

## CHAPTER 6

### *Clerodendrum glandulosum* Lindl. incorporated functional pasta: phytochemical, textural, structural and sensory studies

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#### 6.1. Introduction

Pasta is a fundamental meal that is mostly made by combining water and durum wheat semolina, and it is sold in a wide variety of forms around the world. Due to its numerous desirable qualities, including its vast variety of forms, extended shelf life, good nutritional value, hygienic quality, and due to its low cost, pasta is consumed all over the world. Due to its widespread usage around the world, pasta is one of the healthiest foods. The physical, chemical, and textural features of the pasta are directly influenced by the composition of the raw materials used in its creation. It can be challenging to incorporate non-traditional ingredients without having an adverse influence on the pasta's quality qualities or without having those elements have an effect that is inconsistent with the quality of the pasta. Thus, it is necessary to pay more attention to developing nutrient-rich pasta products with higher-quality features and innovating unique nonconventional components to improve the quality of the dough. In order to create unique pasta products, further study is needed to identify and develop nonconventional components with greater utility and reasonable costs (Nilusha and Jayasinghe, 2019).

The manufacture of pasta presents a number of opportunities for product innovation. According to Laus et al., (2017), pasta is an appropriate vehicle for combining dietary supplements such plant extracts, vitamins, minerals, fatty acids, and dietary fibre. Scientists and manufacturers have been working to create new pasta formulas in recent years so that it cannot only provide nutrients and energy to the body, but also modulate one or more specific bodily functions by enhancing a specific physiological response and/or lowering the risk of disease. Functional pasta products are what these novel formulations are known as. The process of refining flour and semolina results in the loss of phenolic chemicals, therefore commonly consumed pasta does not contain them. The long-term consumption of diets high in plant polyphenols may provide protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases, according to epidemiological studies and related meta-analyses. As a result, a number of methods have been devised to create functional pasta

that is high in phenolic compounds. Antioxidant consumption has been linked to decreased lymphocyte DNA oxidative damage. Similar findings have been reported for foods and beverages high in polyphenols, demonstrating the antioxidant properties of polyphenols (Vitrac et al., 2002). One of the methods recently investigated to produce functional pasta is the use of powders and extracts from plant foods and food byproducts.

Some scientists have been looking into the prospect of enhancing pasta with herbal raw materials in recent years. Padalino et al., (2019) added an extract from the *Salicornia europaea* plant to fresh durum wheat pasta. Enhanced anti-inflammatory and anticancer capabilities are present in this annual herb (Kang et al., 2011). Angiolillo et al., (2019) investigated the impacts of microbiological and sensory quality utilising bioactive ingredients added to freshly filled pasta after being isolated from broccoli byproducts. Saffron extract was added to pasta made from durum wheat flour, and it was discovered that this additive reduced the glycaemic index and starch digestibility of the enriched pasta (Armellini et al., 2019). Additionally, the presence of saffron caused the digestive juices to release crocin, a significant component that gives saffron its colour. Different plant species' leaves are an important source of a variety of bioactive substances with beneficial effects on health that can be employed as food additives. Recently, wheat pasta was made with *Moringa oleifera* L. leaf powder, according to Simonato et al., (2020). This plant's leaves added phenolic chemicals, DF, protein, and minerals to pasta.

An essential member of this family, *Clerodendrum glandulosum* Lindl. (also known as *C. colebrookianum* Walp.), grows profusely in the wild in India's Northeast (NER) as well as the tropical and subtropical regions of the South Asian subcontinent. The genus contains a number of species that are found throughout India in varied agro-ecological zones, from the Himalayan foothills to the Kanyakumari coast. In the North Eastern region of India, there are 18 species and 2 variants of the genus *Clerodendrum*. Nefafu, Phuinum, and Anphui are some of its common names in Assamese, Mizo, and Thadou-Kuki ethnomedicinal traditions, respectively (Nath and Bordoloi, 1991; Kalita et al., 2012; Duary and Haokip, 2021). This plant maintains a unique place in NER folk medicine and culinary customs due to its several strong medicinal effects, and it is frequently taken. For the treatment of several metabolic syndromes (MetS), such as hypertension, high cholesterol, diabetes, obesity, etc. *Clerodendrum glandulosum* (CG) has been mentioned. Extracts obtained from leaves of CG have been reported as antioxidant, hepatoprotective,

anti-inflammatory, cardioprotective, hypolipidemic, antiobesity, anti-hyperglycemic and antidiabetic (Jadeja et al., 2010).

