कम ग्लाइसेमिक क्षमता वाले फाइबर समृद्ध भारतीय चावल किस्मों की पहचान करने के लिए स्टार्च गुणवत्ता मैट्रिक्स विकसित करना

Developing starch quality matrix to identify fibre rich Indian rice varieties with low glycaemic potential

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CERTIFICATE

This is to certify that the thesis entitled, ***‘‘Developing starch quality matrix to identify fibre rich Indian rice varieties with low glycaemic potential”*** submitted to the Faculty of Post Graduate School, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, in partial fulfilment of requirement of degree of **Master of Science in Biochemistry**, embodies the result of a *bona-fide* research work carried out by **Mr. Sohel Rahaman (Roll No. 21105)** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma

It is further certified that any help or source of information, as has been availed of in this work, has been duly acknowledged.

Date: 26/06/2020
Place: New Delhi

Dr. Veda Krishnan
Chairperson
Advisory Committee

Developing starch quality matrix to identify fibre rich Indian rice varieties with low glycaemic potential

By

Sohel Rahaman

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LIST OF TABLES

Figure 1.1. **A schematic representation of starch hierarchical organization.** (A) Starch accumulate in the endosperm of rice grain (B) Scanning electron micrograph of starch granule (C) Starch granule with growth rings radially organized and extending from hilum (D) Blockets, the small units of granules. Blockets consist of super helices comprising (E) Crystalline and (F) amorphous lamellae formed by double helices and branched segments of amylopectin (G) Double helices and branched fragment along with amylose form super helices (H) Glucosyl units showing α-(1,4)- and α-(1,6)-linkages known as amylopectin (I) Two types of amylopectins differing in branching pattern – S & L type (J) Glucosyl units showing α-(1,4) form linear single strand helices known as amylose (K) Amylose exist in two allomorphs – A and B type (L) Glucose, monomeric unit of starch synthesis. [Adopted from Praveen et al., 2017]

Figure 1.2. **Starch digestion and nutritionally relevant fractions.** Figure on the right shows the different components of starch and the location of digestion in the alimentary canal. RDS after digestion in the stomach, results in glucose and insulin spike followed by a period of hypoglycaemia causing hunger and lack of energy. SDS digestion occurs mainly in the duodenum of small intestine and promotes slow release of glucose over a long period of time. This results in a continuous satiety level and retains energy. RS after escaping hydrolysis in small intestine moves to the large intestine, where it is acted upon by gut microbiome producing short chain fatty acids (SCFA) [Adopted from Mondal et al. (2020) in press; science reporter].

Figure 1.3. **Rice starch hydrolysis and factors affecting it.** Rice contains about 78-89% starch and its hydrolysis occurs in two steps in human body. The illustration (left side) represents the action of carbolytic enzymes like amylase and amyloglucosidase. Step 1 in the digestion of starch is catalysed by salivary and pancreatic amylases which produce maltotriose, maltose and glucose. Step 2 of hydrolysis to glucose is carried out by brush bordered disaccharidases or glucosidases. Inherent factors which often interplay and affecting starch digestibility is presented on the right side of the illustration. TS- Total Starch; AC- Amylose content; APC –Amylopectin content; RDS – Rapidly Digestible Starch; SDS– Slowly Digestible Starch; RS- Resistant Starch; GT- Gelatinization temperature; ASV- Alkali Spreading Value; GC- Gel consistency; PV- Peak viscosity; BD- Breakdown viscosity; FV- Final viscosity; SB- Setback viscosity.

Figure 2.1. **Graphs depicting the post prandial glycaemic response of foods rich in rapidly digestible starch (RDS) and slowly digestible starch (SDS).** (A) Quick glycaemic spike within 30-40 min endorse the presence of higher proportion of RDS fraction. (B) Sustained glycaemic response till 240 min

depicts the higher proportion of SDS. Solid line shows the glycaemic response due to rice and dotted line depicts standard glucose. The ratio of glycaemic response, known as glycaemic index (GI) in case of graph A and extended glycaemic index (EGI) in case of graph B.

Figure 2.2. **Diagrammatic representation of *in vitro* starch hydrolyzation kinetics simulating the *in vivo* human digestive system.** Starch fractions are divided into three – rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Starch digestion starts in the oral cavity where salivary amylase breaks down it into maltose units, the bolus moves down to the stomach where the enzyme is inactivated (due to the acidic environment). There is no carbohydrate breakdown in the stomach and the bolus moves to the small intestine. RDS fraction completely digest in the jejunum of small intestine, while SDS further continue till ileum. The pancreatic amylase breaks down the RDS and SDS fractions in the small intestine. RS after escaping digestion from mouth, stomach and intestine, moves to the large intestine (colon) where the RS granules are solubilized or fermented or bio-transformed into short chain fatty acids (SCFA) by the gut microbiome SCFAs. (Partially adopted from (https://www.cell.com/trends/microbiology/fulltext/S0966-842X(19)30239-2?rss=yes)

Figure 2.3. **Scanning electron micrograph depicting microstructure variations affecting starch digestibility.** (A) Full grain depicting the presence of disrupted bran and germ (B) zoomed surface highlighting the bran layer disruption (C) Full grain rice depicting disrupted bran and presence of partial germ (D) Zoomed surface highlighting intact bran layer (E) Overview of endosperm showing multi-layered pericarp, granule packing from periphery towards central zone (F) Variations observed in starch granule shape, size, compound nature and distribution [Partially adopted from Ramyabai et al., 2019].

Figure 2.4. **Starch components having role in its digestibility**. (A) Amylose or long linear chains of α-(1, 4) linked glucans (B) Amylopectin, an extensively branched glucans with α-(1, 4) and α-(1,6) glycosidic linkages [Partially adopted from https://pdb101.rcsb.org]

Figure 2.5. **Physico-chemical attributes affecting starch digestibility as well as overall eating and cooking quality (ECQ).** Illustration depicts the interdependence of parameters like alkali spreading value (ASV), gel consistency (GC), gelatinization temperature (GT) and amylose content (AC).

Figure 2.6. **Pasting profile analysis using Rapid Visco** Analyser (A) principle behind analysis of pasting parameters based on Viscosity measurement (starch in an excess amount of water), applying a temperature profile including a heating and cooling step. 1. Gelatinization onset (TP, pasting temperature), 2. hydration of starch granules, 3. max. intensity of gelatinization (PV, peak viscosity), 4. enzymatic and shear destruction of starch granules, 5. minimum viscosity (HPV, hot paste viscosity), 6. viscosity loss (B, breakdown), 7. final viscosity

(FV), and 8. paste hardening (S, setback). (B) Pasting profile and conventional definitions used in the analysis [Adopted from Schirmer et al., 2015].

Figure 2.7. **Gelatinization and retro gradation mechanism of rice starch.** (A) Gelatinization mechanism of starch. (B) Retro gradation mechanism of rice starch. The dotted lines represent hydrogen bonding [Mechanism adopted from Tako et al., 2014].

Figure 3.1. **Selected rice genotypes for the present study and their possible geographical origin.** Twenty milled rice genotypes selected from different geographies of the country to obtain the expected contrasting starch quality indices.

Figure 3.2. **Texture measurement using Texture profile analyser (TPA) (A)** Texture analyzer model featuring a 5-kg load cell and 35 mm stainless steel flat ended cylindrical probe **(B)** Principle behind texture analysis. Maximum force needed to achieve deformation is measured as hardness while the force required for overcoming the attractive forces between the sample surface and the probe is measured as stickiness **(C)** Force-time curve of the compression test depicting the attachment force as hardness and detachment force to separate probe from sample as stickiness. Positive peak force values are mentioned as hardness and negative peak force as stickiness.

Figure 3.3. **Standard reference curve for the determination of percentage of amylose in selected rice genotypes** (concentration ranged from 5-50%).

Figure 4.1. **Microstructure evaluation of rice genotypes using stereo zoom microscope** (A) Scale ranging from 1-10 developed (*in-house*) to quantify the micro structure aberrations (B) Tabulated observations of the variations in microstructure scores (C) Stereo zoom microscopic images of rice samples a. Karupanel b. IR-78908 c. VLT-6 d. Kamlesh e. DV-85 f. Vasumati g. Longku Labat h. Basmati 802(CANP377) i. Maguraphulla j. Ranbir basmati k. Basmati 5888 l.Basmati 802(ANP377) m. P1568-05-6-4-153 n. Basmati 397 o. Maharaji p. Chimbalate basmati q. Basmati 6141 r. JGL 1798 s. HBC 46 t. BJ-1

Figure 4.2. **Textural properties of selected rice (cooked samples) analysed by Texture Profile Analyzer (TPA). (A&B)** Force-time curve of the compression test depicting the attachment force as hardness and detachment force to separate probe from sample as stickiness. Positive peak force values are mentioned as hardness and negative peak force as stickiness. **(C)** Bar diagram showing hardness and stickiness values, represented as newton (N). Maximum force necessary to attain deformation is measured as hardness while the maximum detachment force required to separate probe from sample is measured as stickiness or adhesiveness. Results are expressed as mean ± SE of three independent experiments. Different letters depict mean significant differences at significance level 0.05 according to Tukey's method.

Figure 4.3. **Total starch and amylose content in the selected rice genotypes** (A) Bar graph depicts the variations observed in total starch content (TSC) among the selected rice genotypes expressed in percentage (W/DW). (B) Bar graph showing percent total amylose content (TAC) the selected rice genotypes expressed in percentage (W/DW). Results are expressed as mean ± SE of three independent experiments. (W-weight; DW-dry weight). Different letters depict mean significant differences at significance level 0.05 according to Tukey's method.

Figure 4.4. **Amylopectin and resistant starch (RS) content in the selected rice genotypes** (A) Bar graph depicts the variations observed in total amylopectin content (TAPC) among the selected rice genotypes expressed in percentage (W/DW). (B) Bar graph showing percent RS content in the selected rice genotypes expressed in percentage (W/DW). Time course starch digestion from 30-180 min using *in vitro* starch digestion simulation involving amylase and amyloglucosidase based on glucometry were performed. The un-digested fraction of starch after 120 min were hydrolyzed using KOH and further measured using glucose-oxidase/peroxidase (GOPOD) reagent for RS measurement. Results are expressed as mean ± SE of three independent experiments. (W-weight; DW-dry weight). Different letters depict mean significant differences at significance level 0.05 according to Tukey's method.

Figure 4.5. **Alkali spreading value (ASV) analysis and indirect analysis of gelatinization temperature (GT) of the selected rice genotypes.** Different milled rice genotypes tested for ASV as whole rice grains after 24 h treatment with 1.8% KOH. a. Karupanel b. IR-78908 c.VLT-6 d. Kamlesh e. DV-85 f. Vasumati g. Longku labat h. Basmati 802(CANP377) i. Maguraphulla j. Ranbir basmati k. Basmati 5888 l. Basmati 802(ANP377) m. P1568-05-6-4-153 n. Basmati 397 o. Maharaji p. Chimbalate basmati q. Basmati 6141 r. JGL 1798 s. HBC 46 t. BJ-1.

Figure 4.6. **Physiochemical attribute of gel consistency (GC) was measured in the selected rice genotypes**. GC, which is a measure of cold paste-viscosity of cooked rice was analyzed by standard procedure using thymol blue dye. Gel length from the bottom of the tube to the end of the gel in millimeters (mm) were measured and depicted here. Results are expressed as average of three independent experiments. a. Karupanel b. IR-78908 c.VLT-6 d. Kamlesh e. DV-85 f. Vasumati g. Longku labat h. Basmati 802(CANP377) i. Maguraphulla j. Ranbir basmati k. Basmati 5888 l. Basmati 802(ANP377) m. P1568-05-6-4-153 n. Basmati 397 o. Maharaji p. Chimbalate basmati q. Basmati 6141 r. JGL 1798 s. HBC 46 t. BJ-1

Figure 4.7. **Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA)**. a. Karupanel b.IR-78908 c.VLT-6 d. Kamlesh

Figure 4.8. **Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA)**. e.DV-85 f. Vasumati g. Longku labat h. Basmati 802(CANP377)

Figure 4.9. **Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA)**. i. Maguraphulla j. Ranbir basmati k. Basmati 5888 l. Basmati 802(ANP377)

Figure 4.10. **Comparative pasting profile of selected rice genotypes measured by (Rapid visco analyzer (RVA)**. m. P1568-05-6-4-153 n. Basmati 397 o. Maharaji p. Chimbalate basmati

Figure 4.11. **Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA)**. q. Basmati 6141 r. JGL 1798 s. HBC 46 t.BJ-1

Figure 4.12. **Starch quality matrix (SQM) -1 developed based on variables affecting inherent glycaemic potential of rice**. Six variables including apparent amylose content (AAC), total amylopectin content (TAPC), total starch (TS), resistant starch (RS), microstructure and gelatinization temperature (GT) were used for association analysis using Pearson's correlation as well as Cramer's V statistics (A) Heat map developed based on correlation. The values of the correlation are expressed in range from –1 to +1. Red colour indicates highest correlation while blue depicts least correlation. (B) Heat map developed based on p value. The values of significance are expressed in range from –1 to +1. Red colour indicates highly significant while blue depicts least significance (C) Scatter plot depicts the idea of the distribution of the data like well-defined positive or negative linear relationships, non-linear relationships or no apparent relationship utilizing the Cartesian coordinate plane. R values show the correlation significance.

Figure 4.13. **Starch quality matrix (SQM) - 2 developed based on physico-chemical variables affecting eating and cooking quality of rice**. Association between 8 continuous variables – gel consistency (GC), alkali spreading value (ASV), peak viscosity (PV), breakdown (BD), final viscosity (FV), setback (SB), hardness and stickiness using Pearson's correlation as well as Cramer's V statistics was analyzed (A) Heat map developed based on correlation. The values of the correlation are expressed in range from –1 to +1. Red colour indicates highest correlation while blue depicts least correlation. (B) Heat map developed based on p value. The values of significance are expressed in range from –1 to +1. Red colour indicates highly significant while blue depicts least significance (C) Scatter plot depicts the idea of the distribution of the data like well-defined positive or negative linear relationships, non-linear relationships or no apparent relationship utilizing the Cartesian coordinate plane. R values show the correlation significance.

Figure 4.14. **A regression model developed using the SAS System ('Local', X64_7PRO) based on SQM 1 to predict the % content of resistant starch (RS) in the rice genotypes under study**. Total RS% is the sum of predicted RS% plus residual RS%. Here only the values of apparent amylose content (AAC or TAC) and total starch (TS) were considered to derive the equation.

Figure 4.15. **Development and validation of the multiple regression model developed to predict the inherent glycaemic potential in terms of resistant starch (RS) content in rice** (A) Analysis of variance (ANOVA) test for significant explanatory variables (TAC and TSC) (B) The distribution of residuals for the log-linear regression model. The normal (N) density estimate of residual in multiple linear regression analysis is depicted in solid line while kernel (K) density in dotted lines (C) Scatterplot of residuals associated with TAC variable tested (D) Scatterplot of residuals associated with TS variable tested (E) Predicted value of RS% using the linear regression model. (F) Observed values of RS% after analysis. [TAC - Total amylose content; TSC - Total starch content]

Figure 4.16. **Analysis of the stability of the model developed through multiple regression for predicting resistant starch (RS) content in rice** (A) Leverage and influence points having impact on the model. Triangle symbol shows the outlier and leverage (B) Q-Q lot of residuals for RS% (C) Residual fit spread plot for RS% (D) box plot depicting residual for RS% (E) The measures of influence of the predicted model was also performed by DFFITS and Cook's D-statistics.

Figure 4.17. ***In vitro* starch hydrolyzation kinetics to simulate gastro-intestinal digestion method with steps, expected time frame and simulated conditions (protocol is partially adopted from INFOGEST2.0).** (A) Flow diagram of in vitro starch hydrolyzation kinetics compartment wise with expected time frame and conditions simulated (B). Time course starch digestion from 30-180 min using *in vitro* starch hydrolyzation kinetics based on glucometry (C) histogram depicting the variations in the fractions of starch – rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and nutraceutical starch (NS). Results are expressed as mean ± SD of three independent experiments.

Figure 5.1. **Inter-relationship among the explanatory variables predicting inherent glycaemic potential derived from starch quality matrix (SQM)-1.** Traits in bold shows significant correlations. (TS-Total starch, TAC-Total amylose content, TAPC-Total amylopectin content, GT-Gelatinization temperature, RS-Resistant starch)

Figure 5.2. **Inter-relationship among the explanatory variables predicting preferred eating and cooking quality derived from starch quality matrix (SQM)-2.** Traits in bold shows significant correlations (GC - gel consistency, ASV-alkali

spreading value, PV - pasting viscosity, BD - break down, FV - final viscosity, SB-set back)

LIST OF ABBREVIATIONS

AAC - Apparent Amylose Content

ALC - Amylose-lipid Complex

ASV - Alkali Spreading Value

ANN - Annealing

BD - Breakdown

BV - Breakdown Viscosity

CT - Cooking Time

DM - Diabetes Mellitus

DNS - Dinitrosalicylic acid

DP - Degree of Polymerization

ECQ - Eating and Cooking Quality

EGI - Extended Glycaemic Index

FAO - Food and Agriculture Organization

GBSS - Granule Bound Starch Synthase

GC - Gel Consistency

GI - Glycaemic Index

GL - Glycaemic Load

GLOPAN - Global Panel on Agriculture and Food Systems for Nutrition

GOPOD - Glucose Oxidase/Peroxidase Reagent

GR - Glycaemic Response

GT - Gelatinization Temperature

HMT - Heat Moisture Treatment

HPV - Hot Paste Viscosity

HFWR - High Fibre White Rice
IIRR - Indian Institute of Rice Research
IGAU - Telangana and Indira Gandhi Agricultural University
JTSAU - Jayashankar Telangana State Agricultural University
MDRC - Madras Diabetic Research Centre
MGAM - Maltase-Glucoamylase
NRRI - National Rice Research Institute
NIR - Near-Infrared Spectroscopy
NS - Nutraceutical Starch
NSPS - Non-Starch Polysaccharides
PV - Peak Viscosity
PT - Pasting Temperature
RDS - Rapidly Digestible Starch
RVA - Rapid Visco Analyzer
RS - Resistant Starch
SB - Setback
SBE - Starch Branching Enzyme
SCFA - Short Chain Fatty Acid
SDS - Slowly Digestible Starch
SDF - Soluble Dietary Fibre
SHK - Starch Hydrolyzation Kinetics
SQM - Starch Quality Matrix
SI - Sucrase-Isomaltase
SR - Swelling Ratio

TAC - Total Amylose content

TAPC - Total Amylopectin content

T1DM - Type 1 Diabetes Mellitus

T2DM - Type 2 Diabetes Mellitus

TDF - Total Dietary Fibre

TPA - Texture Profile Analyzer

TS - Total starch

TSC - Total starch content

TV - Trough Viscosity

WHO - World Health Organization

CHAPTER 1 INTRODUCTION

Role of starch in nutrition is undeniable as the food pyramid recommends 6-11 serving of starch based foods per day, which accounts for 60-70% of the daily per capita energy. Being the major glycaemic carbohydrate, during digestion it depolymerizes through the action of α-amylase and brush bordered amyloglucosidase enzymes into monomeric glucose form in the small intestine (Lehmann and Robin, 2007)**.** Staple cereals like white rice have thus been known to generate high kinetic rate and glycaemic response which relatively increase insulin secretion (Frei et al., 2003). Positive dependence between high glycaemic index (GI), glycaemic load (GL) and glycaemic response (GR) after taking rice diets and thus a major risk for chronic diseases like type 2 diabetes (T2DM) (Seah et al., 2019; Zuniga et al., 2014). There are mounting evidences to share insights that Asians are more prone towards postprandial blood glucose spike with insensitive insulin response than Caucasians even for similar foods ingested, which escalated the risk chance for developing T2DM (Abate and Chandalia, 2007). Diabetic challenge which we face in our country India with regard to controlling carbohydrate consumption is that rice/rice products are part of our culture and not just food for living. Thus even reports suggest that compromising health over food is also common in our country (Lawton et al., 2008). Thus an effective strategy for managing diabetes in India as well as in Asia would be to improve the carbohydrate quality through modifying the inherent glycaemic response of rice along with reducing the carbohydrate quantity. Meta-analysis in this direction, substituting conventional high GI foods with low GI foods endorsed clinical leg-up on glycaemic control (Brand-Miller et al., 2003). Thus to tackle the global economic burden due to chronic hyperglycaemia, tailoring the inherent glycaemic response of starch rich staple cereals like rice and their products is the need of the hour.

Inherent glycaemic response of rice depends on its complex starch hierarchy **(Fig.1.1),** which in turn affect the extent of starch digestibility as well as its absorption in the small intestine. Starch digestibility ability of food like rice is usually defined as GI and white rice is known to be high GI in nature (Kumar et al., 2018).

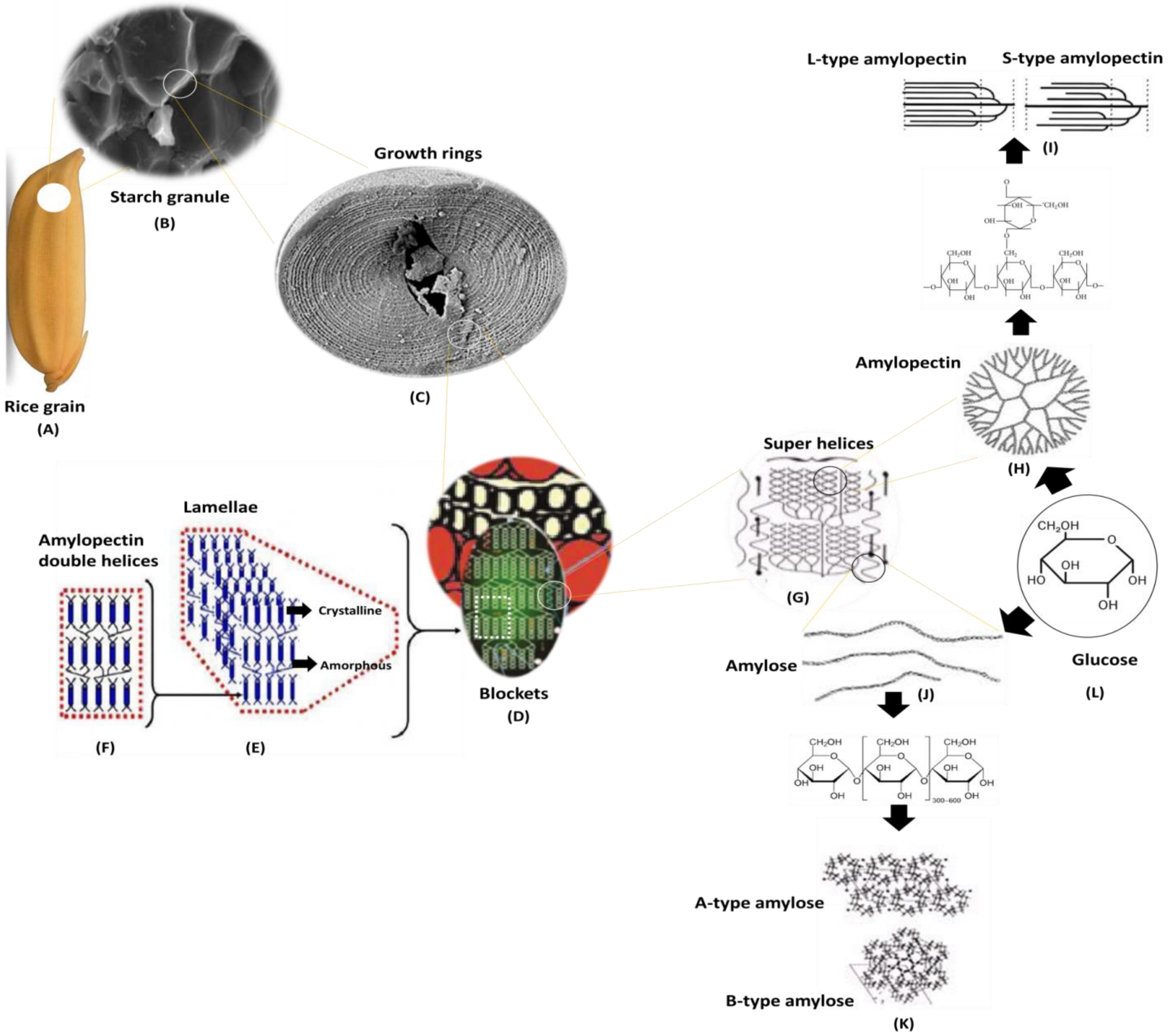


Fig.1.1. A schematic representation of starch hierarchical organization. (A) Starch accumulate in the endosperm of rice grain (B) Scanning electron micrograph of starch granule (C) Starch granule with growth rings radially organized and extending from hilum (D) Blockets, the small units of granules. Blockets consist of super helices comprising (E) Crystalline and (F) amorphous lamellae formed by double helices and branched segments of amylopectin (G) Double helices and branched fragment along with amylose form super helices (H) Glucosyl units showing α-(1,4)- and α-(1,6)-linkages known as amylopectin (I) Two types of amylopectins differing in branching pattern – S & L type (J) Glucosyl units showing α-(1,4) form linear single strand helices known as amylose (K) Amylose exist in two allomorphs – A and B type (L) Glucose, monomeric unit of starch synthesis. [Adopted from Praveen et al., 2017]

Digestibility profile divides starch majorly into three types– Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS is interpreted as the portion of dietary starch digested within 20-30min ($G_{20/30}$), while SDS contributes to the extended GR *i.e.* glucose released in about 240 min (G_{240}). RS is the third portion is represented as the fraction of dietary starch which skips the enzymatic digestion in upper gastrointestinal tract. RS has also examined and known as beneficial, as it evades human digestion and hence food rich in this fraction endorse its low digestibility nature (Butardo and Sreenivasulu, 2016). Over and above, RS shares similar physiology with dietary fibre and act as carbon source for the colon gut microbiome releasing beneficial short chain fatty acids (SCFAs) (Brouns et al., 2002) **(Fig.1.2).**

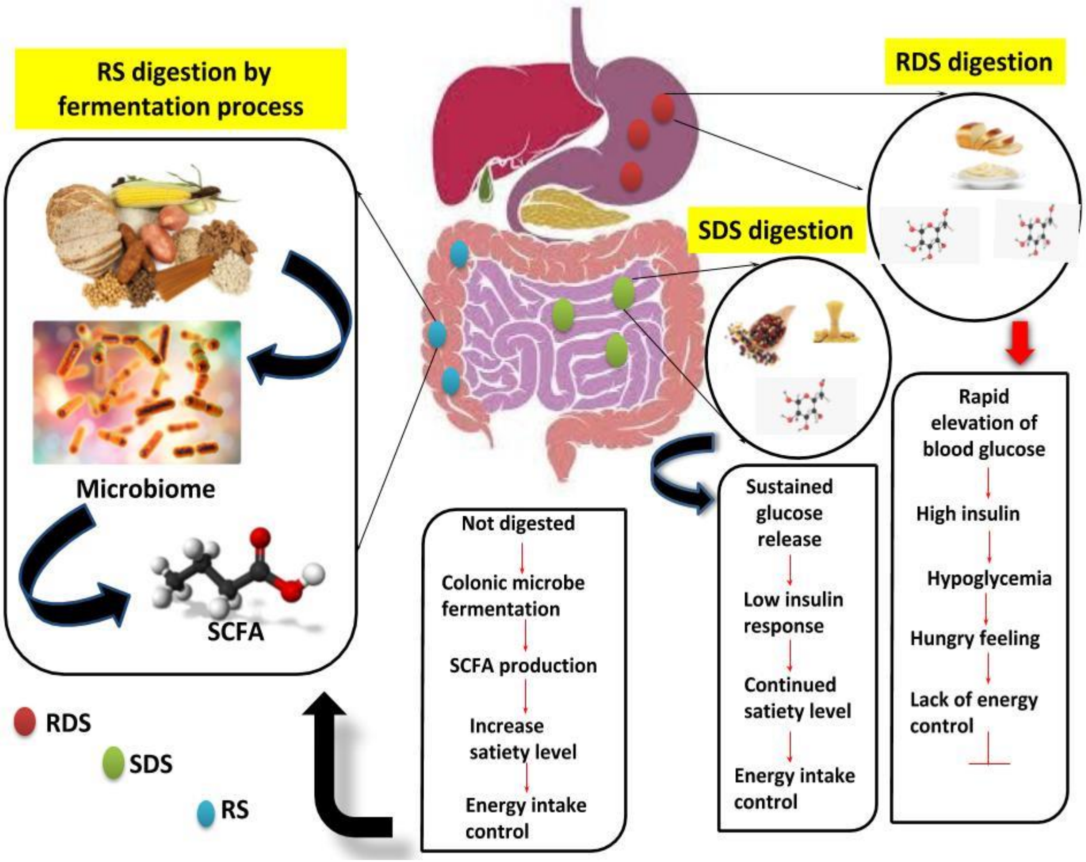


Fig.1.2. Starch digestion and nutritionally relevant fractions. Figure on the right shows the different components of starch and the location of digestion in the alimentary canal. RDS after digestion in the stomach, results in glucose and insulin spike followed by a period of hypoglycaemia causing hunger and lack of energy. SDS digestion occurs mainly in the duodenum of small intestine and promotes slow release of glucose over a long period of time. This results in a continuous satiety level and retains energy. RS after escaping hydrolysis in small intestine moves to the large intestine, where it is acted upon by gut microbiome producing short chain fatty acids (SCFA) [Adopted from Mondal et al. (2020) in press; science reporter].

Most recent publications (Homayouni et al., 2014; Raigond et al., 2015) classified RS into five sub categories: (i) RS1: resistance due to physical entrapment. (ii) RS2: resistance due to inherent high molecular ordered configuration. (iii) RS3: resistance due to change in molecular structure after cooking-cooling process (iv) RS4: processing induced modifications like newer chemical bonds and linkages. (v) RS5: resistance due to formation of amylose-lipid complexes inherently or due to processing. As RDS being positively correlated to GI, rice varieties rich in SDS and RS could solve the aftermath of chronic hyperglycaemia. Even though an inverse relation was expected between GI and RS, previous report by Kumar et al., (2018) mentioned it’s decisive. RS estimated till date in white rice varieties varied from 0.35% to 3.2% (Kumar et al., 2018; Murtagh and Legendre, 2014; Krishnan et al., 2020). As inherent RS content limited to less than 4% reported till date, high through put screening tools as well as efficient strategies have to be deduced in future to analyse and to improve the RS content in rice. Many more RS profiling of rice varieties especially in the heirloom indigenous rice may share novel insights. Recently the role of RS and SDS together called nutraceutical starch (NS) having reported to have role in anti-hyperglycaemia, also underlines the fact that higher content of low digestible starch will be beneficial. Till date, numerous factors have been known to influence the rice starch digestibility like grain type, botanical origin, microstructure, molecular structure, matrix composition/interactions (total starch, amylose, amylopectin, protein, and lipids), rheological attributes, physico-chemical properties, gelatinization parameters and processing conditions **(Fig.1.3).**

Microstructure of the grain modulates the digestibility by either preventing enzyme diffusion towards starch granule (*i.e.* multi-layered pericarp) or enzyme adsorption to the granule, or ultimately the hydrolytic event (Lehmann and Robin, 2007). The size, shape, surface pores, channels as well as cellular layers have known as major microstructural variations among rice types contributing difference in its digestibility. Other than the intactness of the natural grain matrix (due to origin), alterations in the microstructure has also been due to certain processing conditions like milling or cooking; thus has known to contribute towards higher gelatinization rate induced starch digestibility. A possible correlation between the microstructure and starch digestibility was recently reported by Ramyabai et al. (2019) where, incomplete breakages in the bran layer and presence of fissures or cracks on the surface or incomplete disruption to germ was endorsed with increased gelatinization rate and associated glycaemic response. Other than that, cooking conditions also alter the texture of rice which in turn affects their digestibility induced GR. Texture is a multi-factorial sensory property commonly studied as a gold standard for eating and cooking quality, while it also acts as a physico-chemical trait correlating digestibility. The stickiness or hardness, majorly due to the differences in composition (amylose or amylopectin) ultimately affects the molecular structure of starch packaging as well as crystallinity. The amorphous or tight package at nanoscale have known to vary amylolytic digestibility which is in turn is dependent on the degree of polymerisation (DP) of linear glucan (amylose) as well as branched glucan (amylopectin).

