Pomeranz (1989), and Li and Gao (2010) also noticed increased yield of RS at low moisture levels. Thus, maintaining appropriate moisture level during processing is essential to obtain an increased yield of RS. Moreover, as indicated by Chung *et al.* (2009_b) the susceptibility to enzyme hydrolysis decreases or increases after heat moisture treatment depending on source of starch, cultivar and processing conditions.

The effect of cooling conditions after autoclaving rice starch indicated better retention of RS in the samples which were cooled at -20°C when compared to room temperature cooled samples. Maximum retention occurred in rice starch cooled at -20°C at a higher moisture level of 40 per cent and at an autoclaving temperature of 121°C (Table 47). Thus, more RS was noticed in most of the samples cooled at -20°C. Vaidya and Sheth (2011) also indicated increased yield of RS in cereal products stored at low temperature. Yadav (2011) indicated pronounced effect on the RS content of pressure cooked potatoes and sweet potatoes on refrigerated storage. Different studies conducted by Mitsuda (1993), Marsono and Topping (1993), Kavita *et al.* (1998), Bravo *et al.* (1998), Frei *et al.* (2003), Faraj *et al.* (2004), Carreira *et al.* (2004), Agama-Acevedo *et al.* (2004), Kim *et al.* (2006_a) Mitra *et al.* (2007) and Blazek and Copeland (2010) also indicated increased yield of RS in foods stored at low temperature.

Table 47. Variation (%) in RS content with variation in cooling conditions

Cooling conditions / Temperature (°C)	Moisture (%)			
	0	10	20	40
	RT ➡ -20°C	RT ➡ -20°C	RT ➡ -20°C	RT ➡ -20°C
121	+1.32	+2.54	+0.44	+16.71
141	+6.12	-1.31	-6.36	+15.30

RT- Room Temperature

The increase RS noticed in rice starch cooled at -20°C is due to the faster retrogradation of the gelatinized starch which becomes intensified according to the storage conditions as indicated by Rosin *et al.* (2002). At lower temperature, the retrogradation occurs at a faster rate due to better nucleation and crystal growth as suggested by Biliaderis (1991). Moreover, the samples stored at -20°C were allowed to cool at room temperature before freezing. This also favoured better retrogradation of starch gel. Contradictory to these findings Johansson *et al.* (1984), Hasjim and Jane (2009) and Milasinovic *et al.* (2010) did not notice obvious effect on the RS content on any of the starches such as wheat/rye bread, acid modified starch and maize starch respectively when cooled at -20°C after heat treatment.

Among the 48 treatments experimented by varying the temperature and time of autoclaving, moisture application and cooling condition, five treatments with maximum RS content were selected to find out the effect of repeated autoclaving and cooling cycles on RS formation. Among this, two treatments were carried out by autoclaving at a temperature of 141°C for 40 minutes with (T_{37}) and without (T_{31}) moisture and cooling at -20°C. In the other two treatments, autoclaving was carried out at 141°C for 40 (T_{13}) and 60 minutes (T_{14}) with 10 per cent moisture and cooling at room temperature. The fifth one (T_{35}) was autoclaved at 121°C for 60 minutes with 10 per cent moisture and cooled at -20°C.

Among this, highest RS was noticed in T_{31} followed by T_{37}. Thus, it was seen that the ideal conditions with respect to temperature and time of autoclaving to yield more RS is 141°C for 40 minutes. The effective cooling condition was at -20°C. Maximum moisture level to yield more RS was found to be 10 per cent. It was also seen that even a lower temperature of 121°C for a longer duration of 60 minutes of autoclaving yielded more RS with 10 per cent moisture and cooling at -20°C. The effect of heat treatment to yield more RS was indicated by Sajilata *et al.* (2006). Chung *et al.* (2009$_a$) also indicated higher RS with increase heat moisture treatment in corn, pea and lentil. Brumovsky *et al.* (2009) illustrated the significance of combined effect of time and temperature on RS formation.

Thus, it was seen that RS formation depends not on a single factor but on multiple factors like time and temperature of autoclaving, moisture as well as cooling conditions. Studies conducted by Berry (1986), Englyst *et al.* (1987), Eerlingen *et al.* (1993), Sagum and Arcot (2000) and Vaidya and Sheth (2011) also indicated the influence of water content, heating temperature, time of cooling, number of heating and cooling cycles, pH, freezing, drying, storage time and temperature and presence of additives on RS formation in different foods.

5.3. Effect of repeated autoclaving and cooling on *in vitro* digestibility of starch and proximate composition of rice starch

To find out the effect of repeated autoclaving and cooling cycles on *in vitro* digestibility of starch, the selected five treatments with high RS content were subjected to autoclaving and cooling for two, three and four times and compared with the constituents present in raw rice starch.

It was seen that in all these five treatments, RS content increased irrespective of the number of autoclaving and cooling cycles when compared to the RS content of raw rice starch except in T_{35} and T_{14} subjected to two autoclaving and cooling cycles. Among the various repeated autoclaving and cooling cycles, maximum yield of RS occurred in rice starch subjected to four repeated autoclaving and cooling cycles in T_{31} (92.12%) followed by T_{14} (92%), T_{37} (87.24%), T_{13} (85.44%) and T_{35} (83.47%). The percentage increase of RS in these treatments over raw rice starch varied from 6.02 in T_{35} to 17.01 per cent in T_{31} (Table 48). Thus, maximum increase was noticed in rice starch autoclaved at a higher temperature of 141°C for 40 minutes without moisture and cooled at a lower temperature of -20°C with repeated autoclaving and cooling cycles for four times. Though, a slight decrease in RS occurred in T_{35} and T_{14} subjected to two autoclaving and cooling cycles; in the subsequent third and fourth cycles these were compensated by an increased yield of RS. As observed in the present study, Yadav *et al.* (2009) also indicated an increase in yield of RS in legumes, cereals and tubers due to multiple heating and cooling cycles. They observed an increase of 2.51 to 8.16 per cent of RS after three heating and cooling cycles. Sievert and Pomeranz (1989) also indicated the pronounced effect of increased number of autoclaving and cooling cycles on RS formation in cereals, tubers, pulses and amylomaize starch. They noticed a 40 per cent improvement in RS when the number of cycles was stepped upto 20 in amylomaize starch. Similar increase in RS due to repeated heating and cooling cycles were reported by Bjorck *et al.* (1987) in wheat starch, Bjorck *et al.* (1990) in barley, Mangala *et al.* (1999) in ragi and cereal flour ; Hickman *et al.* (2009) and Kim and Kwak (2009) in corn starch; Chung *et al.* (2009_b) in pea, corn and lentil; Milasinovic *et al.* (2010) in maize starch and Ha *et al.* (2012) in rice. Berry (1986), Escarpa *et al.* (1996), Vasanthan and Bhatty (1998), Aparicio - Saguilan *et al.* (2005), Sajilata *et al.* (2006), Hickman *et al.* (2009) and Zhao and Lin (2009) also indicated the pronounced effect on the yield of RS content by repeated autoclaving and cooling cycles. Thus, the number of autoclaving and cooling cycles were decisive of a higher yield of RS.

Autoclaving of starch involves gelatinisation which destroys the molecular order of starch. The dispersed starch polymers reform as a semi crystalline structure when cooled due to retrogradation (Garcia- Alonso *et al.*, 1998 and Singh *et al.*, 2010) finally increasing the yield of RS. This might also be due to the increase in the net crystallinity of RS because of cooling in between successive autoclaving cycles which in turn decreases the hydrolysis limit of pancreatic alpha amylase and finally results in an increased yield of RS. The presence of more linear amylose also increases RS formation upon cooking and cooling due to the aligning or association of amylose molecules with each other during retrogradation. Studies conducted by Haralampu (2000), Brumovsky and Thompson (2001), King and Tan (2005) and Milasinovic *et al.* (2013)

Table 48. Variation (%) in RS content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				RS (%)		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Number of autoclaving and cooling		
					Two	Three	Four
T_{31}	141	40	0	-20°C	+4.37	+4.50	+17.01
T_{37}	141	40	10	-20°C	+2.59	+2.91	+10.81
T_{35}	121	60	10	-20°C	-0.14	+4.98	+6.02
T_{14}	141	60	10	RT	-0.17	+4.12	+16.86
T_{13}	141	40	10	RT	+2.63	+1.65	+8.52

*Resistant starch content in raw rice starch was taken as the base (78.73%)

RT- Room Temperature

also indicated that after debranching of starch the linear chains of amylose contribute to a better RS.