The goal of the current study was to create novel, functional pasta with high antioxidant properties by fortifying it with *Clerodendrum glandulosum* Lindl., and to evaluate how fortification affected the physiochemical, textural, and sensory characteristics of the fortified pasta to increase consumer acceptance of the quick-to-prepare food as a means of disease prevention. This study will help to use underutilized wild plant for development of functional food. This work will open the new opportunities to pasta making industries to add value to their product, attract customers and increase their sale.

## **6.2. Materials and methods**

### **6.2.1. Procurement of raw material**

*Clerodendrum glandulosum* Lindl. leaves were procured from the local market of Churachandpur, Manipur. Leaves were shade dried at ambient temperature ranging from 30 - 35°C without exposure to sun light. The semolina was purchased from the local market of Tezpur, Assam.

### **6.2.2. Chemicals and reagents**

The chemicals Folin–Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium carbonate, ethanol and standards namely, Gallic acid was purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA).

### **6.2.3. Leaf powder and extracts preparation**

The leaves of *Clerodendrum glandulosum* Lindl. were cleaned and air dried. The dried samples were ground into a fine powder (about 250 µm) using a mixer (HL1643/04 600-Watt, Philips), and they were then kept at 4 °C until they were used. To extract active components from the leaf powder, especially polyphenolic compounds, ultrasonic aided extraction was used. It was carried out utilising the Ultrasonicator's (U500, Takashi Electric Co., Ltd, Tokyo, Japan) process parameters with 75 % solvent concentration, 20 percent liquid-to-solids ratio, and 30 minutes of extraction time obtained through

optimized methods. The extract was lyophilized to obtain a dried form. For further investigation, the dried extracts were gathered and stored in a deep freezer.

#### 6.2.4. Pasta formulation and dough preparation

Plant powder and plant extracted freeze dried powder were added in formulation by replacing semolina with 0 %, 2.5 %, 5 %, 7.5 %, and 10 %w/w (Table 1). To achieve equal blending, samples were stirred in a bowl for 10 minutes. Control refers to semolina or pasta that hasn't had plant powder or extract added to it. Each sample received a set amount of water to reach a wet basis moisture content of 40%. To obtain a consistent sheet, each sample was manually kneaded before being run five times from each pass through the pasta machine (Imperia Italy dal 1932, PHP5450). Each dough sheet's final thickness of 2.5 mm was measured using a calliper. Using a pasta machine, fresh pasta was rolled in 2.25 mm diameter thickness. Prior to each test, the samples were placed in sealed plastic bags and allowed to rest for 10 minutes. For subsequent investigation that needed dried samples, additional samples were dried at 45–50 °C for 4–5 h to attain moisture content of 6.0–6.5%. The dried products of various blends were packed in polyethylene bag.

**Table 6.1.:** Different formulations of pasta with raw leaf powder and extract powder

Sample code	Semolina (g/100 g)	Raw (g/100 g)	Extract (g/100 g)
CNT	100	0	0
R-2.5	97.50	2.50	0
R-5.0	95.00	5.00	0
R-7.5	92.50	7.50	0
R-10.0	90.00	10.00	0
E-2.5	97.50	0	2.50
E-5.0	95.00	0	5.00
E-7.5	92.50	0	7.50
E-10.0	90.00	0	10.00

CNT- control; R-raw plant powder; E- extract powder

### 6.2.5. Phytochemical analysis

Total phenolic content (TPC) of the extract was determined by the modified Folin–Ciocalteu method (Hulle *et al.*, 2017). TPC were expressed as mg gallic acid equivalents per gram of crude extract. Determination of DPPH radical scavenging activity was performed with the method suggested by Devi, *et al.*, (2022). The formula for calculating scavenging activity is given in Eq. (6.1).

$$\text{DPPH radical scavenging (\%)} = [1 - (\text{Absorbance}_{517\text{nm control}} / \text{Absorbance}_{517\text{ sample}})] \times 100 \quad (6.1)$$

### 6.2.6. Cooking attributes

#### 6.2.6.1. Moisture content of the pasta

The moisture content (fresh pasta) of the cooked and uncooked pasta formulations and the control were both measured according to AACCC (2000).

#### 6.2.6.2. Optimum cooking time (OCT)

20 g of pasta were divided into equal lengths of 100 mm, and they were then boiled in 300 ml of boiling water. In order to determine the ideal cooking time, the pasta's white centre was squeezed between two transparent glass slides every 30 seconds during the cooking process (AACCC, 2000). The ideal cooking time was determined to be when the white core fully vanished.