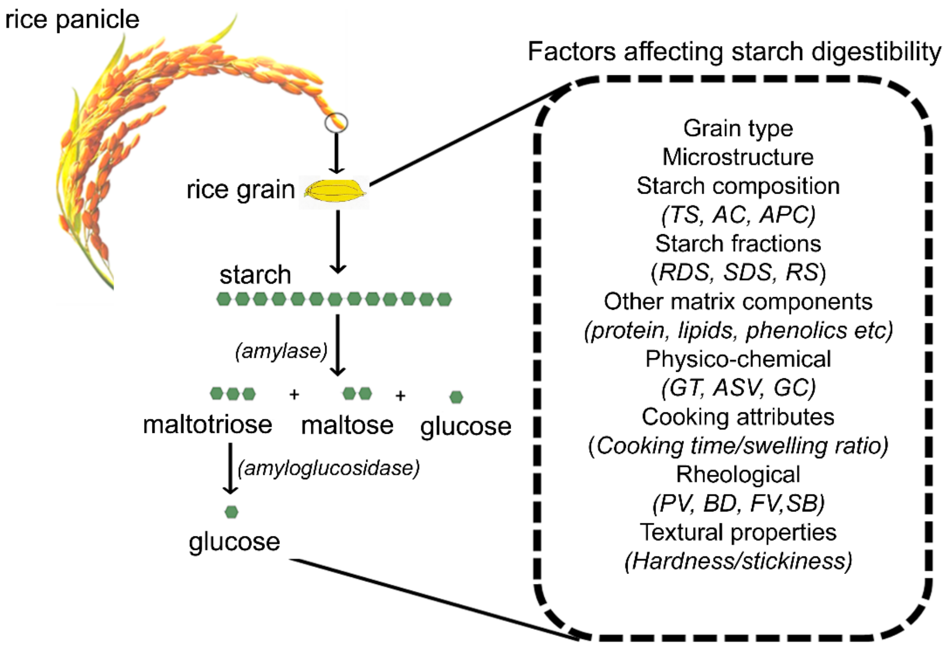


Fig.1.3. Rice starch hydrolysis and factors affecting it. Rice contains about 78-89% starch and its hydrolysis occurs in two steps in human body. The illustration (left side) represents the action of carbolytic enzymes like amylase and amyloglucosidase. Step 1 in the digestion of starch is catalysed by salivary and pancreatic amylases which produce maltotriose, maltose and glucose. Step 2 of hydrolysis to glucose is carried out by brush bordered disaccharidases or glucosidases. Inherent factors which often interplay and affecting starch digestibility is presented on the right side of the illustration. TS- Total Starch; AC- Amylose content; APC –Amylopectin content; RDS – Rapidly Digestible Starch; SDS– Slowly Digestible Starch; RS- Resistant Starch; GT- Gelatinization temperature; ASV- Alkali Spreading Value; GC- Gel consistency; PV- Peak viscosity; BD- Breakdown viscosity; FV- Final viscosity; SB- Setback viscosity.

It has been reported that sticky types of rice are easily digestible and low in amylose content; while higher amylose variants found to be harder and less digestive (Hongyan et al., 2016; Cameron and Wang, 2005). Studies reported to have lower gelatinization temperature (GT), enthalpy value, and molecular crystallinity, their lowered resistance towards enzyme digestion (Zhu et al., 2011). In comparison to low amylose and waxy phenotype, intermediate amylose rice starch showed much more stable molecular order due to higher proportion of stable double helices with stronger crystallites. Such hints correlated a possible relation between amylose and RS (Zhu et al., 2011), while in contrary recent report by Krishnan et al. (2020) justifies the role of other components like amylopectin debranching rate as an indicator towards RS formation. Rice cultivars having similar amylose contents but displaying different stickiness, suggested that proportion of amylose-amylopectin in the leached contents during cooking could be an determinant for texture (Patindol et al., 2010). Hence the microstructure as well as texture depends on the molecular composition and configuration of amylose and amylopectin.

Among the components of starch having key role in digestibility, amylose has been well characterized since 1980s (Bhattacharya and Juliano, 1985). Starch gelatinization occurs during cooking is the initial step towards digestibility. During the process, starch granules absorb water and swells losing its molecular order. Over and above if continued it will melt the crystallites leading to leaching of linear amylose. Such leaching into the solution phase forms a protective layer around the granule prevents further swelling. This in turn reduces the accessibility of enzyme to penetrate further into the granule and reduce the digestibility (Tester and Morrison, 1990). This reduces the susceptibility of starch to digestive enzymes, as the swelling of starch granules increases the accessibility of enzymes to penetrate into the granules. So greater amylose content allows greater resistance to swelling and minimizes hydrolysis. Considering the impeccable role of amylose in starch quality, various molecular biology efforts have been taken to characterize the gene responsible for its synthesis - Granule Bound Starch Synthase (GBSS). The relationship between *GBSS* alleles and amylose content in different rice varieties was initially characterized by Dobo et al., 2010, where different combinations of SNPs in exons 1(G/T), 6(A/C), and 10(G/T) was found to vary the amylose content. Low amylose varieties had TAC and TCC allele, intermediate amylose had GCC allele and high amylose varieties had GAC and GAT allele. The C/A polymorphism in exon 6 caused serine/ tyrosine substitution which destabilize GBSS and result with low amylose phenotype. A positive correlation between RS content and *gbssI* expression in rice cultivars was reported by Kumar et al. (2018). That mutation with in the GBSS could increase the RS content as per Gurunathan et al. (2019). They discovered that wild type GBSSI and SNP in *ssIIa* and *ssIIIa* zoomed the RS level to about 8.68%.

As the molecular configuration of starch is dependent on both components, the role of amylopectin towards digestibility has recently unravelled through advance studies involving size exclusion chromatography, crystallinity and electron microscopy. Li et al. (2016) reported that fine molecular structure of amylopectin including the size and distribution of chain-length are contributing factors towards starch texture and digestibility. Syahariza et al. (2013) highlighted that besides the composition, the intricate structural features of amylopectin may play compelling role in determining the hydrolytic rate of starch in cooked rice grains. Increased percentages of A–type crystalline structure and amylopectin side chains of DP 6–24 both have been shown to increase the rate of digestion (Martens et al., 2018), while B or V type contributed through compact crystalline packaging has shown to inhibit inside out digestion of starch granules. Manipulating amylopectin levels as well as its structure through engineering, various branching and debranching enzymes were carried out. Zhang and others (2006) reported that slow digestion properties of cereal starches are associated with high levels of short amylopectin chains. Recent report by Krishnan et al. (2020) proposed the role of debranching enzyme, pullulanase towards accumulation of RS fraction in rice. Targeted mutagenesis in starch branching enzyme (SBE) *I* and *IIb* in rice through CRISPR/Cas9 has reported to increase RS to 9.8%, also demonstrated the role of amylopectin structure in starch configuration and digestibility (Sun et al., 2017). Besides the significant role of composition, starch digestibility also depends on the physico-chemical attributes which in turn is depended on the structural hierarchy of starch at various scales.

Gelatinization attributes based on composition are well correlated with digestibility. Structural disruption due to gelatinization escalates its susceptibility to enzymatic hydrolysis *in vitro* and thus increases its availability for digestion and absorption (Granfeldt et al., 2000). Thus degree of gelatinization correlates positively to starch digestibility, in contrary gel consistency correlates negatively (Park et al., 2017; Lamsal et al., 2007). Hardness of gel consistency (GC) has reported with higher amylose content and also known as an indicator of longer cooking time. Cooking time being the process of gelatinization, has majorly related with amylose content as amylose act as a diluent and also inhibits swelling of starch, which leads to the rice cultivars having a longer cooking duration. Generally, varieties with shorter cooking time are digested faster as they are gelatinized more easily. Optimum cooking time is positively correlated with thickness of the grain (Mohapatra and Bal, 2006), which is difficult to digest than slender or thin grains. Alkali spreading value (ASV), which gives an estimate of the gelatinization temperature (GT) can also be used get a preliminary idea on eating quality. ASV is negatively correlated with the amylose content and GT. A higher GT has known to have slower *in vitro* starch digestibility (Liu et al., 2016). So rice varieties with lower values of ASV and GC will take longer time to cook digested slowly since it has higher GT **(**Juliano et al., 1981**)**.

Rapid visco-analysis (RVA) based starch pasting profiles have been determined, as they may have the potential to assess the quality of starch. While estimating eating and cooking quality of rice starch using RVA found that, setback (SB) and breakdown (BD) values play significant role as compared to peak viscosity (PV) and others (Bao et al., 2006). Amylose content has reported to be the major determinant of pasting parameters. It has found to be negatively correlated to peak viscosity (PV) and breakdown viscosity (BV) (Hu et al., 2004). A lower peak viscosity implies lower digestibility of gelatinized starch (Hu et al., 2004). It has also been shown that higher setback values are linked to higher amylose in cooked rice (Kurasawa et al., 1972). Lower RVA parameters show a lower amount of liberated glucose (Correa et al., 2012), indicating their slower rate of digestion, promoting less GI. Such parameters can be used to screen rice varieties with lower digestibility and well suited for hyperglycaemic patients.

Rice being more than a food for Asians especially Indians and completely replacing the diet with other types of low digestible cereals is not a viable option. Even though there is a starch dilemma that all rice is hyperglycaemic, India is blessed with a rich diversity of rice varieties but till date starch quality studies are lacking. Many inter-relationship studies were conducted to understand the dependency among parameters affecting starch quality. Even though significant association were found, consistency was the major bottleneck due to the wide germ plasm diversity of rice and the complexity associated with the heritability in the quality parameters (Bao, 1999). The most well associated parameters like amylose, RVA profiles and GC has also found to vary between *indica* and *japonica* subspecies (Shu et al., 1999; Tang, 1981).

Though several attempts were made to correlate various compositional and rheological parameters with rice starch quality using a predictive near-infrared spectroscopy (NIR) by Bao et al. (2001), but many factors like RDS, SDS, RS, cooking time, microstructure and so on remain unaddressed. Many more a comprehensive study involving various explanatory variables contributing towards digestibility is not yet known. The explicit hidden mechanism of resistance of starch granules to hydrolysis is also perplexing because all these factors are often reported to be inter-linked. Hence often the established relationships among these parameters were not dependable or rational. So in the current scenario, it is necessary to test each parameter of rice starch quality considered important as per the objectives of the genetic modification program which is tedious, time consuming and cost-bearing. Thus considering the assessment of parameters affecting rice starch quality as a resource-intensive part in breeding, a predictive tool comprising all the possible variables affecting starch digestibility is the need of the hour. Developing such a starch quality matrix (SQM) will directly assist breeders to screen existing varieties as well as to develop newer lines with better starch quality.

Keeping in view the above prospects, to characterize various parameters modulating starch digestibility in rice, the present study was conducted with the following objectives:

1) ***Nutritional phenotyping of rice varieties for high quality starch and fibre contributing towards low glycaemic potential***.

2) ***Developing rice SQM and validating their inherent glycaemic potential using in vitro assays.***

CHAPTER 2 REVIEW OF LITERATURE

Nutrition is the new global priority and the Global Panel on Agriculture and Food Systems for Nutrition (GLOPAN) commissioned a foresight report in 2015, which highlighted that the food systems should be repositioned to provide high quality diets for all. It's also the need of the hour as epidemiologic studies have revealed correlations between aspects of diet, physical activity and nutrition, with metabolic disorders like diabetes, which is now the fourth leading cause of death worldwide (http://www.who.int/en/news-room/fact-sheets/detail/cancer). Among the glycaemic carbohydrate sources in diet, staple cereals like white rice is one of the pre-eminent source and on top of the list contributing towards hyperglycaemia and associated ailments. The increased frequency of type 2 diabetes mellitus (T2DM), a metabolic syndrome marked by high level of blood glucose well correlated with carbaholic diet, created a '*starch dilemma*' and thus high glycaemic index (GI) commodity like white rice is slowly vanishing from our diet. Various *in vitro* and *in vivo* researches have validated carbohydrates pose a multifaceted health complication when they are hydrolysed and absorbed rapidly (high GI) and more so, when taken in large quantities (high GL). Furthermore, the specialized agency of the United Nations, Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended consuming functional foods having low glycaemic profile, where they modulate post prandial blood glucose levels with reduced glycaemic spike to tackle this problem. The primary strategies of glycaemic control till date is thus aimed to target by either reducing the amount of carbohydrate susceptible to digestion, or reducing of the digestion rate of the food, or reducing the rate of absorption of glucose, or increasing the rate at which glucose is removed from the blood. Of note, reducing the amount of carbohydrate available for digestion being an initial key step, identifying staple cereals like rice of low glycaemic potency or developing processing strategies to further lower down the inherent glycaemic amplitude should be of top priority.

Starch quality based on its digestibility profiles has recently gained attention due to its immense role in modulating the glycaemic response. Carbohydrate quality and quantity is best described through the concepts of GI and GL (Wolever, 2013). GI refers to the glycaemic response induced by a part of food having fifty gram of available carbohydrate, and is expressed as % of the glycaemic response induced by fifty gram of the reference carbohydrate. The reference food is generally a glucose solution or some substitute rich in carbohydrate (like white wheat bread or white rice). A "good" carbohydrate has a low GI (≤55), whereas a "poor" carbohydrate has a high GI (≥70) (GI of glucose = 100g). As the realistic consumption portion sizes affect the glycaemic spike, GL is further taken into account which is worked out by dividing the product of GI and the total available carbohydrate present in a given amount of food by hundred (Augustin et al., 2015; Jenkins et al., 1981). Even though GI of food was considered initially, recent studies highlighted that the structure and composition of food along with matrix components affect its digestibility which in turn affects the glucose absorption rates. Time based *in vitro* kinetics using carbolytic enzymes by Englyst and others (1992) classified starch into 3 types based on the rate of digestion in the small intestine: "rapidly digestible starch" (RDS), "slowly digestible starch" (SDS) and "resistant starch" (RS). RDS has been mostly in direct correlation with glycaemic output; while SDS and RS found beneficial due to the extended glycaemic index (EGI) it imparts **(Fig.2.1).** Even though mounting evidences suggest that food formulated with RS are effective in lowering GI, but expecting an inverse relationship is decisive (Kumar et al., 2018).

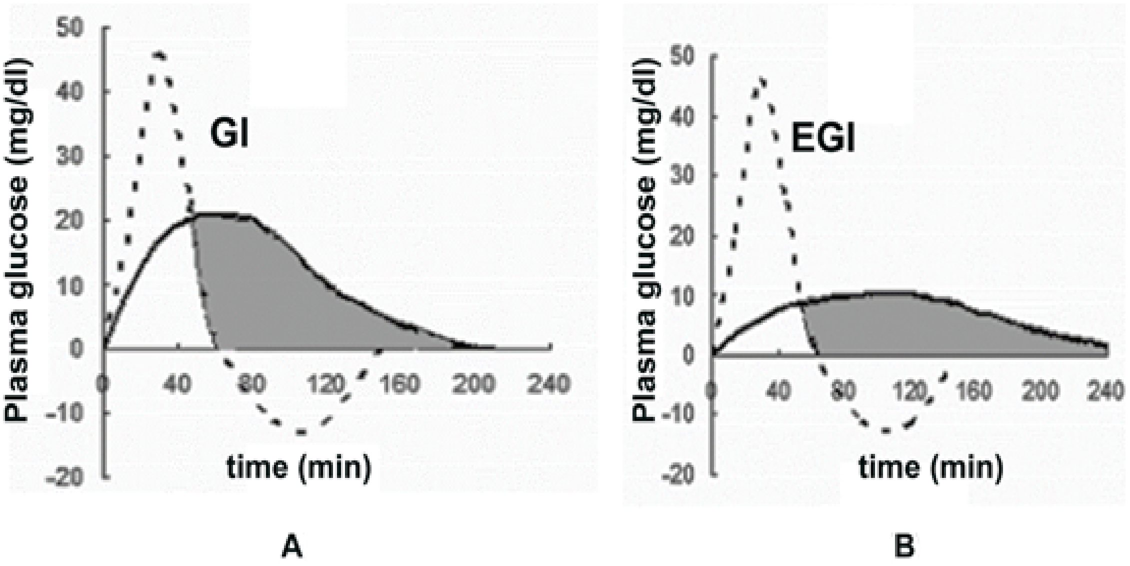


Fig.2.1. Graphs depicting the post prandial glycaemic response of foods rich in rapidly digestible starch (RDS) and slowly digestible starch (SDS) (A) Quick glycaemic spike within 30-40 min endorse the presence of higher proportion of RDS fraction. (B) Sustained glycaemic response till 240 min depicts the higher proportion of SDS. Solid line shows the glycaemic response due to rice and dotted line depicts standard glucose. The ratio of glycaemic response, known as glycaemic index (GI) in case of graph A and extended glycaemic index (EGI) in case of graph B.

Rice mostly known as a high GI food (white rice GI >75; brown rice GI ~65-70), and the usual range found to be between 42-83 (Tamura et al., 2015). Similar reports on milled rice genotypes carried at National Rice Research Institute (NRRI), Cuttack reported that RS and GI varied between 0.35-2.57% and 60.07-70.36% respectively (Kumar et al., 2018). Among the white rice, some low GI varieties like Lalat (53.17), BPT5204 (57.42), Sampada (51), RNR-15048 moniker (51) and Madhuraj (54.025) have been identified by various institutes of repute like Indian Institute of Rice Research (IIRR), Hyderabad; Jayashankar Telangana State Agricultural University (JTASU), Telangana and Indira Gandhi Agricultural University (IGAU), Raipur in India. While the brown and the pigmented heirloom variants have been reported to have higher proportion of low digestible starch variants.

Advances in *in vitro* static digestion models in the last couple of years equipped researches to assess and quantify the starch digestive fractions in rice, to get an understanding of the mechanism behind its glycaemic response. A comparative digestibility study on red, black and white rice revealed that pigmented rice variants had less digestibility (56.10% to 83.43%) as compared to white rice (>87.35%) (Yuliana and Akhbar, 2020). Pasakawee et al. (2018) investigated both milled and un-milled forms of white and pigmented rice (brown, red, purple and black) and reported that GI varied between 47.87–52.22. However, the RS content was lower than 1% in all the varieties analysed. Such conclusions support the fact that inherently RS content and GI relation couldn't be substantial enough to correlate. In line with these reports, Thuengtung et al. (2018) characterized pigmented rice (purple, black and red) and reported that the RS content ranged from 0.29 - 0.3%. The GI of the grain was found to vary between 60.27-65.53% while a much broader range of GI varying between 47.19-69.74 was reported by Meera et al., (2019). Presence of RS in rice, *i.e.* inherent RS has been reported mainly less than 4% (Kumar et al., 2018 and 2020; Deepa et al., 2010). In contrary, a study on 12 long and medium grain rice cultivars in US found that the RS content ranged about 5.86-11.28 %, SDS 13-25.5 % and RDS 64.34-77.84 % (Benmoussa et al., 2007). In some Thai pigmented (black, red, purple) rice cultivars RS ranged from 0.09 - 0.13% (Thuengtung et al., 2018). Another study by Deepa et al. (2010) reported higher RS content and GI between 0.71 - 1.1% and 73.1-74.8 respectively in pigmented rice varieties.

Another low digestibility variant starch, known as SDS has been neglected over the years. Patindol et al. (2010) reported RDS, SDS and RS values from 52.4 - 69.4%, 10.3 - 26.6%, and 1.2% to 9.0% respectively in 16 US rice cultivars. A similar study on long grain brown rice has reported to have 9.2% SDS (Englyst et al., 2006). Krishnan et al., 2020) *in press*) reported that red rice type Njavara had 2.9 times greater SDS than white rice. A strong positive correlation existed between RDS and GI, while GI was found to have a negative association with RS and SDS (Giri et al., 2017). Controlled randomized clinical trials with SDS rich (12g/portion) breakfast is reported to have with efficient glucose metabolism both in terms of rate of appearance and disappearance (Vinoy et al., 2017). Greater proportion of low digestible starch fractions like RS and SDS togetherly known as nutraceutical starch (NS) also found more in pigmented rice than polished white rice (Deepa et al., 2010; Magallanes-Cruz et al., 2017). Recently a high fibre white rice (HFWR) reported to have medium GI (61.3) was developed by Madras Diabetic Research Centre (MDRC), Tamil Nadu, India, where the dietary fibre content was found to have 5-fold higher (8.0 vs. 1.58 %) and RS content was found 6.5-fold higher compared with commercial white rice (Mohan et al., 2016). Studies thus endorsed the fact that pigmented and high fibre rice due to the presence of bran layer which limits to have carbolytic enzymes and due to complex matrix composition, has found to elicit low glycaemic response compared to white rice and thus of medium to low GI in nature. Hence a comprehensive review is included under this chapter comprising the biochemistry of starch digestibility, factors affecting it as well as the existing tools/strategies available till date to predict the nature of starch digestibility in rice.

2.1. Starch digestion

Starch is mainly hydrolysed into monomeric glucose by carbolytic enzymes like amylase [EC no. 3.2.1.1] and amyloglucosidases [EC no. 3.2.1.3]. After mastication, mouth being the initial compartment of starch digestion, salivary α-amylase initiates the process efficiently but also gets rapidly inactivated on reaching the acidic environment in the stomach. Thus the major role of starch digestion is through the carbolytic enzymes existing both in digestive fluids and in the brush border lining of the small intestine (Smith and Morton, 2001). The pancreatic amylase carries out majority of the starch hydrolysis, which is released *via* pancreatic duct into the small intestine. The α-amylase is responsible for catalysing the hydrolysis (endo attack) of α - (1-4) linked glycosidic bonds in linear amylose and in branched amylopectin chains of starch (Lehmann and Robin, 2007). Hydrolysis occur through binding of enzyme with 5 glucose residues next to the terminal reducing glucose unit to specific catalytic sub-sites resulting in the cleavage between the second and third glucosyl residues which are α -1,4-linked (Gray, 1992). The final products obtained from hydrolysis of amylose are mainly maltose, maltotriose, and maltotetrose, while digestion products of amylopectin have reported to be dextrins or branched oligosaccharides. Such products of α-amylase digestion are further digested efficiently by brush border enzymes present in the intestine into monomeric bioavailable glucose. Nutritional fractions of starch based on their time dependent release of glucose is divided into RDS, SDS and RS. RDS contributes majorly to the glycaemic response, SDS to extended glycaemic response and RS act as prebiotic and nourishes the gut microbiota **(Fig.2.2).**

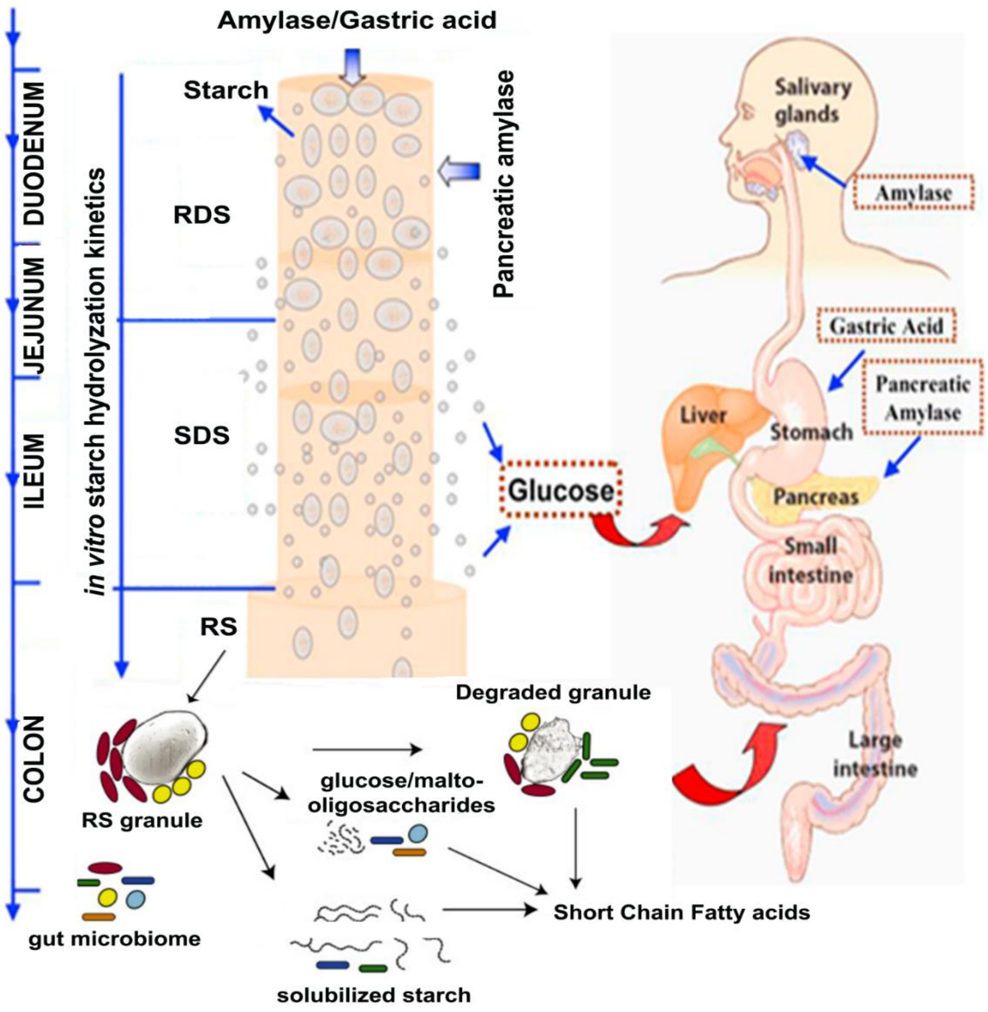


Fig.2.2. Diagrammatic representation of in vitro starch hydrolyzation kinetics simulating the in vivo human digestive system. Starch fractions are divided into three – rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Starch digestion starts in the oral cavity where salivary amylase breaks down it into maltose units, the bolus moves down to the stomach where the enzyme is inactivated (due to the acidic environment). There is no carbohydrate breakdown in the stomach and the bolus moves to the small intestine. RDS fraction completely digest in the jejunum of small intestine, while SDS further continue till ileum. The pancreatic amylase breaks down the RDS and SDS fractions in the small intestine. RS after escaping digestion from mouth, stomach and intestine, moves to the large intestine (colon) where the RS granules are solubilized or fermented or bio-transformed into short chain fatty acids (SCFA) by the gut microbiome SCFAs. [Partially adopted from (https://www.cell.com/trends/microbiology/fulltext/S0966-842X(19)30239-2?rss=yes]

2.2. Factors affecting starch digestibility

Digestibility of starch largely depends on various intrinsic and extrinsic parameters. The inside out digestion process of raw starch granules occurs in three stages: enzyme diffusion towards the starch granule centre, enzyme adsorption (*i.e.* formation of enzyme-starch complex), and finally the hydrolytic event (Lehmann and Robin, 2007)

2.2.1. Intrinsic factors affecting starch digestibility

2.2.1.1. Microstructure and textural attributes

Microstructural characteristics of starch based cereals includes the intactness of bran layer, presence of multi-layered pericarp, cell size/shape/composition etc. act as a major factor influencing the digestibility of starch during gastro-intestinal digestion (Singh et al., 2010; Waldron et al., 1997) (**Fig.2.3**).

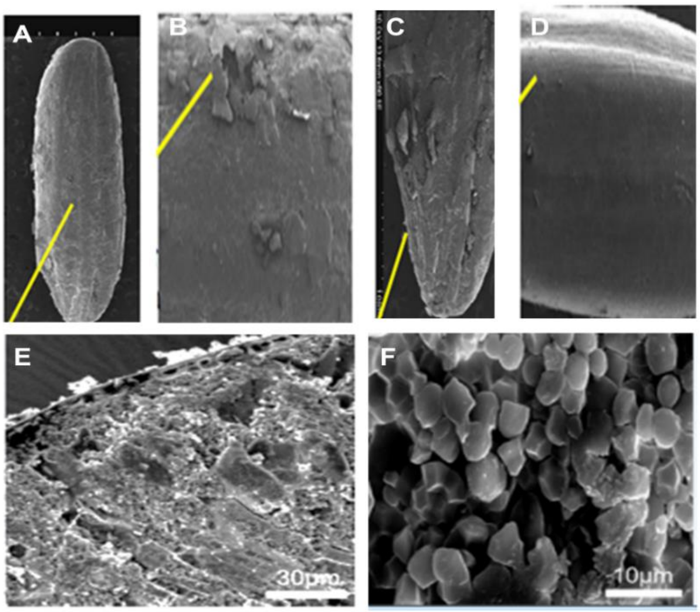


Fig.2.3. Scanning electron micrograph depicting microstructure variations affecting starch digestibility. (A) Full grain depicting the presence of disrupted bran and germ (B) zoomed surface highlighting the bran layer disruption (C) Full grain rice depicting disrupted bran and presence of partial germ (D) Zoomed surface highlighting intact bran layer (E) Overview of endosperm showing multi-layered pericarp, granule packing from periphery towards central zone (F) Variations observed in starch granule shape, size, compound nature and distribution [Partially adopted from Ramyabai et al., 2019]

The intactness of cell wall/membrane components as well as the size and arrangement of starch granules affects the free access of carbolytic enzyme diffusion, which further delay the digestion process. The abundance of starch in the cells along with the shape and size of the starch granules are key factors in deciding the final texture of both cooked as raw natural foods also (Andersson et al., 1994; Van Marle et al., 1997; Martens and Thybo, 2000). Therefore, sufficient knowledge regarding microstructure is needed for better understanding of texture. Type of cultivar, physico-chemical composition, and postharvest storage or other processing are other important factors, which influences the cooking characteristics of rice other than micro-structure (Kaur et al., 2007; Liu et al., 2007). During cooking, continuous heat treatment over a certain period results in changes in the food microstructure and texture. These changes have mainly been related to the gelatinization of starch (Alvarez and Canet, 1998; Ormerod et al., 2002; Singh et al., 2008). Recently a possible correlation between the microstructure and starch digestibility was reported by Ramyabai et al. (2019) where, bran layer disruption and the existence of fissures and cracks on the surface due to partial damage to germ was endorsed with increased gelatinization rate and associated glycaemic response. Various molecular configuration studied over the decades pointed that the microstructure as well as texture depends on the molecular composition and configuration of amylose and amylopectin.

2.2.1.2. Starch and its components

Total starch and its linear (amylose) and branched (amylopectin) components have impeccable role in starch digestibility (**Fig.2.4**). Staple cereals like rice are usually classified into three groups based on their amylose content – low (<20%), medium (20-25%) and high (>25%). Amylose content has been known as a critical factor that control the rates at which starchy foods differ when they are digested and raise both blood glucose as well as insulin responses (Juliano and Goddard, 1986). Low digestibility implicated with high amylose phenotypes is either due to high order molecular configuration or due to interactions with other matrix components. Since amylose is linear in structure, starch abundant in amylose are considered to contain more extensive hydrogen bonding and thus more crystallinity in their structure than low amylose phenotype. Such compactness in structure endorsed by high amylose phenotype has been reported to resist the swelling as well as gelatinization rate on cooking and so logically are more slowly digested which results in lowering of blood glucose and insulin responses. This very reason is the basis of the fact that intake of high-amylose foods has been considered more desirable for individuals with impaired/dysfunctional carbohydrate and lipid metabolism to consume foods high in amylose. (Behall et al., 1988; Behall et al., 1989). Benmoussa et al. (2007) found that higher amylose content promotes a lower digestion rate, while Zhu et al. (2011) suggested that amylase may initially hydrolyse the amylose molecules present in the amorphous regions but the re-association of hydrolysed molecules known as retrograded starch were found to be more resistant. Cai et al. (2015) suggested that "amylose-lipid complexes" (ALC) prevented hydration and thus penetration of amylase in starch granules.

Amylopectin, the major branched component of starch has also reported to influence starch digestibility. The branch diversity as well as degree of polymerization (DP) has been studied in this direction and found to contribute towards low digestible fractions like SDS and RS. Each amylopectin moiety has outer unbranched "A" chains, inner branched "B" chains and a single 'C' chain containing reduced group. The clustered structure owing due to branching and debranching enzyme alters the starch digestibility. Tsuiki and others (2016) reported that amylopectin long chains contributed towards higher RS content in those rice lines which had modified starch biosynthetic pathway to produce higher content of intermediate and long chain amylopectin molecules. It was justified that short amylopectin chains elicited weak points in the crystalline structure of starch, thereby increasing the susceptibility to hydrolysis by digestive enzymes (Jane et al., 1997).

