CHAPTER III

Nutritional, physico-chemical and total antioxidant properties of slowly digestible noodles.
3.1. INTRODUCTION

Starch is the commonest storage carbohydrate in plants and also the largest source of carbohydrates in human food (Jaspreet singh et al., 2010). Starch consists of two types of molecules: amylase (linear polymer of α-D-glucose units linked by α-1, 4 glycosidic linkages) and amylopectin (branched polymer of α-D-glucose units linked by α-1, 4 and α-1, 6 glycosidic linkages). Starch and starchy food products can be classified according to their digestibility, which is generally characterized by the rate and the duration of the glycemic response. Predicting and controlling the glucose absorption due to ingestion of starchy food is of great interest in the context of worldwide health concerns. Most starches contain a portion that digests rapidly (rapidly digesting starch), a portion that digests slowly (slowly digesting starch) and a portion that is resistant to digestion (resistant starch) (Englyst et al., 1999).

Factors such as starch granule morphology, amylose to amylopectin ratio, molecular structure, degree of branching in terms of steric hindrance and consequently mass transfer resistance and their effects on the digestibility and absorption of digested carbohydrates. The physical state of the starch ingested has a major impact on the digestibility therefore effects the processing techniques (thermal processing, extrusion cooking, autoclaving, etc.,) and starch modification.

The other constituents of the food matrix, such as proteins, lipids and polysaccharides, play a significant role during processing which affects the physic-chemical characteristics of digesta and the final digestibility of starch.

Hence, the physico-chemical, viscosity profile and morphological characteristics of starch, the presence of other food components, such as
proteins, fat and non-starch polysaccharides and the changes and interactions occurring during noodle processing and their affect in the enzymatic digestibility of starch, has been studied.

3.2 MATERIALS AND METHODS

3.2.1. Materials

Red-pigmented paddy variety, Jyothi and non-pigmented variety, IR-64 was procured from the Agriculture Produce Market committee (APMC), Bandipalya, Mysore, Karnataka, India. These two paddy varieties were cleaned and stored at 4–6°C until use. Whole chick pea and whole fenugreek seeds were procured from local market. Food grade - xanthan gum and guar gum was procured from Hi-Media, Mumbai. The paddy varieties were de-hulled, the husked (pigmented and non-pigmented) rice, whole Bengal gram and fenugreek seeds were pulverized in a mixer (Johnson Lady Bird plus) and the flour was passed through 60-mesh sieve (250 microns).

3.2.1a. Chemicals

The Folin–Ciocalteu reagent, gallic acid, catechin, pancreatic a-amylase, p-nitrophenyl-a-D-glucopyranoside, 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and ferrozine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

3.2.2. Methods

3.2.2.1. Proximate Composition

Moisture content of the samples were estimated as per the method (AOAC, 2000), the micro Kjeldhal method was employed to determine the total nitrogen and the crude protein was calculated (N x 5.95) (AOAC,
Fat was extracted with petroleum ether (60–80°C) for 12–16 hrs using a Soxhlet apparatus and ash contents (gravimetrically) were determined based on methods outlined in (AOAC, 2000). The total carbohydrate was calculated by difference method.

**3.2.2.2. Determination of Soluble/Insoluble Dietary Fiber**

All samples of raw and parboiled (husked rice and milled rice) were pulverized in a mixer (Johnson Lady Bird plus), and the flour was passed through 60-mesh sieve (250 microns). The flour was gravimetrically defatted prior to fiber analysis by extracting with petroleum ether (60–80°C) for 12–16 hrs using a Soxhlet apparatus (AOAC, 2000). Total dietary fiber was analyzed as per (Asp et al., 1983) with slight modifications. Reagents used were 0.1 mol/L sodium phosphate buffer, pH 6.0; 4 mol HCl/L; 4 mol/L NaOH; 95% of ethanol (95 mL of ethanol + 5 mL of water) and 78% of ethanol (78 mL of ethanol + 22 mL of water). The procedure in brief is as follows:

About one gram of defatted sample was suspended in 25 mL of 0.1 mol/L sodium phosphate buffer (pH 6.0) and treated with 100 mL of α-amylase at boiling water bath temperature for 15 min, and cooled to room temperature. About 20 mL of distilled water was added and the pH was adjusted to 1.5 with 4 mol HCl/L. About 100 mg of pepsin was added and incubated at 40°C in a water bath with agitation for 60 min. Twenty milliliters of distilled water was added and the pH was adjusted to 6.8 with 4 mol/L NaOH. The electrode was rinsed with a few milliliters of water. About 100 mg of pancreatin was added and incubated at 40°C in a water bath with agitation for 60 minutes. The pH was adjusted to 4.5 with 4 mol HCl/L. The contents
were filtered through a dried and weighed crucible (porosity 2) containing 0.5 g of dry Celite as the filter aid.

