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Research Article

Characterization of Salubrious Germinated Rice (*Oryza sativa* L.) Laddus for Physico – Nutritional Attributes and Shelf Life Stability

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Abstract

Cereals constitute a major component of the global diet, with rice serving as the primary staple for a large proportion of the population in developing countries. Rice (*Oryza sativa* L.) is valued for its easy digestibility and therapeutic attributes, making it a suitable base for functional food development. The present study evaluated the physico-chemical characteristics, nutritional composition, shelf stability, and cost implications of value-added laddus prepared from germinated Siddi rice and germinated BPT 5204 rice, using raw rice laddu as the control. Colour analysis indicated that the L*, a*, and b* values of germinated Siddi rice laddu differed by less than 10% from the control, indicating good consumer-acceptable appearance. The total sugars, reducing and non-reducing sugars, total starch, amylopectin, and amylose contents were comparable between the control and experimental laddus. The control and germinated Siddi rice laddus exhibited water activity of 0.77 ± 0.06 and 0.68 ± 0.09, moisture content of 7.84 ± 0.19% and 7.35 ± 0.17%, ash content of 1.74 ± 0.00% and 1.84 ± 0.01%, protein content of 15.37 ± 0.42% and 16.81 ± 0.12%, fat content of 7.41 ± 0.12% and 8.16 ± 0.18%, crude fibre of 1.49 ± 0.00% and 1.42 ± 0.02%, carbohydrate content of 66.31 ± 0.23% and 62.22 ± 0.13%, and energy values of 388.1 ± 0.09 and 385.70 ± 0.08 kcal/100 g, respectively. Statistically significant differences ($p \leq 0.05$) were observed for most parameters, except ash and crude fibre. Overall, germinated Siddi rice laddus exhibited superior nutritional quality compared to those prepared from germinated BPT 5204 rice. Microbial analysis revealed an increase in total bacterial counts during storage; however, values remained within safe limits, with no mould growth observed for up to 15 days at ambient temperature and 30 days under refrigerated conditions in airtight containers. The production cost of the value-added laddus was Rs. 40 per 100 g, marginally higher than the control (Rs. 36 per 100 g). In nutshell. the present findings demonstrate the potential of germinated indigenous rice varieties, particularly Siddi rice, in developing affordable, nutritionally enhanced, and shelf-stable traditional foods. Such value-added products can contribute to dietary diversification, improved nutritional security, and income generation, especially in resource-limited and rice-dependent populations.

Keywords: Siddi, BPT 5204, colour, water activity, proximates, TMC, TBC and shelf life

Introduction

Rice plays an important role in national food security with the slogan "Rice is life" being more appropriate for India as it supports millions of households in rural and urban areas. In Asia, more than 2.0 billion people depended on rice to meet 80.0% of their energy needs as this grain contained 80.0% carbohydrates, 6.0 – 8.0% protein, 3.0% fat and fiber (Chaudhary and Tran, 2001). The world's second-most consumed cereal grain and primary source of protein is rice in many regions of the world particularly Asia (Parmar *et al.*, 2011). The better protein content and adequate amino acid profile of rice added nutritional value to poor man's diet apart from contributing to their protein intake also (Zhang *et al.*, 2011). Germinated cereal grains have softened grain texture prior to drying. When rice is soaked in water for a specific period of time, the embryo developed. Rice's bioactive components increased during germination and cooked faster due to breakdown of complex carbohydrates (Jiamyangyuen and Ooraikul, 2008). Siddi (WGL – 44) is saline tolerant, fine grain type with higher number of grains in panicle, good cooking quality and resistant to gall midge which is a major endemic in rice during kharif season with 135 – 140 days duration (PJTSAU – 50 years of AICRP Report, 2015). Legumes are widely grown all over the world for their nutritional and economic significance. In terms of protein quality, roasted green gram is on par with or better than other legumes and healthy source of carbohydrates, proteins and minerals (Kataria *et al.*, 1989). Pulses are significant crops providing nutrition to billions of people worldwide belonging to Fabaceae (Leguminosae) family and consumed as dry matter seeds known as grain legumes. These pulses have protein of 20 – 25% protein by weight and is second only to cereals in tropical and subtropical areas. The pulses when combined with cereals provided balanced amino acid profile (Tiwari and Singh, 2012). Green gram is on par with or better than other legumes like chickpea, black gram, peas, pigeon pea and also is a healthy source of carbohydrates, proteins and minerals (Jood *et al.*, 1998). Beetroot (*Beta vulgaris* L.) is a root vegetable belonging to family Chenopodiaceae containing vitamins, minerals, phenolics, carotenoids, nitrates, ascorbic acid and betalains that supported health by lowering risk of cardiovascular diseases and hypertension. Hence, using beetroot as an ingredient in various food products has positive impact on human wellbeing and opened up the possibility of creating functional foods (Chhikara *et al.*, 2019). Jaggery commonly called gur in India has excellent nutritional and therapeutic properties that is readily available to rural residents (Rao *et al.*, 2007). It is a well-known natural sweetener produced by concentrating sugarcane juice providing human body quick energy with good amounts of minerals, protein, glucose and fructose (Nath *et al.*, 2015).

Methodology

WGL – 44 (Siddi) used in the present study is procured from Krishi Vigyan Kendra, PJTAU, Wyra, Khammam and is stored under ambient conditions in jute bags till further analysis. The present health mix is selected based on sensory scores using 9-point hedonic scale for colour, appearance, taste and flavour, texture, chewiness and overall acceptability by 20 semi trained panellists from PGRC, PJTAU, Hyderabad The standardised health mixes contained 65.0% of germinated rice in control or germinated and malted Siddi rice or BPT 5204 rice as experimental mix with constant quantity of 15.0% roasted groundnut powder and 20.0% roasted green gram powder. The laddus are prepared using 100g standardised health mixes adding 25.0g of grated jaggery plus 20.0g of grated and dried beetroot. The germinated siddi laddus are compared with germinated BPT 5204 laddus for physico-chemical attributes and shelf-life stability at ambient temperature and under refrigeration.