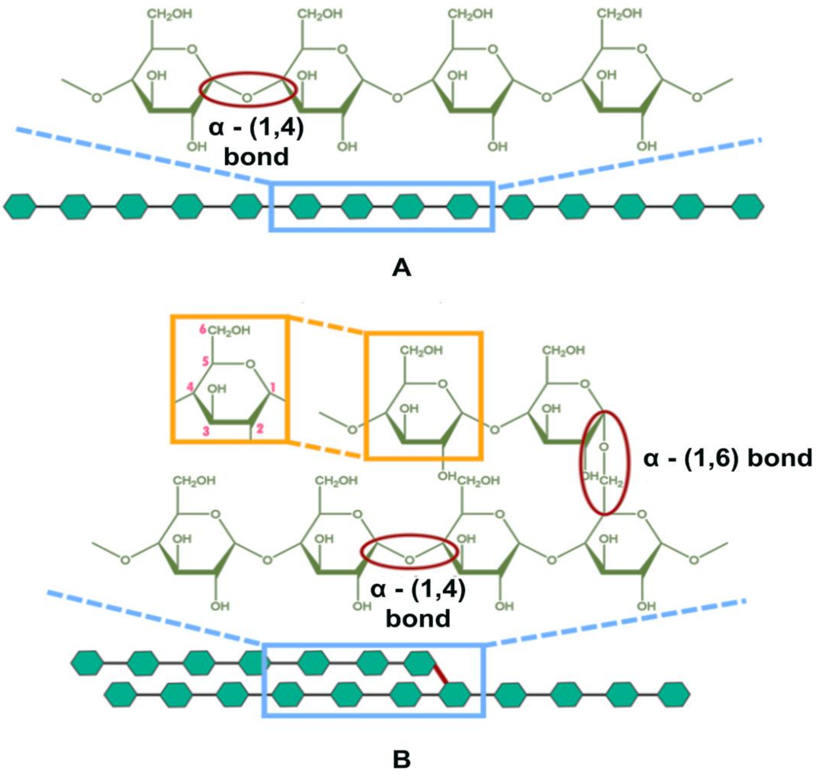


Fig.2.4. Starch components having role in its digestibility. (A) Amylose or long linear chains of α-(1, 4) linked glucans (B) Amylopectin, an extensively branched glucans with α-(1, 4) and α-(1,6) glycosidic linkages [Partially adopted from https://pdb101.rcsb.org]

On the other hand, long amylopectin chains contribute towards more crystalline structure due to increase in hydrogen bonds developed among the chains and thus increase the resistance to enzymatic hydrolysis (Sasaki et al., 2009). In agreement to this, Benmoussa et al. (2007) found RDS was lowest in the cultivars having the highest proportion of long (FrI) and intermediate/short (FrII) amylopectin chains and the lowest proportion of very short chains (FrIII). Cultivars with the highest content of SDS similarly had the highest FrI and Fr II proportions and the lowest Fr III proportion. Their study proved the fact that variability in the fine structure of rice amylopectin can significantly alter the *in vitro* digestion properties of cooked starch. Zhang et al. (2006) reported that the molecular crystallinity of rice starch (A-type) had 3 times the tendency for RDS than potato starch (B-type). In addition, shifting of the rice crystalline polymorph from ‘A’ to ‘C’ to ‘B’ in the same genetic background have been shown to decrease the *in vitro* digestibility of cooked grains in white rice (Butardo et al., 2011). Increased rate of digestion in A-type starches was earlier attributed to a more uneven granular surface structure leading to increased accessible surface area as compared to B-type starches (Williamson et al., 1992). But now the major determining factor for reduced enzymatic hydrolysis of the “B-type” rice starch granules has been attributed to a higher proportion of long chain amylopectins (Butardo et al., 2011; Dhital et al., 2015). This is because it has been found that increased proportions of long amylopectin chains are associated with more stable double helices, and stronger crystallites (Wang et al., 2017) and thus increased GT (Butardo et al., 2011). In a more recent study by Lin et al. (2018), rice starches with same genetic background, similar amylopectin structure but with different amylose content were significantly found with altered digestibility. It was observed that starches having different content of amylose but with the same structure of amylopectin had significant differences in their digestibility. Thus, for different rice varieties, the digestion rates of starch are primarily attributed to the content of amylose, type of amylose fractions and amylopectin fine structure. This is because long chain amylopectin molecules appear to mimic the structure and function of amylose, and therefore can increase the RS content and further cause reduction in the digestibility (Butardo et al., 2011 and 2017).

2.2.1.3. Minor matrix components – Protein, lipids and soluble fibres

Various non-carbohydrate factors like proteins, lipids, phenolics etc present in the rice matrix have also been reported to influence the degree of starch hydrolysis *in vivo*. However very limited studies have been done in this regard. In a food matrix of cereals, the interactions between protein and starch appear to have a role in affecting the rate of starch digestibility (Jenkins et al., 1987). Ye et al. (2018) reported a significant increase in the digestibility of starch in native long-grain *indica* rice flour was observed after the matrix proteins were removed by protease treatment, which underlines the role of proteins in starch digestibility. It was suggested that attachment of endogenous proteins to the surfaces of starch granules restricted the access to the digestive enzymes resulting in a reduction of granular swelling and digestion. Starch-lipid complexes also have been indicated as a major contributing factor towards lowering the glycaemic response of cooked rice (Goddard et al., 1984; Shu et al., 2009). Resistance of complexes to enzymes was demonstrated to increase with increasing crystallinity in structure, degree of polymerization of amylose, length of lipid chain, and complexation temperature (Eliasson and Krog, 1985; Gelders et al., 2005; Tufvesson et al., 2003). Long-chain saturated monoglycerides were found to exhibit more resistance to enzymatic hydrolysis as compared to short-chain saturated as well as unsaturated monoglycerides when complexed with starch of cooked rice (Guraya et al., 1997). Recently, Krishnan et al. (2020) reported the role of cooking fats in modulating the inherent glycaemic potency of rice and proposed a model of lipid induced digestive resistance for the very first time. Other than proteins and lipids, phenolics especially in pigmented rice modulate starch digestibility. Role of phenolic composition as well as types on inhibiting carbolytic enzymes, enhancing intracellular glucose uptake and modulating hepatic glucose homeostasis was validated using *in vitro* models (Boue et al., 2016; Krishnan et al., 2020).

Among the components, soluble fibres are known to forms a gel when mixed with liquids and is associated with lowering cholesterol and controlling blood sugar. Clinical studies on high fibre rice has validated the potential in lowering GR. According to the patent by Leitz and Pusateri (1989), the proportion of SDF in total dietary fibre (TDF) must be at least 10% to be recognized as high quality DF. However, the content of SDF was much lower than this value in the rice bran dietary fibre. But in rice its mostly it is present in bran and thus not available when consumed in the form of polished rice. Soluble fibre fraction comprised of three types, arabinoxylans, beta-glucans and RS. Among which RS is found higher in proportion in endosperm, while arabinoxylans are more concentrated in bran. This highlights the need for food matrix studies involving interaction of major and minor components towards starch bio-accessibility, digestibility and ultimate bioavailability.

2.2.1.4. Nutritional starch fractions – RDS, SDS and RS

Time based digestion profile of starch known as nutritional starch fractions like RDS, SDS and RS were first introduced by Englyst et al. in (1992) through *in vitro* starch digestion experiments. This combined with the works of Wahlquist et al. (1978), Jenkins et al. (1978) and others showed that the physiological form of food and the nature of starch are key determinants of the rate of starch digestion. Englyst et al. (1999; 2003) demonstrated a significant positive relationship between RDS and GI, and found in their study that RDS is responsible for seventy percentage of the remaining variance in glycaemic response. Due to the rapid digestion and absorption of RDS in the duodenum and proximal part of the small intestine, the level of blood glucose suddenly spikes up usually followed by a subsequent occurrence of hypoglycaemia. These sudden and large increments in blood glucose levels can further result in oxidative stress induced cell, tissue and organ damage (Brownlee, 2005). But SDS by virtue of its slow digestion throughout the small intestine, results in a slow and prolonged glucose release into the blood stream, which is coupled to an extended glycaemic response. It may be beneficial in combatting hyperglycaemia related diseases. SDS escapes hydrolysis by salivary α-amylase in the oral cavity, by gastric acids in the stomach and gastric motility mediated vigorous grinding action. Its digestion begins in the small intestine and mostly occurs in the duodenum. SDS is broken down by pancreatic enzymes and converted to small linear oligomers and α-limit dextrins. These products obtained from SDS, mostly made up of disaccharides (maltose), diffuse from the lumen into the brush border membrane, where mucosal enzyme complexes containing sucrase-isomaltase (SI) and maltase-glucoamylase (MGAM) finally digests to produce glucose the) (Heymann et al., 1995; Breitmeier et al., 1997). The final portion RS escapes digestion in the small intestine, but gets fermented like dietary fibre in the colon, which has reported to have potential in preventing colon diseases as well as overall health through gut-brain axis (Englyst et al., 1992; Bjorck et al., 2000; Aston, 2006). RS could lower the rate at which starch is digested but this phenomenon is influenced by various factors such as morphology of starch granules, amylose/

amylopectin ratio, molecular structure, degree of branching in terms of steric hindrance etc. (Alfonso et al., 2011).

Guraya et al. (2001) reported that colorimetric dinitrosalicylic acid (DNS) method can be used to determine the SDS digestibility by measuring the rate of starch hydrolysis due to porcine α-amylase. SDS and RS fractions have been proven to impart a handful of benefits owing to their low digestibility. Foods high in SDS promote better health by lowering the stress on regulatory systems related to homeostasis of glucose (Sievenpiper et al., 2002). Seal et al. (2003) found that when RDS was digested the plasma glucose and serum insulin concentration rose faster, and approximately 1.8 times greater maximum glucose change was recorded, than when SDS was digested. As reported by Ells et al. (2005), consumption of SDS promotes low and continued glycaemic and insulinemic response, which decreases cholesterol levels and incidences of T2DM. Alfonso et al. (2011) found RS and SCFA content were positively correlated and the RS levels significantly affected the production of SCFA regardless of the digestibility of starch in the samples. Fermentation of starch samples *in vitro* showed with increase in the RS content, SCFAs production also increased (Alfonso et al., 2011). RS content and GI has been reported to have a negative correlation, claiming its role in balancing the insulin reaction and in maintaining glucose digestion (Alfonso et al., 2011; Dyson et al., 2019; Mandhania et al., 2019), but in contrary many studies reported it’s decisive.

2.2.1.5. Physico-chemical attributes

As rice cultivars with similar amylose content often varies in quality, certain physico-chemical parameters like alkali spreading value (ASV), gel consistency (GC) and gelatinization temperature (GT) have been used as secondary indices to improve differentiation (**Fig.2.5**).

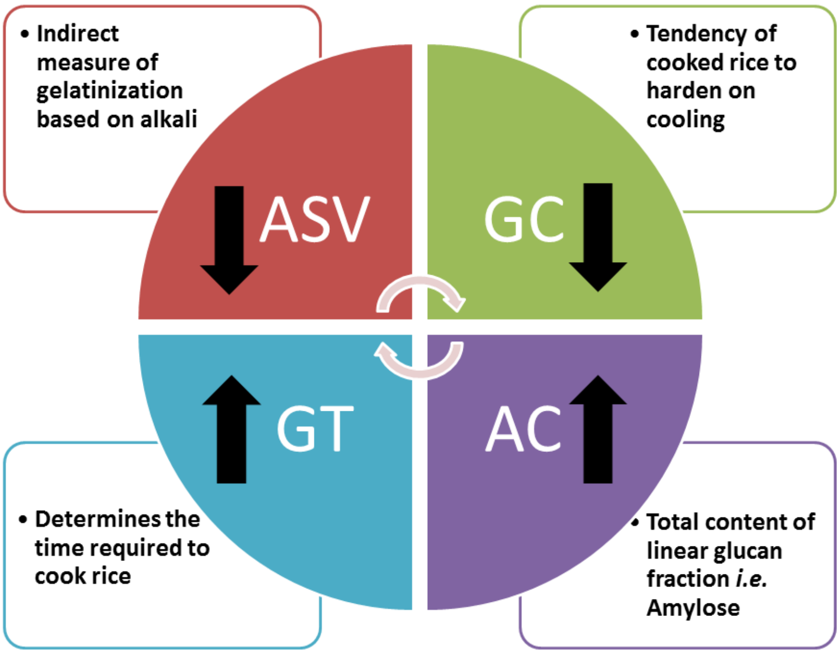


Fig.2.5. Physico-chemical attributes affecting starch digestibility as well as overall eating and cooking quality (ECQ). Illustration depicts the interdependence of parameters like alkali spreading value (ASV), gel consistency (GC), gelatinization temperature (GT) and amylose content (AC)

ASV is simple, inexpensive and a standard method for classifying rice varieties into high, intermediate or low GT types (Mutters and Thompson, 2009). During alkali spreading, 1.7 % KOH gelatinizes starch, particularly its amorphous region, causing degradation of the long linear and branched chains of amylose and amylopectin resulting in rice grain gelatinization. The ASV of the grain is scored according to a scale ranging from 0–7 (Little et al., 1958). In rice, there is a negative correlation between ASV and GT and rice varieties with low starch GT show high ASV scores (Bhattacharya, 1979; Bhattacharya and Sowbhagya, 1972; Juliano et al., 1964; Mariotti et al., 2010; Tan and Corke, 2002). ASVs of 1–3, 3–5 and 5–7 correspond to high (74.5 – 80ºC), medium(70-74ºC) and low GT(<70ºC) types, respectively. GC test is a measure of the stickiness of cooled paste of rice flour mostly depended on the amylose content. Rice varieties could be classified based on the length of GC into 3 groups *viz*. soft, intermediate and hard having >60, 40-60, <40 mm gel length, respectively (Cruz and Khush, 2000). Harder GC is related to harder cooked rice and this characteristic is particularly apparent in high-amylose rice. Such rice is possessing lower digestibility. Rice with soft GC on cooking has a higher level of tenderness and such rice are digested rapidly.

According to the findings by Wickramasinghe and Noda (2008), low amylose starches had lower GT. In the same study it was found that low amylose starch was also easily digestible by enzymes. Such results could indicate that higher GT is associated with lower digestibility. But in contrary another found that the RDS content increased with the increase in the GT, but the content of

RS and SDS decreased (Tamura et al., 2016). *In vitro* digestibility had negative relationships with GC and GT but positive relationships with ASV. Lower GT promotes faster hydration and therefore are digested faster than those varieties that are harder to hydrate, (Panlasigui et al., 1991). Patindol et al. (2010) reported that SDS correlated positively with GC while RDS was not significantly correlated with any of the physicochemical properties.

2.2.1.6. Rheological attributes – pasting parameters

Pasting behaviour based on viscogram indices closely related to starch composition has been used as a potential screening tool towards starch quality (Benmoussa et al., 2007). Hu et al. (2004) reported that viscosity profile analysed by Rapid Visco Analyzer (RVA) is useful in the selection of lines with certified quality in breeding programmes **(Fig.2.6).** Among the variables of pasting profile, final viscosity (FV) is generally used to specify a sample's quality, indicating the behaviour of the mixture on cooking and cooling (Perkin, 2013). Setback viscosity (SB), which is the difference between FV and trough denotes the re-association of starch during cooling, and this has been correlated to the textural characteristics of various products (Perkin, 2013). Lowered digestibility is associated mostly with higher amylose content which corresponds to low peak viscosity (PV) and breakdown (BD) values, and high setback (SB) values. But reports by Bao et al. (2006) showed amylose content significantly correlated with almost all the pasting viscosity properties, except for PV that was found to be greatly influenced by environment.

Kong et al. (2015) found that BD viscosity showed a positive correlation with the amount of short amylopectin fa chains (DP 6-12) which are easily hydrolyzed, but a negative correlation with the amount of medium length amylopectin fb1 (DP 13-24) chains. Previous study also supports the fact that rice starches having greater proportions of fb1 chains had less BD, whereas with greater proportions of fa chains had more BD (Kowittaya and Lumdubwong, 2014). Correlation studies between pasting and starch digestibility by Benmoussa et al. (2007) reported that BD had a high positive correlation with RDS and negative with SDS content. Previous studies endorsed that amylose content and pasting temperature (PT) was positively correlated SB and FV and negatively correlated with PV and BD viscosity. In addition to that SDS and RS contents also had a positive correlation with amylose content. (Chung et al., 2010). Nakamura et al. (2016) used some ratios of RVA parameters and reported that these ratios could be more correlated with the digestibility, than absolute parameters. They reported in their studies that RS had a positive correlation with PT, PV/ FV ratio, PV, BD and negative correlation with SB and SB/Consistency ratios. In pure starches extracted from rice, RS was negatively correlated with BD and positively with FV, SB, PV/FV (Nakamura et al., 2016). Higher SB values could indicate slower digestibility since high SB results from leaching of a high amount of amylose and its reorganization during cooling. (Huynh et al., 2016; Sasaki et al., 2000). Overall there is a great relevance of viscosity variables in digestibility as well as in cooking quality.

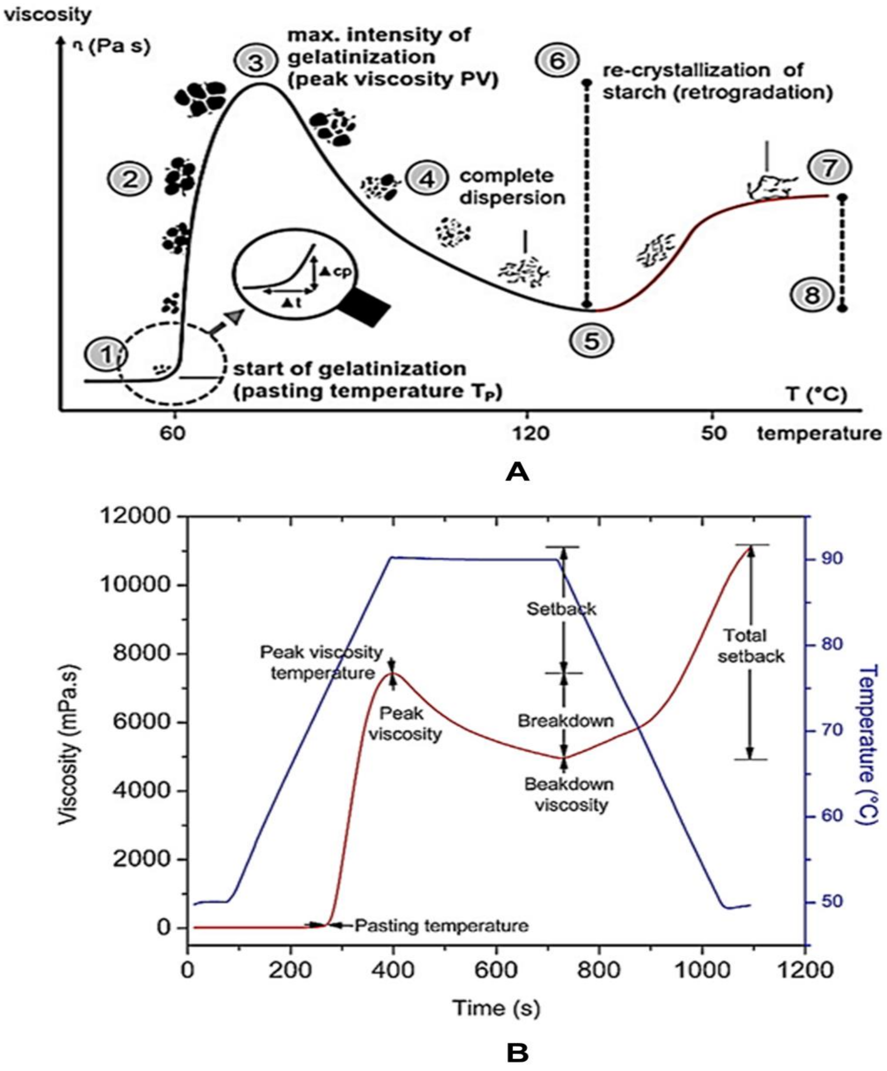


Fig.2.6. Pasting profile analysis using Rapid Visco Analyser (A) principle behind analysis of pasting parameters based on Viscosity measurement (starch in an excess amount of water), applying a temperature profile including a heating and cooling step. 1. Gelatinization onset (TP, pasting temperature), 2. hydration of starch granules, 3. max. intensity of gelatinization (PV, peak viscosity), 4. enzymatic and shear destruction of starch granules, 5. minimum viscosity (HPV, hot paste viscosity), 6. viscosity loss (B, breakdown), 7. final viscosity (FV), and 8. paste hardening (S, setback). (B) Pasting profile and conventional definitions used in the analysis [adopted from Schirmer et al., 2015].

2.2.2. Extrinsic factors affecting starch digestibility

Extrinsic factors include the variables which are not inherent but associated in the processing of grain to plate of rice consume. It includes storage, cooking, gelatinization, retro gradation and parboiling.

2.2.2.1. Storage

Much works regarding the effects of storage on digestibility of rice starch has not been done till date. In one study regarding the ageing mechanism of brown rice, it was found that the storage period of seven months did not affect the starch digestibility of cooked samples (Jaisut et al., 2009). In a recent study on white rice regarding the effects of ageing-related changes, it was found that rice stored at 37°C showed lower digestibility within the first twenty min and overall a lower rate of digestion as compared to that of the rice stored at 4°C. This reduced rate of digestion was attributed to an increase in the strength of cell wall and a reduction in hydration-facilitated disruption of starch granules (Zhou et al., 2016). The study showed that storage could modulate starch digestibility but maybe only when subjected to acceleration of ageing process (e.g. at a high storage temperature of 37°C). Nonetheless, due to limited study design (only three varieties and two storage temperatures), more research is needed to backup this finding. Recent work in our lab found RS decreased during ageing which supports the fact that higher starch hydrolysis to sugar forms might be occurring during ageing.

2.2.2.2. Cooking

Cooking and its variants based on extent and type of cooking has been known to affect starch digestibility. Continued heat treatment while cooking over a definite period causes changes in the food micro-structure and texture and these changes have been mainly related to starch gelatinization (Moughan and Singh, 2008; Singh et al., 2002). Naturally existing pores in the periphery can increase the effective surface area, promoting formation of enzyme-substrate complexes. Cereal starches (including rice) contain peripheral pores that are connected to interior channels, which results in an ‘inside out’ digestibility pattern, having higher rate of hydrolysis compared to the ‘outside in’ pattern in potato and “high amylose maize” starches (Gallant et al.,1997). In one report, the extent of cooking or starch gelatinization was found to have little effect on rice digestibility but rather it was more influenced by the changes in the grain structure resulting from mechanical processing, which might be associated with the chewing/mastication process when cooked rice is ingested (Tamura et al., 2015). Certain studies also support that starch digestibility could be less affected by extent or type of cooking and much more influenced by other variables like mastication, metabolic activity, gastric emptying and so on. Guillen et al. (2018) reported cooking temperature has a significant influence on RS. Effect of the degree of cooking on digestibility of starch between 10 and 20 min reported non-significant effect related to starch gelatinization (Tamura et al., 2016). Thus optimizing efficient cooking strategy to improve SDS or RS content having physico-chemical properties intact will be an interesting study in the future.

2.2.2.3. Gelatinization

Gelatinization Temperature (GT) is an important aspect in breeding for eating and cooking quality (ECQ) traits. During cooking, native starch granules are heated in water and the granules swell by several times of their original size, causing disintegration and are transformed into a paste or a gel. This process called gelatinization, involves the transformation of the structure of native starch from its semi-crystalline phase to an amorphous phase thus getting prone to enzymatic digestion. Bire-fringence, and higher order supramolecular structure are reduced or eliminated by moist heat denaturation. Intermolecular bonds are broken between starch molecules, permitting the hydrogen bonding sites to engage more water molecules. Chung et al. (2006) in their studies found that enzymatic hydrolysis was maximum in completely gelatinised waxy rice followed by partially gelatinised and the native starch. It can be stated that heating to temperature above GT allows recrystallization, leading to more amylopectin retro gradation. This endorses the fact why higher degree of gelatinization increase RS content (Wiruch et al., 2019). As per the mechanism proposed for gelatinization, intermolecular association might take place between the O-6 of amylose and the OH-2 of amylopectin molecules due to hydrogen bonding, as illustrated in **Fig.2.7A**. The short amylopectin chains (A and B1) take part in intermolecular associations. Intermolecular hydrogen bonding between amylose and amylopectin molecules has known to be thermally stable also.

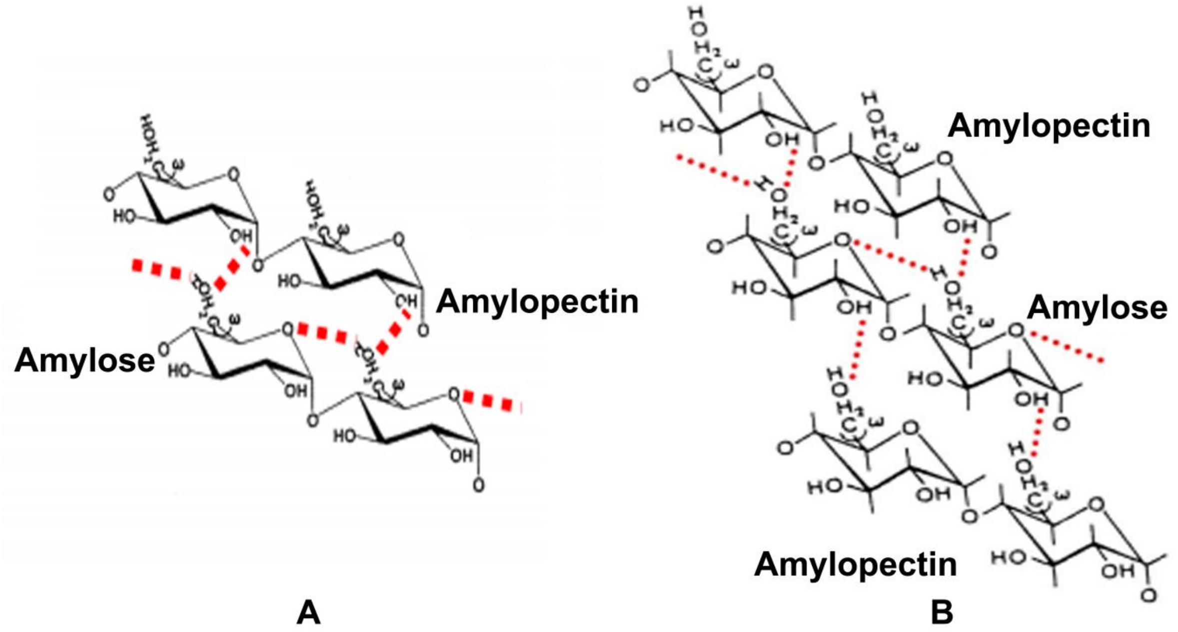


Fig.2.7. Gelatinization and retro gradation mechanism of rice starch. (A) Gelatinization mechanism of starch. (B) Retro gradation mechanism of rice starch. The dotted lines represent hydrogen bonding [Mechanism adopted from Tako et al., 2014]

2.2.2.4. Retro gradation

Retro gradation is the process by which amylose and amylopectin recrystallizes for short and long terms respectively and it is followed by a series of physical changes including increase in viscosity and turbidity of pastes, formation of gel, exudation of water and increase in the degree of crystallinity (Hoover et al., 2010). Susceptibility of retrograded rice starch to enzymatic hydrolysis seems to be largely determined by the extent to which starch undergoes disruption in its structure during gelatinization and the subsequent reordering of the molecules during the process of retro gradation (Wang et al., 2015). Starch digestibility rate can be lowered through retro gradation by cold storage, and reordering of starch molecules. Retro gradation occurs after formation of new intermolecular hydrogen bonding (Borah et al., 2017; Li et al., 2014). Thus, we concluded that another intermolecular hydrogen bond might form between the OH-2 of a D-glucopyranosyl residue of the amylose and the O-6 of a D-glucopyranosyl residue of short side-chain (A and B1) of the amylopectin molecules, as illustrated in **Fig.2.7B**. It can also increase the RS content of cooked rice, mostly classified as type III (Chiu and Stewart, 2013). Reed et al. (2013) attributed the increase in levels of RS observed in fried-rice samples to the retro gradation during the cold storage and to a lesser extent to “amylose-lipid complex” formation. Partially-gelatinized rice starches, with higher amount of crystalline ordering, showed more resistance to enzymatic digestion as compared to retrograded samples (Chung et al., 2006).

2.2.2.5. Parboiling

Parboiling is a process where rice gets partly boiled in the husk, with three steps : soaking, steaming and drying (Miah et al., 2002). Parboiling is an age old practice that is still prevalent in many parts of the world and have shown its significance in lowering the starch digestibility. Parboiling has been proven to cause retro gradation of the gelatinized amylopectin and formation of some low hydrolysable amylose-lipid compounds (Lamberts et al., 2009). Similar report also states that parboiling resulted in the formation of Type II amylose-lipid complex (ALC) which was found to show more resistance to hydrolysis by amylolytic enzymes than the amorphous complex counterpart (type I). More intense parboiling resulted in formation of more heat-stable ALC (Cheng et al., 2019). After parboiling, the aleurone layer and pericarp still remains attached near to the rice grain and since these layers are mainly comprised of cellulose and hemicellulose (not digested in the intestine), it acts as a barrier between starch and digestive enzymes, and eventually lowers the starch digestion. (Tian et al., 2018). Their studies on *in vitro* digestion showed that parboiled rice had a slower digestibility and equilibrium starch hydrolysis than brown rice. An interesting observation was seen after parboiling when native and typical A-type crystalline structure in starch transformed into a combination of A, B and V types. V type starches are known for their resistant to hydrolysis. Gunaratne et al. (2013) found that the parboiling process reduced swelling volume and amylose leaching, which resulted in retaining of amylose. Cheng et al. (2019) reported that parboiling also reduced the estimated GI which was explained in such a way that parboiling caused breakage in the protein structure which acted as a barrier between starch and hydrolytic enzymes.

2.2.2.6. Other processing conditions affecting starch digestibility

Among different processing strategies, heat moisture treatment (HMT) and annealing (ANN) are the hydro-thermal processes directed to change starch properties by manipulating the temperature and moisture levels. Both these involve incubation of starch at temperatures between the glass transition temperature and GT. HMT is carried out at low moisture levels (< 35%) whereas ANN is done in excess of water (> 60%) or at intermediate water levels (40–55%) (Jacobs and Delcour, 1998). Literature search showed uncertain relationship between HMT and rice starch digestibility. HMT had greater, lower or no effect on digestibility. A higher value of digestibility was demonstrated in heated waxy starches than in non-heated samples (Anderson et al., 2002). Similarly, autoclaved HMT rice starches showed higher digestibility than their respective native starches (Zavareze et al., 2010). On the contrary, decreased RDS and increased SDS and RS contents were observed in HMT rice starches (Van Hung et al., 2016; Wang et al., 2018) and heated flours (Silva et al., 2017). Cheng et al. (2019) reported that RS and SDS contents were enhanced to 10.4% and 45.8% respectively by combining HMT with parboiling. The rate of starch digestibility in HMT rice were lower than that of steamed rice (Ito et al., 1988). Anderson et al. (2002) reported that HMT showed no effect on waxy or non-waxy type of rice starches. Longer duration of heating (60 min vs. 30 min) during HMT has been shown to increase digestibility (Anderson et al., 2002; Silva et al., 2017) due to formation of RS (Silva et al., 2017). But an extended heating period of 8 hours was shown to reduce the RS content in waxy rice starch in comparison to native starch (Zeng et al., 2015). Decrease in RDS with increase in RS has been found in HMT starches with high, medium and low amylose content and is assumed to occur because of interactions between amylose and amylopectin formed during HMT (Van Hung et al., 2016). However contradictory results reporting reduction in RDS levels in HMT waxy starches along with reduced RS levels were found in another study (Zeng et al., 2015). This might be because waxy starches lack amylose and have lower proportion of long chain amylopectin (Butardo et al., 2017). Moisture content is also reported to influence the extent to which HMT affects digestibility. HMT starches prepared by autoclaving at different moisture levels (15/20/25%) had more susceptibility to α-amylase, and susceptibility increased with increase in moisture levels (Zavareze et al., 2010). On the contrary, starch digestibility was shown to decrease as moisture content in samples increased (10/20/30%) (Wang et al., 2018).

ANN treatment alone and ANN-HMT combined treatments have reports to influence the rice starch digestibility. ANN treated rice starches were more digestible by α-amylase (Dias et al., 2010), as well as elevated RDS and reduced SDS and RS levels (Zeng et al., 2015). This was ascribed to an increase in porosity of granules and reduced crystalline structure which both facilitate enzyme accessibility to starch (Zeng et al., 2015). In one study, treatment with a combination of ANN and HMT enhanced the RDS and SDS levels and reduced RS levels in waxy rice starch, which was probably due to disruption of crystalline structure by HMT (Zeng et al., 2015). Other studies on acid treated rice starch reported an increased RS content due to ANN treatment (Van Hung et al., 2016) and ANN-cross-linked starch (Song et al., 2011). Since HMT and ANN do not arrive at GT, partial gelatinization could be an important factor driving digestibility.

2.3. Approaches to characterize starch digestibility

At such times when NCDs are rising at an alarming rate and causing widespread effects, both in terms of human suffering and the damage to a country's socioeconomic fabric [World Economic Forum Annual Meeting, 2019], it’s time we use our capabilities, skill set and methodologies required to reverse the advancement of these diseases and achieve quick gains. Improving the quality of starch can be a very potent tool to tackle this pandemic. Even though India is blessed with a rich diversity of rice varieties, till date starch quality studies has been done only on a root level. Factors which often interplay and affect starch digestibility has not yet been fully understood. Individual parameters have been studied worldwide but since there is so much contradiction regarding the results it is incorrect to consider and attribute a single parameter towards affecting starch digestibility. A comprehensive study with several parameters is a solution towards understanding better the genesis of good starch quality. No such predictive tool has been developed till date while limited studies exist. Nondestructive near-infrared reflectance spectroscopy was used by Bao et al. (2001) considering 6 parameters most likely having an effect on starch digestion. But research over the years showed there are more factors involved which are interdependent. A comprehensive study was carried out to develop the relationship of nutritionally important starch fractions in cooked rice with other physico-chemical properties like AC, Crude protein, ASV, GC, pasting and thermal properties. So the need of the hour is to develop such a starch quality matrix with a handful of parameters to facilitate better prediction of starch quality and also assist breeders to develop newer lines with better starch quality.