(a) Residue (insoluble Fiber): Washed with 2 – 10 mL of distilled water and again washed with 2 – 10 mL of 95% of ethanol (95 mL of ethanol + 5 mL of water), it was dried at 105°C to constant weight (or overnight). After cooling it in desiccators (D1), the residue was incinerated at 550°C for at least 5 h. After cooling in a desiccator (I1) it was weighed again.

(b) Filtrate (soluble Fiber): The volume of the filtrate was adjusted to 100 mL. Four hundred millilitres of warm (60°C) 95 g/100 g of ethanol was added. It was allowed to precipitate for 1h (time could be shortened). It was then filtered through a dried and weighed crucible (porosity 2) containing 0.5 g of dry Celite. Washed with 2 x 10 mL of 78% of ethanol (78 mL of ethanol + 22 mL of water), 2x10 mL of 95% of ethanol (95mL of ethanol + 5 mL of water). It was dried at 105° C overnight, weighed after cooling in a desiccator (D2), incinerated at 550°C for at least 5 h and then weighed after cooling in a desiccator (I2).

Blank: Insoluble and soluble blank values were obtained by running the procedure without sample (B1 and B2). The blank values were checked occasionally.

Calculation:

Insoluble dietary fiber (g/100g) = \( \frac{D_1-I_1-B_1}{W} \times 100 \)

Soluble dietary fiber (g/100g) = \( \frac{D_2-I_2-B_2}{W} \times 100 \)

Where, \( W \) = sample weight (g), \( D \) = weight after drying (g), \( I \) = weight after Incineration (g) and \( B \) = weight of ash free blank (g).
3.2.2.3. Amylose Equivalent (AE) Estimation

Sample preparation: Prepared noodles (dry) were pulverized in a mixer (Johnson Lady Bird plus), and the flour was then sieved (60–mesh). De-fatting of samples was carried out with 85% methanol using a Soxhlet apparatus for 18–24 hrs prior to amylose estimation. Amylose was estimated according to the procedure (Singh et al., 2000).

Soluble Amylose: Briefly, ~100 mg (db) of sample was suspended in 20 mL of distilled water in a 100 mL conical flask and heated in boiling water for 20 min with occasional stirring. The sample was cooled to room temperature, transferred to a 100 mL volumetric flask, washed, transferred and finally made up to volume with water and filtered using Whatman filter paper. The filtrate was collected after discarding about 10 mL filtrate. Five millilitres of this filtrate was taken, ~50 mL of water, 0.5 mL of 1 N acetic acid were added and the whole contents were shaken; 2 mL of 0.2% iodine in 2% potassium iodide solution was added, made up to volume with water and kept at room temperature for 20 min. Colour was read at 620 nm as mentioned above, with a blank.

A standard graph was prepared by using different proportions of standard amylose and waxy rice starch, 0% amylose and 100% waxy starch (amylopectin), similarly different proportions and finally 100% amylose and 0% waxy starch (amylopectin), wetted with 1 mL of alcohol followed by 9 mL of alkali and following the same procedure as above for developing the colour. A standard graph was drawn with absorbance on the y–axis and amylose content on the x–axis. A regression equation was
prepared for estimating total and soluble amylose content in the unknown sample.

3.2.2.4. Visco-Amylography Analysis

The gelatinization temperature and viscosity of the native flours and noodle flour were measured in a Micro Visco- amylograph (Model No. 803202, Brabender, Duisburg, Germany) as per the method described by (Itagi and Singh, 2010). The samples were pulverized in a mixer (Johnson Lady Bird plus), and the flour was passed through 60-mesh sieve (microns). The flour samples were converted to 13% slurry. The slurry was then heated from 30 to 92°C at the rate of 7°C/min and held at 92°C for 5 min followed by cooling to 50°C with 1 min holding. The pasting curves obtained were compared and the pasting parameters *viz.* paste viscosity or PV (maximum viscosity during heating phase), hot paste viscosity or HPV (minimum viscosity at 92°C), CPV (final viscosity at 50°C), break down or BD (PV-HPV) and total set back or SBt (CPV-HPV) were recorded. All the viscosity parameters were expressed as BU (Brabender unit).