Microscopic structure of raw and germinated rice starches

1.0 g of finely ground sample is taken in a beaker and added with about 50.0 ml of water. The contents are stirred continuously to break the adhered granules and avoid lump formation. It is allowed to stand for 5-6 min. A drop of the suspension is placed on a microscopic glass slide, pressed with a cover slip taking care that no air is trapped between the slide and cover slip. The excess liquid on the slide is removed with a piece of blotting paper. The prepared slide is examined under the Lawrence and Mayo binocular microscope. Similarly, starch structures of pregelatinized, gelatinized and retrograded rice samples are also examined (FSSAI, 2016).

Physico – nutritional attributes of laddus

The physical properties are analyzed using standard methods for colour (Hunter Lab, 2013). The chemical attributes of laddus analyzed by standard procedures are moisture, ash, protein, fat, crude fibre, carbohydrates and energy (AOAC, 2016), water activity (Abramovic *et al.*, 2008), total, reducing sugars, non-reducing sugars (Somogyi, 1952) along with total starch (Southgate, 1976), amylose and amylopectin (Williams *et al.*, 1958) respectively.

Parameters analysed for laddu during storage:

The parameters analysed for laddu during storage are moisture (AOAC, 2016), free fatty acids and titratable acidity (AACC. 2000) along with pH and TSS (Kathiravan *et al.*, 2014).

Shelf stability of laddus during storage

The shelf stability of laddus are evaluated for its physical parameters, sensory properties and microbial quality on 0^{th}, 7^{th}, 15^{th} and 30^{th} day of storage at ambient temperature and under refrigeration.

Results and Discussion

Initially, health mixes are subjected to microscopic analysis. Then, the laddus are prepared using raw rice, germinated siddi rice and BPT 5204 rice-based health mixes. They are analysed for their physico-chemical and nutritional properties as well as sensory parameters and microbial content during storage.

Microscopic structure of raw and germinated rice

Hydrothermal treatment is one of the most popular techniques used for producing fine grain powders as it involved heterogeneous chemical reactions in aqueous media at over 100°C and high-pressure levels. It is generally achieved using an autoclave oven or pressure cooker (Shandilya and Singh, 2016). The extent of changes in starch due to gelatinization and retrogradation are major determinants of their functional properties for food processing during digestion and in industrial applications. These characteristics determined the quality, acceptability, nutritional value and shelf stability of finished products (Wang and Copeland, 2013). The native, pregelatinised, gelatinised and retrograded starches for raw siddi rice

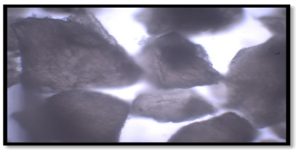
Fig. 1.1: Native RSR

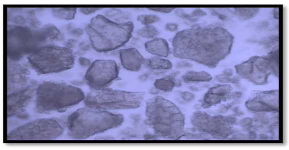
Fig. 1.2: Native GSR

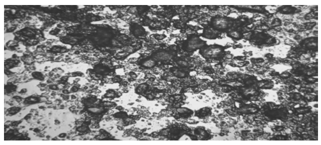
Fig. 1.3: Native GSHM

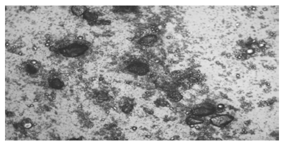
Fig. 1.4: Native MSHM

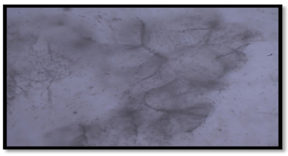
Fig. 2.1: Pregelatinised RSR

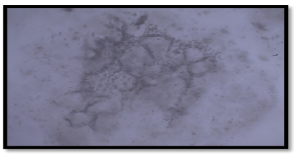
Fig. 2.2: Pregelatinised GSR

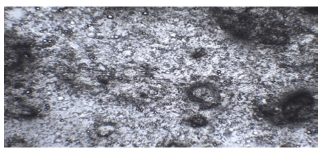
Fig. 2.3: Pregelatinised GSHM

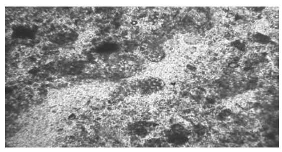
Fig. 2.4: Pregelatinised MSHM

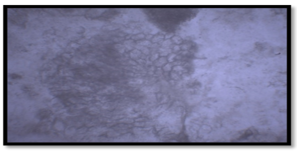
Fig. 3.1: Gelatinised RSR

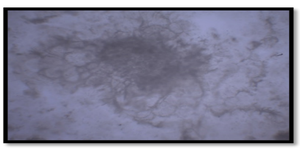
Fig. 3.2: Gelatinised GSR

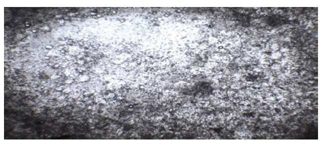
Fig. 3.3: Gelatinised GSHM

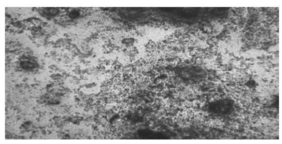
Fig. 3.4: Gelatinised MSHM

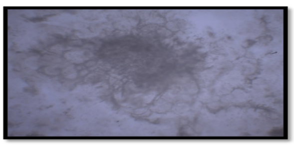
Fig. 4.1: Retrograded RSR

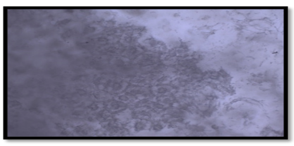
Fig. 4.2: Retrograded GSR

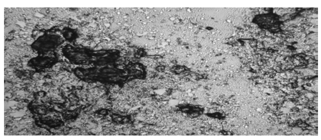
Fig. 4.3: Retrograded GSHM

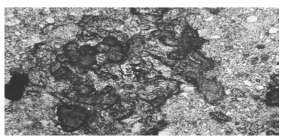
Fig. 4.4: Retrograded MSHM

Fig. 1 – 4: Microscopic structures of raw and germinated rice starches

Note: RSR: Raw siddi rice; GSR: Germinated siddi rice; GSHM: Germinated siddi health mix; MSHM: Malted siddi health mix

(RSR), germinated siddi rice (GSR), germinated siddi health mix (GSHM) and malted siddi health mix (MSHM) are visualised using Lawrence and Mayo binocular microscope to interpret their structural changes after hydrothermal treatments (Fig 1 – 4). The Fig. 1.1, 1.2, 1.3 and 1.4 depicted the structure of native RSR, GSR, GSHM and MSHM starches with clearly demarcated granules in a semi crystalline and amorphous form. In comparison to non-germinated rice, germinated rice produced a significant number of swollen granules with greater compactness. Starch isolated from the native and germinated rice showed similar shaped granules. This indicates that the germination could not affect the shape of rice starch granules. In addition, the starch isolated from the germinated rice exhibited dents surface structure compared with smooth surface of native starch.