3.1. Materials

3.1.1. Experimental materials

For this present study developing starch quality matrix (SQM) based on various parameters, twenty diverse rice varieties were selected and were procured from the Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi (**Fig.3.1**). The rice samples were hulled at 35A, kw 0.2-0.4 rpm using Satake huller (1900, No.554023), followed by milling through Satake Grain Testing Miller (Tm-05, No.554023, Japan) and finally grounded to 100 mesh size particles using an electric blender (Iconic Classic-mixer grinder, India). Samples were stored at RT as well as 4°C for various analyses.

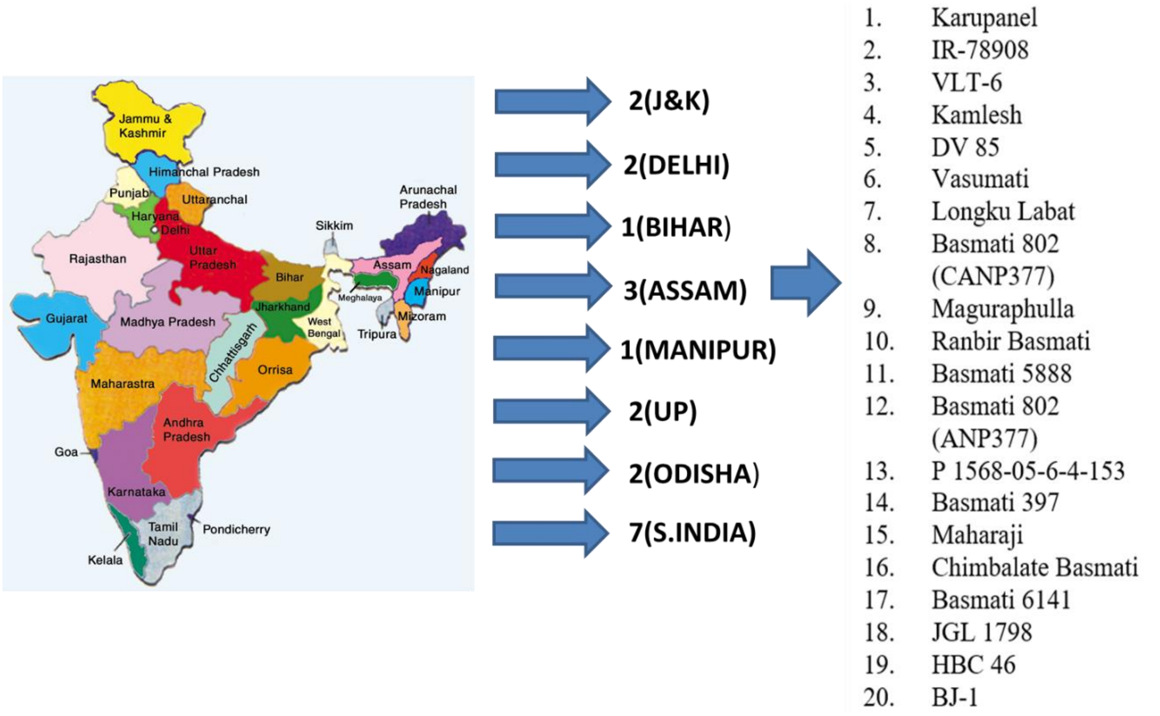


Fig.3.1. Selected rice genotypes for the present study and their possible geographical origin. Twenty milled rice genotypes selected from different geographies of the country to obtain the expected contrasting starch quality indices.

3.1.2. Chemicals and other materials

Most of the reagents used for this study were of analytical grade and procured from Sigma Aldrich (St. Louis Street, MO, USA) and Himedia (Delhi, India). Solvents used were procured from Merck, India. The Resistant Starch (RS), kit was procured from Megazyme International (Ireland, Ltd., Bray, Ireland). All solutions had been prepared in deionized water of resistivity more than 18.2 M Ω/cm.

3.2. Methodology

3.2.1. Experimental design

14 parameters were selected for this study based on the available literature. Raw polished sample was used for 3 parameters *viz*. micro-structure, alkali spreading value (ASV) and gelatinization temperature (GT), powdered form for 9 parameters *viz.* Total starch (TS), apparent amylose content (AAC), resistant starch (RS), gel consistency (GC), peak viscosity (PV), breakdown (BD), final viscosity (FV) and setback (SB) analysis while cooked rice was used for 2 parameters *viz*. hardness and stickiness. 2 different SQM were developed, one with 6 parameters viz. AAC, amylopectin, total TS, RS, microstructure and GT while the other with 8 parameters viz. GC, ASV, PV, BD, FV, SB, hardness and stickiness.

3.2.2. Micro-structure evaluation:

Microstructure variations contributed due to intrinsic and extrinsic factors affects starch digestibility. Integrity of bran layer after milling, leaving with cracks and fissures play role in starch digestibility. Hence, we performed microstructure evaluation of the selected rice grains using stereo-zoom microscope (Leica MZ 16 FA model, Leica, Japan). 4-5 rice kernels were examined and photographed at 12X magnification. To quantify the qualitative differences, a micro-scale was also developed for the very first time based on the extend of fissures and cracks.

3.2.3. Textural profile evaluation:

Textural measurements of cooked rice kernels were carried out using a texture profile analyzer (TPA) (TA-XT Plus, Stable Micro Systems, UK), facility available at the Division of Post-Harvest Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. The model used features a 5-kg load cell. A 35 mm stainless steel flat ended cylindrical probe was used to compress the sample for textural measurements of cooked rice kernels with the device kept in compression mode followed by detachment of the sample in the tension mode. After maintaining the cooked rice for 30 min at 25°C, a single cooked rice grain was kept at the middle of the base plate and uni-axially compressed by a probe at a constant deformation rate (0.5 mm/s) to 90% of its original thickness. For comparing surface hardness and overall hardness, the forces required for 90% strains were used respectively (Okadome et al., 1999). After each test, using a tissue paper the platform and bottom of the probe which came in contact with the samples was cleaned and dried. Cooking was replicated thrice and the average of the readings of technical triplicates of the samples was taken. The hardness and stickiness values based on force were graphed (**Fig 3.2**)

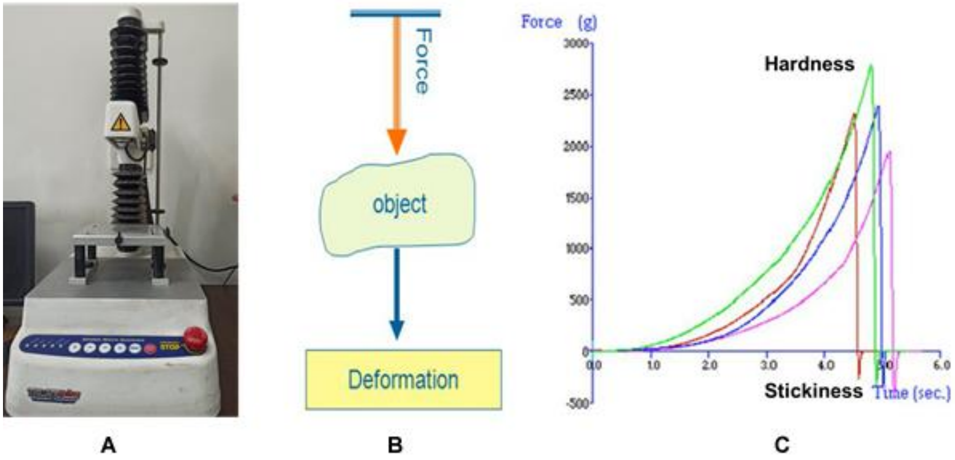


Fig.3.2. Texture measurement using Texture profile analyser (TPA) (A) Texture analyzer model featuring a 5-kg load cell and 35 mm stainless steel flat ended cylindrical probe (B) Principle behind texture analysis. Maximum force needed to achieve deformation is measured as hardness while the force required for overcoming the attractive forces between the sample surface and the probe is measured as stickiness (C) Force-time curve of the compression test depicting the attachment force as hardness and detachment force to separate probe from sample as stickiness. Positive peak force values are mentioned as hardness and negative peak force as stickiness.

3.2.4. Matrix composition analysis:

Major matrix components like starch, amylose, amylopectin and RS having role in starch digestibility was analysed.

Total starch content (TSC): TS of rice samples were estimated using the method reported by Clegg (1956) with slight modifications. 100 mg powdered rice flour was digested with 2 ml ethanol (70%, hot). Centrifugation of the homogenate was done for 10 min at 12000 rpm and the residue was washed with ethanol (70%, hot) followed by drying. The dried residue was next suspended in suspension buffer [water (5 ml) + 52% Perchloric acid (7.5 ml)] and shaken well for 5 min. It was then centrifuged for 10 min at 12000 rpm and the supernatant was retained. The extraction step was repeated with fresh perchloric acid (5 ml) followed by centrifugation for 10 min at 12000 rpm and supernatant was retained. Supernatants were mixed and volume made up to 100 ml using distilled water. Appropriate aliquots were taken in triplicates, combined with anthrone reagent (5 ml), incubated for 10 min in boiling bath water, and then rapidly cooled. Absorbance was measured at a wavelength of 620 nm in UV-Vis spectrophotometer (Evolution 220, Thermoscientific, USA). Glucose concentration was computed using standard curve of glucose and then multiplying by a factor of 0.9 (as 1 g glucose is obtained from 0.9 g starch on hydrolysis), starch content was calculated.

Total Amylose content (TAC): Apparent proportion of amylose in the samples was measured using the amylose-iodine complex colorimetric method (Juliano, 1971). 100 mg rice powder was suspended in suspension buffer containing 95% ethanol (1 ml) and 1N sodium hydroxide (9 ml). The tubes were kept for 15 min in boiling water bath. After cooling, volume made up to 100 ml was done with distilled water, from which aliquots in triplicates of 5 ml were transferred to another 100 ml volumetric flask, mixed with 1N acetic acid (1 ml) and 0.2% iodine solution (2 ml) and kept for 20 min in darkness. Absorbance was recorded at 620 nm against a reagent blank containing potato amylose (Sigma Aldrich, USA) as the standard for reference. 5-50% standard curve was prepared to quantify and classify the genotypes based on amylose content (**Fig.3.3**).

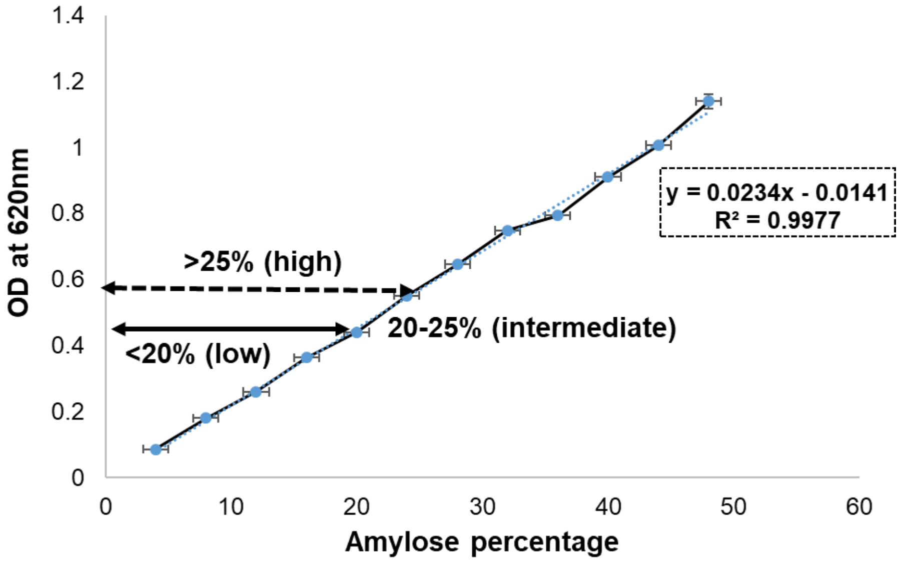


Fig. 3.3. Standard reference curve for the determination of percentage of amylose in selected rice genotypes (concentration ranged from 5-50%).

Total Amylopectin content (TAPC): Amylopectin content for each genotype was worked out from the difference between total starch content and amylose content.

***Resistant starch content (RS)*:** The amount of digestion resistant fraction was quantified using RS assay kit (Megazyme International Ireland Ltd., Bray, Ireland) with little modifications. Pancreatic α-amylase (10 mg/ml) along with amyloglucosidase (3 U/ml) was added to 100 mg of rice powder and kept on shaker (200 rpm) for 16 hr at 37°C. The reaction was terminated by adding an equal volume of absolute ethanol and recovering the RS pellet after centrifugation (3000 rpm) for 10 min. Using 50 % ethanol (v / v), the recovered pellet was washed two times. The final pellet recovered was suspended in 2 M KOH (2ml) and stirred for 20 min in an ice water bath. A continuous stirring was then applied with addition of 8 ml sodium acetate buffer (1.2 M, pH 3.8), followed by 0.1 ml amyloglucosidase (300 U / ml). After vortexing the tubes, they were incubated for 30 min at 50°C followed by centrifugation for 10 min at 3000 rpm). Supernatant was collected, from which 0.1 ml of aliquots were added to 3 ml glucose oxidase/peroxidase reagent (GOPOD) and incubated for 20 min at 50°C. The absorbance was read against reagent blank at 510 nm. The RS% was determined using the formula given on the manufacturer’s protocol.

3.2.5. *Physico-chemical attributes*:

Consumer preferences are majorly shouldered on rice grain's eating and cooking quality, which are indirectly indexed through the determination of their physico-chemical attributes. Exploring starch quality in terms of digestibility is majorly governed by composition and structure of starch. In this paper, 4 physico-chemical parameters were analysed *viz.* ASV, GC, cooking time (CT) and swelling ratio (SR).

Alkali spreading value (ASV): This method is inexpensive, but is not very accurate in determining actual GT of milled rice due to the subjective nature of visual scoring of grain spreading. Ten rice kernels of each variety were kept in a petri-plate having in it 10 ml 1.7% KOH solution (prepared freshly). During alkali spreading, KOH gelatinizes starch, particularly its amorphous region, causing degradation of the long linear and branched chains of amylose and amylopectin; this results in rice grain gelatinization. Uniform space was kept between the grains and petriplate was then covered and kept for incubation at 30°C for 24 hr. Based on the extent of digestion, ASV was assigned to each rice variety. ASVs 1-3, 3-5, 5-7 correspond to high, intermediate and low GT types respectively.

Gel consistency (GC): GC is a measure of how much tendency cooked rice shows to harden on cooling. 250 mg rice flour of each rice genotype was added into corresponding glass tubes. 500 µl ethanol (95%) containing thymol blue (0.025%) was put in each tube and mixed thoroughly to avoid clumping. After gently vortexing the mixture, 2 ml KOH (0.2N) was added and once again vortexed. Glass marbles were used to cover each tube to prevent steam loss and reflex of sample. Tubes were then kept in water bath for 6 min at 92^{o}C, followed by keeping at RT for 5 min and finally for 15 min in an ice bath. Tubes were horizontally laid on a graph sheet on a flat bench for 30 min and the spreaded gel (blue in colour) length in mm was calculated from bottom of the tubes to the end.

Cooking time (CT): Rice satisfies the nutritional need and foundation of the diet over 3 billion people but its acceptance and popularity is solely based on its cooking properties that also include minimum cooking time. 2 g of whole rice kernels were added to 20 ml distilled water and cooked in a boiling water bath. Few kernels were taken out at different time intervals and were pressed between glass plates to check the presence of any white core in the kernels. The time point where no white core remained was the optimum CT. For both the pigmented rice varieties, an initial soaking of 10 min prior cooking was carried out.

Swelling ratio (SR):

2 g of each rice variety was cooked for optimum cooking time as per the standard procedure described earlier, the SR was calculated by the formula:

SR = Average breadth of cooked kernel/Average breadth of raw kernel

3.2.6. Pasting profile analysis using rapid Visco Analyser (RVA):

The RVA is a rotating viscometer that records continuously the viscosity of the sample under controlled temperature and shear conditions. 3.5 g flour slurry (12% dry basis) was added to 25ml water (deionized) in an aluminium canister. Then the paddle was put inside the canister, mixed and sample lumps (if any) were pushed down. The paddle was inserted into the RVA motor coupling. The tower was depressed for lowering the canister into the RVA and let the test begin. A viscosity graph of the sample was generated on the monitor attached to the RVA (Perten, Sweden, RVA 4500). The test ended automatically. The total runtime for each sample was 13 min.

3.2.7. In vitro starch digestibility analysis using Starch Hydrolyzation Kinetics (SHK):

In vitro digestibility is the process to mimic the physiological conditions of the human digestive system in which starch is hydrolysed. Rapidly digestible starch (RDS) and RS amounts were calculated from the SHK experiments. 1 g rice grains in an excess of boiling water were cooked for their optimum cooking time. Using a domestic sieve, the rice was drained and allowed to cool at room temperature for 5 min. This was immediately followed by assaying the intact rice grains by an *in vitro* digestion system which mimics the buccal, gastric and pancreatic food digestion in the human upper gastrointestinal tract. In brief, cooked rice was placed in an Oakridge tube containing 4 ml α-amylase (250 U/ml, pH 7.0). After about 20 s, acidified (0.02 M HCl) pepsin (1 mg/ml) was added to the tubes and incubated in a shaking water bath for 30 min at 37°C. Then after the pH of the digest was adjusted to 6.0 (0.2 M acetate buffer, pH 6.0), 2 ml pancreatin (2 mg/ml) and 2 ml amyloglucosidase (28 U/ml) were added and the digest was left for incubation for another 5 hours on a shaker. After every 30 min interval, 1 ml aliquot was collected after proper shaking of the tubes. The aliquots were boiled at 100º C for 5 min and stored at 4º C for further use. All the aliquots were centrifuged for 10 min at 10000 rpm at RT and the supernatant collected. 30 µl aliquot was added for glucose estimation by DNS reagent. The glucose released in the first 30 min in the RDS. The glucose released from 30-240 min as the SDS content.

3.2.8. ***Starch quality matrix (SQM) development and validation***

Based on the interdependency and non-dependency, we developed two starch quality matrixes based on the parameters analyzed.

For SQM1, for quantifying the inherent glycaemic response, association between 6 variables (microstructure, AAC, APC, TS, RS and GT) were used. As the data from the variables used for SQM development in the present study were of both continuous (quantitative) and categorical (qualitative), different methods of association were used to determine the correlation as well as the strength of correlation between the variables. Among the parameters except microstructure and GT, all were continuous in nature. Association between two continuous variables was estimated using Pearson’s correlation using R software. Association between two categorical variables were analyzed using

Cramer’s V statistics. Further to estimate the association between continuous and categorical variable, correlation ratio was generated. In case of SQM1 association between 6 variables, out of which 4 were continuous and 2 were categorical in nature. Here we found Pearson’s correlation for 4 continuous variables, Cramer’s V statistics for 2 categorical variables and correlation ratio for interdependent association among them.

For developing starch quality matrix with focus on eating and cooking quality, SQM2 was developed. Association between 8 continuous variables (GC, ASV, PV, BD, FV, SB, hardness and stickiness) using Pearson’s correlation was analysed. The values of the correlation coefficient are not expressed in units of the data, but range from –1 to +1. The diagrammatic representation, known as scatterplot provides a picture of the relation of the bivariate data. It depicts the idea of the distribution of the data like well-defined positive or negative linear relationships, non-linear relationships or no apparent relationship. The analyses, heat maps, scatterplot matrix, pairwise correlations, regression model etc were developed using R software.

3.2.9. Statistical Analysis

Data analysis was done with the statistical software SPSS (Version 19.0). To test the significance of selected genotypes towards each parameter separately was analyzed using ANOVA. For multiple comparisons with significance level of 0.05, we performed Tukey’s test. The correlation study of continuous variables was estimated through Pearson correlation co-efficient, categorical using Cramer’s V statistics. Inter-association dependency among continuous and categorical was analyzed using correlation ratio. The analyses, heat maps, scatterplot matrix, pairwise correlations, regression model etc were developed using R software.

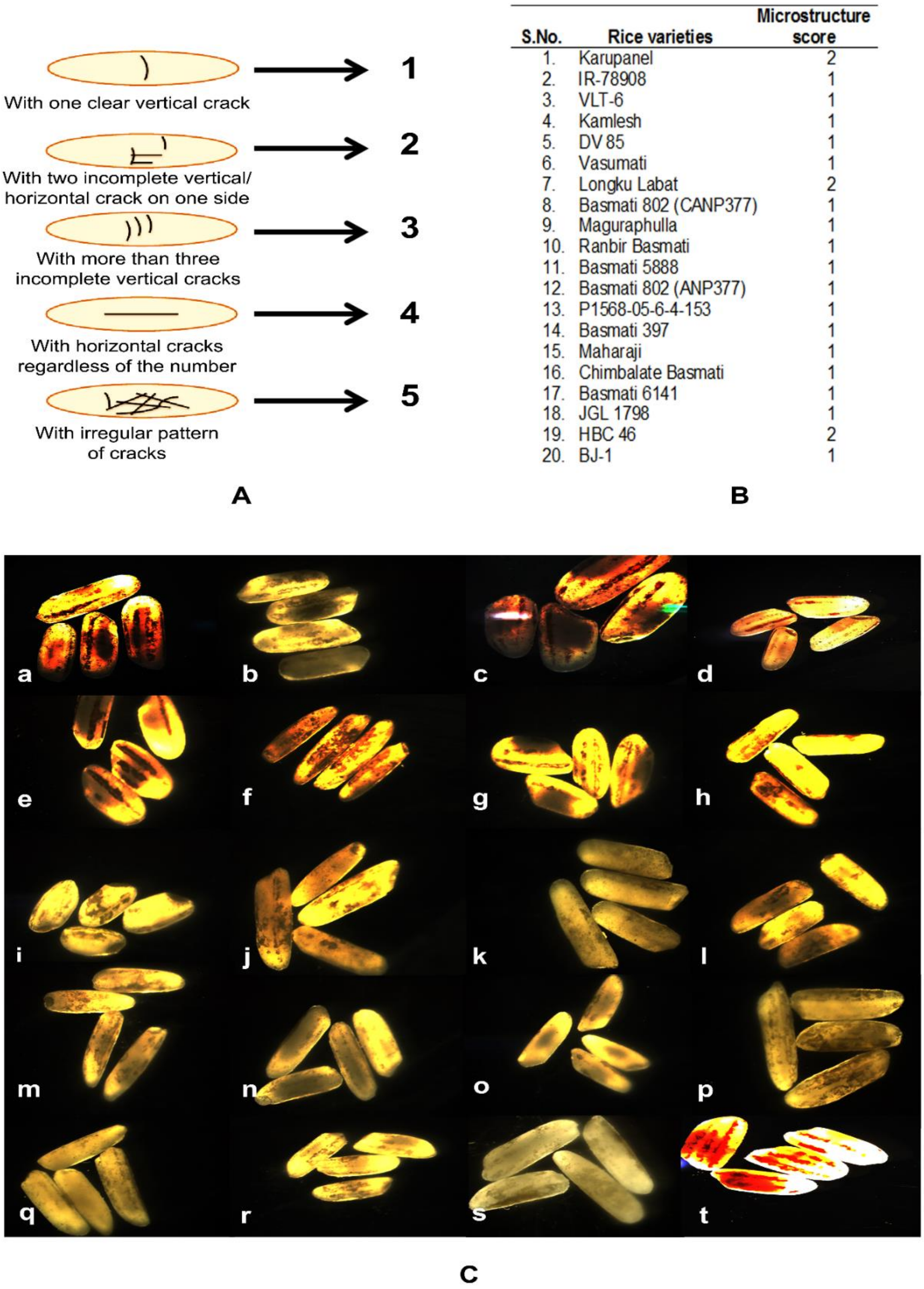
CHAPTER 4 RESULTS

In this chapter, the results obtained under the present investigation entitled "***Developing starch quality matrix to identify fibre rich Indian rice varieties with low glycaemic potential***" is described.

The investigation was carried out to analyse the variations among primary and secondary indices of starch digestibility in twenty selected rice genotypes. Statistically significant variations were observed among the traits and possible inter-relationship were developed. For association analysis through starch quality matrix (SQM), 14 parameters consisting microstructure, composition of starch and its components, nutritional starch fractions, physico-chemical, rheological and textural attributes were investigated**.** As milled grains were used the analysis of indices having role in starch digestibility, minor matrix components like lipids and proteins were not considered under the scope of the present study.

4.1. Variations in the microstructure of rice genotypes affecting starch digestibility

The microscopic evaluation of the rice genotypes was conducted to understand the variations as well as any possible association between microstructure with inherent glycaemic potential**.** A scale was developed to quantify the microstructure variations (**Fig.4.1A**) and rice genotypes were scored based on an average of 20 grains analysed (**Fig.4.1B**). The morphological variations observed and examined under stereo-zoom microscope is depicted in **Fig 4.1C**. The photo micrographs revealed the physical topography and status of bran layers in the grains. The stereo images mostly revealed horizontal fissures in the grain surface along with bran layer remnants. Overall, the rate or degree of milling could also be confirmed through the topography as it has also been known to have an effect on starch digestibility. The disruption of bran layers and fissures or cracks along with partial damage to the germ was also observed (**Fig 4.1C**). Very narrow score range of 1 to 2 was observed among the twenty genotypes analyzed. Based on the microstructure score generated, maximum aberrations were observed in 3 genotypes (Karupanel, Longku Labat and HBC-46).



S.No.	Rice varieties	Microstructure score
1.	Karupanel	2
2.	IR-78908	1
3.	VLT-6	1
4.	Kamlesh	1
5.	DV 85	1
6.	Vasumati	1
7.	Longku Labat	2
8.	Basmati 802 (CANP377)	1
9.	Maguraphulla	1
10.	Ranbir Basmati	1
11.	Basmati 5888	1
12.	Basmati 802 (ANP377)	1
13.	P1568-05-6-4-153	1
14.	Basmati 397	1
15.	Maharaji	1
16.	Chimbalate Basmati	1
17.	Basmati 6141	1
18.	JGL 1798	1
19.	HBC 46	2
20.	BJ-1	1

Fig.4.1. Microstructure evaluation of rice genotypes using stereo zoom microscope (A) Scale ranging from 1-10 developed (*in-house*) to quantify the micro structure aberrations (B) Tabulated observations of the variations in microstructure scores (C) Stereozoom microscopic images of rice samples **a.** Karupanel **b.** IR-78908 **c.** VLT-6 **d.** Kamlesh **e.** DV-85 **f.** Vasumati **g.** Longku Labat **h.** Basmati 802(CANP377) **i.** Maguraphulla **j.** Ranbir basmati **k.** Basmati 5888 l.Basmati 802(ANP377) **m.** P1568-05-6-4-153 **n.** Basmati 397 **o.** Maharaji **p.** Chimbalate basmati **q.** Basmati 6141 **r.** JGL 1798 **s.** HBC 46 **t.** BJ-1

4.2. Variations in the cooking and textural properties of rice genotypes affecting starch digestibility

The cooking characteristics of twenty rice samples are presented in **Table 4.1**. The raw rice samples needed 15 to 26 min to get properly cooked (judged by pressing between glass slides). Among the genotypes IR-78908 had the least cooking time of 14.33±0.47 min. Similarly, Longku Labat recorded the longest cooking time of 26.33±0.47 min. The water absorption capacities are reflected as swelling ratios (SR) and observed with considerable variations among the rice samples. SR ranged from 1.13±0.04 in Ranbir Basmati to a maximum of 1.92±0.03 in Basmati 802 (CANP377). Basmati varieties has known to have lower SR compared to non-basmati and it has been observed in case of Ranbir Basmati (1.13), Basmati 5888 (1.15) while interestingly Basmati 802 variants were observed to have higher SR (>1.9).

Table 4.1. Variations observed among cooking and swelling attributes among the rice genotypes

S. No.	Rice genotypes	Cooking time (CT)	Swelling ratio (SR)
1.	Karupanel	16.16±0.62	1.53±0.04
2.	IR-78908	14.33±0.47	1.54±0.09
3.	VLT-6	20.00±0.81	1.44±0.03
4.	Kamlesh	18.33±0.47	1.17±0.02
5.	DV 85	20.00±0.00	1.65±0.04
6.	Vasumati	23.00±0.81	1.86±0.03
7.	Longku Labat	26.33±0.47	1.44±0.01
8.	Basmati 802 (CANP377)	22.66±0.48	1.92±0.03
9.	Maguraphulla	22.66±0.45	1.50±0.04
10.	Ranbir Basmati	20.66±0.47	1.13±0.04
11.	Basmati 5888	21.00±0.49	1.15±0.01
12.	Basmati 802 (ANP377)	15.33±0.47	1.91±0.04
13.	P 1568-05-6-4-153	19.66±0.45	1.37±0.03
14.	Basmati 397	14.33±0.81	1.08±0.02
15.	Maharaji	18.00±0.92	1.40±0.01
16.	Chimbalate Basmati	20.33±0.47	1.25±0.02
17.	Basmati 6141	18.63±0.49	1.48±0.06
18.	JGL 1798	19.66±0.51	1.27±0.02
19.	HBC 46	24.97±0.83	1.37±0.04
20.	BJ-1	16.66±0.88	1.66±0.03

Results are expressed as mean ± SE of three independent experiments.

Textural properties like hardness and stickiness which acts as secondary indices for starch digestibility, were measured using texture profile analyser (TPA) and quantified based on force-time graph. In this study, all rice genotypes were cooked in the same rice/water ratio to avoid the effect of water content on the textural properties of cooked rice, as it has been shown that greater amounts of water will decrease the rice 's hardness (Bett-Garber et al., 2007). As shown in **Fig. 4.2A** and **4.2B**, cooked rice grains from different rice varieties exhibited significant (P<0.05) differences in their hardness and stickiness. The peak above the X-axis represented hardness while below the X-axis represented the stickiness (**Fig.4.2A&B**). Hardness and stickiness values of all genotypes ranged from 18.31 to 67.19N and from -0.56 to -2.66 respectively **(Fig. 4.2C)**. P1568-05-6-4-153 genotype observed with the lowest hardness of 18.31N, while Basmati 5888 had the highest hardness value of 67.19N. VLT-6 had the lowest stickiness value of -0.08N and Vasumati had the highest stickiness value of -2.94N.

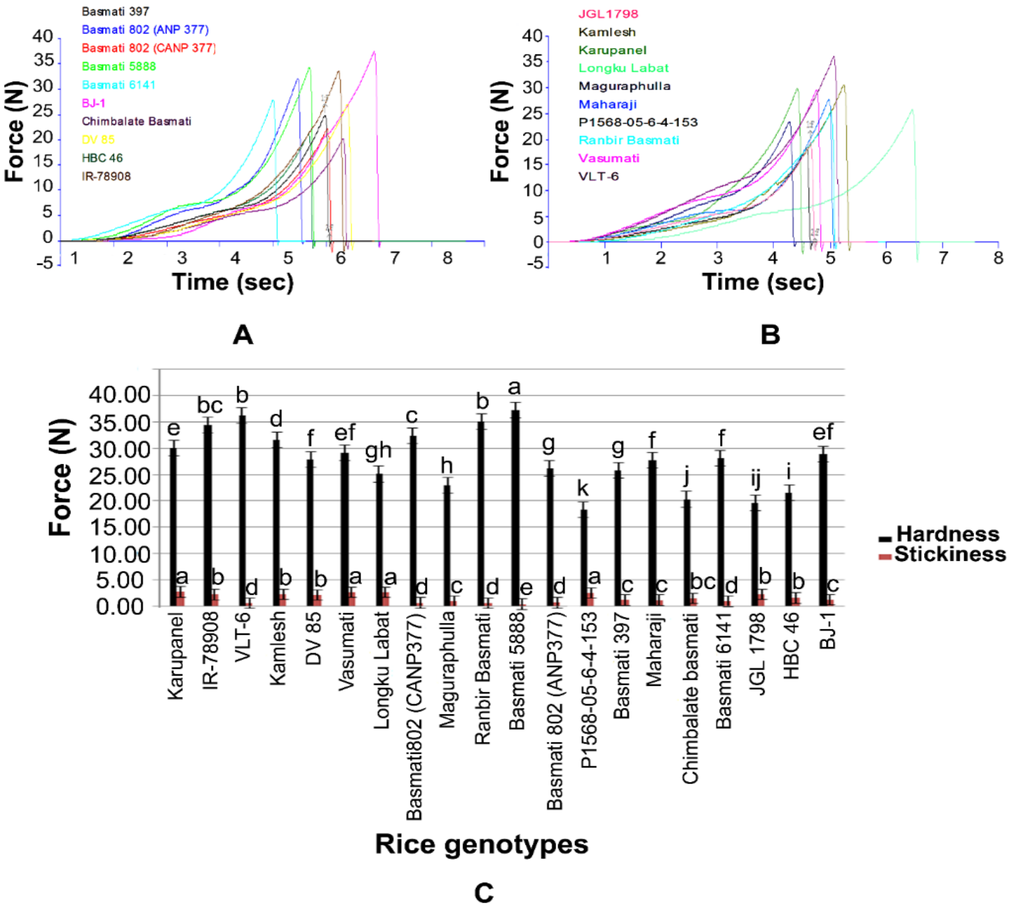


Fig.4.2. Textural properties of selected rice (cooked samples) analysed by Texture Profile Analyzer (TPA). (A&B) Force-time curve of the compression test depicting the attachment force as hardness and detachment force to separate probe from sample as stickiness. Positive peak force values are mentioned as hardness and negative peak force as stickiness. (C) Bar diagram showing hardness and stickiness values, represented as newton (N). Maximum force necessary to attain deformation is measured as hardness while the maximum detachment force required to separate probe from sample is measured as stickiness or adhesiveness. Results are expressed as mean ± SE of three independent experiments. Different letters depict mean significant differences at significance level 0.05 according to Tukey's method.