3.2.2.5. Scanning Electron Microscopy (SEM)

Scanning electron micrographs of native flours and noodles were obtained using LEO 435 VP (LEO Electron Microscopy Ltd. Cambridge, UK) at 20 kV. A double-sided conducting adhesive tape pasted on to a metallic stub was used to mount the samples. Samples were gold-coated and scanned under different magnifications to get clear pattern and used for comparison.
3.2.2.6. Extraction and quantification of total phenolic contents:

Total phenolics from native flours and noodles (PN and NPN) were extracted with 80% aqueous methanol containing 1% HCl (1:50, w/v in a boiling water bath for 30 min. Extracts were centrifuged at 4000 rpm for 10 minutes to collect the supernatants. The procedure was repeated twice, and combined extracts were dried under vacuum in a rotary flash evaporator. The dried extracts were re-dissolved in 80% methanol and used for determining the total phenolic content (TPC) and antioxidant activity.

3.2.2.7. Total phenolic content

Total phenolic content of phenolic extracts was determined spectrophotometrically at 720 nm using Foline-Ciocalteu’s reagent (Singleton et al., 1999). Gallic acid was used as a standard and results were expressed as gallic acid equivalents (mg GAE/100g of sample on wet mass basis).

3.2.2.8. Antioxidant properties:

3.2.2.8a. DPPH free radical scavenging activity:

Free radical scavenging capacities of the samples were determined by the reaction with the 2, 2′- diphenyl–1–picrylhydrazyl (DPPH) radical, according to the method adapted from (Brand-Williams et al., 1995). Briefly, an aliquot of samples (0.1 mL, 10–100 µg/mL) were mixed with 2.9 mL of a methanol solution of DPPH (0.004%). The mixture was shaken vigorously and left to stand at room temperature for 60 min in the dark. The absorbance was measured at 517 nm using Shimadzu UV–1601 spectrophotometer (Shimadzu, Kyoto, Japan) against the blank (mixture without extract). The DPPH radical scavenging activity of extracts was
calculated from the standard curve of trolox and expressed as micromoles of trolox equivalents (TE) per gram of flour. The IC\textsubscript{50} value (µg/mL) was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial amount of DPPH under the experimental conditions given. It was obtained by interpolation from linear regression analysis. Catechin was used as positive controls at 100 µg/mL concentration.

**3.2.2.8b. Ferric Reducing antioxidant power (FRAP):**

Reducing power of phenolic extracts was measured by the direct reduction of Fe\textsuperscript{3+}(CN)\textsubscript{6} to Fe\textsuperscript{2+}(CN)\textsubscript{6} and was determined by measuring absorbance resulting from the formation of the Perl’s Prussian Blue complex followed the addition of excess ferric ions (Fe\textsuperscript{3+}). The ferric reducing antioxidant power method of (Oyaizu, 1986) with slight modification was used to measure the reducing capacity of phenolic extracts. Different concentrations of extracts (5–100 µg/mL in 50% methanol) were mixed with 1.25 mL of 0.2 M, pH 6.6 sodium phosphate buffer and 1.25 mL of potassium ferricyanide [K\textsubscript{3}Fe(CN)\textsubscript{6}] (1%). The mixture was incubated at 50°C for 20 min. After 20 min of incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%), which was then centrifuged at 4000 rpm for 10 min. Finally, supernatant was mixed with 0.5 mL of FeCl\textsubscript{3} (0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Blanks were run in parallel with their absorbance values subtracted from those of the samples. Ascorbic acid and catechin were used as positive controls. The FRAP of extracts was determined as Ascorbic acid equivalent.
3.3. RESULTS AND DISCUSSION

3.3.1. Proximate Composition

The proximate compositions of the pigmented and non-pigmented noodles are presented in (Table 7). The moisture content in both the noodles ranged from 5.56 to 6.40%. The carbohydrate content of the pigmented noodles was ~68% and ~70% in non-pigmented noodles. The ash content (~4%) was high in both the types of noodles.