The impact of repeated autoclaving and cooling on the *in vitro* digestibility of rice starch was determined by estimating the RDS and SDS contents. It was seen that with the increase in the number of autoclaving and cooling cycles, the RDS content decreased in most of the treatments when compared to raw rice starch. In T_{31} though an increase in RDS occurred in rice starch subjected to two autoclaving and cooling cycles, in three and four autoclaving and cooling cycles the RDS content decreased to a greater extent. Here, the RDS content decreased up to 68.52 per cent at the fourth autoclaving and cooling cycles (Table 49) carried out without moisture. As regards the other four treatments with 10 per cent moisture, a decreased yield of RDS was noticed. Chung *et al.* (2009_a) indicated a decrease in the RDS content of the gelatinised starch when compared to native starch. Chung *et al.* (2012) also observed a decrease in the RDS due to heat-moisture treatment of germinated brown rice on parallel with that of native brown rice. Similarly, Mishra *et al.* (2008) also noticed 45 per cent decrease in the RDS content of potatoes subjected to processing and cooling at refrigeration temperature. However, Niba (2003) though observed an increase in RDS upon autoclaving in cereal and tuber flour, upon cooling the RDS decreased to a greater extent. Ovando-Martinez *et al.* (2013) indicated that thermal treatments cause reorganisation of amylose and amylopectin chains of starch which in turn changes their physico-chemical and digestibility properties. The decrease in the RDS content noticed in the present study with increase in the number of autoclaving and cooling cycles is due to the increased yield of RS as suggested by Hagenimana *et al.* (2006) and Pongjanta *et al.* (2008). Niba (2003) indicated increase as well as decrease in RDS content in dietary starches with variations in the cooking method; structure of the starch; heat-moisture treatment applied; germination and source of starch.

The increase in the number of autoclavings upto a threshold level of four

Table 49. Variation (%) in RDS content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				RDS (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Two	Three	Four
T_{31}	141	40	0	-20°C	+12.54	-57.26	-68.52
T_{37}	141	40	10	-20°C	-48.65	+7.55	-38.68
T_{35}	121	60	10	-20°C	-27.35	-8.12	+5.41
T_{14}	141	60	10	RT	-46.08	-31.34	-49.79
T_{13}	141	40	10	RT	-20.23	-25.21	-16.67

*RDS content in raw rice starch was taken as the base (14.04 per cent)

RT- Room Temperature

in general favoured a higher output of RS; almost striking a balance towards the addition of moisture from a dry step, as also favouring a least cooling necessity down from room temperature. At this juncture, when the output of RDS is measured, a decrease in the number of autoclaving with an increased cooling effect becomes a necessity for a better realisation of RDS. It may be concluded that a balance between a desirable output of RS with a higher measurable RDS is necessary when the four parameters namely temperature, time, moisture and cooling effect are put together aiming at a standard output of RS with an enriched RDS.

When compared to the unprocessed starch, the SDS content increased in all the samples which were autoclaved and cooled twice except in T_{31} which was processed without moisture. The SDS content increased up to 90.87 per cent in T_{14} (Table 50). Though, a rapid increase in SDS was noticed in T_{37} subjected to two autoclaving and cooling cycles, during the three and four autoclaving and cooling cycles it slumped down. A pronounced decrease in the SDS content of rice starch was noticed only in samples autoclaved and cooled four times in various treatments.

When compared to the increase of RS content during repeated autoclaving and cooling cycles, the SDS content decreased with increase in the number of cycles. Patindol *et al.* (2010) also indicated that RS and SDS in foods are not co-linear. However, Hagenimana *et al.* (2006) indicated positive correlation between RS and SDS content of foods. Veena *et al.* (1995) also observed a decrease in the SDS content in fermented, germinated, pressure cooked and roasted pulses. As observed in the case of RDS content, Niba (2003) also indicated variation in the SDS content depending upon the processing method, storage condition, heat-moisture treatment, granular/ gelatinised form of starch.

The moisture content of rice starch significantly decreased during repeated

Table 50. Variation (%) in SDS content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				SDS (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Two	Three	Four
T_{31}	141	40	0	-20°C	-71.78	+62.24	-52.14
T_{37}	141	40	10	-20°C	+65.98	-45.78	-42.60
T_{35}	121	60	10	-20°C	+53.53	-38.17	-75.52
T_{14}	141	60	10	RT	+90.87	+16.04	-86.86
T_{13}	141	40	10	RT	+10.24	+30.84	-60.72

*SDS content in raw rice starch was taken as the base (7.23 per cent)

RT- Room Temperature

autoclaving and cooling cycles when compared to the moisture content of native starch (Table 51). By varying the temperature and time of autoclaving and cooling conditions, along with moisture and without moisture application, the moisture content of rice starch varied significantly in all treatments subjected to two and four autoclaving and cooling cycles. In the samples subjected to three autoclavings also the moisture content varied significantly except in T_{35} and T_{13}. A steady decrease in the moisture content was also noticed when the number of autoclaving and cooling cycles were increased to four from two cycles in almost all samples. When compared to the RS content of rice starch subjected to repeated autoclaving and cooling cycles, it was seen that with an increase in the RS content, the moisture content decreased gradually in all the samples.

When compared to raw rice starch, protein content decreased significantly during repeated autoclaving and cooling cycles. Maximum decrease was noticed in rice starches which were subjected to two autoclaving and cooling cycles in which the protein content decreased up to 95.95 per cent (Table 52). In three and four autoclaving and cooling cycles the per cent decrease varied from 1.35 per cent (T_{13}) to 12.16 per cent (T_{31} and T_{14}) and 1.35 per cent (T_{14}) to 21.17 per cent (T_{13}) respectively. When compared to the increase in the RS content of rice starch here also it could be seen that with an increase in the number of autoclaving and cooling cycles an increase in the protein content occurred irrespective of the treatments at three autoclaving and cooling cycles when compared to two cycles. When the number of autoclaving was increased to four, a slight decrease occurred in almost all treatments except in T_{31} and T_{14}. The increase might be due to cleavage of hydrogen bonds of protein-carbohydrate or protein-protein due to autoclaving which could also expose some of the polar amino acid groups buried within the folded protein structure inducing increased hydrophilicity and

Table 51. Variation (%) in moisture content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				Moisture (%)		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Number of autoclaving and cooling		
					Two	Three	Four
T_{31}	141	40	0	-20°C	-23.26	-33.40	-43.33
T_{37}	141	40	10	-20°C	-20.17	-34.71	-45.68
T_{35}	121	60	10	-20°C	-18.86	-45.59	-39.68
T_{14}	141	60	10	RT	-55.07	-51.41	-45.03
T_{13}	141	40	10	RT	-25.61	-45.97	-33.40

*Moisture content in raw rice starch was taken as the base (10.66 per cent)

RT- Room Temperature

Table 52. Variation (%) in protein content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				Protein (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Two	Three	Four
T_{31}	141	40	0	-20°C	-77.48	-12.16	-12.16
T_{37}	141	40	10	-20°C	-94.59	-4.05	-8.11
T_{35}	121	60	10	-20°C	-95.95	-4.05	-9.46
T_{14}	141	60	10	RT	-93.24	-12.16	-1.35
T_{13}	141	40	10	RT	-88.29	-1.35	-21.17

*Protein content in raw rice starch was taken as the base (2.22 per cent)

RT- Room Temperature

extractability as indicated by Wu *et al.* (1998). Babu and Parimalavalli (2014) also noticed increased protein content in sweet potato starch subjected to repeated autoclaving and cooling cycles.

In the case of total carbohydrate, it was seen that when compared to native starch, the total carbohydrate content of rice starch subjected to two, three and four autoclaving and cooling cycles increased significantly in all the five treatments (Table 53). With an increase in the number of autoclaving and cooling cycles also the total carbohydrate content increased to a greater extent in almost all samples. When compared to the RS content, the total carbohydrate content also increased gradually by increasing the number of autoclaving and cooling cycles. Thus, a positive relationship was observed between the carbohydrate content and RS content in repeatedly autoclaved and cooled samples.