4.3. Variations among the matrix components of the rice genotypes affecting starch digestibility

Major matrix components having direct role in starch digestibility like total starch content (TSC), total amylose content (TAC), total amylopectin content (TAPC) and resistant starch (RS) were quantified. TSC ranged from 63.52 to 84.00 % **(Fig 4.3 A).** HBC 46 had the lowest TSC of 63.52% and IR-78908 had the highest TS content of 84.00 %.

The TAC analyzed based on the iodine binding efficiency, showed significant variations ($p<0.05$) among the samples. In the studied milled rice flours, TAC ranged from 10.78% to 29.82% with a mean of 19.5% **(Fig 4.3 B).** Basmati-397 was found to have the lowest TAC of 10.78% and VLT-6 had highest AAC of 29.82%. Among the genotypes investigated, ten genotypes were having <20% amylose and thus classified as low amylose type. Nine genotypes exhibited amylose range between 20-25 and thus medium type while one genotype, VLT-6 categorized as high amylose type. **(Table.4.2)**

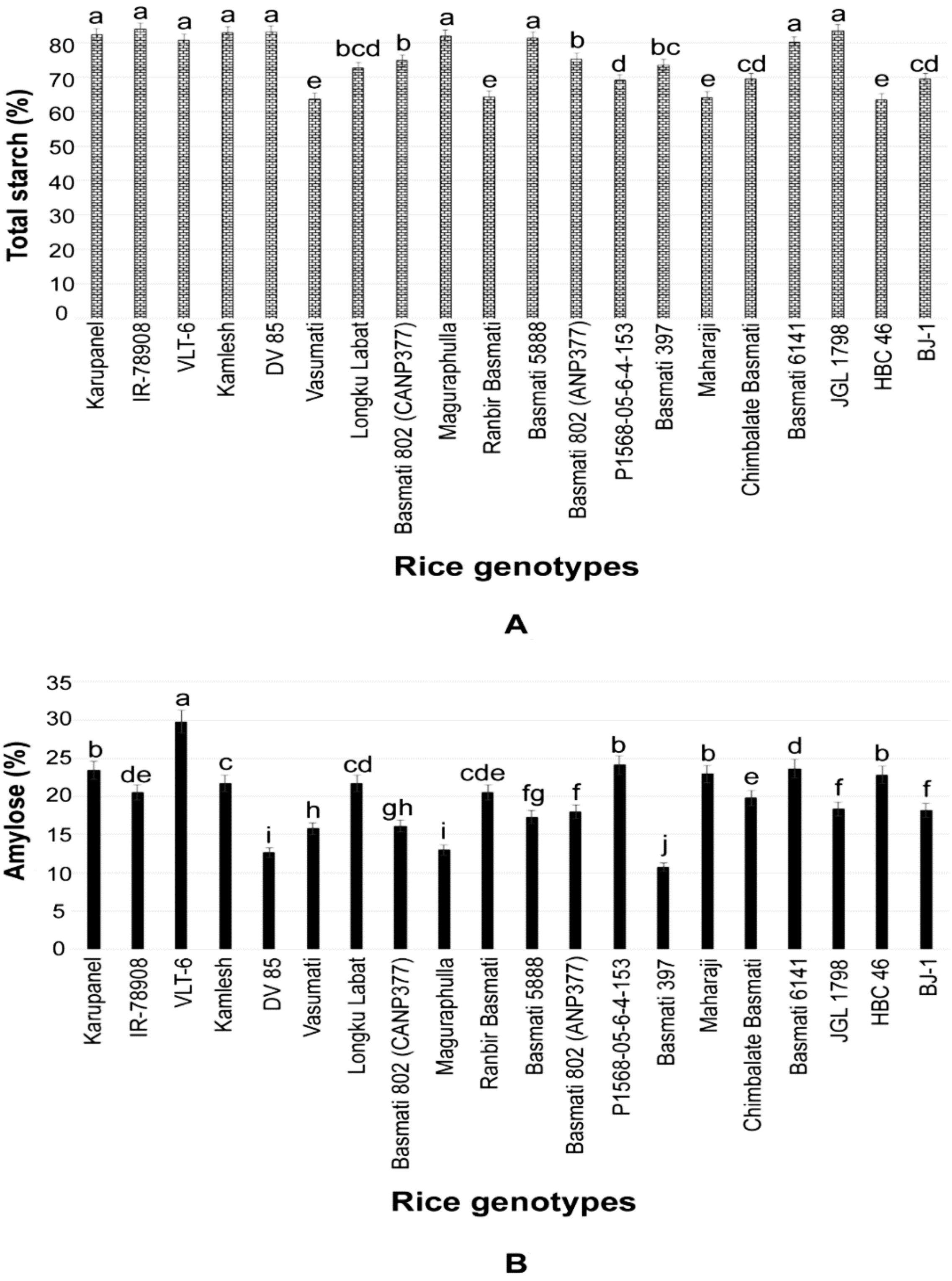


Fig.4.3. Total starch and amylose content in the selected rice genotypes (A) Bar graph depicts the variations observed in total starch content (TSC) among the selected rice genotypes expressed in percentage (W/DW). (B) Bar graph showing percent total amylose content (TAC) the selected rice genotypes expressed in percentage (W/DW). Results are expressed as mean ± SE of three independent experiments. (W-weight; DW-dry weight). Different letters depict mean significant differences at significance level 0.05 according to Tukey's method.

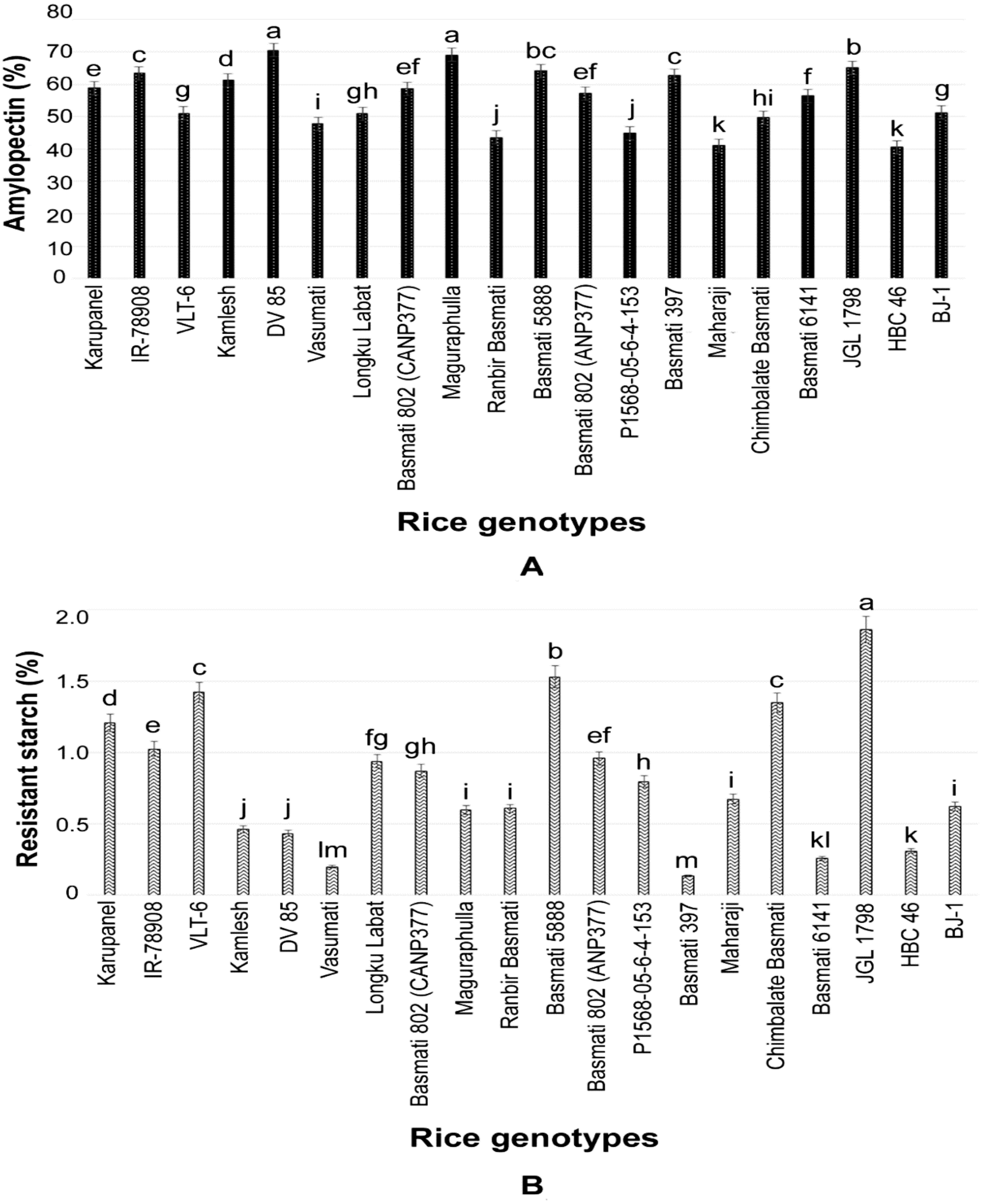


Fig.4.4. Amylopectin and resistant starch (RS) content in the selected rice genotypes (A) Bar graph depicts the variations observed in total amylopectin content (TAPC) among the selected rice genotypes expressed in percentage (W/DW). (B) Bar graph showing percent RS content in the selected rice genotypes expressed in percentage (W/DW). Time course starch digestion from 30-180 min using *in vitro* starch digestion simulation involving amylase and amyloglucosidase based on glucometry were performed. The un-digested fraction of starch after 120 min were hydrolyzed using KOH and further measured using glucose-oxidase/peroxidase (GOPOD) reagent for RS measurement. Results are expressed as mean ± SE of three independent experiments. (W-weight; DW-dry weight). Different letters depict mean significant differences at significance level 0.05 according to Tukey's method.

Table 4.2: Classification of the genotypes into 3 different groups (low, medium and high) based on the range of amylose content.

Low (<20%)	Medium (20-25%)	High (>25%)
Basmati 397	Ranbir basmati	VLT-6
DV 85	IR-78908	
Maguraphulla	Kamlesh	
Vasumati	Longku Labat	
Basmati 802 (CANP377)	HBC46	
Basmati 5888	Maharaji	
Basmati 802 (ANP377)	Karupanel	
BJ-1	Basmati 6141	
JGL1798	P1568-05-6-4-153	
Chimbalate Basmati		

The amylopectin content in all the samples ranged from 40.66% to 70.6% **(Fig. 4.4 A).** The lowest amylopectin content was found in HBC46 with 40.66 % and DV-85 had the highest amylopectin content of 70.6%. For RS analysis, time course starch digestion from 30-180 min using *in vitro* starch digestion simulation involving amylase and amyloglucosidase based on glucometry were performed. The un-digested fraction of starch after 120 min were hydrolyzed using KOH and further measured using glucose-oxidase/peroxidase (GOPOD) reagent for RS measurement. Discrete significant ($P<0.05$) difference was observed in the RS content among the genotypes. RS content ranged from 0.2-1.8%, with minimum in Vasumati and maximum in JGL1798 (**Fig.4.4B**). A medium dependency towards amylose and low dependency towards amylopectin content was observed with RS trait.

4.4. Variations in the physico-chemical attributes of the rice genotypes affecting starch digestibility

Evaluating eating and cooking quality (ECQ) is imperative as the physico-chemical attributes influence multitude of uses in both domestic and industrial fronts. Among the physico-chemical attributes, alkali spreading value (ASV), gel consistency (GC) and gelatinization temperature (GT) were investigated. After exposure of alkali, samples were observed with very few to varied significant changes in their surface structure (**Fig 4.5**). The ASV scale developed by International Rice Research Institute (IRRI), Philippines with 7 categories encompasses the degree of disintegration of milled rice grains in the alkaline solution (potassium hydroxide), indirectly also gives an estimate of GT was used. Grains un-affected in ASV estimation were given score of 1 and grains that were dispersed completely were given score of 7. ASV score ranged from 1.5 in DV-85 (least disintegrated) to 6.8 in VLT-6 (most disintegrated). An inversely proportional relationship being existed between ASV and GT, the genotypes with low ASV found to have high GT. Further based on the degree of disintegration of ten kernels of each rice genotype, the scores assigned were averaged and genotypes were accordingly classified into three different GT groups (**Table 4.3**). GT was classified as High

(>74°C), intermediate (70-74ºC) and low (55-69ºC). A high ASV corresponds to a low GT and vice versa was observed consistently among the genotypes.

Table.4.3. shows the classification based on ASV of rice genotypes into different GT groups. High GT (>74ºC); intermediate (70-74ºC) and low (55-69ºC). ASV results are expressed as mean ± SE of three independent experiments. Gel consistency (GC) range varied from 32 mm in VLT-6 to 75 mm in Basmati 397. According to their GC values the rice grain samples, investigated in the present study could be categorized as soft *i.e* >60mm [DV 85 (68mm), Vasumati (61mm), Maguraphulla (64mm) & Basmati397 (75mm)], Medium *i.e* 41-60 mm [Karupanel (45mm), IR-78908 (51mm), Kamlesh (47mm), Longku Labat (45mm), Basmati 802 (57mm), Ranbir Basmati (48mm), Basmati 5888 (58mm), Basmati 802 (55mm), Maharaji (43mm), Chimbalate Basmati (50mm), JGL 1798 (54mm), HBC 46 (43mm) & BJ-1 (51mm)] and Hard *i.e* <40mm [(VLT-6 (32mm), P 1568-05-6-4-153 (36mm) & Basmati 6141 (40mm)]. Results are expressed as mean ± SE of three independent experiments; High GT (>74ºC); intermediate (70-74ºC) and low (55-69ºC).

Table. 4.3. Classification based on alkali spreading value (ASV) of rice genotypes into different gelatinization temperature (GT) groups.

Low (ASV:6-7; GT:55-69ºC)	Intermediate (ASV:4-5; GT:70-74ºC)	High (ASV:1-3; GT:>74ºC)
VLT-6	Karupanel	Kamlesh
	IR-78908	DV 85
	Longku Labat	Vasumati
	Basmati 802 (CANP377)	Basmati 802 (ANP377)
	Maguraphulla	Maharaji
	Ranbir Basmati	Chimbalate Basmati
	Basmati 5888	Basmati 6141
	P 1568-05-6-4-153	
	Basmati 397	

Among the analyzed 35% genotypes were of soft GC, 60% with intermediate GC and 5% with hard GC phenotype which are given in **Table. 4.3.** The hard GC phenotype observed in VLT6 is due to its formation of rigid rice gel as a result of association of starch polymers in the aqueous phase. The high amylose nature of VLT6 (*i.e.* 29.82%), could possibly the reason behind its comparatively higher leaching when starch granules are heated and thus subsequently resulted with harder network once the gel cools. **Table.4.4** tabulates the ASV values as well as classifications.

Table 4.4. Classification of the genotypes under study into 3 different groups of GT (low, intermediate & high) based on the range of ASV.

S. No.	Rice genotypes	Alkali spreading value (ASV)	Classification	Gelatinization temperature (GT)
1.	Karupanel	4.3±0.20	Intermediate	Intermediate
2.	IR-78908	4.2±0.18	Intermediate	Intermediate
3.	VLT-6	6.8±0.12	High	Low
4.	Kamlesh	1.8±0.18	Low	High
5.	DV 85	1.5±0.21	Low	High
6.	Vasumati	2.5±0.21	Low	High
7.	Longku Labat	3.9±0.22	Intermediate	Intermediate
8.	Basmati 802 (CANP377)	4.0±0.20	Intermediate	Intermediate
9.	Maguraphulla	4.6±0.29	Intermediate	Intermediate
10.	Ranbir Basmati	4.6±0.25	Intermediate	Intermediate
11.	Basmati 5888	3.9±0.26	Intermediate	Intermediate
12.	Basmati 802 (ANP377)	3.7±0.14	Intermediate	Intermediate
13.	P 1568-05-6-4-153	4.5±0.25	Intermediate	Intermediate
14.	Basmati 397	3.8±0.27	Intermediate	Intermediate
15.	Maharaji	3.1±0.22	Low	High
16.	Chimbalate Basmati	2.6±0.25	Low	High
17.	Basmati 6141	2.2±0.27	Low	High
18.	JGL 1798	2.7±0.31	Low	High
19.	HBC 46	3.9±0.22	Intermediate	Intermediate
20.	BJ-1	3.8±0.27	Intermediate	Intermediate

Table 4.5. tabulates the classification of the genotypes under study into three different groups (soft, medium, hard) based on the range of the GC length. Four genotypes Vasumati, Maguraphulla, DV85, Basmati397 were soft GC length (>60mm), medium consistency is BJ1, JGL1798, Chimbalate Basmati, Basmati 802 (CANP377), IR-78908, Ranbir Basmati, Kamlesh, Longku Labat, HBC 46, Maharaji, Karupanel, Basmati 802 (ANP377), Basmati 5888. Among high consistency, it includes VLT-6, P-1568-05-6-4-153 and Basmati6141.

Table 4.5. Classification of the genotypes under study into 3 different groups (soft, medium and hard) based on the range of GC length)

SOFT (>60mm)	**Medium (40-60mm)**	**Hard (<40mm)**
Vasumati	BJ1	VLT-6
Maguraphulla	JGL1798	P-1568-05-6-4-153
DV85	Chimbalate Basmati	Basmati6141
Basmati397	Basmati 802 (CANP377)	
	IR-78908	
	Ranbir Basmati	
	Kamlesh	
	Longku Labat	
	HBC 46	
	Maharaji	
	Karupanel	
	Basmati 802 (ANP377)	
	Basmati 5888	

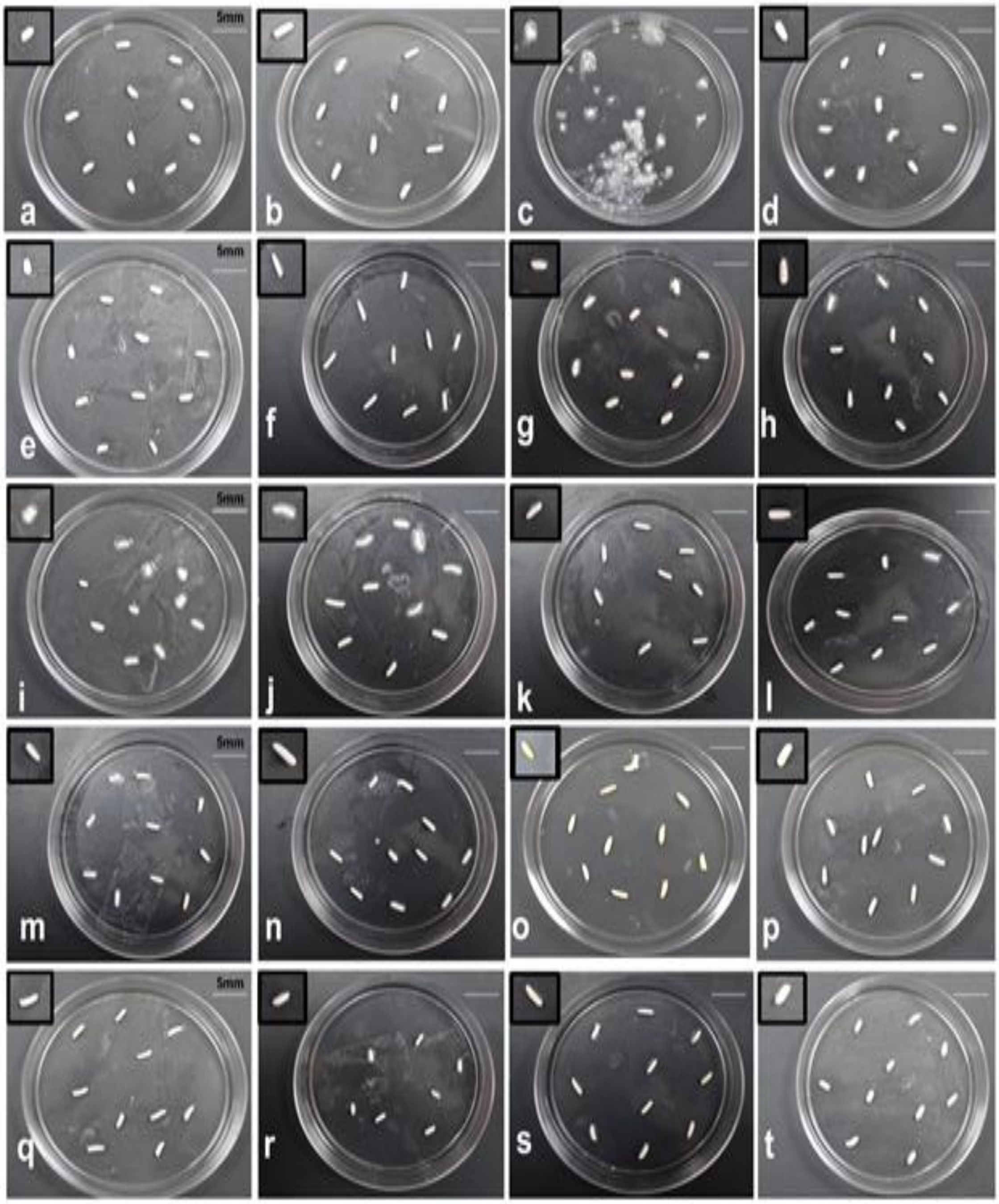


Fig.4.5. Alkali spreading value (ASV) analysis and indirect analysis of gelatinization temperature (GT) of the selected rice genotypes. Different milled rice genotypes tested for ASV as whole rice grains after 24 h treatment with 1.8% KOH. **a.** Karupanel **b.** IR-78908 **c.**VLT-6 **d.** Kamlesh **e.** DV-85 **f.** Vasumati **g.** Longku labat **h.** Basmati 802(CANP377) **i.** Maguraphulla **j.** Ranbir basmati **k.** Basmati 5888 **l.** Basmati 802(ANP377) **m.** P1568-05-6-4-153 **n.** Basmati 397 **o.** Maharaji **p.** Chimbalate basmati **q.** Basmati 6141 **r.** JGL 1798 **s.** HBC 46 **t.** BJ-1.

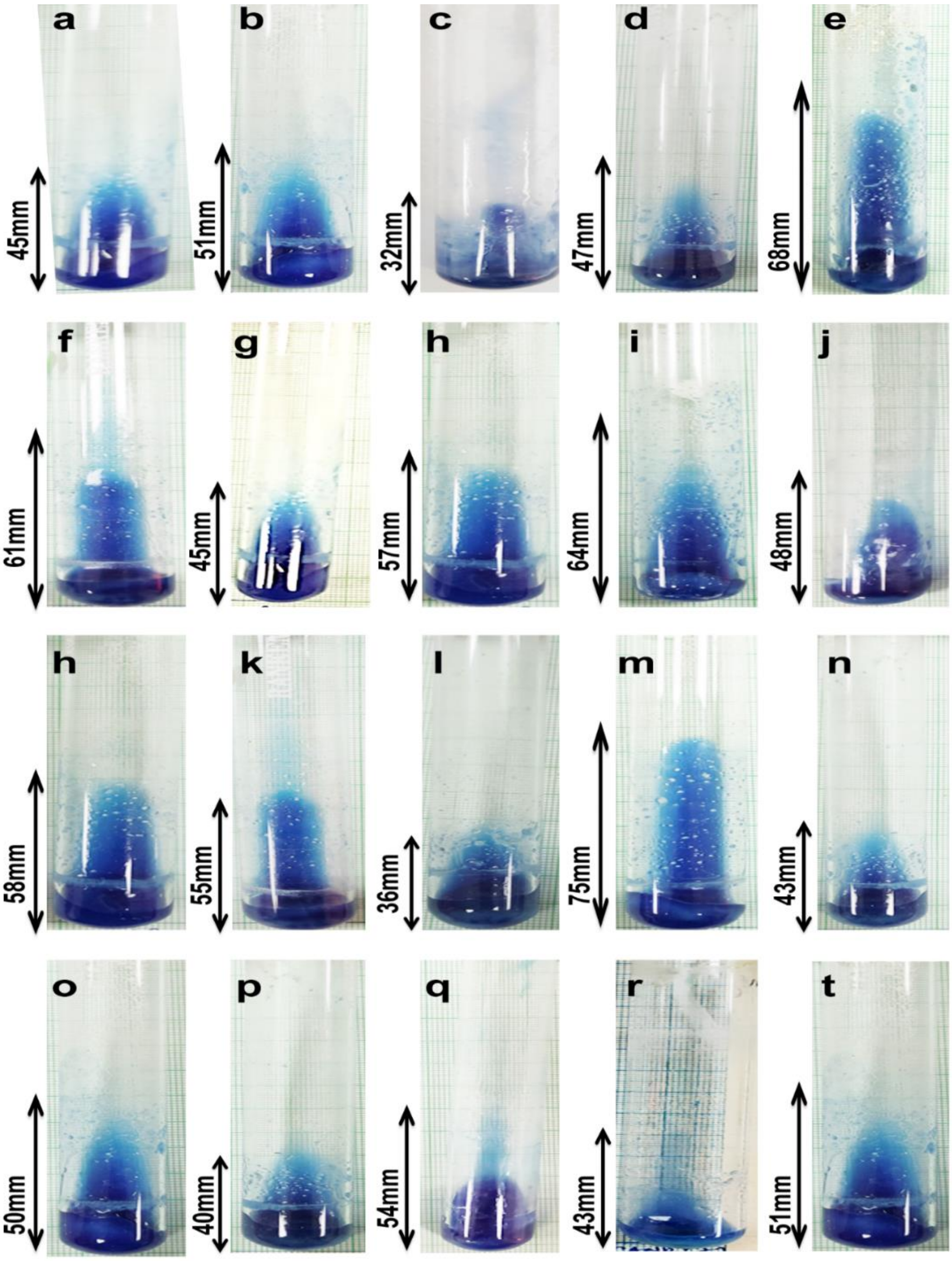


Fig.4.6. Physiochemical attribute of gel consistency (GC) was measured in the selected rice genotypes. GC, which is a measure of cold paste-viscosity of cooked rice was analyzed by standard procedure using thymol blue dye. Gel length from the bottom of the tube to the end of the gel in millimeters (mm) were measured and depicted here. Results are expressed as average of three independent experiments. **a.** Karupanel **b.** IR-78908 **c.**VLT-6 **d.** Kamlesh **e.** DV-85 **f.** Vasumati **g.** Longku labat **h.** Basmati 802(CANP377) **i.** Maguraphulla **j.** Ranbir basmati **k.** Basmati 5888 **l.** Basmati 802(ANP377) **m.** P1568-05-6-4-153 **n.** Basmati 397 **o.** Maharaji **p.** Chimbalate basmati **q.** Basmati 6141 **r.** JGL 1798 **s.** HBC 46 **t.** BJ-1

4.5. Variations in the rheological attributes of the rice genotypes affecting starch digestibility

The graphical representation of pasting profiles of rice genotypes is presented in **Fig.4.7-4.11** and pasting properties are tabulated in **Table.4.6.**

Table.4.6. Pasting properties of rice genotypes analysed using rapid visco analyzer (RVA)

S. No.	Rice genotypes	PV	BD	FV	SB
1.	Karupanel	3897.0±89.1	1209.5±50.2	5606.0±67.9	2918.5±29.0
2.	IR-78908	3589.0±48.1	1285.5±46.0	5163.5±20.5	2860.0±22.6
3.	VLT-6	5003.5±92.6	369.0±28.3	8516.0±128.7	3881.5±64.3
4.	Kamlesh	3438.5±37.5	796.0±11.3	5441.0±2.8	2798.5±29.0
5.	DV 85	3465.5±61.5	1276.5±26.2	4760.0±106.0	2571.0±70.7
6.	Vasumati	3081.0±28.3	262.5±26.2	6986.0±2.8	4167.5±4.9
7.	Longku Labat	3377.5±03.5	786.5±3.5	6147.0±36.8	3556.0±36.7
8.	Basmati 802 (CANP377)	3328.0±36.7	820.0±8.5	5105.0±24.0	2662.0±26.8
9.	Maguraphulla	1047.0±19.8	58.5±10.6	2368.5±17.7	1380.0±8.5
10.	Ranbir Basmati	1996.5±58.7	84.5±26.2	4892.0±15.5	2980.0±17.0
11.	Basmati 5888	2268.0±251.7	264.5±101.1	4994.0±449.7	2990.5±299.1
12.	Basmati 802 (ANP377)	3287.0±25.5	860.0±17.0	5530.0±2.8	2907.0±33.9
13.	P 1568-05-6-4-153	303.06±43.8	606.0±2.8	5106.0±67.9	2440.5±10.6
14.	Basmati 397	2886.0±172.5	424.0±101.8	5398.0±19.8	2936.0±51.0
15.	Maharaji	2518.0±32.5	276.0±15.5	5075.5±37.5	2830.5±16.3
16.	Chimbalate Basmati	2463.0±87.7	227.5±26.2	5134.0±21.2	2895.5±36.0
17.	Basmati 6141	2858.0±12.7	441.0±2.82	5300.5±37.5	2883.5±27.6
18.	JGL 1798	3560.5±19.1	319.0±46.7	7969.5±13.4	4728.0±79.2
19.	HBC 46	2627.5±54.4	469.5±12.0	2245.5±50.2	6.5±2.1
20.	BJ-1	3192.0±56.6	688.0±29.7	5256.5±72.8	2752.5±46.0

Results are expressed as mean ± SE of three independent experiments; PV-Pasting viscosity; BD-Break down viscosity; FV- Final viscosity; SB-Set back viscosity

Among the rheological parameters, peak time ranged from 5.4±0.02 min to 7.0±0.05 min. The pasting temperature (PT), representing the temperature of the initial viscosity increase (beginning of gelatinization) ranged from 79.85 to 92.9°C with an average of 87.6°C for the samples analysed. Maximum PT of 92.9°C was observed in Maguraphulla while least of 79.85°C was observed in Karupanel. Peak viscosity (PV) indicates the water-holding capacity of a sample, while final viscosity, the most commonly used parameter to define a particular sample's quality, indicates the ability of the material to form a viscous paste or gel after cooking and cooling. PV ranged from 1547-

5003.5RVU averaging 3084.7 RVU. Maximum PV was observed in VLT6 while least was observed in Maguraphulla. Trough viscosity (TV), representing the lowest viscosity upon heating was highest at 4634.5 RVU for VLT6, while the lowest value of 988.5 RVU was observed in Maguraphulla. The average for the entire samples analyzed were 2489.9 RVU. Final viscosity (FV) range recorded was from 2368.5-8516 RVU. Maguraphulla had the lowest FV while, VLT6 recorded the highest FV. Huge variation in the breakdown (BD) values were observed among the rice samples which ranged from 58.5 RVU (Maguraphulla) to 1285.5 RVU (DV85). HBC46 exhibited the lowest set back (SB) value of 1125 RVU while highest observed in JGL1798 genotypes with 4728RVU. The mean SB viscosity of 3005 RVU of the genotypes in the present study was higher than the mean reported by Bao et al. 2006 (*i.e.* 69.2) as well as 108.4 RVU reported by Gayin et al., (2015).