#### 6.2.6.3. Cooking loss (CL)

According to the formula for calculating the amount of solids lost in cooking water (AACCC, 2000). 300 ml of boiling water was used to cook 10 g of pasta for the recommended amount of time (6 min). In order to calculate the pasta's cooking loss, 100 ml of cold water was rinsed off of them. The cooking water was collected in an aluminium container and heated to 105 °C in an air oven so that it would evaporate and reach a constant weight. The percentage of starting materials for the residual was calculated based on its weight.

#### 6.2.6.4. Swelling index and water absorption index

According to Cleary & Brennan (2006) approach, the swelling index (SI) of cooked pasta (g water per gram dry pasta) was calculated. After cooking, pasta (100 g) was weighed and dried at 105 °C until a constant weight was achieved. The swelling index was determined using Eq. (6.2).

$$SI = \frac{w_c - w_d}{w_d} \quad (6.2)$$

Where,  $W_c$  is weight of cooked pasta (g) and  $W_d$  is weight of pasta after drying (g).

The water absorption index (WAI) (g per 100 g) was determined using Eq. (6.3).

$$WAI = \frac{w_c - w_u}{w_u} \quad (6.3)$$

$W_c$  is weight of cooked pasta (g) and  $W_u$  is weight of uncooked pasta (g).

#### 6.2.7. Texture profile analysis of cooked pasta

Twenty grams of pasta were cooked in one liter of boiling water for the recommended amount of time (6 min). Before conducting a texture examination, the pasta was rinsed with 1 L of distilled water and left to acclimate for 6 minutes in plastic containers at room temperature. To measure the texture characteristic, a dependable micro system TA-XT2 texture analyzer (texture technology corp., UK) equipped with a 25 mm cylindrical probe was used. TPA parameters, hardness (g) (the peak force that occurs during the first compression), cohesiveness (calculated as the ratio of  $A_2/A_1$ , where  $A_1$ : area under the peak of the first bite and  $A_2$ : area under the peak of the second bite), and springiness (cm) distance<sup>2</sup> (Bourne, 2002). The instrument had a P5 cylinder probe with settings of 75 percent strain, auto (Force), trigger type, 1 mm/s pre-test speed, 5 mm/s test speed, and 10 mm/s post-test speed. The trigger time was set at 5 sec. At a time, one pasta stripe was examined. The mean peak compression force, given in  $g/mm^2$ , was taken into consideration for hardness, springiness, and chewiness values (Fiorda et al., 2013).

### 6.2.8. Colour

Using a Color Measurement Spectrophotometer (Ultra Scan VIS, Hunter Lab, a41-1013-504, Reston, VA), the L\*, a\*, and b\* colour values of the various samples were assessed. "L\*" stands for degree of lightness or darkness (L\*=0 indicates perfect black and L\*=100 indicates most perfect white); 'a\*' indicates degree of redness (+) and greenness (-); whereas 'b\*' indicates degree of yellowness (+) and blueness (-). Hue angle and Chroma were identified. The total colour difference  $\Delta E$  between the formulated samples and the control sample was calculated using the Eq. (6.4).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (6.4)$$

### 6.2.9. Sensory evaluation of cooked pasta

At Tezpur University in Assam, twenty panellists evaluated the cooked pasta samples based on their sensory quality. Pasta products were cooked in boiling water without the addition of salt at the optimal cooking time, rinsed, and stored in warm conditions until testing. Using a five-point scale score card with scores ranging from Poor/Not adequate to Excellent, the sensory evaluation of pasta was done. Flavor, taste, scent, texture, appearance, and general acceptability were the factors that were analysed.

### 6.2.10. Thermal gravimetric analysis (TGA)

Thermogravimetric analysis was used to study thermal stability (STA 6000, Perkin Elmer, USA). Using a nitrogen environment and a heating rate of 20°C min<sup>-1</sup>, TGA measurements were carried out in the temperature range of 25–600°C with a sample mass of 8 mg. In a twin Calvet calorimeter, two open ampoules suspended from a balance's arms were placed inside of its parallel cylindrical cavities (Schiraldi *et al.* 1996).

### 6.2.11. Differential scanning calorimetry (DSC)

The method described by Aravind *et al.* (2012) was modified to test the starch gelatinization characteristics of raw and cooked pasta samples using a differential scanning calorimeter (DSC-60, Thermal analyzer, Shimadzu, Japan). The Differential Scanning Calorimeter (DSC) pan was filled with 0.01ml of sample water and ground samples (5 mg) after being weighed. The pan was hermetically covered, and the samples were left to

equilibrate for an entire night at room temperature. A pan's lid and its empty interior served as the reference sample. Processing was carried out at a rate of 10 °C/min between 10 °C and 110 °C. Measurements were made twice and a DSC heating curve was produced. Temperatures for the transition's enthalpy (H), peak gelatinization, endset, and beginning (onset) were also measured.