In the case of amylose, it was seen that in processed rice starch the content was lower than the native starch (Table 54). The decrease noticed in processed starch might be due to the leaching of amylose from the intact starch granules as indicated by Schweizer *et al.* (1986) and Sagum and Arcot (2000). However, with repeated autoclaving and cooling cycles a steady increase in the amylose content occurred in the case of T_{37}, T_{35} and T_{14}. In the other two treatments also a noticeable increase in the amylose content occurred in the samples which were subjected to three autoclaving and cooling cycles, when compared to two autoclaving and cooling cycles. Study conducted by Babu and Parimalavalli (2014) also indicated increase in the amylose content of sweet potato starch subjected to autoclaving and cooling cycles. In consonance with the increase of RS content due to repeated autoclaving and cooling cycles, the amylose content also increased parallel. Various studies conducted by Sievert and Pomeranz (1989), Annison and Topping (1994), Erlingen and Delcour (1995), Escarpa *et al.*(1996), Haralampu (2000), Skrabanja *et al.* (1999), Sagum and Arcot (2000), Rosin *et al.*(2002), Frei *et al.* (2003), Hu *et al.* (2004), Walter *et al.* (2005), Kim *et al.*(2006$_a$), Morita *et al.* (2007), Yadav *et al.* (2009), Li and Gao

Table 53. Variation (%) in total carbohydrate content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				Total carbohydrate (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Two	Three	Four
T_{31}	141	40	0	-20°C	+1.61	+2.15	+3.76
T_{37}	141	40	10	-20°C	+4.30	+4.84	+4.30
T_{35}	121	60	10	-20°C	+2.15	+2.69	+3.23
T_{14}	141	60	10	RT	+1.61	+2.15	+2.69
T_{13}	141	40	10	RT	+1.08	+3.23	+2.15

* Total carbohydrate content in raw rice starch was taken as the base (93 per cent)

RT- Room Temperature

Table 54. Variation (%) in amylose content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				Amylose (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Two	Three	Four
T_{31}	141	40	0	-20°C	-11.80	-2.25	-6.18
T_{37}	141	40	10	-20°C	-4.49	-3.37	-2.25
T_{35}	121	60	10	-20°C	-19.66	-10.67	-8.43
T_{14}	141	60	10	RT	-12.92	-12.92	-2.81
T_{13}	141	40	10	RT	-7.30	-6.74	-13.48

* Amylose content in raw rice starch was taken as the base (35.6 per cent)

RT- Room Temperature

(2010), Patindol *et al.* (2010), Vaidya and Sheth (2011), Dundar and Gocmen (2013) and Kouadio *et al.*(2013) indicated the positive relationship of amylose and yield of RS.

In most of the processed starch, the amylopectin content was found to be higher when compared to the content in native starch (Table 55). However, the variation was found to be statistically insignificant. In repeated autoclaving and cooling cycles, the amylopectin content decreased steadily with an increase in the number of autoclaving and cooling cycles in T_{37}, T_{35} and T_{14}. Schweizer *et al.* (1986) also indicated changes in the proportion of amylose and amylopectin content of starch during processing.

The NSP content of raw rice starch was comparatively lower than the processed starch in most of the treatments. With repeated autoclaving and cooling cycles, NSP increased up to 40.22 per cent in samples autoclaved and cooled three times when compared to native starch (Table 56). Study conducted by Periago *et al.* (1996) also revealed higher amount of NSP in pea varieties after thermal processing. This might be due to the loss of other nutrients, most probably sugars during cooking, since NSP cannot be generated through food processing. Cheung and Chau (1998) also noticed increase in the NSP to various extent with increase in the cooking time of legume flours.

Among the five treatments studied with respect to the effect of RS content of rice starch subjected to repeated autoclaving and cooling cycles, two treatments with maximum RS content was selected to study the quality characteristics of rice flour incorporated with different levels of RS. The treatments selected were T_{31} and T_{14}. In T_{31} rice starch was autoclaved at 141°C for 40 minutes without moisture and cooled at -20°C with four repeated autoclaving and cooling cycles. In the second treatment also, the rice starch was subjected to autoclaving at 141°C for 60 minutes with 10 per cent moisture and cooled at room temperature in four cycles.

Table 55. Variation (%) in amylopectin content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				Amylopectin (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Two	Three	Four
T_{31}	141	40	0	-20°C	+6.52	+1.24	+3.42
T_{37}	141	40	10	-20°C	+2.48	+1.86	+1.24
T_{35}	121	60	10	-20°C	+10.87	+5.90	+4.66
T_{14}	141	60	10	RT	+7.14	+7.14	+1.55
T_{13}	141	40	10	RT	+4.04	+3.73	+7.45

* Amylopectin content in raw rice starch was taken as the base (64.4 per cent)

RT- Room Temperature

Table 56. Variation (%) in non starch polysaccharides content of rice starch due to multiple autoclaving and cooling cycles

Treatments	Processing conditions				Non starch polysaccharides (%)		
					Number of autoclaving and cooling		
	Temperature (°C)	Time (mts)	Moisture (%)	Cooling	Two	Three	Four
T_{31}	141	40	0	-20°C	+8.12	+14.27	+17.34
T_{37}	141	40	10	-20°C	+38.99	+40.22	+38.99
T_{35}	121	60	10	-20°C	+9.10	+30.38	+26.69
T_{14}	141	60	10	RT	-23	-6.27	+20.42
T_{13}	141	40	10	RT	+12.30	+26.69	+3.94

* Non starch polysaccharides content in raw rice starch was taken as the base (8.13 per cent)

RT- Room Temperature

5.4. Quality characteristics of RSRF and RS supplemented rice products

The selected two starches were incorporated into the *puttu* and *idiappam* flour so as to deliver 10, 15 and 20g RS per 100g of rice flour and various quality characteristics of RSRF and products were evaluated.

5.4.1. Physical qualities

Bulk density is a property of powders, granules and other divided solids especially used in reference to mineral component, chemical substance, ingredients, food stuffs or any other mass of corpuscular or particulate matter. It is defined as the mass of many particles of the material divided by the total volume they occupy (Buckman and Brady, 1960) and used in quality control.

The BD of *puttu* flour supplemented with RS was found to be on par with the non-supplemented flour. However, in the case of *idiappam* flour the BD varied significantly in $T14_{(1)}$ and $T14_{(2)}$ and other three treatments were on par with the non-supplemented flour. Though, much variation was not noticed in *puttu* flour supplemented with RS when compared to non-supplemented flour, in *idiappam* flour, BD decreased in the RS supplemented flour. An increase in the BD was noticed by Anderson *et al.* (1981); Moore *et al.* (1990); Rye *et al.* (1993) and Jim *et al.* (1994) in flour with an increase in the quantity of sugar and dietary fibres used to replace starch. Meuser and Wiedmann (1989) also indicated an increase in BD as a result of sugar liquefaction during heating. The low bulk density or no change observed in the bulk density of the RS supplemented and non-supplemented flour might be due to the low amount of sugar and insoluble dietary fibre in the flour.

Water absorption index measures the amount of water absorbed by starch and is used as an index of gelatinisation which corresponds to the volume of the gel formed (Anderson *et al.*, 1969 and Mason and Hoseney, 1986). WAI depends on the availability of hydrophilic groups and on the capacity of gel formation of the macromolecules (Gomez and Aguilera, 1983). The ability of the flour to absorb water is very important in food preparations (Mbofung *et al.* 2006). Kaur *et al.* (2002) indicated that water absorption by flour is mainly due to swelling and solubility of starch granules, which in turn depends on amylose content.

The WAI of all the RS supplemented *puttu* and *idiappam* flour were found to be significantly lower than the non-supplemented flour. Decrease in WAI may be due to the incorporation of RS as observed by Abiodun *et al.* (2010) in which the authors indicated a decrease in the WAI in yam flour with the incorporation of cassava starch. The high WAI of non-supplemented flour might be due to the loose association of the starch polymers in the granules as indicated by Ekwu *et al.* (2005). The increased amylose content of the flour due to the incorporation of RS might have caused a decreased destruction of the granule structure and hence a decrease in WAI as indicated by Nakorn *et al.* (2009). Fernandez-Gutierrez *et al.* (2004) indicated that low hydration capacities favoured by the formation of inter and intra molecular protein bonds with amylose and amylopectin will also lower WAI.

Water solubility index is used as an indicator of the destruction of starch components (Ding *et al.* 2005 and Reddy *et al.*2014). Water solubility index is related to the quantity of water soluble molecules and is associated to dextrinisation: WSI is related to the amount of soluble solids. WSI give the amount of soluble components that has leached out from the granules of starch (Guha *et al.* 1997 and Mitchell *et al.*1997). Resistant starch supplemented *puttu* and *idiappam* flour had higher WSI than non-supplemented flour.