Changes in the structural and physicochemical properties of quinoa starch during germination exhibited more pinholes and obvious crimples than native starch. Germination decreased the relative crystallinity of starch, but it did not change its crystalline structure type (Xing *et al.*, 2021).

The RSR, GSR, GSHM and MSHM starch granules are not only moist but swollen to a greater extent and no longer intact when the starches are hydrothermally treated for pregelatinisation as shown in Fig 2.1, 2.2, 2.3 and 2.4. Th pre-gelatinization is a hydrothermal process that initiated with boiling and ended with drying. It is an effective way to increase physicochemical and functional properties by a modified granule structure.

Pre-gelatinization of starch results in increase of solubility and swelling properties (Majzoobi *et al.*, 2011). Both sprouting and pregelatinization increased the amylose content of sorghum flour. The amylose increased with rise in the pregelatinization temperature and time as well as sprouting time in both treatments. This increase could be as a result of the broke down of the starch by the amylolytic enzymes inherent in the seeds during sprouting into simple sugars as amylose (Mbaeyi and Onweluzo, 2010).

Prolonged hydrothermal treatment for further starch gelatinization caused the remaining hydrogen bonds to break down leading to loss of birefringence, solubilization of starches and transformation into viscous paste. The germinated rice gel showed less air bubbles than raw rice gel indicating the compactness of gel due to presence of simple sugar formed during germination as depicted in Fig 3.1, 3.2, 3.3 and 3.4.

Gelatinization is an important phenomenon when it comes to the cooking properties, texture, and palatability of starch-based food products. Starch gelatinization disrupts the molecular orderliness within the granule and results in granular swelling, crystallite melting, loss of birefringence, increase in viscosity and solubilisation (Chakraborty *et al.*, 2022). The obtained results may help in understanding the influence of germination on functional properties of rice starch and choosing appropriate applications to promote utilization of rice starch in food industry.

When gelatinized starches are completely cooled, the disintegrated starch chains reorganised into partially ordered structures that are different from native granules as depicted in Fig 4.1, 4.2, 4.3 and 4.4 and considered as retrograded starches. The retrogradation is more visible in germinated rice gel than the raw rice gel. Retrogradation of starches is frequently thought to have negative effect on foods and is a significant cause of the staling of breads and other starch-rich foods causing decreased shelf life and consumer acceptance (Collar and Rosell, 2013). However, due to intended change of structural, mechanical and sensory properties, it is beneficial in specific applications for the production of breakfast cereals, parboiled rice and dehydrated mashed potatoes (Karim *et al.*, 2000). Additionally, it is favoured for its nutritional value since retrograded starches controlled the release of glucose into the blood stream due to slower enzymatic digestion (Copeland *et al.*, 2009; Shandilya and Singh, 2016).

Physico-nutritional characteristics of control, germinated siddi and germinated BPT 5204 laddu:

The parameters like colour, total sugars, reducing sugars, non-reducing sugars, total starch, amylose and amylopectin of developed value added laddu with control (CL), germinated siddi (GSL) and BPT 5204 (GBL) health mixes are evaluated and presented in Tables 1 and 2.

Colour scores of CL, GSL and GBL laddu are presented as L*, a* and b* values after analysing using Munsell colour charts (Table 1). The L* and b* values for CL with 42.77±0.21, 19.64±0.45 respectively, for GSL with 41.63±0.15, 11.42±0.13, and 57.60±0.36 respectively and for GBL laddu with 40.45±0.13, 16.72±0.11 indicating more lightness with yellow character for siddi laddu.

Table 1: Colour analysis of laddus

Sample	L*	a*	b*
CL	42.77^{c}±0.21	10.62^{a}±0.27	19.64^{c}±0.45
GSL	41.63^{b}±0.15	11.42^{b}±0.13	18.00^{b}±0.25
GBL	40.45^{a}±0.13	12.92^{c}±0.10	16.72^{a}±0.11
Mean	41.63	11.65	18.12
SE of Mean	0.16	0.18	0.27

Note: Values are expressed as mean ± standard deviation of three determinations; Means within the same column followed by a common letter do not significantly differ at p ≤ 0.05; CL: Control laddu; GSL: Germinated Siddi laddu; GBL: Germinated BPT 5204 laddu.

While the maximum a* values are noticed for GBL laddu with 12.92±0.10 when compared to GSL laddu with 11.42±0.13 and least for CL laddu with 10.62±0.27 indicating more redness and higher colour intensity for GBL laddu as perceived by human eye. There is statistically significant difference for all colour values of samples (p≤0.05).

The total sugar content of CL laddu is 28.12±0.36%, GSL laddu is 26.13±0.36% and for GBL laddu is 24.40±0.39% as shown in Table 2. The reducing sugar content of CL laddu is 2.26±0.11% and GSL laddu is 2.15±0.12% and for GBL laddu is 1.99±0.02%. There is statistically no significant difference (p≤0.05) between CL and GSL but statistically significant difference (p≤0.05) is observed between GSL and GBL. The non-reducing sugar content of CL laddu is 26.96±0.40%, GSL laddu is 22.98±0.30% and GBL laddu is 22.42±0.20%.