#### **6.2.12. FT-IR Spectroscopy**

Using Nicolet Impact 410, Thermoscientific, UK's Fourier transform infrared spectroscopy, functional group presence in formulated pasta with *Clerodendrum glandulosum* Lindl. was identified. In a mortar and pestle, the samples were crushed into a fine powder (2 mg), completely combined with 50 mg of desiccated KBr, and then made into pellets using a hydraulic press. Scan wavenumbers ranged from 4000 to 400 cm<sup>-1</sup> (Dutta and Mahanta 2012). Spectral analysis was performed by using Origin software 8.5.

#### **6.2.13. X-Ray diffraction analysis (XRD)**

All cooked and uncooked fortified pasta samples, along with the control sample, underwent X-ray diffraction analysis to determine the X-ray diffraction (XRD) pattern using an X-ray diffractometer (D8 Focus, Bruker AXS, Germany) with an acceleration potential of 35 kV and 25 mA current. A curve fitting technique described by Lopez-Rubio et al., (2008) was used to analyse the data. The proportion of crystallinity was calculated. The area under the baseline minus the area from background scattering from the instrument and air were used to calculate the total area, which was used to determine crystallinity. The total area was then expressed as a percentage.

#### **6.2.14. SEM analysis**

SEM was used to examine the pasta's microstructure (JEOL JSM-6390 LV, SEM, USA). Using a sputter gold coater, samples were carefully adhered to a metal stud using double-sided tape. With an accelerating voltage of 10 kV and magnifications of 100X and 1000X, the morphological structure of the sample graphs was seen.



### 6.2.15. Statistical analysis

Experimental results were analyzed through a one-way analysis of variance (SPSS 18.0, SPSS Inc., Chicago, USA), and significant differences within treatments ( $P < 0.05$ ) were evaluated using the Tukey's HSD multiple-range test. The Origin 8.5 (Origin Lab Corporation, Northampton, USA) software was used for figures analysis.

## 6.3. Results and discussion

### 6.3.1. Phytochemical analysis

Radical scavenging activities are crucial to prevent the harmful effects of free radicals in different diseases, including cancer. A standard method for evaluating the antioxidant activity of plant extracts is DPPH free radical scavenging (Rahman et al., 2015). Since the radical molecule is stable and does not need to be manufactured, the DPPH assay is thought to be a valid accurate, simple, and cost-effective approach to assess the radical scavenging ability of antioxidants. It is a quick, easy, affordable, and popular method for determining a compound's capacity to act as a hydrogen donor or free radical scavenger, as well as for assessing the antioxidant activity of foods. Antioxidants in complicated biological systems can also be quantified using this method using solid or liquid samples. This procedure is simple and used to assess the overall antioxidant capacity. When comparing the control sample and the fortified samples, it was found that the sample treated with R-10(UP) extracted leaves powder had a DPPH scavenging percentage that was three times (67.56 %) higher than the untreated control sample (22.25 %). Cooking the samples revealed that the treated samples' scavenging percentage i.e., highest: E-10 (CP) with 74.19% was significantly higher than that of the control sample (20.96 %). This result is consistent with earlier research on enriched pasta including grape marc as well as an oregano and carrot leaf conducted by Marinelli et al., (2015) and Boroski et al., (2011).

The experiments of the phenolic content analysis were expressed in Table 6.2, as mg gallic acid per g of dry plant powder/extract. The phenolic content of the formulated samples was higher in each stage of the pasta production process than the control sample. In instance, the pasta formed using powder made from 10% extracts had the most bioactive chemicals (49.51 mg GAE/g). All of the *Clerodendrum glandulosum* Lindl. supplemented samples showed considerably greater TPC as compared to the durum wheat semolina

pasta. TPC rose along with the concentration of leaves powder, which went from 0% to 10%. With enhanced plant fortification, the TPC of the wheat semolina pasta rose from 13.96 to 49.51 mg GAE/g. The Table 6.2. shows that the phenolic content fell as the percentages dropped; in fact, cooked pasta showed a lower phenolic value than uncooked samples, likely as a result of the drying and cooking processes combined. The effect of heating on phenols varies depending on the kind of bioactive chemical and type of product was also reported (Abdel-Aal and Rabalski, 2013).