In the present study *puttu* and *idiappam* flour were roasted to a particular level and during roasting dextrinisation of starch will take place leading to the degradation of the starch components and hence a higher WSI. The higher WSI noticed in RS supplemented flour when compared to non-supplemented flour might be due to the degradation occurred in the starch during the autoclaving process.

5.4.2. Organoleptic evaluation of products prepared with RSRF

Two rice based breakfast products namely *puttu* and *idiappa*m prepared with RS incorporated rice flour were evaluated for various organoleptic attributes and compared with the organoleptic qualities of respective products prepared without RS supplemented rice flour. The organoleptic characteristics were evaluated through the judgement of 10 panelists in three replications. The panelists were asked to evaluate the two products for five quality attributes as well as overall acceptability on a nine point hedonic scale. The value of the scales ranged from nine (like extremely) to one (dislike extremely) for each of the organoleptic attributes.

High mean and rank scores for all the quality attributes namely appearance, colour, flavour, texture, taste and over all acceptability was noticed in both the products prepared without RS supplementation. The products prepared with RS supplemented rice flour were also found to be organoleptically acceptable with a mean score above seven. Munoz *et al.* (1992) indicated that in a hedonic scale of nine points, a score of six can be considered as a limit for acceptability. Here, highest mean and rank scores for all the quality attributes was obtained for *puttu* prepared with a supplementation of 10g RS per 100 g rice flour in T_{14}. With an increase in the quantity of RS incorporation, a slight decrease in most of the quality attributes including over all acceptability occurred for *puttu.* In case of *idiappam,* among the RS incorporated products highest mean and rank scores for various quality attributes was noticed in *idiappam* prepared with rice flour incorporated with 15g RS per 100 g in T_{31}. Here also, the lowest scores were obtained for *idiappam* prepared with the maximum RS incorporation of 20g per 100g of rice flour ($T_{31(3)}$ and $T_{14(3)}$). It was also seen that maximum per cent of judges accepted the two products by giving a score in between 8-9 indicating that they like the product very much or extremely (Table 57 and 58). The RS incorporated *puttu* and *idiappam* were found to be on par with control with respect to acceptability. All the judges graded *puttu* and *idiappam* prepared without RS supplementation with a score of eight and nine indicating that they liked the products very much or extremely. In the case of RS supplemented *puttu* also, more than 70 per cent of the judges ranked the product by giving a score of eight and nine especially the *puttu* prepared with 10 and 15 g RS supplementation. For *puttu* prepared with 20g RS supplementation, only 45 per cent of judges gave the same score, especially with respect to flavour, texture and taste. In the case of *idiappam* also the same trend was noticed for various quality attributes. Here, more than 60 per cent of judges graded *idiappam* by giving a score of eight and nine indicating its likeability for various quality attributes. In the case of 20g supplementation, the score was found to be slightly lower and it was found to be six and seven especially with respect to flavour, texture and taste indicating that the product is only moderately acceptable. As the per cent of RS increased, the product became hard due to the retrogradation of starch taken place during steaming and then cooling before conducting the organoleptic evaluation. Mir *et al.* (2013) indicated increased hardness in rice flour mixtures with an increase in the amount of maize starch containing high amount of RS. Sagum and Arcot (2000) and Arora (2011) also indicated the impact of gelatinisation during steaming and the association of amylose molecules during cooling of the product so as to form a rigid gel due to retrogradation. It was also seen that none of the judges disliked the two RS supplemented products.

Studies conducted by Leszczynski (2004) indicated that the incorporation of RS to food products did not produce any negative impact on the organoleptic property. Aigster *et al.* (2011) also indicated the feasibility of incorporation of moderate levels of RS in cereal based products without compromising their acceptability. As observed in the present study, Maziarz, *et al.* (2013) also did not observe any significant alteration in the consumers acceptability of RS incorporated muffins, bread and chicken curry. In a study conducted by

Table 57. Judge's evaluation (%)* of organoleptic qualities of *puttu* prepared with RSRF

Treatments / Characters	T_{31} (1)		T_{31} (2)		T_{31} (3)		T_{14} (1)		T_{14} (2)		T_{14} (3)		Control	
	Scores													
	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9
Appearance	6.67	93.33	26.67	73.33	6.67	93.33	6.67	93.33	6.67	93.33	20	80	-	100
Colour	3.33	96.67	33.33	66.67	20	80	6.67	93.33	20	80	30	70	-	100
Flavour	16.67	83.33	26.67	73.33	53.33	46.67	6.67	93.33	20	80	26.67	73.33	-	100
Texture	3.33	96.67	23.33	76.67	53.33	46.67	13.33	86.67	23.33	76.67	50	50	-	100
Taste	10	90	13.33	86.67	53.33	46.67	10	96	20	80	53.33	46.67	-	100
Overall acceptability	-	100	20	80	40	60	6.67	93.33	20	80	43.33	56.67	-	100

* Per cent of judges out of 30 ratings

6-7 indicates like slightly and like moderately respectively

8-9 indicates like very much and like extremely respectively

Table 58. Judge's evaluation (%)* of organoleptic qualities of *idiappam* prepared with RSRF

Treatments / Characters	$T_{31 (1)}$		$T_{31 (2)}$		$T_{31 (3)}$		$T_{14 (1)}$		$T_{14 (2)}$		$T_{14 (3)}$		Control	
	Scores													
	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9	6-7	8-9
Appearance	-	100	3.33	96.67	33.33	66.67	3.33	96.67	13.33	86.67	3.33	96.67	-	100
Colour	6.67	93.33,	10	90	53.33	46.67	3.33	96.67	13.33	86.67	36.67	63.33	-	100
Flavour	36.67	63.33	10	90	40	60	3.33	96.67	16.67	83.33	63.33	36.67	-	100
Texture	33.33	66.67	6.67	93.33	60	40	13.33	86.67	20	80	66.67	33.33	-	100
Taste	26.67	73.33	6.67	93.33	70	30	3.33	96.67	16.67	83.33	60	40	-	100
Overall acceptability	23.33	76.67	-	100	56.67	43.33	-	100	13.33	86.67	53.33	46.67	-	100

* Per cent of judges out of 30 ratings

6-7 indicates like slightly and like moderately respectively

8-9 indicates like very much and like extremely respectively

Sankhon *et al.* (2013) it was seen that incorporation of RS prepared from Parkia flour to wheat flour did not produce any detrimental effect on the sensory properties of bread. This is in conformance with the study of Aravind *et al.* (2013) where the incorporation of 10 and 20 per cent RS in semolina did not produce any significant effect on the sensory properties of pasta. Majzoobi *et al.* (2014) concluded that a maximum level of incorporation of 20 per cent of RS in sponge cake will produce an acceptable product. However, studies conducted by Kim *et al.* (2001) indicated maximum level of 12.5 per cent incorporation of RS to produce excellent quality yellow layer cake.

5.4.3. *In vitro* starch digestibility of the products prepared with RSRF

The percentage increase in the RS content of products supplemented with RS varied from 35.74 per cent to 141.79 per cent in *puttu* and 0.42 to 26.72 per cent in *idiappam* (Table 59) prepared with RS incorporated flour. When compared to the products prepared without RS supplementation, more yield of RS was recorded in products prepared with RS incorporation at different levels. However, more percentage of RS was retained at a lower supplementation of 10g RS per 100g rice flour in both the products ($T_{31\,(1)}$ and $T_{14\,(1)}$) in contrast to RS augmented supplementation from 10g to 15g and then to 20g.

When RS content of *puttu* and *idiappam* were compared, it was seen that in all the treatments including control more RS content was found in *idiappam.* This might be due to the addition of more amount of boiled water during the preparation of *idiappam* dough and later the retrogradation taking place during cooling of the dough. Moreover, during the preparation of the product, dough was extruded using '*Sevanazhi*' during which slight modification of RS content could have occurred. As observed in the present study, Kim *et al.* (2006_b) also noticed higher yield of RS in extruded products. During further cooking of the extruded *idiappam* also there is having a possibility of an increase in RS content of the product. In the preparation of the *puttu,* only small quantity of water was sprinkled in the flour for moistening the flour before cooking *puttu* by steaming.