Table 2: Sugars and starch composition of value added laddus

Sample	Total sugars	Reducing sugars	Non-reducing sugars	Total starch	Amylose	Amylopectin
CL	28.12^{c}±0.36	2.26^{b}±0.11	26.96^{c}±0.40	56.67^{c}±0.36	36.71^{d}±0.22	21.04^{b}±0.18
GSL	26.13^{b}±0.36	2.15^{b}±0.12	22.98^{b}±0.30	58.83^{d}±0.46	35.02^{c}±0.19	23.78^{c}±0.38
GBL	24.40^{a}±0.39	1.99^{a}±0.02	22.42^{b}±0.20	59.20^{e}±0.32	33.50^{b}±0.27	25.70^{d}±0.46
Mean	26.22	2.13	24.12	58.24	35.08	23.50
SE of Mean	0.36	0.08	0.30	0.38	0.22	0.32

Note: Values are expressed as mean ± standard deviation of three determinations; Means within the same column followed by a common letter do not significantly differ at p ≤ 0.05. Unit of measurement is %. CL: Control laddu; GSL: Germinated Siddi laddu; GBL: Germinated BPT 5204 laddu

The total starch content of CL laddu is 56.67±0.36% and GSL laddu is 58.83±0.46% and GBL laddu is 59.20±0.32% indicating quicker gelatinisation.The amylose content of CL laddu is 36.71±0.42%, GSL laddu is 35.02±0.19% and GBL laddu is 33.50±0.27% with the grand mean ± SE of 35.08±0.22% and statistically significant difference (p≤0.05) between samples.The amylopectin content of CL laddu is 21.04±0.98%, GSL laddu is 23.78±0.38% and GBL laddu is 25.70±0.46% with grand mean ± SE of 23.50±0.32% and statistically significant difference (p≤0.05) between samples. The lower total starch in CL laddu compared to GSL and GBL laddus is due to the germination process that broke down complex carbohydrates leading to higher availability of simple sugars during analysis as shown in Table 2. The water activity of CL laddu is 0.72±0.01 and GSL laddu is 0.68±0.09 and GBL laddu is 0.77±0.06 with a grand mean ± SE of 0.72±0.06 and statistically significant difference (p≤0.05) is present between the samples. The monitoring of water activity is an important method to control the growth of moulds from spoiling foods (Mwikya *et al.*, 2000).

The mean scores of proximates for CL, GSL and GBL laddu are tabulated in Table 3. The moisture content of CL laddu is 7.55±0.13%, GSL laddu is 7.35±0.17% and GBL laddu is 7.84±0.19% with a grand mean ± SE of 7.58±0.16% and statistically significant difference (p≤0.05) is observed for them. The ash content of CL laddu is 1.80±0.01%, GSL laddu is 1.84±0.01% and GBL laddu is 1.74±0.00% with a grand mean ± SE of 1.79±0.01% and statistically no significant difference (p≤0.05) for the samples is observed.

The protein content of CL laddu is 17.65±0.26% and GSL laddu is 16.81±0.12% and GBL laddu is 15.37±0.42% with statistically significant difference ($p \leq 0.05$) between them. The fat content of CL laddu is 7.88±0.16%, GSL laddu is 8.16±0.18% and GBL laddu is 7.41±0.12% with statistically significant difference ($p \leq 0.05$) between them.

Table 3: Proximate analysis of value-added laddus

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Crude fiber (%)	Carbohydrates (%)	Energy (Kcal/100g)
CL	7.55^{b}±0.13	1.80^{a}±0.01	17.65^{c}±0.26	7.88^{b}±0.16	1.68^{b}±0.10	63.63^{c}±0.20	395.20^{c}±0.12
GSL	7.35^{a}±0.17	1.84^{a}±0.01	16.81^{b}±0.12	8.16^{c}±0.18	1.42^{a}±0.02	62.22^{b}±0.13	385.70^{a}±0.08
GBL	7.84^{c}±0.19	1.74^{a}±0.00	15.37^{a}±0.42	7.41^{a}±0.12	1.49^{a}±0.00	66.31^{c}±0.23	388.10^{b}±0.09
Mean	7.58	1.79	16.62	7.82	1.53	64.06	389.66
SE of Mean	0.16	0.01	0.26	0.16	0.04	0.16	0.08

Note: Values are expressed as mean ± standard deviation of three determinations; Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$; CL: Control laddu; GSL: Germinated Siddi laddu; GBL: Germinated BPT 5204 laddu

The crude fiber content of CL laddu is 1.68±0.10% and GSL laddu is 1.42±0.02% and GBL laddu is 1.49±0.00% with statistically significant difference ($p \leq 0.05$) is observed between CL and GSL but statistically no significant difference ($p \leq 0.05$) between GSL and GBL.The carbohydrate content of CL laddu is 63.63±0.20%, GSL laddu is 62.22±0.13% and GBL laddu is 66.31±0.23% and statistically significant difference ($p \leq 0.05$) observed between the samples. The loared protein and fat content of GBL laddu increased its carbohydrate content. The energy content of CL laddu is 395.20±0.12 Kcal/100g, GSL laddu is 385.70±0.08 Kcal/100g and GBL laddu is 388.10±0.09 Kcal/100g. There is statistically significant difference ($p \leq 0.05$) between the samples. The loared water activity for GSL laddu may keep the product shelf stable for longer duration than GBL laddu. The GSL laddu provided higher protein and fat than GBL laddu making it suitable for underweight people and can be included in protein deficit diets. The disruption of starch structure during pregelatinisation released amylopectin that is partially responsible for starch swelling in health mixes (Thomas and Atwell, 1999). Seed sprouting involved energy use from break down of starch to sugars and lipids to free fatty acids resulting in a shift of nutrient profile (Peer and Leeson, 1985).