**Table 6.2:** Total phenolic content and Antioxidant activity in uncooked and cooked Pasta

Sample Code	TPC (mg GAE/g)	DPPH Activity (% inhibition)
Uncooked Pasta (UP)		
CNT(UP)	9.28±0.16 <sup>a</sup>	22.25±1.88 <sup>a</sup>
R-2.5(UP)	13.96±1.84 <sup>b</sup>	42.01±1.40 <sup>d</sup>
R-5.0(UP)	16.32±1.08 <sup>c</sup>	45.05±0.95 <sup>e</sup>
R-7.5(UP)	18.29±2.49 <sup>cd</sup>	47.28±1.23 <sup>e</sup>
R-10.0(UP)	35.47±2.01 <sup>g</sup>	53.10±1.84 <sup>f</sup>
E-2.5(UP)	23.02±1.17 <sup>e</sup>	40.02±2.43 <sup>d</sup>
E-5.0(UP)	40.70±2.39 <sup>h</sup>	57.57±1.84 <sup>g</sup>
E-7.5(UP)	44.54±1.31 <sup>i</sup>	63.40±1.31 <sup>h</sup>
E-10.0(UP)	49.51±1.84 <sup>j</sup>	67.56±2.01 <sup>i</sup>
Cooked Pasta (CP)		
CNT(CP)	7.57±0.43 <sup>a</sup>	20.96±1.89 <sup>a</sup>
R-2.5(CP)	13.52±1.32 <sup>b</sup>	32.24±2.07 <sup>b</sup>
R-5.0(CP)	14.72±1.57 <sup>b</sup>	36.27±1.03 <sup>c</sup>
R-7.5(CP)	16.88±0.21 <sup>c</sup>	44.59±2.01 <sup>e</sup>
R-10.0(CP)	22.79±2.39 <sup>de</sup>	51.45±2.31 <sup>f</sup>
E-2.5(CP)	19.13±1.16 <sup>d</sup>	33.41±2.31 <sup>b</sup>
E-5.0(CP)	26.13±1.43 <sup>f</sup>	51.95±1.81 <sup>f</sup>
E-7.5(CP)	34.11±1.91 <sup>g</sup>	68.16±2.14 <sup>i</sup>
E-10.0(CP)	43.55±2.55 <sup>i</sup>	74.19±2.58 <sup>j</sup>

CNT- control; R-raw plant powder; E- extract powder, GAE = Gallic Acid Equivalent

Means with different letters in the same row indicate that there is significant difference between the samples ( $p \leq 0.05$ )

### **6.3.2. Cooking attributes of pasta**

Cooking quality is an important parameter for any pasta evaluation. The cooking performances of the investigated pasta samples in terms of optimum cooking time, cooking loss, and water absorption, swelling index and moisture percentages are shown in Table 3. As can be observed from table there was no difference in optimum cooking time. In fact, for all the samples studied, the optimum cooking time was around 6 minutes, therefore the optimum cooking time of the samples were fixed. Unlike many other studies where it showed a lower cooking loss than the CNT sample, in the present study a contrasting observation could be seen where there is an increased in cooking loss.

Formulated samples recorded lower values for the swelling index and water absorption than the control sample. The samples fortified with the highest percentage (10%) showed the lowest value (Table 6.3). These findings concur with those of Rizk and Tolba (2002), who noted that pasta enhanced with carotenoids from tomato peels had a swelling index value that was comparable to the control sample (100% wheat flour). Since this information is needed for various subsequent tests, determining the moisture content is a crucial initial step in analysing the quality of wheat or flour. When the percentages of substituting leaves powder for semolina flour were raised, a decrease in moisture content was noticed, which is a favourable sign that they may have a longer shelf life. The addition of Moringa leaves to pasta causes the moisture content to drop, according to findings reported in a study (Getachew, *et al.*, 2020). Another study showed that, pasta enriched with 2.5, 5, 7.5 and 10% herbs was showed significant difference in cooking quality of pasta (Bhandari *et al.*, 2023).

#### **6.3.2.1. Texture Profile Analysis of pasta**

The primary determinant of pasta quality that affects consumer approval is its texture (Susanna and Prabhakar, 2013). Using Texture Analyzer, the pasta's texture was examined. The Table 6.3 gives the hardness, springiness, and chewiness values. All of the formulated samples were softer than the cooked control sample in terms of hardness. The table makes it evident that the hardness of the samples decreases significantly when the percentages of the formulations from 2.5 to 10 % are raised. Similar results were seen from a study that fortified pasta with white button mushrooms (Chauhan *et al.*, 2017). Pasta prepared with

increasing levels of chestnut flour also delivered a softer texture which was related to the greater cooking loss (Alinovi et al., 2023). Semolina's hardness decreased when leaves powder was added as a supplement. As the formulation percentages were increased, the springiness and chewiness likewise decreased. Pasta underwent significant microstructural changes when cooking. The diffusion of water from the outside to the centre begins to alter the consistency of dry pasta. The alterations were more pronounced near the pasta strand's surface, where protein matrix began to denature and starch granules were no longer intact as they were in the centre (Sozer et al., 2007). The protein and starch content of the leaves powder-supplemented pasta, which affects the textural qualities like hardness, springiness and chewiness, may be the cause of the drop in pasta strength.