Table 59. Variation (%) in the RS content of the product prepared with RSRF in comparison with control

Treatments	Effective yield of RS (g/100g of rice flour)	RS (%)	
		Puttu	*Idiappam*
$T_{31\ (1)}$	10	+114.20	+15.42
$T_{31\ (2)}$	15	+47.26	+2.15
$T_{31\ (3)}$	20	+37.60	+1.32
$T_{14\ (1)}$	10	+141.79	+26.72
$T_{14\ (2)}$	15	+97.32	+9.03
$T_{14\ (3)}$	20	+35.74	+0.42

With respect to the RDS content, it was seen that with a decrease in RS content of the products, RDS content increased in most of the treatments including control (Table 60). RDS content was found to be more in *puttu* than in *idiappam.* When compared to control, the RDS content was lesser in all the RS supplemented *puttu.* The RDS was more in the two products prepared with the non-supplemented flour as observed by Menon *et al.* (2012) in spaghetti prepared with 85 per cent maida.

The slowly digestible starch was found to be more in *idiappam* in almost all treatments including control (Table 61). Here also, it could be seen that with an increase in the level of RS in the different treatments in *puttu* and *idiappam,* the SDS content also parallelly increased especially in *puttu.*

Table 60. Variation (%) in the RDS content of the product prepared with RSRF in comparison with control

Treatments	Effective yield of RS (g/100g of rice flour)	RDS (%)	
		Puttu	*Idiappam*
$T_{31\ (1)}$	10	-39.65	+2.71
$T_{31\ (2)}$	15	-12.40	-14.13
$T_{31\ (3)}$	20	-6.92	-17.52
$T_{14\ (1)}$	10	-23.02	-9.85
$T_{14\ (2)}$	15	-14.39	+0.13
$T_{14\ (3)}$	20	-3.29	-1.45

Table 61. Variation (%) in the SDS content of the product prepared with RSRF in comparison with control

Treatments	Effective yield of RS (g/100g of rice flour)	SDS (%)	
		Puttu	*Idiappam*
$T_{31\ (1)}$	10	+274.23	-26.52
$T_{31\ (2)}$	15	+73.99	+54.55
$T_{31\ (3)}$	20	+29.69	+69.08
$T_{14\ (1)}$	10	+82.07	+12.66
$T_{14\ (2)}$	15	+42.40	-9.64
$T_{14\ (3)}$	20	+45.13	+5.42

5.5. Effect of storage on the quality aspects of RSRF and RS supplemented products

On the basis of the mean organoleptic scores of *puttu* and *idiappam*, one product each with highest mean organoleptic scores were selected for further investigation. The flour used for the preparation of each of the products was packed separately in polythene bags of 200 gauge thickness for a period of six months under ambient condition. Physical qualities, proximate composition, microbial and insect infestation of the flour were evaluated initially and at the end of six months of storage. *Puttu* and *idiappam* were prepared using the selected RS incorporated flour initially and at the end of the storage and evaluated the *in vitro* starch digestibility and organoleptic qualities of the two products to notice the changes occurred in the various quality attributes during storage of the flour. All the quality attributes were compared with the corresponding qualities of the non-supplemented flour. The results pertaining to these aspects are discussed in this section.

5.5.1. Physical qualities

All the three physical quality parameters namely bulk density, water absorption index and water solubility index of *puttu* and *idiappam* flour supplemented with and without RS decreased during six months of storage (Fig.11). The decrease was found to be statistically significant only in the case of WAI of *puttu* flour without RS supplementation. When compared to the control flour, the BD as well as WAI of the RS incorporated *puttu* as well as *idiappam* flour was found to be slightly lower. The decrease was found to be statistically significant only in the case of WAI. However, WSI was found to be slightly higher in RS supplemented *puttu* as well as *idiappam* flour than non-supplemented flour.

Fig.11. Changes (Initial → Final) in the physical qualities of RSRF during storage

Physical qualities	*Puttu* flour ($T_{14\ (1)}$)		*Idiappam* flour ($T_{31\ (2)}$)	
	RSRF	Control	RSRF	Control
Bulk density	⇩	⇩	⇩	⇩
Water absorption index	⇩	⬇	⬌	⇩
Water solubility index	⇩	⇩	⇩	⇩

Decrease - ⇩

Significant decrease- ⬇

No change- ⬌

The decrease in the bulk density noticed during storage in the flour is due to the increase in the moisture content as indicated by Lawal *et al.* (2014). Studies conducted by Kunhimon (2010) and Lakshmy (2011) also indicated decrease in the bulk density, water absorption index and water solubility index of bamboo seed flour and rice flour during storage. Lawal *et al.* (2014) also noticed a significant decrease in the bulk density of yam flour during storage. Similar to the findings of the present study, a decrease in the water absorption index with an increase in the storage time was noticed in autoclaved banana starch by Gonzalez-Soto *et al.* (2007). The decrease occurring in the water absorption capacity of the flour during storage might be due to the conversion of starch to sugars on storage. This decrease in the starch content might have influenced the water absorption capacity of the flour.

5.5.2. Proximate composition

The moisture content of *puttu* and *idiappam* flour prepared with and without RS supplementation increased during storage (Fig.12). The percentage increase varied from 17.45 per cent to 20.99 per cent in the two types of flours prepared with RS incorporation. In the case of non-supplemented flour it was found to be 12.22 per cent (*puttu* flour) and 26.77 per cent (*idiappam* flour) (Table 62). The increase occurred in the moisture content of the flour during storage may be due to the hygroscopic properties of the flour and the moisture pickup by the flour as suggested by Butt *et al.* (2004), Balasubramanian and Sadasivan (1989) and Sharif *et al.* (2003). Similar findings were also reported by Hamed *et al.* (1973) and Chellammal (1995) in sweet potato flour, Liya (2001) in taro flour and Adejumo (2013) in wheat flour.

The protein content of *puttu* as well as *idiappam* flour prepared with and without RS supplementation decreased during storage. When compared to RS supplemented flour, the decrease in the protein content during storage was more in non-supplemented *puttu* (41.46%) and *idiappam* flour (18.48%) flour

(Table 62). The decrease in the protein content could also be due to the browning reactions taken place in the flour during storage as indicated by Sharif *et al.* (2003). The increase in the moisture content of the flour during storage might have favoured increased proteolytic activity leading to a decrease in the protein content. Different studies conducted by Leelavathi *et al.* (1984), Upadhyay *et al.* (1994), Misra and Kulshrestha (2003), Sharon (2010), Lakshmy (2011) and Raj (2011) also indicated a decrease in the protein content of cereal flour, tuber flour and processed food mixtures during storage.

Table 62. Variation (%) in proximate composition RS supplemented *puttu* and *idiappam* flour

Proximate composition	*Puttu* flour ($T_{14\,(1)}$)		*Idiappam* flour ($T_{31\,(2)}$)	
	RSRF	Control	RSRF	Control
Moisture (g/100g)	+20.99	+12.22	+17.45	+26.77
Protein (g/100g)	-22.36	-41.46	-6.02	-18.48
Total carbohydrate (g/100g)	-1.66	-1.36	-1.53	-0.41

A similar trend of decrease in the total carbohydrate content of *puttu* and *idiappam* flour prepared with and without RS supplementation occurred during storage. However, the decrease was found to be below two per cent ((Table 62). Studies conducted by Kungu *et al.* (2003), Raj (2011), Lijitha (2012) and Lawal *et al.* (2014) also indicated a decrease in the carbohydrate content of cereal and pulse flour, seed flour, and tuber flour during storage. The slight decrease in the carbohydrate content might be due to the lowering of the hydrocarbon content of the flour during storage as indicated by Losser (1987) and due to hydrolysis occurred in the polysaccharides as indicated by Upadhyay *et al.* (1994) and Pillai (2001).