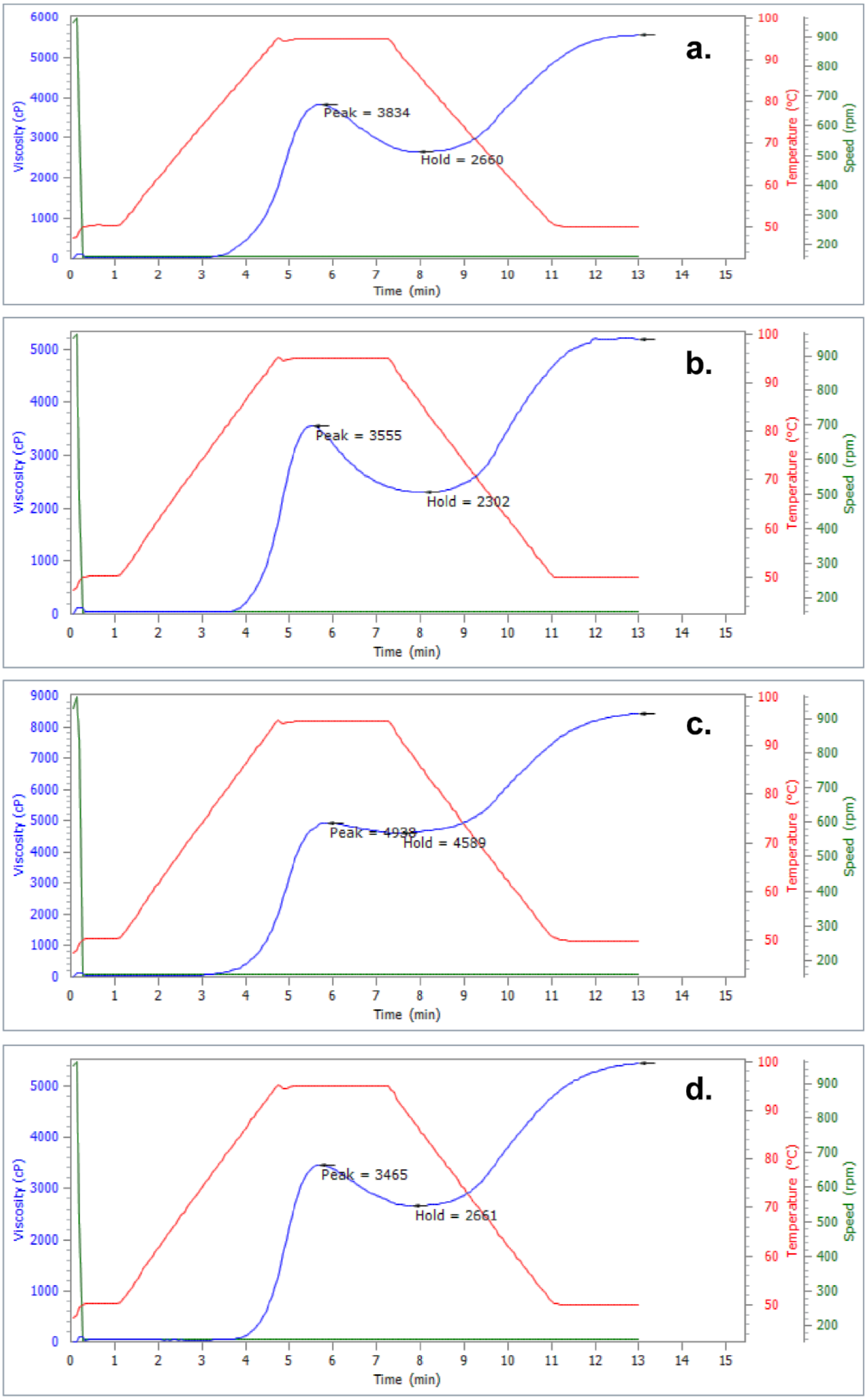


Fig.4.7. Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA). a. Karupanel **b.**IR-78908 **c.**VLT-6 **d.** Kamlesh

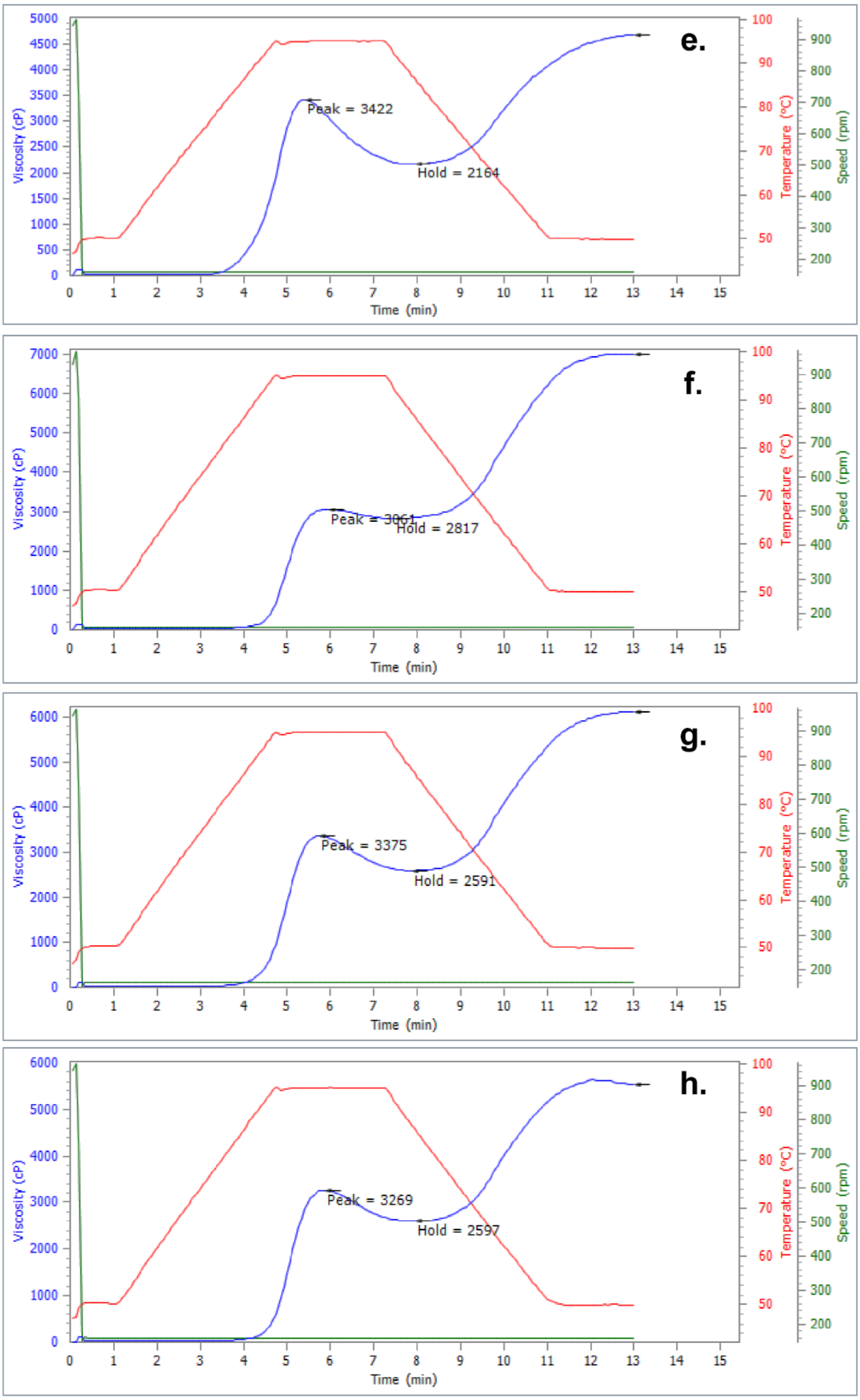


Fig.4.8. Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA). **e.**DV-85 **f.** Vasumati **g.** Longku labat **h.** Basmati 802(CANP377)

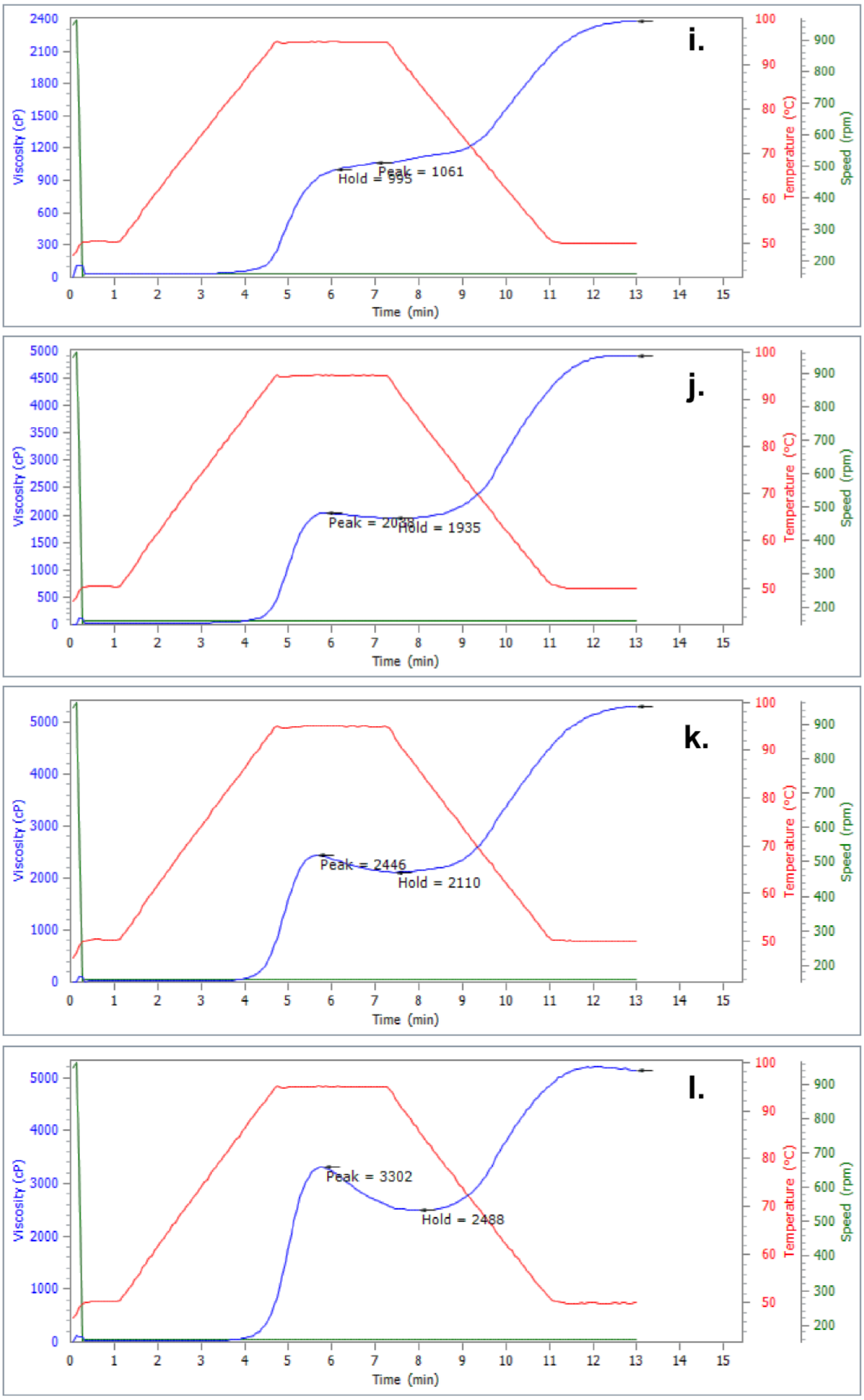


Fig.4.9. Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA). **i.** Maguraphulla **j.** Ranbir basmati **k.** Basmati 5888 **l.** Basmati 802(ANP377)

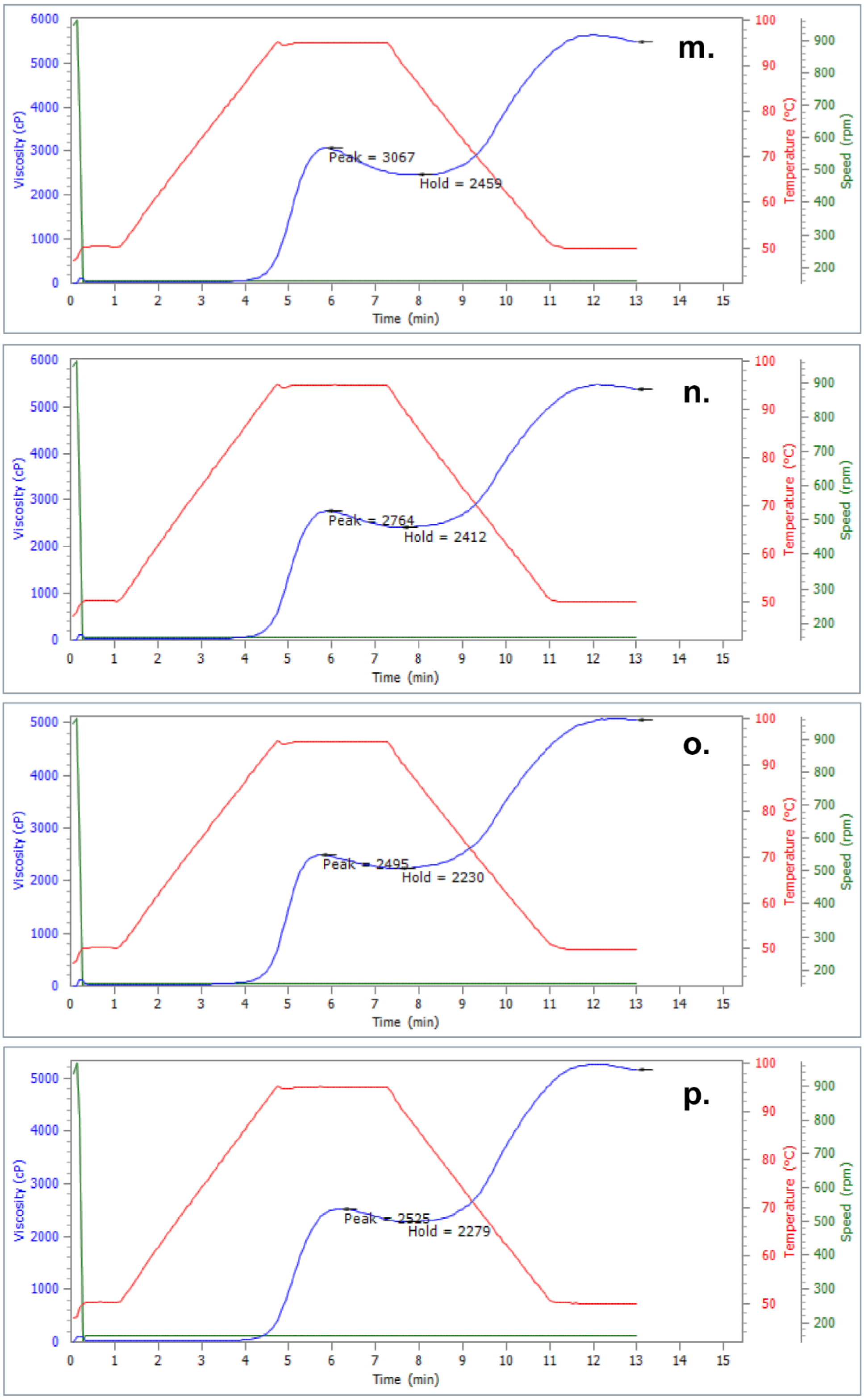


Fig.4.10. Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA). **m.** P1568-05-6-4-153 **n.** Basmati 397 **o.** Maharaji **p.** Chimbalate basmati

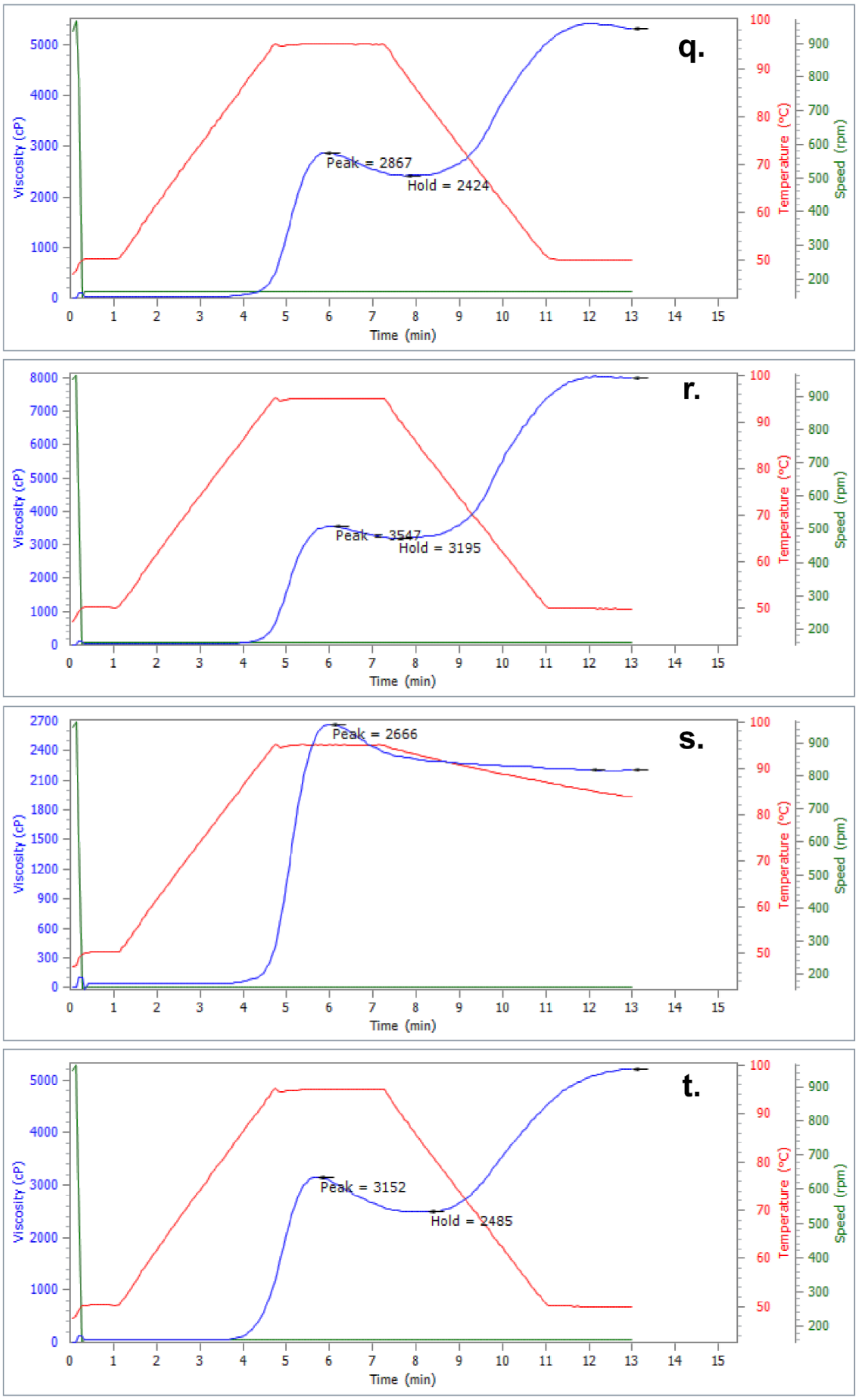


Fig.4.11. Comparative pasting profile of selected rice genotypes measured by Rapid visco analyzer (RVA). q. Basmati 6141 **r.** JGL 1798 **s.** HBC 46 **t.**BJ-1

4.6. Starch quality matrix development

Two types of Starch quality matrixes (SQMs) were developed based on primary and secondary index variables affecting inherent glycaemic potential as well as ECQ of rice.

For SQM1, six variables including TAC, TAPC, TS, RS, microstructure and GT were reconstituted for association analysis using Pearson's correlation as well as Cramer's V statistics based on the continuous and categorical nature of variables. A heat map representing correlation values ranging between –1 to +1 was developed (**Fig.4.12A**). Red colour indicates the highest correlation while blue depicts least correlation. Heat map developed based on p value developed underlines the values of significance and also expressed in range from –1 to +1(**Fig.4.12B**). Similar to correlation matrix, red colour indicates highly significant while blue depicts least significance Scatter plot which is a diagrammatic representation of correlation depicts the idea of the distribution of the data like well-defined positive or negative linear relationships, non-linear relationships or no apparent relationship utilizing the Cartesian coordinate plane. R values shows the correlation significance (**Fig.4.12C**). Among the traits, microstructure was positively correlated with TS (r=0.0751) and TAC (r=0.2426) and negatively with amylopectin (r=-0.0886), RS (r=-0.0061) & GT (r=-0.2301). TSC showed mild positive correlation with GT (r=0.0015), TAC (r=0.0208) & microstructure (r= 0.0751); a medium positive correlation with RS (r= 0.1842) but a high positive correlation with amylopectin (r= 0.7898). Interestingly, no negative correlation was found between TS and other parameters. TAC was positively correlated with TS (r=0.0208) & microstructure (r=0.2426) and negatively with amylopectin (r=-0.5968), RS (r=-0.0069) & GT (r=-0.3656). Amylopectin was positively correlated with TS (r=0.7898), RS (r=0.1521) & GT (r=0.2255) and negatively with TAC (r=-0.5968) & microstructure (r=-0.0886). RS in this study showed positive correlation with amylopectin (r=0.1521) & TS (r=0.1842) and very low negative correlations with AAC (r=-0.0069), microstructure (r=-0.0061) & GT (r=-0.0669).

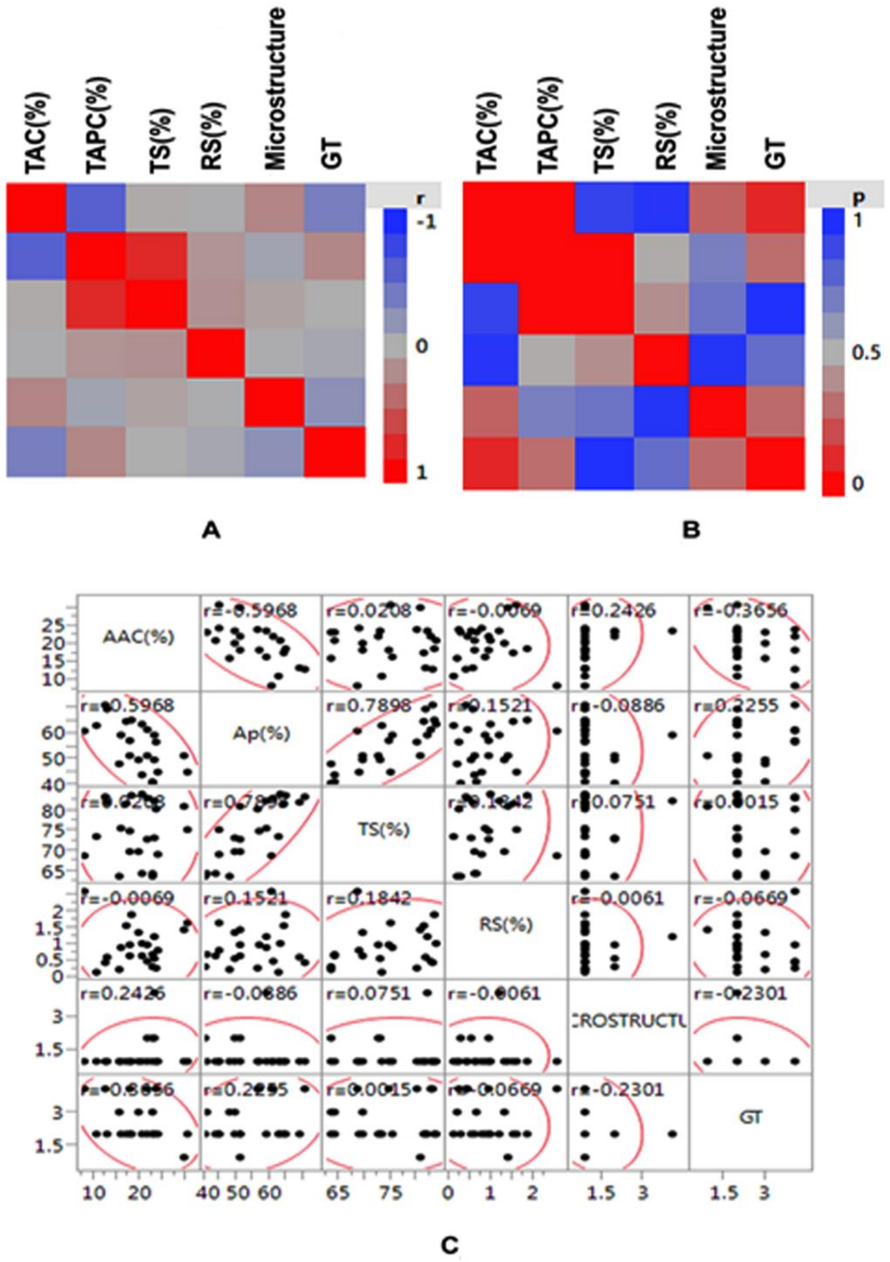


Fig.4.12. Starch quality matrix (SQM) -1 developed based on variables affecting inherent glycaemic potential of rice. Six variables including apparent amylose content (AAC), total amylopectin content (TAPC), total starch (TS), resistant starch (RS), microstructure and gelatinization temperature (GT) were used for association analysis using Pearson's correlation as well as Cramer's V statistics (A) Heat map developed based on correlation. The values of the correlation are expressed in range from –1 to +1. Red colour indicates highest correlation while blue depicts least correlation. (B) Heat map developed based on p value. The values of significance are expressed in range from –1 to +1. Red colour indicates highly significant while blue depicts least significance (C) Scatter plot depicts the idea of the distribution of the data like well-defined positive or negative linear relationships, non-linear relationships or no apparent relationship utilizing the Cartesian coordinate plane. R values show the correlation significance.

As it's crucial that rice varieties with low glycaemic amplitude should also be of preferred ECQ, Starch quality matrix (SQM) - 2 was developed based on physico-chemical variables.

Association between 8 variables like GC, ASV, PV, BD, FV, SB, hardness and stickiness were analysed similarly using Pearson's correlation as well as Cramer's V statistics. To elucidate the inter-relationship among the variables, correlation analysis was carried out and depicted as heat map based on p value, which underlines the values of significance (range from –1 to +1) (**Fig.4.13A**). Similar to correlation matrix, red colour indicates highly significant while blue depicts least significance Scatter plot which is a diagrammatic representation of correlation depicts the idea of the distribution of the data like well-defined positive or negative linear relationships, non-linear relationships or no apparent relationship utilizing the Cartesian coordinate plane. R values shows the correlation significance (**Fig.4.13B**).

ASV was found to be positively correlated with PV (r=0.0672), FV (r=0.2484), SB (r=0.1790) & stickiness (r=0.3219) and negatively correlated with GC (r=-0.3973), BD (r=-0.3862) & hardness (r=-0.0140). GC was found to be positively correlated with BD (r=0.1913) & hardness (r=0.1479) and negatively with ASV (r=-0.3973), PV (r=-0.1557), FV (r=-0.0860), SB (r=-0.1391) & stickiness (r=-0.1892). PV was positively correlated with ASV (r=0.0672), BD (r=0.5904), FV (r=0.7430) & SB (r=0.5150) and negatively correlated with GC (r=-0.1557), hardness (r=-0.0845) & stickiness (r=-0.0866). BD was positively correlated with GC (r=0.1913), PV (r=0.5904), FV (r=0.0758) & hardness (r=0.0069) and negatively with ASV (r=-0.3862), SB (r=-0.0084) & stickiness (r=-0.3773). FV was positively correlated with ASV (r=0.2484), PV (r=0.7430), BD (r=0.0758) & SB (r=0.9358) and negatively correlated with GC (r=-0.1892), hardness (r=-0.0799) & stickiness (r=-0.0329). SB was positively correlated with ASV (r=0.1790), PV (r=0.5150) & FV (r=0.9358) and negatively correlated with GC(r=-0.0860), BD (r=-0.0084), hardness (r=-0.0484) & stickiness (r=-0.1200). Hardness was positively correlated with GC (r=0.1479), BD (r=0.0069) and negatively correlated with ASV (r=-0.0140), PV (r=-0.0845), FV (r=-0.0799), SB (r=-0.0484) and stickiness (-0.3362). Stickiness was found to be positively correlated with ASV (r=0.3219) and negatively correlated with GC (r=-0.1391), PV (r=-0.0866), BD (r=-0.3773), FV (r=-0.0329), SB (r=-0.1200) and (hardness (r=-0.3362).

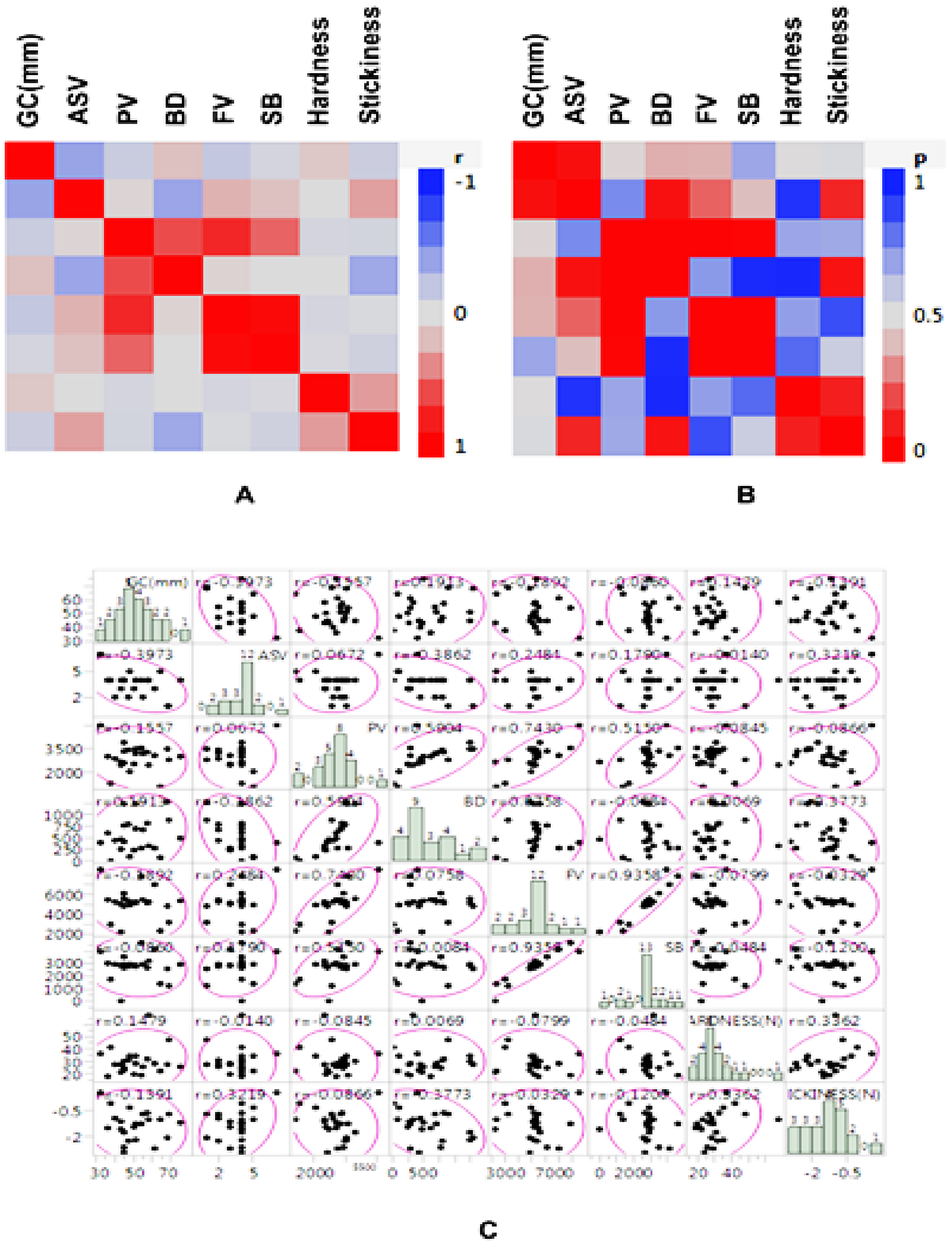
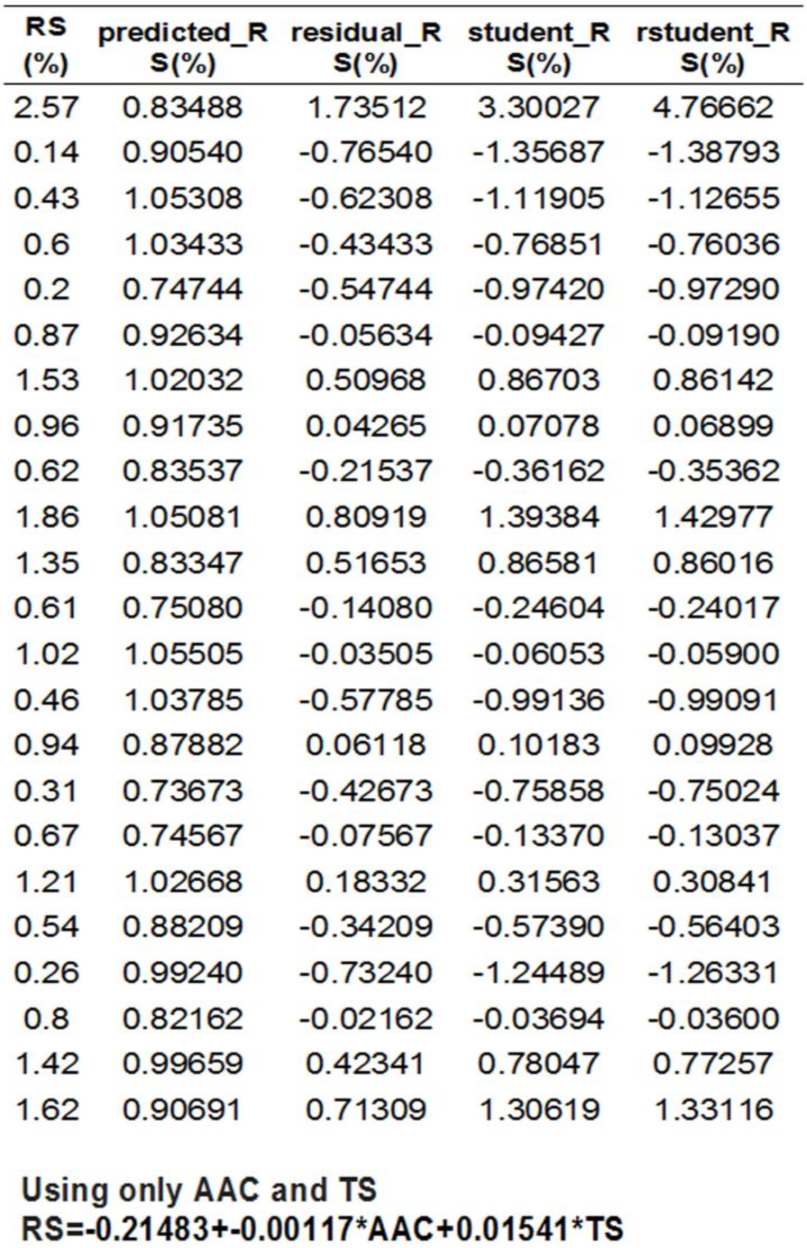


Fig.4.13. Starch quality matrix (SQM) - 2 developed based on physico-chemical variables affecting eating and cooking quality of rice. Association between 8 continuous variables – gel consistency (GC), alkali spreading value (ASV), peak viscosity (PV), breakdown (BD), final viscosity (FV), setback (SB), hardness and stickiness using Pearson's correlation as well as Cramer's V statistics was analyzed (A) Heat map developed based on correlation. The values of the correlation are expressed in range from –1 to +1. Red colour indicates highest correlation while blue depicts least correlation. (B) Heat map developed based on p value. The values of significance are expressed in range from –1 to +1. Red colour indicates highly significant while blue depicts least significance (C) Scatter plot depicts the idea of the distribution of the data like well-defined positive or negative linear relationships, non-linear relationships or no apparent relationship utilizing the Cartesian coordinate plane. R values show the correlation significance.