Shelf life is referred to the duration of consumer's acceptance of a foodstuff. Any food developed can undergo deterioration due to spoilage, develop off flavours due to microbes and auto-oxidation by natural enzymes present in them (Balasubramanian and Viswanathan, 2010). This may lead to development of health hazards in the consumers. Hence, food storage and its safety become an integral part of food processing and product development (Geetha *et al.*, 2020). The developed rice laddus are further evaluated for moisture, titratable acidity, pH, TSS, free fatty acids, sensory parameters and microbial quality (total bacterial and mould counts) on 0th, 7th and 15th at ambient temperature and 0th, 7th, 15th and 30th day under refrigeration for shelflife stability. The moisture content of laddu increased over the time from 0th to 30th day. The moisture content of GSL laddu at ambient temperature and RGSL laddu under refrigeration ranged from 7.35±0.01 to 10.62±0.03 and 7.35±0.01 to 8.82±0.12 and for GBL laddu and RGBL laddu are between 7.84±0.24 to 10.74±0.18 and 7.84±0.12 to 9.22±0.23 respectively as depicted in Tables 4a and 4b. The moisture content of laddu increased gradually for all samples during storage at ambient temperature and under refrigeration. For GSL and RGSL laddu, it increased by 10.06, 31.15, 44.48% respectively and 0.95, 14.28, 20.00% respectively whereas GBL and RGBL laddu, it increased by 4.85, 23.45 and 36.98% respectively and 1.02, 10.46 and 17.6% respectively on 7th, 15th and 30th day of storage compared to fresh laddu. The laddu are found to be hygroscopic during storage. The laddus are found to be hygroscopic when stored in air tight containers during storage. The titratable acidity of GBL laddu at ambient temperature and refrigeration ranged from 9.90±0.08 to 10.68±0.07 and 9.90±0.08 to 10.58±0.27% LA with statistically significant difference ($p \leq 0.05$) between samples as depicted in Table 4a. While the titratable acidity of GSL laddu at ambient temperature and refrigeration ranged from 8.60±0.16 to 10.26±0.06% and 8.60±0.16 to 10.18±0.03% LA from 0th to 30th day of storage with statistically significant difference ($p \leq 0.05$) between samples as depicted in Table 4b.

The titratable acidity increased gradually for all samples. The GSL laddu showed increase of 1.16, 6.97, 19.3 respectively whereas for RGSL laddu increased by 1.39, 6.74, 18.37% respectively by 30th day of storage at ambient temperature. For GBL laddu, it increased to 3.33, 5.65, 7.88% and for RGBL laddu increased to 2.22, 3.33, 6.87% by 30th day of storage compared to fresh sample under refrigeration. The titratable acidity decreased in samples stored under refrigeration due to acidic hydrolysis of polysaccharides where acids are utilised to convert non-reducing sugars into reducing sugars (Bhardwaj and Pandey, 2011).

The pH of GBL laddu at ambient temperature and refrigerated storage ranged from 7.31±0.25 to 7.20±0.13 and 7.31±0.25 to 7.06±0.08 respectively with statistically significant difference ($p \leq 0.05$) between samples as depicted in Table 4a. The pH of GSL laddu at ambient temperature and refrigerated storage ranged from 7.39±0.45 to 6.78±0.25 and 7.39± 0.12 to 6.68±0.23 respectively with grand mean ± SE of 7.04±0.42 and 6.99±0.12 and statistically significant difference ($p \leq 0.05$) is observed between samples from 0th to 30th day of storage as depicted in Table 4b. The pH decreased gradually for all samples GSL and RGSL laddu by 2.57 – 9.06 % while for the GBL and RGBL laddus pH decreased by 0.42 – 3.42% on 7th, 15th and 30th day of storage making them slightly acidic. Since acidity and pH are inversely correlated, an increase in titratable acidity caused a pH drop. Acetic acid and lactic acid generation during storage may be the cause of high acidity and loared pH caused by breakdown of sugars (Hariharan and Mahendran, 2016).

The formation of acid is due to breakdown of simple sugars formed during germination. The storage of millets and its products resulted in complex reactions *viz.* lipid hydrolysis, oxidation and polymerization leading to quality loss. The lipoxygenase (LOX) in millet kernels oxidised the unsaturated fatty acid with deterioration in flavour and quality. The products with a_w below 0.60 are stable against microbial growth still chemical and enzymatic reactions can occur resulting in development of off-flavours and product deterioration during storage (Jensen and Risbo, 2007; Wang *et al.*, 2014).

There is a gradual increase in TSS for both laddus as depicted in Tables 4a and 4b. This may be because of starch and other polysaccharide degradation in the laddus to soluble sugars during storage (Meena *et al.*, 2018). The TSS of GBL and RGBL laddu ranged from 2.03±0.16 to 3.86±0.06 and 2.03±0.12 to 3.76±0.12 °Brix with statistically significant difference ($p \leq 0.05$) between samples. Likewise, the TSS of GSL laddu at ambient temperature and under refrigeration are 2.28±0.08 to 3.86±0.16 °Brix and 2.28±0.08 to 3.66±0.06 °Brix with grand mean ± SE of 3.52±0.26 and 3.19±0.13 °Brix and statistically significant difference ($p \leq 0.05$) between them during 0th to 30th day of storage. The TSS increased in stored laddu due to deteoration of carbohydrates by hydrolysis or by bacteria as shown in shelf-life studies. As storage time extended, the TSS rose gradually which may have been caused by the hydrolysis of polysaccharides into monosaccharides and oligosaccharides (Bhardwaj and Mukherjee, 2011).The TSS increased gradually for all samples. The GBL laddu showed TSS increase by 11.33, 21.18, 90.14% and RGBL laddu by 22.16, 58.62, 85.22% respectively, while for GSL laddu increase is by 32.90, 42.98, 69.29% respectively and for RGSL laddu, it increased by 15.35, 34.21, 60.52% respectively during 7th, 15th and 30th day of storage compared to fresh laddu. The TSS increased in stored laddu due to deterioration of carbohydrates.

The free fatty acid content of laddus increased during storage. The free fatty acid content of GSL and RGSL laddu ranged from 0.53±0.13 to 0.92±0.23 and 0.53±0.13 to 0.72±0.10 and for GBL and RGBL laddu are 0.56±0.14 to 0.94±0.08 and 0.56±0.14 to 0.82±0.03 respectively at ambient temperature and under refrigeration (Tables 4a and 4b) with statistically significant difference ($p \leq 0.05$). The increased FFA in refrigerated samples than for ambient temperature samples indicated that moisture may influence formation of FFA. The increased TSS may be due to the laddus being hygroscopic and absorbing due to refrigeration. The free fatty acids of laddu increased gradually for all samples. For GSL and RGSL laddu, it increased as 11.32, 24.52, 35.85% and 9.43, 13.2 and 16.98% respectively and for GBL and RGBL laddu it increased by 14.28, 25.00, 32.14% and 12.5, 14.28, 28.57% respectively during 7, 15 and 30 days of storage compared to freshly prepared laddu. The increase FFA for refrigerated samples is higher than at ambient temperature indicating that moisture may influence formation of FFA. The increased TSS may cause the laddus to be hygroscopic.