Fig.12. Changes (Initial → Final) in the proximate composition of RSRF during storage

Proximate composition	*Puttu* flour ($T_{14\ (1)}$)		*Idiappam* flour ($T_{31\ (2)}$)	
	RSRF	Control	RSRF	Control
Moisture	↑	↑	↑	↑
Protein	↓	↓	↓	↓
Total carbohydrate	⇩	⇩	⇩	⇩

Significant increase- ↑

Significant decrease- ↓

Decrease - ⇩

5.5.3. Total microflora

With respect to the microbial count of the flour during storage, it was seen that the count of bacteria, yeast and fungi increased at the end of storage (Fig.13). The increase in the microbial load is due to the increase in the moisture content of the flour as indicated by Kapoor and Kapoor (1990). In the present study, microorganisms might have multiplied during storage due to the various favourable environmental factors and storage conditions. Various extrinsic and intrinsic factors like moisture, relative humidity, storage temperature, type of samples, packaging etc. might have affected the microbial count of flour during storage. Studies conducted by Kapoor and Kapoor (1990) in sweet potato flour, Sharon (2003) in bread fruit flour, Misra and Kulshrestha (2003) in potato flour, Bhatiwada (2007) in grain amaranth flour, Hanmant (2010) in mango seed kernel flour, Kunhimon (2010) in bamboo seed flour, Lakshmy (2011) in parboiled rice flour and Lijitha (2012) in cycas seed flour also indicated increase in the microbial load during storage.

5.5.4. Insect infestation

The insect infestation was not at all observed initially as well as at the end of storage due to the proper drying given to the flour and appropriate storage conditions used for the study. Though, a slight increase in the moisture content was noticed during storage in both types of flour, the moisture content was retained below seven percent till sixth month of storage. As observed in the present study Bhatiwada (2007) Kunhimon (2010), Lakshmy (2011) and Lijitha (2012) did not notice any insect infestation during storage in grain amaranth flour, bamboo seed flour, rice flour and cycas seed flour.

Fig.13. Changes (Initial ➡ Final) in the total micro flora of RSRF during storage

Total microflora	*Puttu* flour ($T_{14\ (1)}$)		*Idiappam* flour ($T_{31\ (2)}$)	
	RSRF	Control	RSRF	Control
Bacteria	↑	↑	↑	↑
Yeast	↑	↑	↑	↑
Fungi	↑	↑	↑	↑

Increase- ↑

5.5.5. Organoleptic qualities of the products prepared with RSRF

Puttu and *idiappam* prepared with and without RS supplementation obtained a mean score above seven when the products were prepared using the flour stored for six months. This indicates that both the products prepared with the stored flour also are acceptable to the judges. However, the scores for different quality attributes as well as overall acceptability decreased slightly when the products were prepared with the stored flour when compared to the products prepared with fresh flour (Fig.14 and 15). Here, greater decrease was noticed in the products prepared with the non-supplemented flour. Moreover, it was also seen that nearly 83 per cent of judges who evaluated the RS incorporated *puttu* judged the product by giving scores in between eight and nine (Table 63). In the case of non-supplemented *puttu,* only 63 per cent gave the same score. In the case of *idiappam,* all the judges gave a higher score for the non-supplemented *idiappam* prepared with the stored flour (Table 64). For RS supplemented *idiappam* also, more than 96 per cent judged the product by giving a score 8 and 9 indicating its likeability even when it was prepared with the stored flour. It was also seen that RS supplemented *idiappam* was more acceptable to the judges when compared to RS supplemented *puttu*. This might be due to the hard texture of *puttu* prepared using RS incorporated flour.

5.5.6. *In vitro* starch digestibility of RSRF products during storage

The *in vitro* digestibility of *puttu* and *idiappam* prepared with the stored flour showed a slight increase in the RS content in both the products when they were prepared with stored flour. Aigster *et al.* (2011) also noticed an increase in the RS level during storage in cereal based products due to retrogradation of amylose chain. A slight increase in the RDS content and a decrease in the SDS content also occurred in both products when they were prepared with the stored flour (Fig.16).

Table 63. Judge's evaluation (%)* of organoleptic qualities of *puttu* prepared with RSRF ($T_{14\ (1)}$) during storage

Characters	RSRF		Control	
	Scores			
	6-7	8-9	6-7	8-9
Appearance	13.33	86.67	13.33	86.67
Colour	13.33	86.67	23.33	76.67
Flavour	33.33	66.67	53.33	46.67
Texture	20	80	23.33	76.67
Taste	23.33	76.67	50	50
Overall acceptability	16.67	83.33	36.67	63.33

* Per cent of judges out of 50 ratings

6-7 indicates like slightly and like moderately respectively

8-9 indicates like very much and like extremely respectively

Table 64. Judge's evaluation (%)* of organoleptic qualities of *idiappam* prepared with RSRF ($T_{31\ (2)}$) during storage

Characters	RSRF		Control	
	Scores			
	6-7	8-9	6-7	8-9
Appearance	-	100	-	100
Colour	10	90	6.67	93.33
Flavour	20	80	16.67	83.33
Texture	26.67	73.33	13.33	86.67
Taste	13.33	86.67	13.33	86.67
Overall acceptability	3.33	96.67	-	100

* Per cent of judges out of 50 ratings

6-7 indicates like slightly and like moderately respectively

8-9 indicates like very much and like extremely respectively

Fig.14. Changes (Initial ➡ Final) in the mean score for the organoleptic qualities of *puttu* prepared with RSRF ($T_{14\ (1)}$) during storage

Rice flour used	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
RSRF	⬇	⬇	⬇	⬇	⬇	⬇
Rice flour	⬇	⬇	⬇	⬇	⬇	⬇

Decrease- ⬇

Fig. 15. Changes (Initial ➡ Final) in the mean score for the organoleptic qualities of *idiappam* prepared with RSRF ($T_{31\ (2)}$) during storage

Rice flour used	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
RSRF	⬇	⬇	↕	⬇	⬇	⬇
Rice flour	⬇	⬇	↕	⬇	⬇	⬇

Decrease- ⬇

No change- ↕

Fig.16. Changes (Initial➡Final) in the *in vitro* starch digestibility of RSRF during storage

***In vitro* starch digestibility (%)**	***Puttu* flour ($T_{14\ (1)}$)**		***Idiappam* flour ($T_{31\ (2)}$)**	
	RSRF	**Control**	**RSRF**	**Control**
Resistant starch	⇧	⬌	⇧	⬌
Rapidly Digestible Starch	⇧	⬆	⇧	⇧
Slowly Digestible Starch	⬇	⬇	⬇	⇩

Increase - ⇧

Significant decrease- ⬇

Significant increase- ⬆

No change- ⬌

5.6. Glycaemic response of RSRF products

Glycaemic response is a measure of the impact of a food on blood sugar (Hubrich and Nabors, 2006). Postprandial glycaemic responses of *puttu* and *idiappam* supplemented with RS was assessed among diabetic and non-diabetic individuals in comparison with non-supplemented products. The increase in blood glucose level was relatively in a lower range when RS supplemented *puttu* and *idiappam* were given to both diabetic as well as non-diabetic subjects when compared to the products without RS supplementation. Here, it could be seen that the percentage increase in glucose is at a lower rate after the consumption of RS supplemented food products. Thus, the percentage increase in blood glucose level was found to be high in subjects who consumed non supplemented products. The percentage increase in blood sugar level was found to be 37.09 per cent and 17.83 per cent when RS supplemented *puttu* was given to diabetic and non-diabetic subjects when compared to a percentage increase of 44.75 per cent and 20.21 per cent in non-supplemented *puttu* (Table 65). In the case of *idiappam* also, the similar trend was observed in which the percentage increase was found to be 32.59 per cent (diabetic) and 17.5 per cent (non-diabetic) after consumption of RS supplemented food product. In the case of non-supplemented product this was found to be 42.90 per cent and 26.50 per cent respectively. The percentage increase in blood sugar level in diabetic subjects was relatively in a lower rate when compared to normal subjects after the consumption of RS supplemented food products. Several studies conducted by Behall *et al.* (1989), Liljeberg and Bjorck (1994), Raben *et al.* (1994), Achour *et al.* (1997), Reader *et al.* (1997 and 2002), Akerberg *et al.* (1998), Behall and Hallfrisch (2002), Brighenti *et al.* (2006), Robertson *et al.* (2005), Mitra *et al.* (2007), Li *et al.* (2010), Kendall *et al.* (2010), Johnson *et al.* (2010), Anderson *et al.* (2010), Brites *et al.* (2011), Maki *et al.* (2012) and Alexander (2012) also confirmed the beneficial effect of consuming RS incorporated foods in lowering post prandial glycaemic response. As indicated by Raben *et al.* (1994), Reader *et al.* (1997), Haralampu (2000), Nugent (2005) and Sajilata *et al.* (2006) in the small intestine RS is slowly absorbed resulting in decreased postprandial glucose response. Though, earlier studies had postulated that retrograded amylose is nonnutritive, studies conducted by Haralampu (2000) indicated that amylases slowly attack the structure and consequently glucose and other oligosaccharides are released from retrograded starch over a sustained period through normal digestive process. Modulation of glucose release and uptake in humans could be considered important in the use of RS in food products suggested for diabetic subjects.