**Table 6.3:** Optimal cooking time, cooking loss, swelling index, water absorption, moisture and texture profile analysis of pasta

Sample code	OCT (min)	Cooking loss (%)	Swelling index (SI)	Water absorption (%)	Moisture (%)	Hardness (g)	Springiness	Chewiness
CNT	06.00	2.24±0.42 <sup>a</sup>	1.32±0.02 <sup>b</sup>	57.55±1.36 <sup>c</sup>	57.73±1.13 <sup>c</sup>	63.34±1.31 <sup>f</sup>	1.16±0.06 <sup>b</sup>	99.69±0.37 <sup>s</sup>
R-2.5	06.00	2.63±0.30 <sup>a</sup>	1.06±0.05 <sup>a</sup>	52.86±2.15 <sup>c</sup>	55.79±1.53 <sup>c</sup>	25.98±0.11 <sup>a</sup>	0.91±0.01 <sup>b</sup>	38.59±0.56 <sup>c</sup>
R-5.0	06.00	2.53±0.18 <sup>a</sup>	1.09±0.05 <sup>a</sup>	47.94±0.65 <sup>b</sup>	52.53±0.54 <sup>b</sup>	34.81±0.58 <sup>c</sup>	0.80±0.13 <sup>b</sup>	55.29±1.84 <sup>c</sup>
R-7.5	06.00	2.55±0.13 <sup>a</sup>	1.23±0.01 <sup>b</sup>	46.01±1.91 <sup>ab</sup>	48.57±0.85 <sup>a</sup>	49.02±0.32 <sup>d</sup>	0.91±0.04 <sup>b</sup>	71.02±1.46 <sup>f</sup>
R-10.0	06.00	3.38±0.18 <sup>b</sup>	1.14±0.02 <sup>a</sup>	44.67±1.93 <sup>a</sup>	47.45±0.53 <sup>a</sup>	55.86±0.61 <sup>c</sup>	0.88±0.07 <sup>b</sup>	106.75±1.13 <sup>h</sup>
E-2.5	06.00	2.73±0.29 <sup>ab</sup>	1.31±0.02 <sup>b</sup>	54.19±1.65 <sup>d</sup>	53.75±1.83 <sup>c</sup>	24.72±1.19 <sup>a</sup>	0.87±0.01 <sup>a</sup>	42.16±1.97 <sup>d</sup>
E-5.0	06.00	3.32±0.44 <sup>a</sup>	1.28±0.01 <sup>b</sup>	51.83±1.38 <sup>c</sup>	51.32±0.78 <sup>b</sup>	30.00±1.43 <sup>b</sup>	0.88±0.02 <sup>b</sup>	42.61±2.34 <sup>d</sup>
E-7.5	06.00	3.48±0.19 <sup>b</sup>	1.22±0.05 <sup>b</sup>	49.01±0.39 <sup>c</sup>	49.78±0.32 <sup>ab</sup>	25.87±1.54 <sup>a</sup>	0.83±0.08 <sup>b</sup>	35.80±0.49 <sup>b</sup>
E-10.0	06.00	4.45±0.69 <sup>c</sup>	1.23±0.03 <sup>b</sup>	46.61±0.56 <sup>ab</sup>	48.58±1.51 <sup>a</sup>	27.24±1.92 <sup>a</sup>	0.05±0.42 <sup>a</sup>	17.84±1.29 <sup>a</sup>

CNT- control; R-raw plant powder; E- extract powder. Means with different letters in the same coloumn indicate that there is significant difference between the samples ( $p \leq 0.05$ )