Table 4a: Parameters for shelf stability of Siddi laddus

Day	Moisture (%)		Titratable acidity (%)		pH		TSS (ºBrix)		Free fatty acid (%)	
	GSL	RGSL	GSL	RGSL	GSL	RGSL	GSL	RGSL	GSL	RGSL
0th day	$7.35^{a}\pm0.01$	$7.35^{a}\pm0.01$	$8.60^{a}\pm0.16$	$8.60^{a}\pm0.16$	$7.39^{a}\pm0.45$	$7.39^{a}\pm0.45$	$2.28^{a}\pm0.08$	$2.28^{a}\pm0.08$	$0.53^{a}\pm0.13$	$0.53^{a}\pm0.13$
7th day	$8.09^{b}\pm0.06$	$7.42^{b}\pm0.02$	$8.70^{b}\pm0.12$	$8.72^{b}\pm0.08$	$7.20^{b}\pm0.24$	$7.18^{b}\pm0.08$	$3.03^{b}\pm0.09$	$2.63^{b}\pm0.09$	$0.59^{b}\pm0.03$	$0.58^{b}\pm0.12$
15th day	$9.64^{c}\pm0.12$	$8.40^{c}\pm0.11$	$9.20^{c}\pm0.07$	$9.18^{c}\pm0.07$	$6.82^{c}\pm0.05$	$6.72^{c}\pm0.12$	$3.26^{c}\pm0.22$	$3.06^{c}\pm0.12$	$0.66^{c}\pm0.16$	$0.60^{c}\pm0.06$
30th day	$10.62^{d}\pm0.03$	$8.82^{d}\pm0.12$	$10.26^{d}\pm0.06$	$10.18^{d}\pm0.03$	$6.78^{d}\pm0.25$	$6.68^{d}\pm0.23$	$3.86^{d}\pm0.16$	$3.66^{d}\pm0.06$	$0.92^{d}\pm0.23$	$0.72^{d}\pm0.10$
Mean	8.92	7.98	9.19	10.18	7.04	6.99	3.52	3.19	0.62	0.58
SE of mean	0.08	0.10	0.10	0.08	0.42	0.12	0.26	0.13	0.12	0.08

Note: Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$; GSL: Germinated Siddi laddu at ambient temperature; RGSL: Germinated Siddi laddu under refrigeration; GBL: Germinated BPT 5204 laddu at ambient temperature; RGBL: Germinated BPT 5204 laddu under refrigeration

Table 4b: Parameters for shelf stability of BPT 5204 laddu

Day	Moisture (%)		Titratable acidity (%)		pH		TSS (ºBrix)		Free fatty acid (%)	
	GBL	RGBL	GBL	RGBL	GBL	RGBL	GBL	RGBL	GBL	RGBL
0th day	$7.84^{a}\pm0.24$	$7.84^{a}\pm0.12$	$9.90^{a}\pm0.08$	$9.90^{a}\pm0.08$	$7.31^{a}\pm0.25$	$7.31^{a}\pm0.25$	$2.03^{a}\pm0.16$	$2.03^{a}\pm0.12$	$0.56^{a}\pm0.14$	$0.56^{a}\pm0.14$
7th day	$8.22^{b}\pm0.03$	$7.92^{b}\pm0.06$	$10.23^{b}\pm0.12$	$10.12^{b}\pm0.09$	$7.28^{b}\pm0.23$	$7.18^{b}\pm0.24$	$2.26^{b}\pm0.08$	$2.48^{b}\pm0.24$	$0.64^{b}\pm0.03$	$0.63^{b}\pm0.06$
15th day	$9.68^{c}\pm0.10$	$8.66^{c}\pm0.26$	$10.46^{c}\pm0.04$	$10.23^{c}\pm0.07$	$7.24^{c}\pm0.12$	$7.14^{c}\pm0.12$	$2.46^{c}\pm0.18$	$3.22^{c}\pm0.18$	$0.70^{c}\pm0.09$	$0.64^{c}\pm0.06$
30th day	$10.74^{d}\pm0.18$	$9.22^{d}\pm0.23$	$10.68^{d}\pm0.07$	$10.58^{d}\pm0.27$	$7.20^{d}\pm0.13$	$7.06^{d}\pm0.08$	$3.86^{d}\pm0.06$	$3.76^{d}\pm0.12$	$0.94^{d}\pm0.08$	$0.82^{d}\pm0.03$
Mean	9.12	8.41	10.32	10.20	7.25	7.17	3.89	3.62	0.66	0.63
SE of mean	0.07	0.09	0.28	0.38	0.04	0.08	0.18	0.13	0.12	0.08

Note: Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$; GSL: Germinated Siddi laddu at ambient temperature; RGSL: Germinated Siddi laddu under refrigeration; GBL: Germinated BPT 5204 laddu at ambient temperature; RGBL: Germinated BPT 5204 laddu under refrigeration

Sensory analysis of laddus during storage

The sensory evaluation is conducted to know the changes in value added product laddus as shown in Tables 5a and 5b. On 0th to 15th day of storage, it is found that both laddus received acceptable sensory scores under ambient temperature and refrigeration. Results showed that taste, flavour and overall acceptability are high for GSL laddu. The appearance scores are 8.76 and 8.73 respectively for GSL and GBL laddu. The colour score for GBL laddu is 8.86 and GSL laddu is 8.82. Taste and flavour of GSL is 8.78 and for GBL is 8.72. Texture scores are 8.88 and 8.62 respectively for GSL and GBL laddu. Chewiness scores of GSL and GBL laddu are 8.80 and 8.78 respectively. The overall acceptability scores are 8.82 and 8.86 for GSL and GBL laddu on 0th day. The sensory scores of laddu decreased under ambient temperature from 0th to 15th day of storage. Under refrigeration sensory scores declined from 0th to 30th day of storage. All sensory parameters decreased over the course of the storage period due to increased moisture, TSS and FFA content under ambient and refrigerated conditions. The percentage decrease in sensory parameters for GSL laddu is 1.36 to 14.05% for colour. 1.14 to 15.30%, for appearance, for taste and flavour sensory scores decreased from 1.82 to 19.60% respectively, for texture 5.85 to 25.45% respectively, for chewiness 2.04 to 27.72% respectively and overall acceptability decreased from 2.70 to 27.53% respectively on 7th and 15th day of storage which is gradual decreased as the duration increased (Table 5a). The percentage decrease in sensory parameters is gradual for RGSL laddu and decreased by 0.68, 1.58 and 17.68% respectively for colour, 0.92, 3.88 and 18.72% respectively for appearance, for taste and flavour sensory scores decreased by 0.68, 6.37 and 24.6% respectively, for texture by 5.18, 9.23, 29.95% respectively, for chewiness by 2.04, 10.45 and 28.86% respectively and overall acceptability by 2.03, 10.60 and 32.02% respectively from 7th to 30th day of storage (Table 5a).