Table 65. Increase (%) in blood sugar level after consuming RS supplemented food products

Volunteers	*Puttu*		*Idiappam*	
	RSRF *puttu*	Control *puttu*	RSRF *idiappam*	Control *idiappam*
Normal subjects	17.83	20.21	17.5	26.50
NIDDM subjects without medication	37.09	44.75	32.59	42.90

Contradictory to this, Kim *et al.* (2003) and Bodinham *et al.* (2010) did not notice any significant effect on the postprandial glycaemic response in healthy adults by consuming RS supplemented products. Studies conducted by Hoebler *et al.* (1999), Robertson *et al.* (2003 and 2005), Yamada *et al.* (2005), Behall *et al.* (2006), Hasjim *et al.* (2010), Kendall *et al.* (2010), Kwak *et al.* (2012), and Kimura (2013) indicated that higher doses of RS are needed to produce significant decline in postprandial glucose. Mitra *et al.* (2007) observed a decrease in fasting blood sugar level in subjects by giving 150 g of rice containing 8 to 10g RS for 12 weeks. As noticed in the present study, Behall and Howe (1995) indicated decrease in blood glucose levels in both normal and hyperinsulinaemic subjects by giving amylose starch. However, Behall and Scholfield (2005) in their study among hyperinsulinaemic and normal individuals noticed better glucose response in hyperinsulinaemic subjects by feeding high amylose chips and muffins containing RS as observed in the present study. Hence, it may be concluded that RS incorporated products are ideal with a relatively better response in diabetic subjects to control the disease in a natural way. Continued use of RS supplemented foods in the long run is also ideal to manage the problem of diabetes mellitus.

Summary

6. SUMMARY

The present study entitled "Optimisation and utilisation of resistant starch for value addition in rice products" was undertaken with the objectives of standardising processing treatments for optimising resistant starch (RS) formation in rice starch and evaluating the changes in rice starch properties. The study also aimed to standardise rice flour supplemented with RS, to evaluate its quality attributes during storage and to study the nutritional and organoleptic qualities of RS supplemented rice products. The effect of RS on postprandial glycaemic response was also evaluated.

In the present study, starch was isolated from rice variety Uma. Parboiled rice was prepared by conventional method using hot soaking process. Starch was isolated from raw as also parboiled rice after powdering. Starch content of parboiled rice was found to be slightly higher (66.6%) than that of raw rice (65.47%). The yield of RS from raw rice (78.73%) was more when compared to parboiled rice (76.29%).

The effect of processing conditions on the RS content of raw rice starch was studied with respect to temperature and time of autoclaving over different moisture levels and two cooling conditions. The raw rice starch was autoclaved at 121°C and 141°C for 20, 40 and 60 minutes with 0, 10, 20 and 40 per cent moisture. The samples after autoclaving were cooled at room temperature as also at -20°C. When the effect of temperature on RS content of raw rice starch was studied, it was seen that the autoclaved samples had comparatively low RS content both at 121°C and 141°C irrespective of the degree of cooling as compared with the RS content of raw rice starch. However, with an increase in the temperature of autoclaving from 121°C to 141°C, the RS content increased to a greater extent in both the samples which were cooled at room temperature as well as at -20°C. With an increase in the time of autoclaving, a decrease in the RS content occurred in rice starch especially in samples which were processed without moisture and cooled at room temperature. The effect of increased time of autoclaving (20→40, 40→60) on RS formation was positive especially in samples cooled at -20°C. The effect of moisture application on RS formation indicated that with an increase in the moisture content from zero to 10 per cent, the yield of RS increased both in room temperature and -20°C cooled samples. However, with a further increase of moisture from 10 to 20 per cent and from 20 to 40 per cent, the yield of RS decreased considerably in samples autoclaved both at 121°C and 141°C. Greater decrease occurred at the maximum moisture application of 40 per cent. The effect of cooling conditions after autoclaving indicated better retention of RS in the samples which were cooled at -20°C.

Among the 48 treatments experimented by varying the temperature and time of autoclaving, moisture application and cooling conditions, five treatments with maximum RS content was selected to find out the effect of repeated autoclaving and cooling cycles on RS formation. In these selected treatments (T_{31}, T_{37}, T_{35}, T_{14} and T_{13}) RS content varied from 78.41 to 82.82 per cent. The selected treatments indicated that the effective condition with respect to temperature and time of autoclaving to yield more RS was 141°C for 40 minutes. The effective cooling condition was found to be -20°C. Maximum moisture level to yield more RS was found to be 10 per cent. It was also seen that at a lower temperature of 121°C, autoclaving for duration of 60 minutes yielded more RS with added moisture of 10 per cent and a cooling temperature of -20°C. From the above results it may be concluded that chain effect of factors *viz* time and temperature of autoclaving, moisture and cooling condition leads to effective RS formation.

To find out the effect of repeated autoclaving and cooling cycles on *in vitro* digestibility of starch and proximate composition, the selected starches from the selected five treatments with high RS content were subjected to autoclaving and cooling for two, three and four times and compared with the constituents present in raw rice starch.

The RS content of all the samples which were autoclaved and cooled three and four times with and without moisture were found to be significantly higher than that of raw rice starch. An increase in the RS content was noticed with an increase in the number of autoclaving and cooling cycles in all the samples irrespective of the temperature and time of autoclaving, moisture content and cooling conditions. In the case of RDS, a steep decrease was noticed under four autoclavings in relation to the RDS content of control (14.04 %). It was seen that with the increase in the number of autoclaving and cooling cycles, the RDS content decreased in most of the treatments. Parallelly, the SDS content also decreased with increase in the number of autoclaving and cooling cycles.

The moisture and protein content of rice starch significantly decreased during repeated autoclaving and cooling cycles when compared to the native starch. A steady decrease in the moisture content was also noticed when the number of autoclaving and cooling cycles were increased to four from two in almost all the samples. In the case of total carbohydrates, it was seen that as compared to native starch the total carbohydrate content of rice starch subjected to two, three and four autoclaving and cooling cycles increased significantly in all the five treatments. In processed rice starch the amylose content was lower than that of native starch. However, with repeated autoclaving and cooling cycles a steady increase in the amylose content occurred in the case of T_{37}, T_{35} and T_{14}. In most of the processed starches, the amylopectin content was found to be higher when compared to that in native starch. In repeated autoclaving and cooling cycles, the amylopectin content decreased steadily with an increase in the number of autoclaving and cooling cycles in T_{37}, T_{35} and T_{14}. The NSP content of raw rice starch was comparatively lower than the processed starch in most of the treatments. With repeated autoclaving and cooling cycles, NSP increased up to 40.22 per cent in samples autoclaved and cooled upto three times.

From the five treatments subjected to repeated autoclaving and cooling cycles for a higher output of RS, two treatments yielding relatively higher RS was selected as the basis for formulating the resistant starch supplemented rice flour.

The selected two starches (T_{31} and T_{14}) were incorporated into the *puttu* and *idiappam* flour so as to deliver 10, 15 and 20g RS per 100g of rice flour. After incorporation, the physical qualities such as bulk density (BD), water absorption index (WAI) and water solubility index (WSI) of both the flours were studied and compared with the physical qualities of non-supplemented flour. *Puttu* and *idiappam* were prepared using the selected flour and organoleptic qualities of the products were evaluated. Apart from this, *in vitro* digestibility of both the products was also determined with respect to RS, RDS and SDS content.

The BD of *puttu* flour supplemented with RS was found to be on par with the non-supplemented flour. As regards the *idiappam* flour, BD decreased when RS was supplemented. The WAI of both the RS supplemented *puttu* and *idiappam* flours were found to be significantly lower than the non-supplemented flour. Resistant starch supplemented *puttu* and *idiappam* flour had higher WSI than non-supplemented flour.