#### 6.3.4. Colour evaluation of different formulated pasta

One of these sensory qualities that may be assessed the quickest is colour. When evaluating the aesthetic appeal and market worth of food goods, colour is a crucial component. Food is evaluated by consumers not just for its nutritional value or possible benefits to health, but also for its sensory qualities, which have a direct impact on consumer preferences, selection, and desires (Shim et al., 2011). When evaluating the aesthetic appeal and market

worth of food goods, colour is a crucial component. For samples of cooked and uncooked pasta, colour values were measured. One of these sensory qualities that may be assessed the quickest is colour. When plant powder and extract powder were used to make pasta, there were noticeable colour differences that persisted even after the pasta had been cooked. As we increased the percentages of the leaves powder, the formed pasta became noticeably darker and displayed lighter to darker shades of green (Table 6.4). Pasta made from durum semolina that wasn't cooked had the highest lightness  $L^*$  value (82.24), followed by pasta made from cooked control CNT (UP) with 72.01. All the formulation samples' pasta (apart from the control sample) had an increased  $L^*$  value after cooking, and this increase in lightness may be the result of colour loss during cooking. Furthermore, the colour balance of samples treated with extracted plant powder was pushed toward red (positive  $a^*$ ) whereas samples of enriched pasta treated with raw plant powder were all shifted toward green (negative  $a^*$ ). The highest  $a^*$  was found in uncooked samples E-10 (UP). This might be because of the pigments in leaves having a water-soluble nature. Such colour alterations in pasta samples may be caused by pigment swelling and conversion during cooking.

**Table 6.4:**  $L^*$ ,  $a^*$ ,  $b^*$  and  $\Delta E$  values of the Pasta samples

Sample	$L^*$	$a^*$	$b^*$	$\Delta E$
<b>Uncooked Pasta (UP)</b>				
R-2.5(UP)	54.92±0.96 <sup>g</sup>	-0.33±0.11 <sup>b</sup>	22.80±0.31 <sup>d</sup>	28.36±1.01 <sup>a</sup>
R-5.0(UP)	43.45±1.46 <sup>c</sup>	-0.05±0.01 <sup>a</sup>	19.11±0.67 <sup>c</sup>	38.99±0.67 <sup>de</sup>
R-7.5(UP)	37.98±0.94 <sup>bc</sup>	-0.62±0.07 <sup>b</sup>	17.30±0.98 <sup>b</sup>	43.13±2.08 <sup>f</sup>
R-10.0(UP)	41.32±0.96 <sup>d</sup>	-1.21±0.11 <sup>c</sup>	22.24±1.56 <sup>d</sup>	43.09±2.04 <sup>f</sup>
E-2.5(UP)	54.12±2.08 <sup>g</sup>	1.34±0.05 <sup>f</sup>	33.62±0.32 <sup>f</sup>	33.66±1.61 <sup>bc</sup>
E-5.0(UP)	47.50±0.55 <sup>f</sup>	3.35±0.29 <sup>g</sup>	35.12±2.18 <sup>f</sup>	41.11±1.89 <sup>c</sup>
E-7.5(UP)	43.50±1.22 <sup>c</sup>	3.44±0.54 <sup>g</sup>	28.05±0.4 <sup>c</sup>	40.98±1.28 <sup>c</sup>
E-10.0(UP)	41.07±0.85 <sup>d</sup>	3.01±0.06 <sup>g</sup>	25.75±0.99 <sup>c</sup>	42.63±0.58 <sup>ef</sup>
<b>Cooked Pasta (CP)</b>				
R-2.5(CP)	44.43±2.14 <sup>ef</sup>	-1.84±0.04 <sup>c</sup>	18.47±0.47 <sup>b</sup>	28.11±2.04 <sup>a</sup>
R-5.0(CP)	38.48±0.61 <sup>c</sup>	-1.76±0.15 <sup>c</sup>	22.93±2.37 <sup>d</sup>	35.01±0.87 <sup>c</sup>
R-7.5(CP)	36.65±0.61 <sup>b</sup>	-2.49±0.15 <sup>d</sup>	17.73±0.85 <sup>b</sup>	35.7±0.49 <sup>c</sup>
R-10.0(CP)	33.31±1.15 <sup>a</sup>	-2.63±0.21 <sup>d</sup>	11.16±1.38 <sup>a</sup>	39.18±1.29 <sup>d</sup>
E-2.5(CP)	52.17±1.88 <sup>g</sup>	-1.17±0.11 <sup>c</sup>	26.81±0.95 <sup>c</sup>	31.17±4.95 <sup>b</sup>
E-5.0(CP)	45.17±1.19 <sup>f</sup>	0.76±0.81 <sup>c</sup>	27.51±0.18 <sup>c</sup>	34.28±2.54 <sup>c</sup>
E-7.5(CP)	38.41±0.98 <sup>c</sup>	0.58±0.20 <sup>c</sup>	19.67±0.95 <sup>c</sup>	27.23±6.61 <sup>a</sup>
E-10.0(CP)	36.70±0.83 <sup>b</sup>	1.36±0.10 <sup>f</sup>	19.85±1.57 <sup>c</sup>	32.36±3.80 <sup>b</sup>