The percentage decrease in sensory parameters for GBL laddus are 2.03 to 4.28% for colour, 3.55 to 5.38% for appearance, taste and flavour sensory scores decreased from 5.73 to 8.02%, for texture 5.80 to 10.78%, for chewiness 10.25 to 20.04% and overall acceptability decreased from 6.58 to 27.21% on 7th and 15th day of storage (Table 5b). The percentage decrease in sensory parameters of RGBL laddu are 2.03, 4.96 and 18.73% respectively for colour, 3.55, 4.23 and 19.70% respectively for appearance, for taste and flavour sensory scores decreased from 2.3, 6.88 and 26.6% respectively, for texture 4.17, 6.96 and 28.53% respectively, for chewiness 7.06, 10.13 and 29.04% respectively and overall acceptability decreased from 7.93, 10.65 and 33.22% respectively from 7th to 30th days of storage compared to fresh samples (Table 5b). There is statistically no significant difference ($p \leq 0.05$) for colour, appearance and texture but, statistically significant difference for taste, flavour, chewiness and overall acceptability at $p \leq 0.05$ of GSL and GBL laddus from 0th to 15th days of storage. There is statistically no significant difference ($p \leq 0.05$) for colour, appearance, texture, taste and flavour of RGSL and RGBL laddus from 0th to 30th day of storage with statistically significant difference for chewiness and overall acceptability of both samples at $p \leq 0.05$ during storage.

Total bacterial and mould count of laddus during storage

The analysis of total bacterial count is done to detect their presence in these laddus stored at ambient temperature and under refrigeration. No bacterial growth is found up to 7th day of storage period at ambient temperature. From 15th day of storage, the bacterial growth gradually increased at ambient temperature. The shelf life increased to 30th day under refrigeration for laddus placed in air tight container. The bacterial growth gradually increased as shown in Table 6 may be due fluctuations in temperatures and poor handling during storage. There is no mould growth up to 15th day of storage at ambient temperature and 30th day of storage for refrigerated laddus placed in air tight containers may be due to lower water activity as shown in Table 3. Storing products at low temperatures helped preserve food products for longer period by slowing down processes like bacterial growth and oxidation (Kilima *et al.*, 2014). The TBC of fresh millet-based diabetes mix is found to be $1.86 \pm 0.30 \times 10^3$ CFU which increased significantly to $5.97 \pm 0.58 \times 10^3$ CFU as the storage period increased to 90 days. The increase is within safer limit and with no detection of moulds in both the fresh and stored samples during storage (Geetha *et al.*, 2020).

Table 5a: Sensory analysis of siddi value added laddus during storage at ambient temperature and under refrigeration

Day	Colour		Appearance		Taste and flavour		Texture		Chewiness		Overall acceptability	
	GSL	RGSL	**GSL**	**RGSL**	**GSL**	**RGSL**	**GSL**	**RGSL**	**GSL**	**RGSL**	**GSL**	**RGSL**
0^{th}	$8.82^{a}\pm0.15$	$8.82^{c}\pm0.14$	$8.76^{ab}\pm0.12$	$8.76^{b}\pm0.18$	$8.78^{c}\pm0.17$	$8.78^{ab}\pm0.18$	$8.88^{b}\pm0.12$	$8.88^{a}\pm0.16$	$8.80^{c}\pm0.15$	$8.80^{d}\pm0.16$	$8.86^{c}\pm0.16$	$8.86^{d}\pm0.14$
7^{th}	$8.70^{a}\pm0.18$	$8.76^{c}\pm0.12$	$8.66^{ab}\pm0.16$	$8.68^{b}\pm0.24$	$8.62^{b}\pm0.24$	$8.72^{ab}\pm0.12$	$8.36^{b}\pm0.45$	$8.42^{a}\pm0.22$	$8.62^{b}\pm0.14$	$8.62^{c}\pm0.12$	$8.62^{b}\pm0.22$	$8.68^{c}\pm0.16$
15^{th}	$7.58^{a}\pm0.12$	$8.68^{c}\pm0.08$	$7.42^{ab}\pm0.24$	$8.42^{b}\pm0.22$	$7.06^{a}\pm0.16$	$8.22^{ab}\pm0.03$	$6.62^{b}\pm0.32$	$8.06^{a}\pm0.14$	$6.36^{a}\pm0.12$	$7.88^{b}\pm0.18$	$6.42^{a}\pm0.12$	$7.92^{b}\pm0.12$
30^{th}	-	$7.26^{c}\pm0.06$	-	$7.12^{b}\pm0.12$	-	$6.62^{ab}\pm0.06$	-	$6.22^{a}\pm0.08$	-	$6.26^{a}\pm0.22$	-	$6.02^{a}\pm0.08$
Mean	8.36	8.38	8.28	8.25	8.15	8.08	7.95	7.90	7.92	7.89	7.96	7.87
SE of mean	0.32	0.12	0.16	0.24	0.18	0.12	0.17	0.18	0.15	0.16	0.16	0.09

Note: Values are expressed as mean ± standard deviation of fifteen determinations; Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$; GSL: Germinated Siddi laddu at ambient temperature; RGSL: Germinated Siddi laddu under refrigeration; GBL: Germinated BPT 5204 laddu at ambient temperature; RGBL: Germinated BPT 5204 laddu under refrigeration

Table 5b: Sensory analysis of BPT 5204 value added laddus during storage at ambient temperature and under refrigeration