Both types of noodles (PN and NPN) had similar high proportion of protein (~17%) probably the combination of rice (pigmented/non-pigmented) and chickpea improved the levels of quality protein in the noodles. When cereals and legumes are combined, the quality scores of the combined protein may be much higher than each of the individual values (Hegarty, 1995). Grain legumes are valuable sources of protein (18-25%). Several studies have been published on the in vitro and in vivo digestion of starch in legumes and the resistant starch (RS) formation during cooking and storage of legume-based food products (Bravo et al., 1998; 1999; Hoover and Zhou, 2003; Jenkins et al., 1982; Osorio-Diaz et al., 2005; Tovar et al., 1992a; Tovar and Melito, 1996; Velasco et al., 1997). The high RS content in legumes explains, at least partly, why the starch digestion rate and therefore the release of glucose into the blood stream are slower after the ingestion of legumes, resulting in reduced glycemic and insulminemic postprandial responses. Hence usage of chickpea in the formulation, as a good source of protein and presence of resistant starch, may lead to slow release of glucose.

The fat content in both types of noodles were ~3.5%, may be contributed from the whole grains used in the formulation. The fat content in
the formulation may also helps in lowering the rate of starch digestion, as one of the factors in slow digestion is lipid-starch complexes. Complexes between fatty acids such as lauric acid and amylase can form rapidly under physiological conditions which contribute to the formation of resistant starch (Seligman et al., 1998). The formation of such complexes with lipids may result in significant changes in the behaviour of the starch, including decreased solubility, increased gelatinization temperature and delayed retrogradation and resistance towards the action of digestive enzymes.

Enzymatic resistance of the pure amylase and lipid complexes has also been reported in the literature (Gelders et al., 2005; Holm et al., 1983). With the help of in vitro and in vivo digestibility studies on amylase-lipid complexes, (Holm et al., 1993) observed that, complexed amylase is hydrolysed and absorbed in the gastrointestinal tract to the same extent as free amylase but at a somewhat slower rate.

Dietary fiber in these preparations is important due to its functional effects in the gut, for instance, viscous fiber-containing foods may elicit low postprandial glycemic response due to delayed glucose absorption (Bravo et al., 1999; Jenkins et al., 1982; Tovar et al., 1992b).

Insoluble fiber has been reported to be effective in the glycemic control in dogs with insulin-dependent diabetes mellitus (Kimmel et al., 2000). Insoluble fiber of pre-germinated brown rice has been reported to lower both postprandial blood glucose and insulinemic responses in normal Wistar rats (Seki et al., 2005). Fiber concentrates of rice bran were effective in lowering serum glucose and cholesterol in both type 1 and 2 diabetics (Qureshi et al., 2002). It is likely that the beneficial effect of fenugreek seed
mucilage is due to some of the bioactive compound present in the mucilage, known to facilitate insulin secretion (Sauvaire et al., 1998). Dietary fiber present in the noodles (PN and NPN) is mostly insoluble in nature (~15-17%) and hence the anti-hyperglycemic action of the PN and NPN in the present study could also have been contributed by dietary fiber present in the noodles.

Table 7. Proximate composition of pigmented and non-pigmented noodles.

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>Noodles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pigmented</td>
</tr>
<tr>
<td>Moisture</td>
<td>6.40 ± 0.03^a</td>
</tr>
<tr>
<td>Carbohydrate*</td>
<td>68.34±0.51^b</td>
</tr>
<tr>
<td>Protein</td>
<td>17.47 ± 0.28^a</td>
</tr>
<tr>
<td>Fat</td>
<td>3.48 ± 0.18^a</td>
</tr>
<tr>
<td>Ash</td>
<td>4.32 ± 0.02^b</td>
</tr>
<tr>
<td>Total Dietary fiber</td>
<td>22.82 ± 0.28^a</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>17.03 ± 0.33^a</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>5.80 ± 0.62^b</td>
</tr>
</tbody>
</table>

* Carbohydrate is by difference method.
Values are mean ± standard deviation of three determinations (n=3).
Values within the same column with different letters are significantly different at p< 0.05.
3.3.2. Amylose Equivalent (AE) Estimation

The amylose content of the starch granules varies with the botanical source of the starch which may be attributed to differences in the activities of enzymes involved in the biosynthesis of linear and branched components. Total amylose content (Table 8) in both the types of noodles was around 28 to 29%. More than 14% insoluble amylose content was observed in both the types of noodles.