4.7. Development and validation of regression models to predict the inherent glycaemic potential in terms of resistant starch (RS) content in rice

The ultimate goal of the present study was to develop a predictive model based on explanatory variables and such a fitted model could be used to develop prediction for RS content in rice.

In this study, regression analysis which is a relationship analysis deduced between a dependent variable, (*i.e.* RS content in the present study) analyzed based on multiple explanatory variables (six variables analyzed). As multiple explanatory variables were in the present study, multiple linear regression was performed (**Fig.4.14**). Among the six variables, the present efficient model was developed based on maximum value of the coefficient of determination (R^2). Thus the analysis yielded a predictive value for RS resulting from a linear combination of fitted variables *i.e.* TSC and TAC. R^2 coefficient estimates the amount of variance in the criterion score accounted for by a linear combination of the predictor values. The model equation deduced was RS=-0.21483+-0.00117*TAC+0.01541*TS. Further the developed model was fitted and performed for stability analysis. **Fig.4.15A** tabulates the mean square, which is just the sum of squares divided by its degree of freedom (DF) and F value is the ratio of the mean square variation between the variables and mean squares with in the variables so as its outcome is determined for p value. The R^2 value of 0.03040 and probability values for TAC (0.9615) and TSC (0.4114) signifies the significant influence of these parameters on RS regression analysis revealed that both TS and TAC have significant effect on RS% as regression coefficients were significant (**Fig.4.15A**).



RS (%)	predicted_RS(%)	residual_RS(%)	student_RS(%)	rstudent_RS(%)
2.57	0.83488	1.73512	3.30027	4.76662
0.14	0.90540	-0.76540	-1.35687	-1.38793
0.43	1.05308	-0.62308	-1.11905	-1.12655
0.6	1.03433	-0.43433	-0.76851	-0.76036
0.2	0.74744	-0.54744	-0.97420	-0.97290
0.87	0.92634	-0.05634	-0.09427	-0.09190
1.53	1.02032	0.50968	0.86703	0.86142
0.96	0.91735	0.04265	0.07078	0.06899
0.62	0.83537	-0.21537	-0.36162	-0.35362
1.86	1.05081	0.80919	1.39384	1.42977
1.35	0.83347	0.51653	0.86581	0.86016
0.61	0.75080	-0.14080	-0.24604	-0.24017
1.02	1.05505	-0.03505	-0.06053	-0.05900
0.46	1.03785	-0.57785	-0.99136	-0.99091
0.94	0.87882	0.06118	0.10183	0.09928
0.31	0.73673	-0.42673	-0.75858	-0.75024
0.67	0.74567	-0.07567	-0.13370	-0.13037
1.21	1.02668	0.18332	0.31563	0.30841
0.54	0.88209	-0.34209	-0.57390	-0.56403
0.26	0.99240	-0.73240	-1.24489	-1.26331
0.8	0.82162	-0.02162	-0.03694	-0.03600
1.42	0.99659	0.42341	0.78047	0.77257
1.62	0.90691	0.71309	1.30619	1.33116

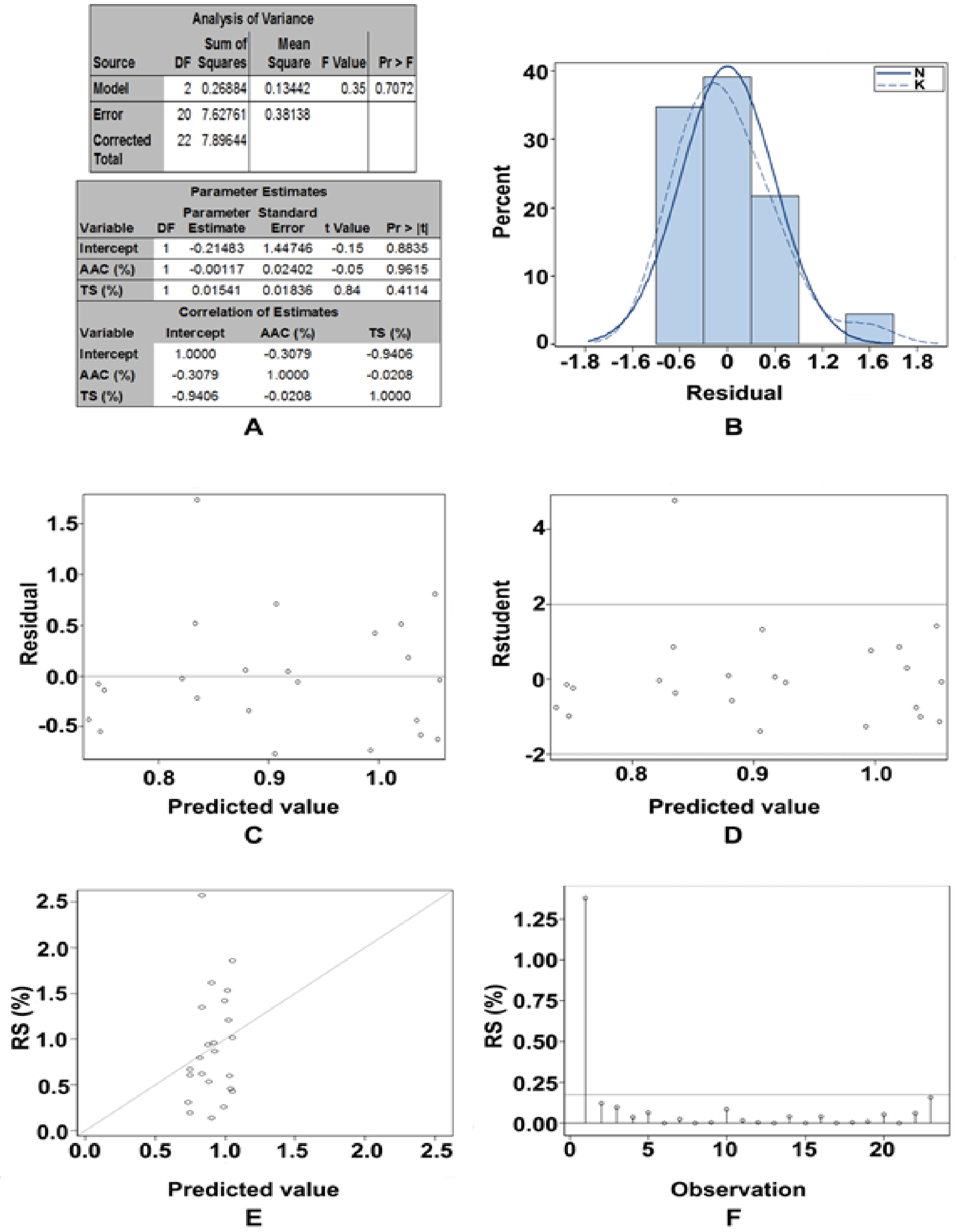
Using only AAC and TS
RS=-0.21483+-0.00117*AAC+0.01541*TS

Fig.4.14. A regression model developed using the SAS System ('Local', X64_7PRO) based on SQM 1 to predict the % content of Resistant Starch (RS) in the rice genotypes under study. Total RS% is the sum of predicted RS% plus residual RS%. Here only the values of apparent amylose content (AAC or TAC) and total starch (TS) were considered to derive the equation.

Fitting values to the model and checking the model are important steps after model development. The raw materials of model checking are the residuals which is defined as the differences between observed and fitted values. The distribution of residuals for the log-linear regression model is depicted in **Fig.4.15B**. The normal density estimate of residual in multiple linear regression analysis is depicted in solid line while kernel density in dotted lines (**Fig.4.15B).** Scatterplot of residuals associated with variables tested is shown in **Fig.4.15C &D**. Actual and predicted RS values depicted based on the regression models with basic quality indices like TAC

(**Fig.4.15C)** and TSC (**Fig.4.15D).** Predicted value of RS% using the linear regression model is shown in **Fig.4.15E** and the observed values of RS% after analysis is in **Fig.4.15F**.

As a most efficient and accurate model for prediction determined, it is prudent that the model should be assessed for stability, which is performed through cross validation models. Leverage and influence points having impact on the model was also analyzed and illustrated in **Fig.4.16A)**. A second type of diagnostic aid used in the present study is the probability plot, a graph of the residuals versus the expected order statistics of the standard normal distribution. This graph known as Q-Q Plot because it plots quantiles of the data versus quantiles of a distribution. The Q-Q lot of residuals for RS% is shown in **Fig.4.16B**. Residual fit spread plot for RS% (**Fig.4.16C**). In the R-F spread plot for the model, the right-hand plot is taller than the left-hand plot ((**Fig.4.16C**). This shows that there is a lot of variation that is not explained by the model. Furthermore, the residual distribution does not appear to be normally distributed. The right tail of the residual distribution is long, and the distribution is skewed. Box plot depicts the residual for RS% (**Fig.4.16D**) The measures of influence of the predicted model was also performed by DFFITS and Cook's D-statistics (**Fig.4.16E)**.



Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.26884	0.13442	0.35	0.7072
Error	20	7.62761	0.38138		
Corrected Total	22	7.89644			

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-0.21483	1.44746	-0.15	0.8835
AAC (%)	1	-0.00117	0.02402	-0.05	0.9615
TS (%)	1	0.01541	0.01836	0.84	0.4114

Correlation of Estimates			
Variable	Intercept	AAC (%)	TS (%)
Intercept	1.0000	-0.3079	-0.9406
AAC (%)	-0.3079	1.0000	-0.0208
TS (%)	-0.9406	-0.0208	1.0000

Fig. 4.15. Development and validation of the multiple regression model developed to predict the inherent glycaemic potential in terms of resistant starch (RS) content in rice (A) Analysis of variance (ANOVA) test for significant explanatory variables (TAC and TSC) (B) The distribution of residuals for the log-linear regression model. The normal (N) density estimate of residual in multiple linear regression analysis is depicted in solid line while kernel (K) density in dotted lines (C) Scatterplot of residuals associated with TAC variable tested (D) Scatterplot of residuals associated with TS variable tested (E) Predicted value of RS% using the linear regression model. (F) Observed values of RS% after analysis. [TAC-Total amylose content; TSC-Total starch content]

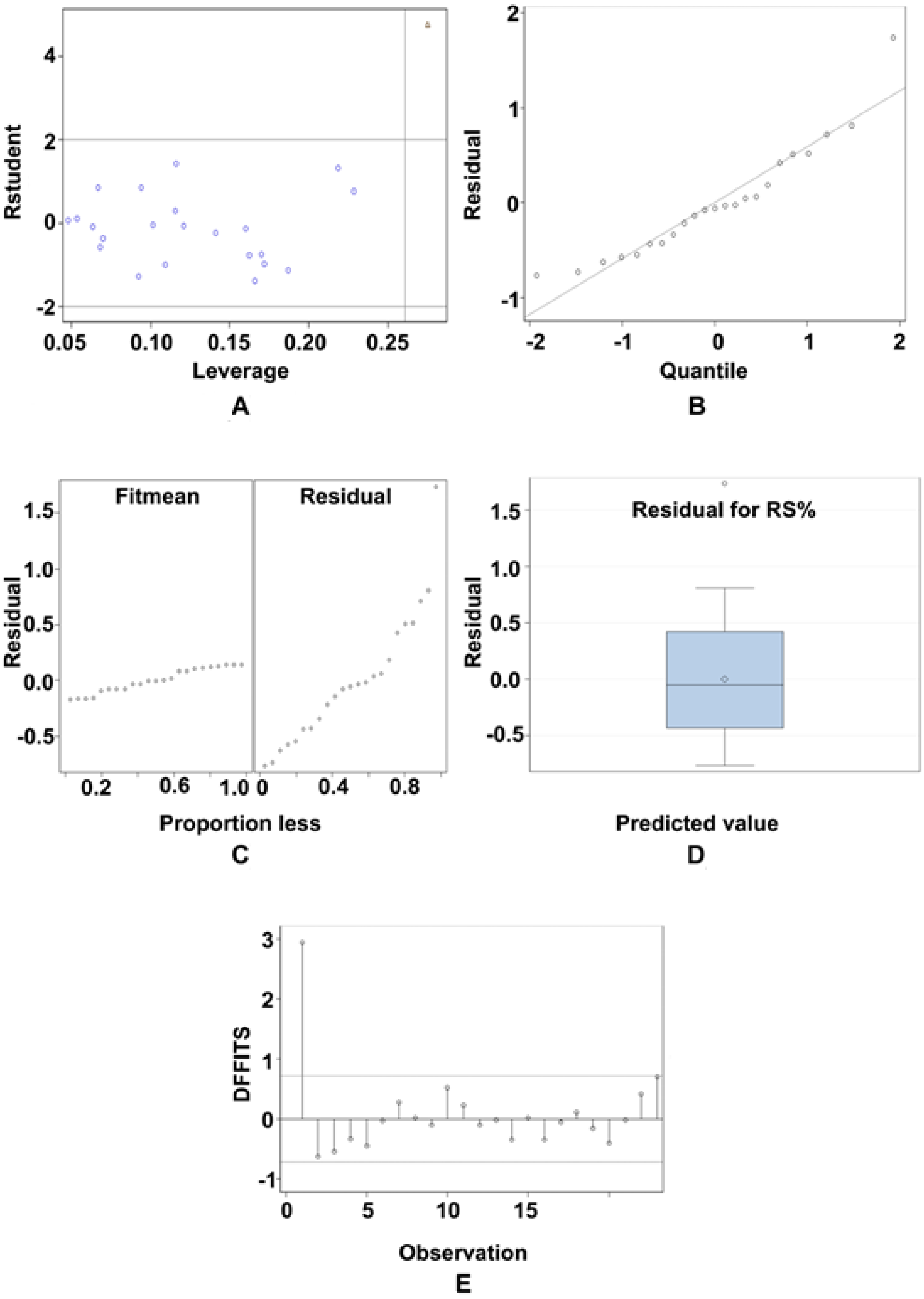


Fig. 4.16. Analysis of the stability of the model developed through multiple regression for predicting Resistant Starch (RS) content in rice (A) Leverage and influence points having impact on the model. Triangle symbol shows the outlier and leverage (B) Q-Q lot of residuals for RS% (C) Residual fit spread plot for RS% (D) box plot depicting residual for RS% (E) The measures of influence of the predicted model was also performed by DFFITS and Cook's D-statistics**.**

4.8. In vitro starch digestibility analysis using starch hydrolyzation kinetics (SHK):

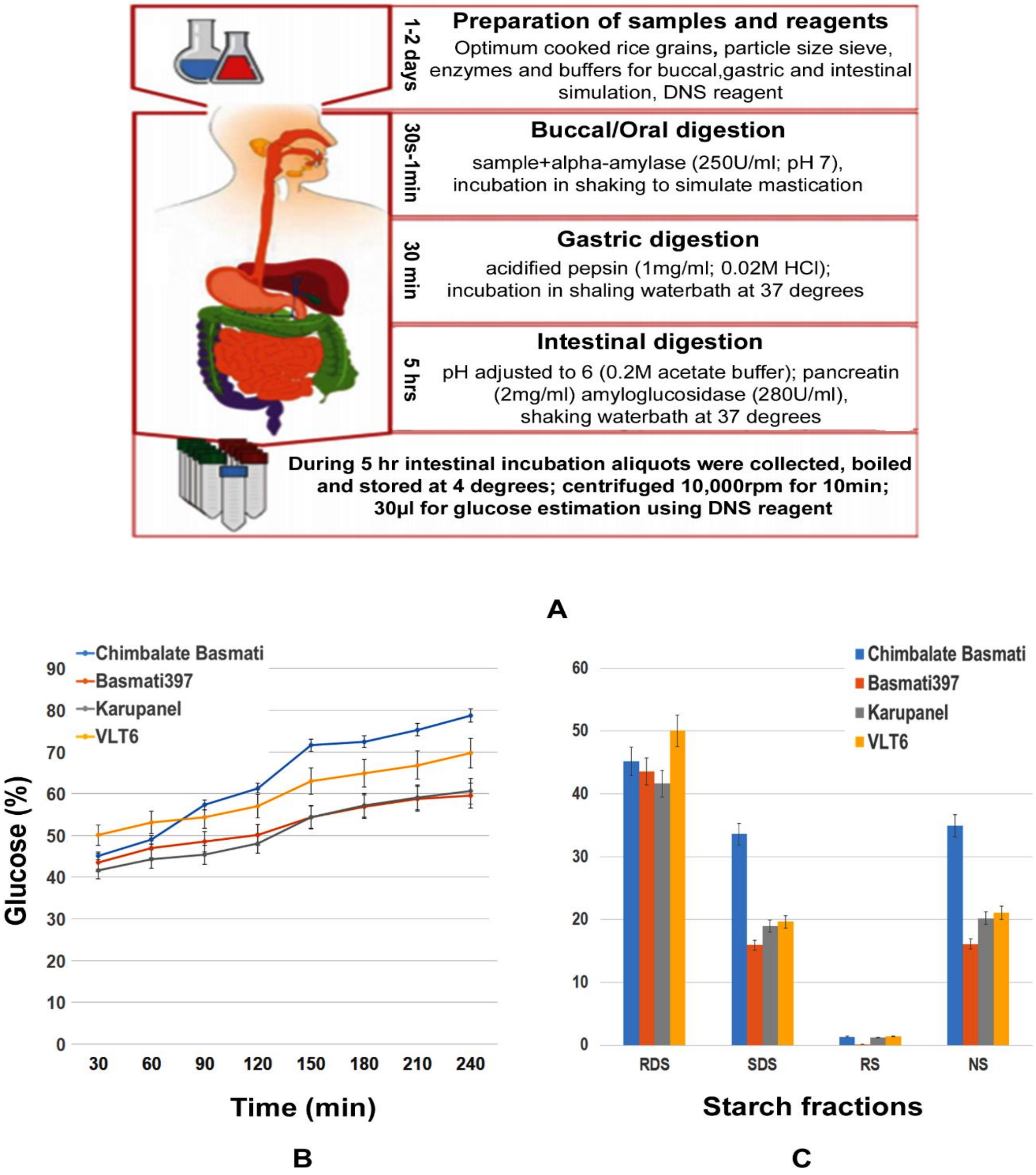


Fig.4.17. *In vitro* starch hydrolyzation kinetics to simulate gastro-intestinal digestion method with steps, expected time frame and simulated conditions [protocol is partially adopted from INFOGEST2.0]. (A) Flow diagram of in vitro starch hydrolyzation kinetics compartment wise with expected time frame and conditions simulated (B). Time course starch digestion from 30-180 min using *in vitro* starch hydrolyzation kinetics based on glucometry (C) histogram depicting the variations in the fractions of starch – rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and nutraceutical starch (NS). Results are expressed as mean ± SD of three independent experiments.

Based on the predicted regression model for inherent glycaemic potential *i.e.* SQM1 and easting cooking qualities *i.e.* SQM2, we selected few candidates to validate by *in vitro* starch hydrolyzation kinetics. *In vitro* methods are used as cost effective tools to evaluate the glycaemic property of foods simulating human digestive conditions and thus act as less complex, easy and effective as for screening studies. Here, we optimized an in house *in vitro* static starch hydrolyzation kinetics focussing compartment specific digestion using enzyme system suitable for cooked white rice (**Fig.4.17A**). Time course digestion based on glucometry revealed significant ($P<0.05$) variation among the selected genotypes. VLT-6, Basmati 397, Karupanel and Chimbalate basmati were selected based on comparative lower digestibility and preferred cooking qualities based on SQM1 and SQM2. Based on the digestogram performed from 0-240min, varieties showed GR ranging from 41-80% (**Fig.4.17B**). Analysis of nutritional starch fractions, revealed that Karupanel, Chimbalate Basmati, Basmati 397 and VLT6 showed 41.62, 45.14, 43.56 and 50.06% RDS respectively. In line with that, SDS % was observed to be 19, 33.59, 15.96 and 19.65% in Karupanel, Chimbalate Basmati, Basmati 397 and VLT6 respectively.

The digestion resistant RS fraction was also estimated by hydrolysing the pellet after centrifugation and following the manufacturer's protocol as per RS megazyme kit. The RS content varied from 0.14-1.42%, with an average of 1.03%. Least RS content was found in Basmati 397 while maximum was observed VLT6 (**Fig.4.17C**). Nutraceutical starch (NS) which is a combination of SDS and RS was also calculated and found to range from 16.10-34.94%. Similar to RS, least NS was observed in Basmati 397. But in contrary, maximum NS was found in Chimbalate Basmati. From the data obtained from the present study, it is clear that Chimbalate Basmati is the most suitable candidate among the analysed candidates having optimal digestibility as well as eating and cooking qualities followed by Karupanel and VLT-6.

CHAPTER 5 DISCUSSION

Globally there is an increased incidence of Diabetes mellitus (DM), a metabolic syndrome marked by high level of blood glucose. Type 1 diabetes mellitus (T1DM) is induced by a multifactorial mode of inheritance characterized by the destruction of insulin-secreting islet of Langerhans or by insulin resistance, whereby the primary insulin target organs are unresponsive to insulin action (type 2 diabetes mellitus, T2DM). This global menace has risen about 7 fold since 1950 and further expected to about 591.9 million by 2035 (Beagley et al., 2014). Even though the aetiology behind is multifactorial, long term over eating of high carbaholic diet which majorly composed of rapidly digestible starch (RDS), stimulate pancreatic insulin secretion and predisposes to T2DM. Thus the impaired tolerance with compromised glucose regulatory system by the overwhelming oxidative stress induced in pancreas is the most critical factor and hence controlling this in any means will reduce risks of diabetic complications. As there is no cure, the existing management strategies majorly focused on low glycaemic index (GI) diet to control glucose release.

White rice is the staple food for more than half of the global population to meet their 1/5th calorie requirement, but also notorious for its high glycaemic amplitude. Even though low glycaemic variants like brown rice and pigmented variants are capturing a niche elite market, unfortunately the truth is that they can't replace white rice. The glycaemic profile in turn depended on the starch quality, has initially known to be biochemically governed by the ratios of amylose and amylopectin fractions. Based on starch digestogram, three fractions have been well studied *i.e.* RDS, slowly digestible starch (SDS) and resistant starch (RS). Where RDS has found positively associated with glycaemic response (GR) and SDS and RS to be negatively associated. Despite the evidences highlight that there are many primary and secondary indices of starch quality known to affect the trajectory of digestion course of starch under *in vivo* conditions. But limited information is available on comprehensive studies probing link and inter-relationship as well as on available existing prediction tools to select varieties with low glycaemic potency. Hence the goal of the present study was to analyse various primary and secondary indices *i.e.* 14 intrinsic variables including microstructure, matrix composition (starch, amylose, amylopectin, RS), physico-chemical parameters (ASV, GC, GT), textural (hardness, stickiness) and rheological attributes (PV, BD, FV, SB) of starch digestibility to reconstitute a starch quality matrix (SQM) based on twenty rice (*Oryza sativa*. L) genotypes. Further we developed a fitted regression model for predicting RS content with required accuracy.

Among the variables, microstructure of food is a recent addition, still few reports endorsed the relationship between microstructure and *in vitro* starch digestibility. The assessment of microstructure intactness through stereo-zoom microscopy conducted revealed the morphological and structural variations especially the intactness of bran layer as well as germ. Overall degree of milling or inherent variations based on the differences in their biological origin could be the possible reasons (Ramyabai et al., 2019). The scale developed to quantify the microstructure variability based on the vertical or horizontal fissures is first of its kind to characterize such variations. The disruption of bran layers and fissures or cracks along with partial damage to the germ has been previously reported as a possible cause for rapid enzyme diffusion as well as enzymatic digestion. Such microstructural attributes also affect kernel disruption as well as result in fast enzyme hydrolysis. It has been stated by Christensen et al. (2013), Kasprzak et al. (2012) and Juntunen et al. (2013) that intact microstructure along with complex matrix encapsulating starch and protect it from enzymatic digestion. Hence we suspected a higher swelling ratio as well as lowered RS but the trend was not significant due to the narrow range observed in the current study. Bravo et al. (2019) concluded that the rougher microstructure due to fibre content could be behind the observed low digestibility (Bravo et al., 2019). Also in agreement to the previous point, confocal laser scanning microscopic studies showed that starch granule surface area of the flours beard cluster-shaped protein–non-starch polysaccharides (NSPS) or soluble fibres could be preferentially absorbing less water, preventing swelling as well as gelatinization (Schuchardt, et al., 2016). A possible correlation between the microstructure and starch digestibility was recently reported by Ramyabai et al. (2019) where, incomplete breakages in the bran layer and presence of fissures or cracks on the surface or incomplete disruption to germ was endorsed with increased gelatinization rate and associated glycaemic response. The cooking parameters like cooking time (CT) and swelling ratio (SR) indirectly correlated with the microstructural aberration variations in few studies, but the narrow range observed in the present study couldn’t substantiate that. The reports suggested that the longer time required for cooking could be due to lesser degree of milling, complex matrix involving proteins and lipids which interact with starch thus preventing absorbing and gelatinization.

Previous reports by Sood and Singh (2006) reported that SR observed to be less in basmati varieties compared to non-basmati types. In the current work, we observed similar trend in Ranbir Basmati and Basmati 5888 with SR of 1.13 and 1.15 respectively. The results are in agreement to already reported work by Ahuja et al. (1995) and Deka and Sood (2003). While interestingly Basmati 802 (CANP and ANP) were observed to have higher SR. Regarding the relationship between CT and SR, mostly negatively correlated with total amylose content (TAC) as amylose act as a diluent as well as inhibitor for swelling. But we couldn’t have observed such a negative correlation consistently in all the genotypes analyzed. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. Arisaka and Yoshi (1999) as well as Ritika et al. (2010) revealed that the degree of gelatinization, swelling power and solubility of low-amylose starch paste were higher than those of high-amylose starch pastes. A positive correlation has been deduced also between amylopectin and swelling power which clearly justifies the association observed.

Textural perception of the cooked rice is another preferential parameter which vary widely in India. Harder grains are preferred in south, fluffy long in north and sticky in north eastern states. Thus texture in terms of hardness and stickiness are determinants of not only palatability but also digestibility and associated GR. Using texture profile analyser (TPA), the hardness index was determined as the amount of force required by the cylindrical probe to compress the rice grains and determines the palatability of cooked rice (Bello et al., 2006). Based on the composition, molecular structure, matrix composition and so on, the ability to absorb, swell and disrupt vary among rice types leading to different hardness and stickiness index. Furthermore, it is well-documented that the linear amylose leached during gelatinization, are likely to contribute to the stickiness of cooked rice (Leelayuthsoontorn and Thipayarat, 2006). In the present study, VLT-6 had the lowest stickiness value of -0.08N which also correlates well with the highest TAC observed (29.82%). Vasumati had the highest stickiness value of -2.94N was low amylose phenotype (15%). Previously Juliano et al. (1981) reported similar observation in milled rice. In the same study, hardness was also positively correlated with amylose content, whereas stickiness was negatively correlated with TAC (Juliano et al., 1981). This is consistent with other reports (Cameron and Wang, 2005; Patindol et al., 2010). In contrary, Roy et al. (2010) reported that the hardness and stickiness of cooked rice depends majorly on the moisture content along with forms and variety of rice providing an acceptable texture ranges *i.e.* 66-69% (for soft rice) and about 75% moisture content (for optimally cooked rice). Amylose content and GT of rice starch are other variables positively correlated with hardness attributes of cooked rice (Mestres et al., 2011). Along with high TAC, long chain of amylopectin has shown to contribute much harder texture. Stickiness another attribute, defined as cohesion strength has been positively correlated with solubilized starch/amylose. Amylose leakage observed to be higher with increasing temperature and such high leaching levels may get concentrated enough to act as a coated film to increase stickiness (Leelayuthsoontorn and Thipayarat, 2006). The first molecular determinant for the palatability trait was reported by Li et al. (2017) where they mentioned it to be increased with decreasing amylose content in the whole grain, and, in the leachate along, with increasing total amount of amylopectin, the proportion of short amylopectin chains, and amylopectin molecular size.

Other than texture and microstructure, the major factor controlling gelatinization through preventing swelling rate is complex matrix. Starch digestion rate is directly related to starch components – amylose, amylopectin. Along with them RS fraction has routinely analyzed expecting a negative correlation with glycaemic response. Presence of higher proportion of RS, which is a prebiotic soluble fibre has known to lower the glycaemic spike as well as in improving the cholesterol metabolism and gut health. Other than RS, total starch and amylose are undoubtedly the major factors having an established absolute relation with starch digestibility. Previous reports by Pang et al.

(2018) reported a range of 9-34% while Krishnan et al. (2020) and Bao et al. (2006) reported 7-33% TAC. Comparing those, considerably narrow range was observed in the present study (10.7-29.8%). Roferos et al. 2006 also reported on milled rice where amylose content ranged between 12 to 33%. Other studies on rice by Tan and Corke, 2002 and Champagne et al. (1999) reported AAC in the range of 6.3-28.2% and 10.3-24.9%, respectively. The *sativas* as compared to the *glaberrimas* seem to have a wider range of TAC which includes waxy rice.

In the present study, the presence of branched glucans, known as amylopectin were analyzed based on classical method. Further the results were validated using specific conconavalin based extraction and estimation. Amylopectin is the predominant fraction and it varied from 70-80%, while wider range like 39.94-61.87% has reported by Krishnan et al. (2020). Significant correlations are observed between starch digestion rate and amylopectin content as well as molecular structural characteristics like branching diversity as well as chain length. The *in vitro* digestion rate tends to increase with longer amylopectin branches compared to short amylopectin branches, although the statistical analyses show that further data are needed to establish this unambiguously (Syahariza et al., 2013). Increased percentages of A–type crystalline structure and amylopectin side chains of DP 6–24 both have been shown to increase the rate of digestion (Martens et al., 2018), while B or V type contributed through compact crystalline packaging has shown to inhibit inside out digestion of starch granules. Manipulating amylopectin levels as well as its structure through engineering various branching and debranching enzymes were carried also out to alter starch digetsibility. Zhang and others (2006) reported that slow digestion properties of cereal starches are associated with high levels of short amylopectin chains. Recent report by Krishnan et al. (2020) proposed the role of debranching enzyme, pullulanase towards accumulation of RS fraction in rice highlight a novel model of RS synthesis *i.e.* “Pullulanase-amylopectin trimming model”.