Day	Colour		Appearance		Taste and flavour		Texture		**Chewiness**		**Overall acceptability**	
	GBL	**RGBL**	**GBL**	**RGBL**	**GBL**	**RGBL**	**GBL**	**RGBL**	**GBL**	**RGBL**	**GSL**	**RGSL**
0^{th}	$8.86^{a}\pm0.16$	$8.86^{c}\pm0.07$	$8.73^{ab}\pm0.08$	$8.73^{b}\pm0.16$	$8.62^{b}\pm0.18$	$8.72^{ab}\pm0.09$	$8.72^{c}\pm0.22$	$8.62^{a}\pm0.12$	$8.78^{c}\pm0.12$	$8.78^{d}\pm0.12$	$8.82^{c}\pm0.03$	$8.82^{d}\pm0.22$
7^{th}	$8.68^{a}\pm0.12$	$8.68^{c}\pm0.12$	$8.42^{ab}\pm0.12$	$8.42^{b}\pm0.06$	$8.12^{b}\pm0.24$	$8.52^{ab}\pm0.06$	$8.22^{b}\pm0.20$	$8.26^{a}\pm0.16$	$7.88^{b}\pm0.06$	$8.16^{c}\pm0.24$	$8.24^{b}\pm0.06$	$8.12^{c}\pm0.18$
15^{th}	$8.48^{a}\pm0.06$	$8.42^{c}\pm0.15$	$8.26^{ab}\pm0.18$	$8.36^{b}\pm0.08$	$7.69^{b}\pm0.26$	$8.12^{ab}\pm0.03$	$8.02^{a}\pm0.16$	$8.02^{a}\pm0.22$	$7.02^{a}\pm0.14$	$7.89^{b}\pm0.22$	$6.42^{a}\pm0.10$	$7.88^{b}\pm0.24$
30^{th}	-	$7.20^{c}\pm0.13$	-	$7.01^{b}\pm0.12$	-	$6.40^{ab}\pm0.12$	-	$6.16^{a}\pm0.14$	-	$6.23^{a}\pm0.22$	-	$5.89^{a}\pm0.18$
Mean	8.68	8.30	8.47	8.13	8.14	7.95	8.32	7.75	7.90	7.76	7.82	7.68
SE of mean	0.12	0.14	0.24	0.16	0.32	0.10	0.28	0.12	0.16	0.24	0.08	0.28

Note: Values are expressed as mean ± standard deviation of fifteen determinations; Means within the same column followed by a common letter do not significantly differ at $p \leq 0.05$; GSL: Germinated Siddi laddu at ambient temperature; RGSL: Germinated Siddi laddu under refrigeration; GBL: Germinated BPT 5204 laddu at ambient temperature; RGBL: Germinated BPT 5204 laddu under refrigeration

Table 6: Total bacterial count of Siddi and BPT 5204 laddus during storage

Dilution	TBC (CFU/g) for GSL and GBL			TBC (CFU/g) for RGSL and RGBL			
	0th day	7th day	15th day	0th day	7th day	15th day	30th day
10^{-1}	Nil	Nil	Too many to count	Nil	Nil	18.28±0.66** 24.46±0.02##	Too many to count
10^{-2}	Nil	Nil	108.68±1.49* 128.88±1.89#	Nil	Nil	6.38±1.50** 8.26±0.32##	118.48±1.89** 126.45±0.29##
10^{-3}	Nil	Nil	1.46±0.02* 2.43±0.19#	Nil	Nil	Nil	20.26±0.12** 24.26±0.42##
10^{-4}	Nil	Nil	Nil	Nil	Nil	Nil	Nil
10^{-5}	Nil	Nil	Nil	Nil	Nil	Nil	Nil
10^{-6}	Nil	Nil	Nil	Nil	Nil	Nil	Nil
10^{-7}	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Note: Values are of three determinations; *GSL: Germinated siddi laddu at ambient temperature; #GBL: Germinated BPT 5204 laddu at ambient temperature; **RGSL: Germinated siddi laddu under refrigeration; ##RGBL: Germinated BPT 5204 laddu under refrigeration

Costing of the developed laddus

The price of germinated rice, roasted green gram and groundnut are calculated based on the prevailing price of ingredients used in formulation along with processing charges. The total cost expended for the preparation of laddus is calculated for 100 g (Table 7). The developed laddus costed between Rs. 36.00 to 40.00 for the various rice based laddus which are affordable and cost-effective than the commercial laddus. Besides this, the developed products are nutritionally rich and made with easily available food ingredients. The cost of laddus can be decreased when prepared in bulk as more quantities will be purchased in bulk.

Table 7: Cost of laddus

Ingredients	Quantity of ingredients used (g)	Cost in Rs. / Kg for			Cost of 100 g in Rs. for		
		CL	GSL	GBL	CL	GSL	GBL
Rice	65.0	68.00	68.00	72.00	4.45	4.45	4.68
Green gram	20.0		137.00			2.74	
Groundnuts	15.0		156.00			2.34	
Jaggery	25.0		130.00			3.25	
Beetroot	20.0		40.00			0.80	
Fuel	-	15.00	15.00	15.00	10.00	14.00	14.00
Labour	-	12.00	12.00	12.00	12.00	12.00	12.00
Total					**36.00**	**40.00**	**40.00**

Note: CL – Germinated Siddi rice; GSL – Germinated and malted Siddi rice; GBL – Germinated and malted BPT 5204 rice

Conclusions

The present study concluded that the increased temperature altered cell wall structure, promoting fibre depolymerization and solubilization during roasting. Germinated Siddi laddu (GSL) showed improved colour attributes, higher starch and amylopectin, and distinct nutritional differences compared to control laddu (CL). Storage led to increased moisture, TSS and free fatty acids with a decline in sensory quality over time. The developed composite laddus remained shelf-stable for 30 days under refrigeration and 15 days at ambient conditions.

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Author Contributions

AA carried out the research work, compiled the data and wrote the original manuscript, JSW conceived, presented the idea, monitored the research work and edited the draft, BAK reviewed the progress of the research work, JHK

has helped in procuring raw rice samples and MS helped in carrying out of statistical analysis.

Conflict of Interest

There is no conflict of interest as declared by all the authors.

Ethical Approval

The article doesn't contain any study involving ethical approval.

Use of Generative AI or AI assisted technologies

Authors declare that no Generative AI or AI assisted technologies have been used in preparation of this manuscript.

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