Amylose content of the starches varies from 14 to 31% for normal genotypes. Raw starches high in amlopectin have been shown to digest more quickly than those high in amylose. Legumes contain 30 to 40% amylase and 60 to 70% amlopectin in their starch granules, while most other food starches contain 25 to 30% amylase and 70 to 75% amlopectin (Hoover and Zhou, 2003). Amylopectin is a much larger molecule than amylase with average molecular weight at $10^5$ and $10^6$, whereas the average molecular weight for amylase is $10^4$. Therefore, amylopectin has a much larger surface area per molecule than amylase, which makes it a preferable substrate for amylolytic attack. Indeed, raw legume starch with high amylase has been shown to be less digestible than corn starch in rats and the rate of hydrolysis of legumes starch in vitro is less than that of corn starch (Thorne et al., 1983).

Furthermore, the glucose chains of amylose starch are more bound to each other by hydrogen bonds making them less available for amylolytic attack than amlopectin which has many branched chains of glucose. For these reasons the nature of the starch in carbohydrate foods is important to consider the expected glycemic response.
Higher amylose content as observed in both types of noodles may contribute to lower the starch digestion because a positive correlation exists between amylose content and resistant starch formation.

Table 8. Amylose content of pigmented and non-pigmented noodles.

<table>
<thead>
<tr>
<th>Noodles</th>
<th>Total amylose (%)</th>
<th>Soluble amylose (%)</th>
<th>Insoluble amylose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigmented</td>
<td>28.59±0.88^a</td>
<td>13.85±0.52^a</td>
<td>14.74±1.40^a</td>
</tr>
<tr>
<td>Non-pigmented</td>
<td>27.70±0.06^a</td>
<td>12.59±0.18^b</td>
<td>15.11±0.25^a</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three determinations (n=3). Values within the same column with different letters are significantly different at p< 0.05.
3.3.3. Viscosity behaviour of native flours and noodles

The pasting profiles of the native flours and noodles are presented in (Table 9). Gelatinization temperature (GT), a physical property of starch, is the temperature at which 90% of starch granules swell irreversibly in hot water with loss of crystalline structure and birefringence. The GT was highest in whole chickpea flour (~70°C) followed by pigmented (~68°C) and non-pigmented (~67°C) raw husked rice. The higher GT for the whole chickpea might be largely due to the reduced swelling ability of starch granules.

The peak viscosity (PV), indicating the amount of swelling of starch granules was apparently lower in whole chickpea (~210 BU). But there was markedly higher PV observed in non-pigmented raw husked rice (~562 BU) followed by pigmented rice (~394 BU). This data indicates that the starch granules in whole chickpea swells less compared to that of rice (pigmented/non-pigmented). Thus, the greater extent of retrogradation in the legume-chickpea in the formulation might help to explain its low and delayed peak viscosity, reflecting greater resistance to breakdown.

Hot paste viscosity (HPV) is the viscosity where, on heating the maximum swollen starch granules do not have space to swell more in the given volume, but the swollen granules bombard each other and break down and hence viscosity decreases from peak viscosity. HPV was high in non-pigmented raw husked rice (407 BU) followed by pigmented raw husked rice (320 BU), indicating the phenomenon that these rice (pigmented/non-pigmented) behave like cross linked starches. The low HPV was observed in whole chickpea (~187 BU).
Cold paste viscosity (CPV) was high in non-pigmented raw husked rice (~830 BU), indicating the non-coloured rice starch swells to a greater extent while cooling the sol formed during heating phase. In the case of raw pigmented husked rice the CPV was (~699 BU). But, the cold paste viscosity decreased in whole chickpea ~254 BU.

Starch breakdown (BD) differed significantly (p<0.05) among native flours. BD in non-pigmented rice (~155 BU) was high compared to pigmented rice (~74 BU) and the extent was quite less in whole chickpea (~24 BU). Breakdown viscosity measures the tendency of swollen starch granules to rupture when held at high temperature and continuous shearing and is indicative of the stability of the starch granules on heating (Patindol et al., 2005).

The set back (SB) values are indicative of the retrogradation tendency of starch. Since the initial gel network development is dominated by amylose gelation (Miles et al., 1985), set back is more likely related to the retrogradation tendency of amylose. The SB values were higher in the native rice flours, than in chickpea. SB increased in all the native flour samples (Fig 9).