The breakfast products namely *puttu* and *idiappa*m prepared with RS incorporated rice flour were evaluated for various organoleptic attributes and compared with the organoleptic qualities of respective products prepared with raw rice flour. High mean and rank scores for all the quality attributes namely appearance, colour, flavour, texture, taste and over all acceptability was noticed in both the products prepared without RS supplementation. The products prepared with RS supplemented rice flour were also found to be organoleptically acceptable with a mean score above seven. Here, highest mean and rank scores for all the quality attributes was obtained for *puttu* prepared with a supplementation of 10g RS per 100 g rice flour in T_{14}. In the case of *idiappam*, among the RS incorporated products, highest mean and rank scores for various quality attributes was noticed in *idiappam* prepared with rice flour incorporated with 15g RS per 100 g in T_{31}.

When compared to the products prepared without RS supplementation, more yield of RS was recorded in products prepared with RS incorporation at varying levels. However, more percentage of RS was retained at a lower supplementation of 10g RS per 100g rice flour in both the products in contrast to augmented RS supplementation from 10g to 15g and then to 20g. When RS content of *puttu* and *idiappam* were compared, it was seen that in all the treatments including control more RS content was found in *idiappam*. With respect to the RDS content it was seen with a decrease in RS content of the products, RDS content increased in most of the treatments including control. With an increase in the level of RS in the different treatments in *puttu* and *idiappam,* the SDS content also parallelly increased especially in *puttu*.

On the basis of the mean organoleptic scores of *puttu* and *idiappam*, one product each with highest mean organoleptic scores were selected for further investigation. The flour used for the preparation of each of the products was packed separately in polythene bags of 200 gauge thickness and stored for six months under ambient condition. Physical qualities, proximate composition, microbial and insect infestation of the flour were evaluated initially and at the end of six months of storage. *Puttu* and *idiappam* were prepared using the selected fresh RS incorporated as also stored flour and evaluated for *in vitro* starch digestibility and organoleptic qualities. All the quality attributes were compared with that of the products prepared using non-supplemented flour.

All the three physical qualities namely bulk density, water absorption index and water solubility index of *puttu* and *idiappam* flour supplemented with and without RS decreased during six months of storage.

The moisture content of *puttu* and *idiappam* flour prepared with and without RS supplementation increased during storage. The protein and total carbohydrate content of both flour decreased during storage.

With respect to the microbial count of the flour during storage, it was seen that the count of bacteria, yeast and fungi increased at the end of storage. The insect infestation was not at all observed at any storage time.

Puttu and *idiappam* prepared with and without RS supplementation obtained a mean score above seven when the products were prepared using the stored flour. However, the scores for different quality attributes as well as overall acceptability decreased slightly when the products were prepared with the stored flour when compared to fresh flour. Here, greater decrease was noticed in the products prepared with the non-supplemented flour.

A slight increase in the RS and RDS contents with a decrease in the SDS content occurred in the two products when they were prepared with the stored flour.

Postprandial glycaemic response of *puttu* and *idiappam* supplemented with RS was assessed among diabetic and non-diabetic individuals in comparison with non-supplemented products. The increase in blood glucose level was relatively in a lower range when RS supplemented *puttu* and *idiappam* were given to both diabetic as well as non-diabetic subjects as compared to the products without RS supplementation.The effect was found to be better among diabetic subjects.

The cost of production of 1kg *puttu* flour supplemented with 10g resistant starch and 1kg *idiappam* flour supplemented with 15g resistant starch were found to be Rs. 83.83 and Rs. 92.65 respectively.

Future line of work

Determination of RS content in important foods
Effect of household cooking methods on RS content
Detailed study on RS supplementation in diabetic subjects
Effect of RS supplementation on other life style diseases

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Appendices

APPENDIX- I

Score card for organoleptic evaluation of resistant starch supplemented rice flour (RSRF) products

Name of the product:
Name of the judge:
Date :

Sl.No.	Characteristics	I	II	III	IV
1.	Appearance				
2.	Colour				
3.	Flavour				
4.	Texture				
5.	Taste				
6.	Overall acceptability				

Evaluate the product on the basis of the scores given below

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Please mention specific comments if any:

Signature

APPENDIX-II

Cost of production for 1 kg *puttu* flour supplemented with 10g resistant starch

Sl.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Rice (Paddy@ Rs.28/Kg)	2.02 kg	56.50
	Alkali (NaOH)	6.23g	1.95
	Polypropylene bags	1Nos.	0.35
2.	**Other items**		
	Electricity charge	0.57 unit	1.72
	Milling charge	2.01 kg	3.03
	Powdering and roasting charge	1.3 kg	16.91
	Labour @Rs. 250 for 8 hours	5.4 minutes	3.37
	TOTAL		83.83

Cost of production for 1 kg *idiappam* flour supplemented with 15g resistant starch

Sl.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Rice (Paddy@ Rs.28/Kg)	2.12 kg	60.51
	Alkali (NaOH)	9.34g	2.92
	Polypropylene bags	1Nos.	0.35
2.	**Other items**		
	Electricity charge	0.88 unit	2.63
	Milling charge	2.16 kg	3.24
	Powdering and roasting charge	1.40 kg	17.95
	Labour @Rs. 250 for 8 hours	8.07 minutes	5.05
	TOTAL		92.65

OPTIMISATION AND UTILISATION OF RESISTANT STARCH FOR VALUE ADDITION IN RICE PRODUCTS

By

LILIA BABY

(2010-24 -101)

ABSTRACT OF THE THESIS

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ABSTRACT

The present study was undertaken to standardise processing treatments for optimising resistant starch (RS) formation in rice starch and to evaluate the changes in rice starch properties. The study also aimed to standardise rice flour supplemented with RS, to evaluate its quality attributes during storage and the effect of RS supplemented food products on postprandial glycaemic response.

Starch was isolated from the raw and parboiled rice of variety Uma. Raw rice had lower starch content and higher RS, compared to parboiled rice.

The effect of processing conditions on the RS content of rice starch was studied in 48 treatments by autoclaving at 121°C and 141°C for 20, 40 and 60 minutes without moisture and with 10, 20 and 40 percentage moisture levels. After autoclaving, the samples were cooled at room temperature and at -20°C. The yield of RS was found to be maximum in samples autoclaved at 141°C for 40 minutes with 10 per cent moisture and cooled at -20°C. Increasing the duration of autoclaving up to 60 minutes at a lower temperature of 121°C was also found to be effective in improving the RS content of rice starch. RS formation not only depends on a single factor but also on multiple factors like time and temperature of autoclaving, moisture as well as cooling conditions.

To study the effect of repeated autoclaving and cooling, five treatments yielding maximum RS content were selected. The RS content increased with increase in the number of autoclaving and cooling cycles irrespective of the temperature and time of autoclaving, moisture content and cooling conditions. Repeated autoclaving and cooling lowered the content of rapidly and slowly digestible starch.

The moisture and protein content of repeatedly autoclaved and cooled samples were significantly lower than the native starch. The total carbohydrate and amylose content increased during processing and found to be maximum in samples autoclaved at 141°C for 40 minutes and cooled at -20°C.

The standardisation of resistant starch supplemented rice flour (RSRF) was carried out by incorporating 10, 15 and 20g of RS to 100g of *puttu* and *idiappam* flour. For this, two treatments with maximum RS content were selected. Physical qualities of the flour, *in vitro* starch digestibility and organoleptic qualities of the products were evaluated. Incorporation of RS decreased the bulk density (BD) and water absorption index (WAI) of flour whereas water solubility index (WSI) increased. *In vitro* starch digestibility was found to be inversely proportional to the RS content of rice flour. The *puttu* and *idiappam* prepared with RS supplemented rice flour were found to be organoleptically acceptable.

The storage studies of RSRF were carried out by selecting RS incorporated *puttu* and *idiappam* flour having better organoleptic acceptance of their products. The flour was stored for six months under ambient condition after packing in polythene bags.

The physical qualities like BD, WAI and WSI of *puttu* and *idiappam* flour decreased during six months of storage. A decrease in protein and total carbohydrate content was noticed with an increase in the moisture level. The RS content of flour improved during storage. The count of bacteria, yeast and fungi increased towards the end of storage in both the flour. Insect infestation was not observed throughout the storage period. The organoleptic qualities of the *puttu* and *idiappam* decreased slightly, when they were prepared from stored flour.

Postprandial glycaemic responses of *puttu* and *idiappam* supplemented with RS was assessed among diabetic and non-diabetic individuals in comparison with non-supplemented products. The increase in blood glucose level was relatively at a lower rate when RS supplemented *puttu* and *idiappam* were given to diabetic as well as non-diabetic subjects. Better response was observed among diabetic subjects.

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