Among the minor matrix components, proteins, lipids and soluble fibres have known to affect starch digestibility, however very limited studies have been done in this regard. Jenkins et al. (1987), one of the initial report that addressed the interactions between starch and proteins to have a role in affecting the rate of digestibility of starch (Jenkins et al., 1987). Ye et al. (2018) reported that a significant increase in starch digestibility in flour of native long-grain *indica* rice was observed after the matrix proteins were removed by protease treatment, which underlines the role of proteins in starch digestibility. It was suggested that endogenous proteins are attached to the surfaces of starch granules, restricting the access to the digestive enzymes resulting in a reduction of granular swelling and digestion. Starch-lipid complexes have also been indicated as a major factor contributing towards lowering the glycaemic response of cooked rice (Goddard et al., 1984; Shu et al., 2009). But in the present work as we used milled rice with very narrow range in protein variation (8-10%), hence not considered the protein content as a major deciding index affecting digestibility. Presence of lipids or lysophospholipids to form amylose-lipid complex forming RS5 has enlightened the role of matrix lipids in starch digestibility. Among the types, long-chain saturated monoglycerides were found to exhibit more resistance to enzymatic hydrolysis as compared to short-chain saturated as well as unsaturated monoglycerides when complexed with starch of cooked rice (Guraya et al., 1997). Recently, Krishnan et al. (2020) reported the role of cooking fats in modulating the inherent glycaemic potency of rice and proposed a model of lipid induced digestive resistance for the very first time. As lipids are known to present in the bran layer and in the present study polished rice were used for analysis, we have not quantified or considered the variation in lipid content towards starch digestibility. The other major factor in white rice are the soluble fibres which majorly includes, RS, arabinoxylans and beta glucans. We quantified arabinoxylans and β-glucans and found to be less than 0.5% and hence conformed RS as the major soluble fibre fraction in polished rice.

Due to immense importance and unique physical functions, RS has been investigated broadly in the past three decades and the inherent RS content reported to be limited with <4% in rice (Kumar et al., 2018; Krishnan et al., 2020). In line with that, 0.2-1.8%, was observed in the present study. A positive dependency has been expected and reported among RS and TAC as high amylose genotypes were inherently rich in RS content. In line with that Li et al. (2015) reported starch samples from the same botanic source observed to have high levels of amylose which well correlated positively with the proportion of in vitro RS measured. In contrary Kumar et al. (2018) reported that it’s decisive but not always rational to correlate RS content with glycaemic index of rice. In contrary, Krishnan et al. (2020) reported a medium dependency towards amylose and low dependency towards amylopectin content related to RS trait. These *in vitro* results were supported by several *in vivo* studies, establishing an in consistent association among TAC, RS and the blood glucose response (Granfeldt et al., 1994). This highlights the need for food matrix studies involving interaction of major and minor components towards starch bio-accessibility, digestibility and ultimate bioavailability.

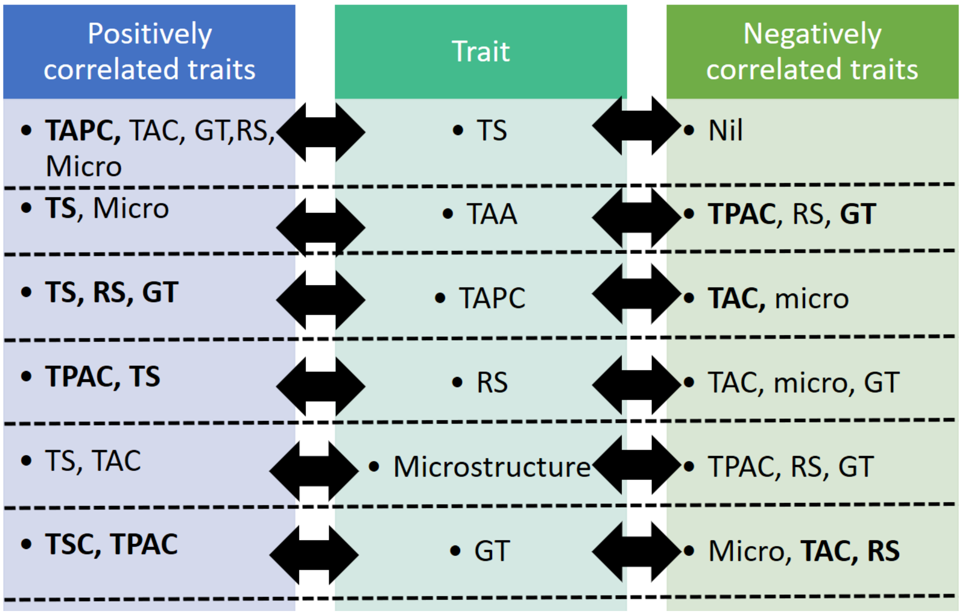
The physico-chemical attributes are the other major indices having impeccable influence on digestibility as well as ECQ which in turn depend on the components of starch consisting of amylose and amylopectin (Wang et al., 2002). The present study categorized the genotypes based on seven category scale and similar classification have been previously reported in Thai rice as well as other rice cultivars (Prathepha et al., 2005; Chemutai et al., 2016). The least affected score observed in DV85 could be attributed to the presence of more long amylopectin chains (B2 and B3) compared to the highest ASV VLT6 genotype. It’s tempting to speculate that SSIIa activity could be more in DV85 than VLT6 as it plays role in extending amylopectin chains and found prior to be more in low ASV clusters (Waters et al., 2006). Among the parameters, alkali spreading value (ASV) gives an estimatation of the gelatinization temperature on whose basis the genotypes could be categorized into low (55- 69°C), intermediate (70 - 74°C) and high (75 – 79°C) GT type. As per the genotypes analyzed in the present research, 5% were categorized as low GT, 60% as intermediate GT and 35% as high GT. A possible dependence between TAC and GT has been shown prior (Pang et al., 2018). It has also shown that GT affects the uptake of water by rice grains, their volume of expansion as well as gelatinization (Vanaja and Babu, 2003). Among the components, amylopectin had been reputed to play major role in determining the starch granule crystallinity and amylose for lowering the melting temperature of these crystalline structures (Flipse et al., 1996). The low GT phenotype observed in VLT6 having the highest amylose content (>29%) supports the concept that, higher TAC lead to less crystalline lamellar structure having lower GT. Our observation is consistent with Bhat and Riar (2016), as the cultivars with the higher mean values of amylopectin indicated higher GT and vice versa. But certain studies also suspect that more than the total content, size or chain length could be playing major role. They found that high-amylose corn starch with up to 90% amylose, long chain amylose molecules could form double helices and contribute to the better crystalline structure, concluding with high GT (conclusion temperature up to 130°C) (Jiang et al. 2010).

GC test is a quick way to assess the hardness of the starch after gelling. It has been often correlated with amylose content and confirmed the hard gelling nature is due to high amylose phenotype. In this study, rice genotypes were classified into the soft, intermediate and hard GC based on the gel migration in a horizontal tube when labelled with thymol blue. Among them 5% found hard GC, 60% with intermediate GC and 35% with soft GC. The hard GC observed in VLT-6 justifies with its high amylose content (29.82%) while the soft GC varieties ranged their TAC from 10-17%. The variations in TAC which resulted in GC difference could be as a result of the different expression levels of *GBSS* gene or variations in the locus (Wanchana et al., 2003; Tran et al., 2011). The intermediate GC observed in 60% genotypes could be due to the effects of gene interaction between synthases (*GBSS*) with branching (*BEIII*) or debranching enzymes (*pullulanase*) (He et al., 2006). Soft GC i.e. gel length of more than 61mm has observed in 30% genotypes in this study. Similar to hard GC, soft GC has also well attributed to the biallelic variability in *GBSS* gene (Wanchana et al., 2003). Classification similar to the current study has been previously reported in 63 non-waxy rice varieties (Tan and Corke, 2002; Kim and Kim, 2016).

Rheological attributes like pasting variables also act as secondary induces of starch digestibility. Starch viscosity and pasting profiles are a reflection of the physical changes occurring during the processing of starch based products and often used to analyze the eating and processability of rice (Chen et al., 2003). The viscosity changes of starch pastes during the cooking and cooling process are generally measured by a Rapid Visco Analyzer (RVA). The onset of viscosity commences when starch is heated to 65◦C (in excess of water). At near 65◦C, amylopectin crystals start dissolving (Slade and Levine, 1988) and proteins begin to hydrate (Matveev et al., 2000). As the temperature is further raised to 95◦C, it reaches the peak viscosity marked by rapid increase in viscosity due to swelling of starch and leaching out of amylose (Tsai et al., 1997).Also there is complex formation with lipids and the proteins are denatured (Fitzgerald et al., 2003). The setback viscosity, a measurement of retrogradation of starch, varied greatly among the samples. The PT observed for this study were much greater than what was reported by Bao et al. 2006 (67.5 to 81.5 °C) averaging 75 °C for some rice varieties. The PV range was 47 units narrower than earlier as reported by Tan and Corke 2002 (92 - 319 RVU), Bao et al. 2006 (90.1 - 305.4 RVU) and Roferos et al. 2006 (105 - 308 RVU). The final viscosity range observed in this study is broader than earlier reports by Tan and Corke, 2002 (99 to 365 RVU) and Bao et al., 2006 (111.4 to 412.6 RVU). Peak viscosity is a measure of the water-holding capacity of a sample, while final viscosity is the viscous paste or gel forming ability after being cooked and cooled. Hu et al. (2004) identified a high-amylose rice cultivar with low paste viscosity profiles (peak, hot paste, and cool paste) but high in RS. On the contrary, a recent study on rice starch indicated that breakdown (BD) viscosity correlated positively with RDS, negatively with SDS, and insignificantly with RS (Benmoussa et al., 2007).

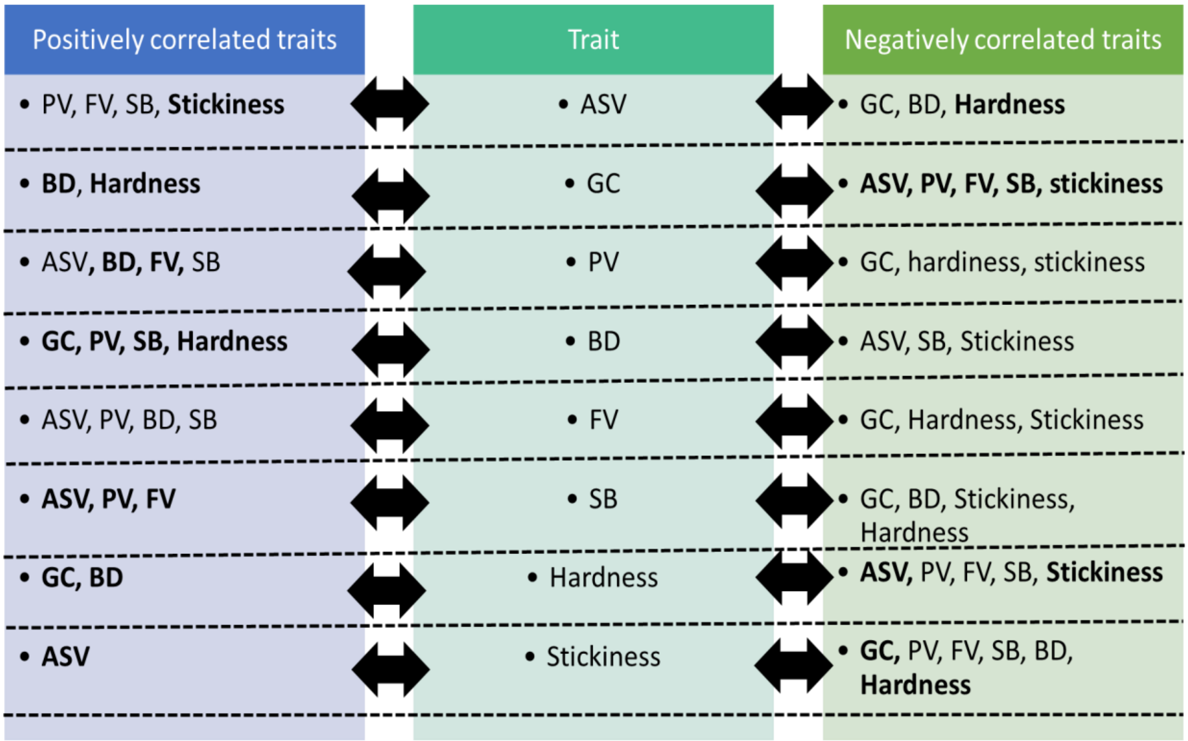
Starch digestibility is well correlated with the structural milieu, bestowed due to components, molecular order as well as other attributes. Few inter-relationship studies have been conducted on understanding the dependency among the starch quality parameters. Even though significant association found, consistency was the major bottleneck due to the wide diversity of rice germ plasm as well as the complexity associated with the inheritance in the quality parameters (Bao, 1999). The most well associated parameters like amylose, RVA profiles and GC has also found to vary between *indica* and *japonica* subspecies (Shu et al., 1999; Tang, 1981). Though some attempts were made to correlate various compositional and rheological parameters with rice starch quality using a predictive near-infrared spectroscopy (NIR) by Bao et al. (2001), but many factors remained unaddressed like RDS, SDS, RS, cooking time, microstructure and so on. Relationship of cooked-rice nutritionally important starch fractions with other physicochemical properties was performed by Patindol et al. (2010) where chemo metric tools were used to establish the association of starch fractions with physicochemical properties. Literature is lacking concerning potential associations of cooked rice nutritionally rich starch fractions with secondary rice quality indices (e.g., viscosity and pasting characteristics, thermal properties, and genetic markers). The explicit hidden mechanism of resistance of starch granules to hydrolysis is perplexing because all these factors are often inter-linked. Hence often the established relationships among these parameters are not dependable or rational. So it is necessary to test each parameter of rice starch quality that is considered important as per the objective of the genetic modification program. Being the assessment of rice starch quality parameters is a resource-intensive part in breeding, a predictive tool comprising all the possible variables affecting starch digestibility is the need of the hour. Developing such a starch quality matrix (SQM) will thus directly assist breeders to screen existing varieties as well as to develop newer lines with better starch quality. In the current study, the biochemical analyses performed revealed that discrete differences existed among the 14 parameters among the genotypes. Notably the dependence as well as inter-relationship also varied among the parameters. Shortlisting the most significant traits, 6 variables like (total starch (TS), total amylose content (TAC), total amylopectin content (TAPC), resistant starch

(RS), microstructure, gelatinization temperature (GT) were reconstituted to develop SQM1 to predict the inherent glycaemic potential. Similarly, 8 explanatory variables [gel consistency (GC), alkali spreading value (ASV), pasting viscosity (PV), break down (BD), final viscosity (FV), set back (SB)] were reconstituted for SQM2 for preferred ECQ. We propose that the regression fitted model developed in the current work could predict RS% in rice with 60-70% efficiency. We would also like to endorse that the present prediction tool developed is a virgin step in this direction and future work will be required to fine tune the model for better accuracy for widely adopting it as a prediction tool in breeding programmes focussed to improve starch quality.



Positively correlated traits	Trait	Negatively correlated traits
TAPC, TAC, GT,RS, Micro	TS	Nil
TS, Micro	TAA	**TPAC**, RS, **GT**
TS, RS, GT	TAPC	**TAC,** micro
TPAC, TS	RS	TAC, micro, GT
TS, TAC	Microstructure	TPAC, RS, GT
TSC, TPAC	GT	Micro, **TAC, RS**

Fig.5.1. Inter-relationship among the explanatory variables predicting inherent glycaemic potential derived from starch quality matrix (SQM)-1. Traits in bold shows significant correlation. (TS-Total starch, TAC-Total amylose content, TAPC-Total amylopectin content, GT-Gelatinization temperature, RS-Resistant starch)



Positively correlated traits	Trait	Negatively correlated traits
• PV, FV, SB, **Stickiness**	• ASV	• GC, BD, **Hardness**
• **BD, Hardness**	• GC	• **ASV, PV, FV, SB, stickiness**
• ASV, **BD, FV,** SB	• PV	• GC, hardiness, stickiness
• **GC, PV, SB, Hardness**	• BD	• ASV, SB, Stickiness
• ASV, PV, BD, SB	• FV	• GC, Hardness, Stickiness
• **ASV, PV, FV**	• SB	• GC, BD, Stickiness, Hardness
• **GC, BD**	• Hardness	• **ASV,** PV, FV, SB, **Stickiness**
• **ASV**	• Stickiness	• **GC,** PV, FV, SB, BD, **Hardness**

Fig.5.2. Inter-relationship among the explanatory variables predicting preferred eating and cooking quality derived from starch quality matrix (SQM)-2. Traits in bold shows significant correlation (GC-gel consistency, ASV-alkali spreading value, PV-pasting viscosity, BD-break down, FV-final viscosity, SB-set back)

Being a prediction tool, validating further with *in vitro* biochemical methods is also critical. *In vitro* digestibility assays has been used since Englyst's method as a cost effective tool to evaluate the glycaemic property of foods. Even though comparative studies have shown that it well correlate with *in vivo* data, it still couldn't reflect the *in vivo* physiology completely. But due to the high cost, complexity and ethical concerns, *in vitro* tools are better alternatives for population screening or selection. It will assist breeders as a quick effective tool in selecting cultivars with favourable digestibility profiles for further breeding programmes. Among the *in vitro* models, static models using compartment specific enzymatic cocktail simulated assays are less complex in design, fabrication and execution and hence a similar assay has been selected for the validation in the present study. The higher content of RS, SDS in turn NS observed Chimbalate Basmati could be due to better intact microstructure (score 1), matrix composition (<20 TAC, 69% TS, 49.7% TPAC) as well as intermediate physico and pasting attributes, affecting the bio-accessibility of starch for enzymatic digestion (Roman and Martinez, 2019). Thus to test the fundamental link among the reported primary and secondary explanatory variables affecting starch digestibility, the present study was conducted. The study thus also comprehended with the developed tool to assess the digestibility of varieties based on two variables – TS and TAC. The refined and fitted model will thus way pavement to breeders and researchers which actively involved in improving the starch quality especially in rice. The efficiency of this model needs to be further refined by increasing sample size and with further more robust fitting models in future, which could also be used in other food crops.

CHAPTER 6 SUMMARY & CONCLUSION

Managing the chronic hyperglycaemia and its induced complications are the global burden and staple cereals are known to have major positive association with it. India currently being a diabetic volcano, its high time for us to intensify our fight against this on our very own tables by increasing our intake of quality starch and thus lowering the post prandial glycaemic amplitude. Anent nutrition and glycaemic response (GR) characterized starch into three fractions differing quality - rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS) having positive and negative correlation to GR. As SDS and RS being found beneficial and in contrary RDS as harmful, identifying rice cultivars with higher proportion of low digestible starch [RS and SDS, in combination known as nutraceutical starch (NS)] is the need of the hour. Directed research is thus required to understand the existing variations in the inherent glycaemic potential as well as to develop further newer varieties of low glycaemic potency.

Available *in-vitro* and *in-vivo* models existing are tedious and time consuming. Evidences highlights the role of primary and secondary indices of starch quality affecting the trajectory of digestion course of starch, but most of the studies were focused on one or few traits. Limited information is thus available on comprehensive studies probing link and inter-relationship among the indices, which could reveal newer insights on starch digestibility. Starch digestibility is well correlated with the molecular structure, components, as well as other physico-chemical and pasting attributes. The explicit hidden mechanism of resistance of starch granules to hydrolysis is perplexing, because all these factors are often inter-linked. Few studies have been conducted probing link among the starch quality parameters till date and even though significant association have been found, consistency was the major bottleneck due to the wide diversity of rice germ plasm as well as the complexity associated with the inheritance in the quality parameters. Hence often, the established relationships among these parameters were not dependable or rational. So it is necessary to test each parameter of rice starch quality that is considered important as per the objective of the genetic modification program. Being the assessment of rice starch quality parameters is a resource-intensive part in breeding, a predictive tool comprising all the possible variables affecting starch digestibility is the need of the hour. Developing such a starch quality matrix (SQM) will thus directly assist breeders to screen existing varieties as well as to develop newer lines with better starch quality. Hence in the present study, we analysed 14 intrinsic variables including microstructure, matrix composition (starch, amylose, amylopectin, RS), physico-chemical parameters (ASV, GC, GT), textural (hardness, stickiness) and rheological attributes (PV, BD, FV, SB) of starch digestibility to reconstitute starch quality matrix (SQM) based on twenty rice (*Oryza sativa*. L) genotypes. Further we developed a fitted regression model for predicting RS content with required accuracy.

Earlier reports have endorsed relationship between intact bran layer microstructure and *in vitro* starch digestibility, which hinder the enzyme diffusion and digestion. The assessment of microstructure intactness through stereo-zoom microscopy conducted revealed the morphological and structural variations especially the intactness of bran layer as well as germ. The virgin step took to develop a scale to quantify the microstructure variability based on the vertical or horizontal fissures or aberrations characterized the discrete variations. A narrow range in microstructural variations were observed, couldn't substantiate as a major variable towards digestibility. Cooking parameters like cooking time (CT) and swelling ratio (SR) has indirectly correlated with the microstructural aberrations. Commonly, SR has observed to be less in basmati varieties compared to non-basmati types due to its vertical elongation behaviour. In the current work, we observed a similar trend in case of Ranbir Basmati and Basmati 5888 with low SR of 1.13 and 1.15 respectively. In contrast, Basmati 802 (CANP and ANP) were observed to have higher SR (>1.9) which could possibly due to being a landrace and its inherent variations. Regarding the relationship between CT and SR, mostly negatively correlated with total amylose content (TAC) as amylose act as a diluent as well as inhibitor for swelling. But we couldn't have observed such a negative correlation consistently in all the genotypes analyzed. But interestingly, a positive correlation was deduced between total amylopectin content (TAPC) and SR which clearly justifies the observed negative correlation. The observed inter-relationship among physico-chemical attributes and RVA variables are included in **Fig.4.18.**

Based on the composition, molecular structure, matrix composition and so on, the ability to absorb, swell and disrupt vary among rice types leading to different hardness and stickiness index. In the present study, VLT-6 had the lowest stickiness value of -0.08N which Among the genotypes, Vasumati had the highest stickiness value of -2.94N was low amylose phenotype (15%). Hardness was found positively correlated with TAC and negatively correlated to stickiness. Other than texture and microstructure, the major factor controlling gelatinization affecting digestibility is through preventing swelling rate. Starch digestion rate is directly related to starch components amylose, amylopectin and RS. We found 10-29% TAC, 40-69% TPAC and 0.2-1.86% RS variations in the analyzed genotypes. RS more than a digestion resistant fraction, is also known as the major soluble fibre in polished rice which act as a prebiotic. Other soluble fibres like arabinoxylans and β-glucans were less than 0.5% in the polished rice (data not shown). Even though contradicting dependency has been reported for RS with starch components, major reports have supported TAC as vital; while we observed a medium dependency with TAPC than TAC. This also underlines our previous observation on the role of an amylopectin debranching enzyme, pullulanase as a key determinant for RS content in rice (Krishnan et al., 2020). These *in vitro* results were also supported by several *in vivo* studies, which majorly highlights the role of food matrix complexity affecting physico-chemical attributes as well as digestibility.

The physico-chemical attributes are widely adopted as quick indices to understand starch quality and well correlated with the components of starch. Based on the international acclaimed ASV scale, least affected score was observed for DV85 among the genotypes, which could be attributed to the presence of more long amylopectin chains (B2 and B3) compared to the highest ASV VLT6 genotype. It's tempting to also speculate that SSIIa activity could be more in DV85 than VLT6 as it plays role in extending amylopectin chains and found prior to be more in low ASV clusters. Based on ASV, GT phenotype were predicted and we found 5% were of low, 60% of intermediate and 35% of high GT phenotype. The low GT phenotype observed in VLT6 having the highest amylose content (>29%) supports the concept that, higher TAC lead to less crystalline lamellar structure having lower GT. Gel consistency (GC) analysis revealed 5% having hard GC, 60% with intermediate GC and 35% with soft GC. The hard GC observed in VLT-6 justifies with its high TAC (29.82%) while the soft GC varieties ranged their TAC from 10-17%. The variations in TAC which resulted in GC difference could be as a result of the different expression levels of *GBSS* gene could be a possible reason behind the variations. The intermediate GC observed in 60% genotypes could be due to the effects of gene interaction between synthases (*GBSS*) with branching (*BEIII*) or debranching enzymes (*pullulanase*).

Rheological attributes like pasting variables also act as secondary induces of starch digestibility. The viscosity changes of starch on cooking and cooling were recorded by a Rapid Visco Analyzer (RVA) for various pasting attributes. The onset of viscosity occurred when starch was heated to 65◦C (in excess water). At near 65◦C, the amylopectin crystals began to dissolve (Slade and Levine, 1988). As the temperature was raised to 95◦C, it reached the peak viscosity (PV), marked by rapid increase in viscosity caused due to starch granules swelling and the leaching out of amylose (Tsai et al., 1997). The setback (SB) viscosity, which is the tendency of retrogradation of starch pastes, differed vastly among the samples as well as break down (BD) were also characterized. The observed inter-relationship among physico-chemical attributes and RVA variables are included in **Fig.4.19.**

In the current study, the biochemical analyses performed revealed that discrete differences existed among the 14 parameters among the genotypes. Notably the dependence as well as inter-relationship also varied among the parameters. Shortlisting the most significant traits, 6 variables like (TSC, TAC, TAPC, RS, microstructure, gelatinization temperature (GT) were reconstituted to develop SQM1 to predict the inherent glycaemic potential. Similarly, 8 explanatory variables (GC, ASV, PV, BD, FV, SB, hardness and stickiness) were reconstituted for SQM2 for preferred ECQ. We propose that the regression fitted model developed in the current work could predict RS% in rice with 60-70% efficiency. The inter-relationship was assessed and correlated which revealed certain stable associations between TAC, GT and pasting properties which might be derived from their physico-chemical properties. Further Pearson's correlation and Cramer's V statistics were used to develop starch quality matrices (SQMs) for low inherent glycaemic potential (SQM1) and for preferred eating and cooking quality (SQM2). A regression model was further developed based on most significant variables TSC and TAC. As the predictability indices were the sum of the squares of residuals, coefficient of determination and so on, further to limit outrageous prediction the model was refined and fitted using outlier analysis, Q-Q plots and Crook's distance analysis. Based on this, 4 most promising candidates (Karupanel, Chimbalate Basmati, Basmati 397 and VLT6) were shortlisted and validated further using *in vitro* starch hydrolyzation kinetics simulating human gastro-intestinal conditions. The high percentage of slowly digestible starch (SDS), RS and nutraceutical starch (NS) quantified exemplified Chimbalate Basmati as the most preferred genotype with low glycaemic amplitude. Thus our study for the first time showed a perspective relationship among 14 traits affecting starch digestibility and developed SQM based prediction tool which would assist in trials to breed high quality-low glycaemic rice varieties in future. We would also like to endorse that the present prediction tool developed is a virgin step in this direction and future work will be required to fine tune the model for better accuracy for widely adopting it as a prediction tool in breeding programmes focussed to improve starch quality.

Developing starch quality matrix to identify fibre rich Indian rice varieties with low glycaemic potential

Abstract

Rice, is a global staple food, feeding half of the world's population. Even though white rice (WR) dominates in consumption and attracting foreign exchange, it is a major contributor to the glycaemic load and associated with increasing type 2 diabetes mellitus (T2DM). Directed research is thus required to understand the existing variations in the inherent glycaemic potential as well as to develop further newer varieties of low glycaemic potency. Even though the starch digestibility is well correlated with the structural milieu, bestowed due to components, molecular order as well as other attributes, few inter-relationship studies have been conducted probing a link among the starch quality parameters. Hence the present study was carried out in twenty rice genotypes analysing their primary and secondary indices of starch digestibility (fourteen traits comprising total starch (TS), total amylose content (TAC), total amylopectin content (TAPC), resistant starch (RS), microstructure, gelatinization temperature (GT), gel consistency (GC), alkali spreading value (ASV), pasting viscosity (PV), break down (BD), final viscosity (FV), set back (SB), hardness and stickiness) for developing a predicting matrix model. The inter-relationship was assessed and correlated which revealed certain stable associations between TAC, GT and pasting properties which might be derived from their physico-chemical properties. Further Pearson's correlation and Cramer's V statistics were used to develop starch quality matrices (SQMs) for low inherent glycaemic potential (SQM1) and for preferred eating and cooking quality (SQM2). A regression model was further developed based on most significant variables TSC and TAC. As the predictability indices were the sum of the squares of residuals, coefficient of determination and so on, further to limit outrageous prediction the model was refined and fitted using outlier analysis, Q-Q plots and Crook's distance analysis. Based on this, 4 most promising candidates (Karupanel, Chimbalate Basmati, Basmati 397 and VLT6) were shortlisted and validated further using *in vitro* starch hydrolyzation kinetics simulating human gastro-intestinal conditions. The high slowly digestible starch (SDS), RS and nutraceutical starch (NS) % quantified, exemplified Chimbalate Basmati as the most preferred genotype with low glycaemic amplitude. Thus our study for the first time showed a perspective relationship among 14 traits affecting starch digestibility and developed SQM based prediction tool which would assist in trials to breed high quality-low glycaemic rice varieties in future.

कम ग्लाइसे मिक क्षमता वाले फाइबर समृद्ध भारतीय चावल किस्मों की पहचान करने के लिए स्टार्च गुणवत्ता मैट्रिक्स विकसित करना

सार

चावल, एक वैश्विक प्रधान भोजन है, जो दुनिया की आधी आबादी को खिलाता है। भले ही सफेद चावल (डब्ल्यूआर) खपत में हावी है और विदेशी मुद्रा को आकर्षित करता है, यह ग्लाइसेमिक लोड में एक प्रमुख योगदानकर्ता है और बढ़ती टाइप 2 मधुमेह मेलेटस (टी 2 डीएम) से जुड़ा हुआ है। इस प्रकार निर्देशित अनुसंधान के लिए अंतर्निहित ग्लाइसेमिक क्षमता में मौजूदा बदलावों को समझने के साथ-साथ कम ग्लाइसेमिक क्षमता की नई किस्मों को विकसित करना आवश्यक है। भले ही स्टार्च पाचनशक्ति को प्रभावित करने के लिए माइक्रोस्ट्रक्चर,मैट्रिक्स कंपोजिशन,टेक्सचर प्रोफाइल,फिजियो-केमिकल, डाइजेस्टिबिलिटी जैसे विभिन्न मापदंडों की भूमिका बताई गई हो, लेकिन व्यापक अध्ययन में अभी भी कमी है। इसलिए वर्तमान अध्ययन स्टार्च पाचनशक्ति के विभिन्न प्राथमिक और माध्यमिक सूचकांकों को शामिल करते हुए किया गया। चौदह लक्षण जिनमें कुल स्टार्च (टीएस), कुल एमाइलोज सामग्री (टीएसी),कुल एमाइलोपेक्टिन सामग्री (टीएपीसी),प्रतिरोधी स्टार्च (आरएस),माइक्रोस्ट्रक्चर, जिलेटिनाइजेशन तापमान (जीटी), जेल स्थिरता (जीसी), क्षार प्रसार मूल्य (एएसवी) ,चिपचिपाहट (पीवी), ब्रेक डाउन (बीडी), अंतिम चिपचिपाहट (एफवी), सेट बैक (एसबी), कठोरता और लसलसाहट शामिल हैं,उनका विश्लेषण बीस चावल जीनोटाइप में किया गया। अंतर-संबंध का आकलन और सहसंबद्ध किया गया जिसमें टीएसी, जीटी और चिपकाने वाले गुणों के बीच कुछ स्थिर संघों का पता चला जो उनके भौतिक-रासायनिक गुणों से प्राप्त हो सकते हैं। इसके अलावा पीयरसन के सहसंबंध और क्रैमर के वी आँकड़ों का उपयोग कम गुणवत्ता वाले ग्लाइसेमिक क्षमता (SQM1) के लिए और पसंदीदा खाने और खाना पकाने की गुणवत्ता (SQM2) के लिए स्टार्च क्वालिटी मैट्रिसेस (SQMs) को विकसित करने के लिए किया गया। एक प्रतिगमन मॉडल को सबसे महत्वपूर्ण चर टीएससी और टीएसी के आधार पर आगे विकसित किया गया । जैसा कि पूर्वानुमान सूचकांकों में अवशिष्टों के वर्गों का योग था, निर्धारण का गुणांक और इसी तरह, अपमानजनक भविष्यवाणी को सीमित करने के लिए मॉडल को परिष्कृत किया गया और बाहरी विश्लेषण, क्यू-क्यू प्लॉट्स और क्रुक के दूरी विश्लेषण का उपयोग करके फिट किया गया। इसके आधार पर, 4 सबसे होनहार उम्मीदवारों (कारुपानेल, चिम्बलेट बासमती, बासमती 397 और वीएलटी 6) को कृत्रिम परिवेशीय (*in vitro*) स्टार्च हाइड्रोलाइजेशन कैनेटीक्स में मानव गैस्ट्रो-आंत्र की स्थिति का अनुकरण करते हुए आगे सूचीबद्ध किया गया। उच्च धीरे-धीरे सुपाच्य स्टार्च (एसडीएस), आरएस और न्यूट्रास्युटिकल स्टार्च (एनएस) की % मात्रा निर्धारित की गई , कम ग्लाइसेमिक आयाम के साथ सबसे पसंदीदा जीनोटाइप के रूप में चिम्बलेट बासमती का उदाहरण दिया गया है। इस प्रकार पहली बार किए गए हमारे अध्ययन में स्टार्च पाचनशक्ति को प्रभावित करने वाले 14 लक्षणों के बीच एक परिप्रेक्ष्य संबंध दिखाया गया है और SQM आधारित पूर्व-सूचना साधन विकसित किया गया है जो भविष्य में उच्च गुणवत्ता वाले कम ग्लाइसेमिक चावल किस्मों को प्रजनन करने के लिए परीक्षणों में मदद करेगा।

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