During the processing of noodles, it undergoes several hydrothermal stages, hence the values of all the parameters of viscography will be less (Fig 10). Gelatinization temperature decreases, as seen from the respective viscograms (Fig 9). Even whole system behaves like a cross linked starch, as there was no break down. Very less precipitation of linear molecules takes place, indicating less degree of retrogradation. Finally, the noodles behaved like highly cross linked starch.
Further more, the viscosity of noodles containing gums can be correlated with the study carried out by (Jaspreet Singh et al., 2010), stating that the postprandial effect of guar gum in the product as a processing ingredient, may contribute to increase the viscosity of digesta within the gastrointestinal tract due to the enlargement of fully hydrated galactomannan chains. This whole phenomenon reduces the rate of digestion and absorption of carbohydrates and therefore lowers the postprandial rise in blood glucose.
Fig 9 (A-C). Viscogram of Native Flours

(A). Jyothi, pigmented husked rice (raw)

(B). IR-64, non-pigmented husked rice (raw)

(C). Whole chickpea (raw)
Fig 10. Viscogram of noodles (pigmented & non-pigmented).
Table 9. Pasting properties of native flours and noodles.

<table>
<thead>
<tr>
<th>Samples</th>
<th>GT</th>
<th>PV</th>
<th>HPV</th>
<th>CPV</th>
<th>BD</th>
<th>SB</th>
<th>SBt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jyothi, pigmented husked rice (raw)</td>
<td>68.45±0.21</td>
<td>394±1.41</td>
<td>320±1.41</td>
<td>699±21.21</td>
<td>74±0.0</td>
<td>305±19.80</td>
<td>379±19.8</td>
</tr>
<tr>
<td>IR-64, non-pigmented husked rice (raw)</td>
<td>67.45±0.21</td>
<td>561.5±71.42</td>
<td>407±33.94</td>
<td>829.5±27.58</td>
<td>154.5±37.48</td>
<td>268±43.84</td>
<td>422.5±6.36</td>
</tr>
<tr>
<td>Whole chickpea (raw)</td>
<td>69.5±0.28</td>
<td>210.5±26.16</td>
<td>186.5±7.78</td>
<td>253.5±4.95</td>
<td>24±18.38</td>
<td>43±21.21</td>
<td>67±2.83</td>
</tr>
<tr>
<td>Pigmented noodles</td>
<td>57.75±0.21</td>
<td>100.5±2.12</td>
<td>100.5±2.12</td>
<td>184.5±2.12</td>
<td>0</td>
<td>0</td>
<td>84±0</td>
</tr>
<tr>
<td>Non-pigmented noodles</td>
<td>56.85±0.07</td>
<td>124.5±7.78</td>
<td>123.5±9.19</td>
<td>220±7.07</td>
<td>1±1.41</td>
<td>95.5±0.71</td>
<td>96.5±2.12</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three determinations (n=3).
Values within the same column with different letters are significantly different at \( p < 0.05 \).

3.3.4. Scanning Electron Microscopy (SEM) of native flours and noodles.

The morphological characteristics of starches from various botanical sources vary with the genotype. The variation in the morphology such as size and shape of starch granules is attributed to the biological origin (Singh et al., 2006; Singh et al., 2007). Firstly, Langworthy and Deuel (1992) reported an apparent direct negative relationship between large size granules and starch digestibility. Many studies were carried out afterwards to validate this relationship. Lindeboom et al., (2004) reported that the small barley and wheat starch granules hydrolyze faster than the large granules. Kaur et al., (2007) observed that significant differences exist among the enzymatic hydrolysis values for different native potato starches and their separated small, medium and large fractions. Among native starches, potato starch with a higher percentage of small granules showed a higher hydrolysis rate than those containing large or medium size granules. The separated large granule fraction showed a lower hydrolysis rate than medium and small granule fractions. The lower susceptibility of large granule starches to enzymatic hydrolysis has been suggested to be due to their smaller granule specific surface area, which may decrease the extent of enzyme binding and ultimately result in less hydrolysis than small granules (Tester et al., 2006). The surface characteristics of the starch granules have been observed to affect their enzymatic digestion. A comprehensive account of the enzymatic digestibility of native uncooked starches from different sources has been reported by (Dreher et al., 1984). There is a higher digestibility for the cereal starches compared to tuber and legume starches. This may be attributed to the presence of numerous pinholes on the surface layer and pores that
penetrates towards the interior of the granules from cereal sources, such as maize. The pores in the granule facilitate the entry of the amylases for digestion. The presence of some non-starchy substances such as proteins over the granule surface may also limit the rate of enzymatic hydrolysis. Granule surface proteins and lipids can reduce surface accessibility by blocking the adsorption sites and therefore influences enzyme binding (Oates, 1997).

From Fig 11(A & B), we can see that the individual granules of rice starch appears to develop into compact spherical bundles or clusters, known as compound granules, which fill most of the central space within the endosperm cells. Whereas in case of whole chickpea, there is a visibility of separated large granule fraction as shown in Fig 12, which are covered by fibrous portions of seed coat. Even granules are seen with different sizes and shapes, fibrous lumps are covered with cell wall materials. The inherited property of chickpea, which contains large granule fraction leads to lower the hydrolysis rate, as mentioned above.

There is a formation of numerous pin holes on the surface layer of food matrix-noodles (Fig 13 & 14), and pores which penetrates the entry of the amylases for digestion. There is a formation of solid mass along with fibrous particles, after undergoing various steps of processing. The mass also informs the strong and coherent mixing and formation of uniform mass during the processing steps. The presence of some non-starchy substances such as proteins over the granule surface is visible which may also limit the rate of enzymatic hydrolysis.
Finally, the compact structure of noodles, the encapsulation of starch by proteins and the physical structure of starch are mainly responsible for the reduced enzymic susceptibility of starch in cooked noodles.

**Fig 11 (A & B).** Scanning Electron Micrographs (SEM) of pigmented and non-pigmented husked rice (raw)
Fig 12. Scanning Electron Micrographs (SEM) of whole chick pea (raw)
(X100; scale bar = 100µm)  (X5000; scale bar = 3µm)

(X150; scale bar = 100µm)  (X5000; scale bar = 3µm)

Fig 13. Scanning Electron Micrographs (SEM) of pigmented noodles.
**Fig 14.** Scanning Electron Micrographs (SEM) of non-pigmented noodles.
3.3.5. Total Phenolic Content

Phenolic compounds, which are predominant plant secondary metabolites, are known to act as antioxidants. Standard curve used for the determination of TPC is shown (Fig 15). The contents of phenolic compounds (mg/100g) in native flours and noodles are presented in (Table 10). The total phenolic compounds in raw pigmented husked rice (Jyothi), raw non-pigmented husked rice (IR-64) and whole chickpea flours were found to be 173.9, 68.4 and 300.8 mg GAE/100 g respectively. However, higher TPC was observed in noodles prepared from Jyothi-chickpea (417.9 mg GAE/100 g) and IR-64- chickpea (460.7 mg GAE/100 g) combinations. Recently, (Prabhu and Jayadeep, 2015) reported 278 and 502.5 mg FA/ 100 g in brans of IR-64 and Jyothi rice varieties, respectively. TPC in raw red-pigmented husked rice is ~3 times higher than the non-pigmented raw husked rice and is composed largely by soluble phenolic compounds, represented by proanthocyanidin and anthocyanin. Heath promoting functional food-ingredients and additives used in the preparation of noodles may have contributed to the higher TPC in noodles.
Fig 15. Standard curve of Gallic acid.

\[ y = 0.0294x - 0.0012 \]

\[ R^2 = 0.9721 \]
Table 10. Total phenolic content of native flours and noodles.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic Content (mg GAE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jyothi, pigmented husked rice (raw)</td>
<td>173.9±29.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>IR-64, non-pigmented husked rice (raw)</td>
<td>68.4±14.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whole chick pea (raw)</td>
<td>300.8±8.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pigmented noodles</td>
<td>417.9±16.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Non-pigmented noodles</td>
<td>460.7±26.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three determinations (n=3). Values within the same column with different letters are significantly different at p< 0.05.
3.3.6. Antioxidant properties

The evaluation of the total antioxidant capacity of foods is getting more importance, since it has been found that, phenolic compounds are one of the most effective antioxidants. The concept of the total antioxidant capacity, which describes the ability of different food antioxidants in scavenging preformed free radicals, has been suggested as a tool for investigating the health effects of antioxidant-rich foods.

3.3.6a. DPPH radical scavenging capacity

The DPPH assay has been widely used to test the free radical scavenging ability of various natural products because it is the simplest method that measures the ability of antioxidants to intercept free radicals. The DPPH radical scavenging capacity of extracts from native flours and noodles were expressed as trolox equivalents (TE) by using standard curve shown in (Fig 16). Noodles prepared from Jyothi-chickpea and IR-64- chickpea combinations were (0.75 and 0.79 µmol/g) respectively. Noodles showed higher TE than the native flours (Table 11). Non-pigmented raw husked rice (IR-64) showed least TE values (0.19 µmol/g). In addition, all the extracts showed concentration dependent radical scavenging activity, the activity increased as the concentration increased for each extract (Fig 17).

IC$_{50}$ values for the DPPH radical scavenging activity is shown in (Table 11). Strong radical scavenging activity was observed in red-pigmented raw husked rice (IC$_{50}$, 26.5 µg/mL), noodles prepared from Jyothi-chickpea (IC$_{50}$, 18.7 µg/mL) and IR-64- chickpea (IC$_{50}$, 23.61 µg/mL) combinations. IC$_{50}$ values of non-pigmented raw husked rice and chickpea were 76.98 and 56.4 µg/mL, respectively.
Fig 16. Standard curve of Trolox.

y = -0.0408x + 1.0286
R² = 0.9752
Fig 17. DPPH radical scavenging activity of native flours and noodles.

The data points are the means ±SD from three experiments.
Table 11. Trolox equivalent and IC₅₀ values of native flours and noodles.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Trolox equivalent (µmol/g sample)</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jyothi, pigmented husked rice (raw)</td>
<td>0.56</td>
<td>26.52±2.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IR-64, non-pigmented husked rice (raw)</td>
<td>0.19</td>
<td>76.98±2.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whole chick pea (raw)</td>
<td>0.53</td>
<td>56.46±1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pigmented noodles</td>
<td>0.75</td>
<td>18.71±1.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Non-pigmented noodles</td>
<td>0.79</td>
<td>23.61±1.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three determinations (n=3). Values within the same column with different letters are significantly different at p< 0.05.
3.3.6b. Ferric Reducing Antioxidant Power (FRAP):

Phenolic compounds which are known as powerful chain breaking antioxidants inhibit bio-molecule oxidation by decreasing concentration of peroxyl radicals via hydrogen atom transfer from the antioxidant to the peroxyl radical, deactivating peroxyl radicals by single electron transfer (Wagh et al., 2012). Therefore, reducing activity of native flours and noodles extracts were investigated and compared with catechin. (Fig 18) shows the calibration curve of ascorbic acid. Noodles prepared from red-pigmented and non-pigmented rice in combination with chick pea were 0.56 and 0.51 µmol ascorbic acid equivalent/g respectively. Noodles showed higher reducing power than native flours (Table 12). Fig 19 (A & B) [pigmented, non-pigmented and chickpea and (noodles)] show the reductive abilities of extracts in comparison with catechin. FRAP activity increased with increasing amount of phenolic extract. However, the FRAP activity of catechin was relatively more pronounced than that of phenolic extract of samples. These results suggest that, phenolic extracts of native flours and noodles (pigmented/non-pigmented) were able to reduce Fe $^{3+}$ to Fe $^{2+}$. Since the FRAP reaction involves a single electron on hydrogen transfer mechanism, functional groups in predominant phenolic compounds play a crucial role in reducing the oxidized intermediate of peroxidation (Monica et al., 2004).
Fig 18. Standard curve of Ascorbic acid.

$y = 0.0103x + 0.0813$

$R^2 = 0.9946$
Table 12. Ascorbic acid equivalent of native flours and noodles.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Ascorbic acid equivalent (µmol/g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jyothi, pigmented husked rice (raw)</td>
<td>0.24</td>
</tr>
<tr>
<td>IR-64, non-pigmented husked rice (raw)</td>
<td>0.188</td>
</tr>
<tr>
<td>Whole chick pea (raw)</td>
<td>1.6</td>
</tr>
<tr>
<td>Pigmented noodles</td>
<td>0.56</td>
</tr>
<tr>
<td>Non-pigmented noodles</td>
<td>0.51</td>
</tr>
</tbody>
</table>
Fig 19 (A & B). Ferric Reducing Antioxidant Power (FRAP) of native flours (a) and noodles (b).
3.4. CONCLUSIONS

Factors acting on starch digestibility are multiple. The important factors that can be considered to have an influence on the catalytic efficiency of the enzymes responsible during *in vitro* starch hydrolysis are the starch characteristics, the physical access of the enzyme to the starch, the availability of water needed for the hydrolysis of the glycosidic linkage and the lowered rates of diffusion of substrates due to the relatively high viscosity. The effects of gums and other factors such as processing techniques, the presence of other food components like proteins, lipids also affect starch digestibility to a significant extent.