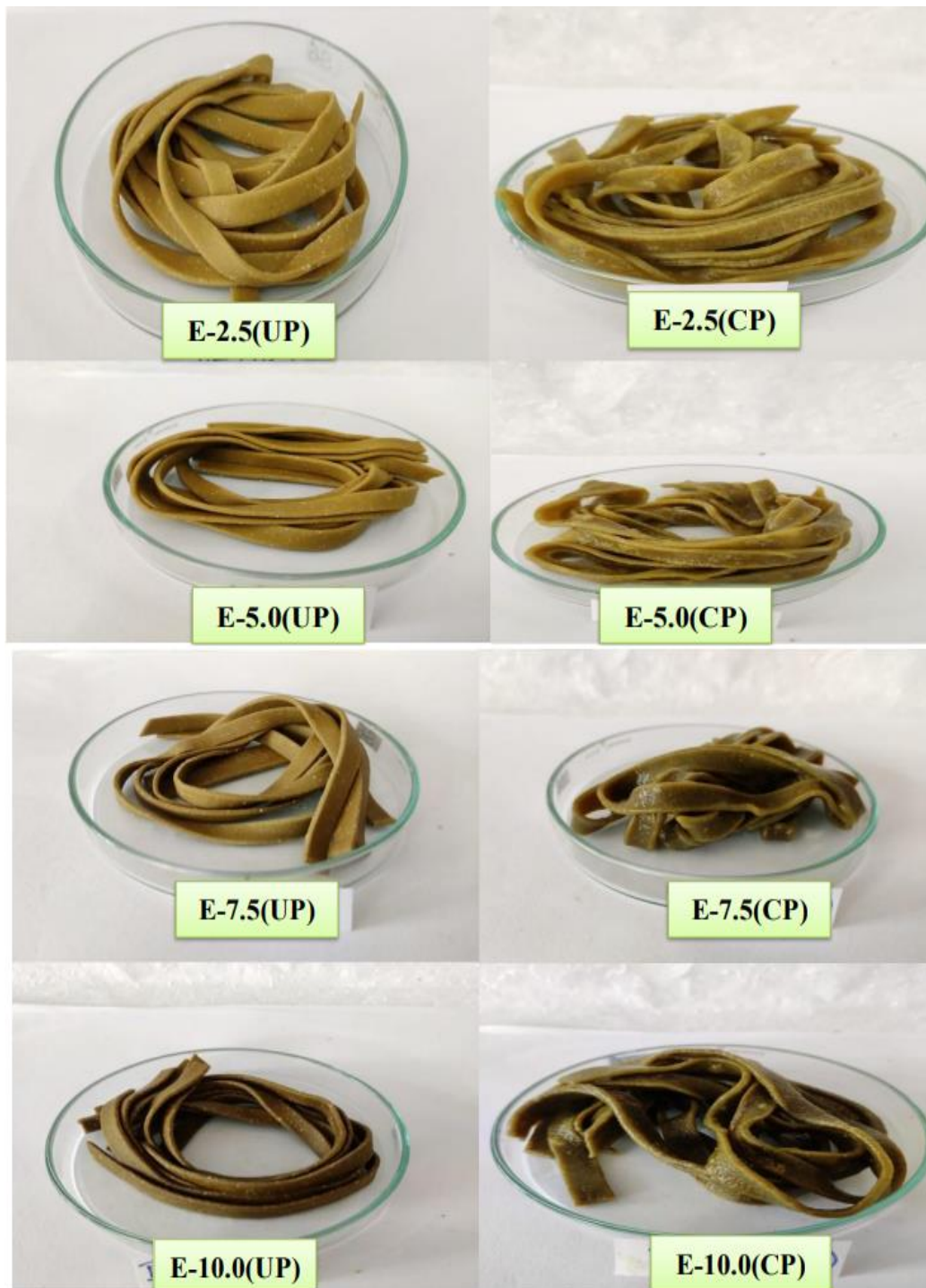
Means with different letters in the same column indicate that there is significant difference between the samples ( $p \leq 0.05$ )

The pasta samples fortified with 2.5 percent extracted plant powder (E-2.5 (UP) had the highest  $b^*$  (yellowness) rating. All the examined pasta samples had E values that were greater than 25. This shows that the colour change caused by the additive is significant enough to be noticeable. The consumer appeal may decline as a result of darker, browner colour and pronounced colour difference (Alinovi et al., 2023; Costell et al., 2009).

### 6.3.5. Sensory Evaluations

The cooked pasta strands were judged based on their appearance, colour, flavour, texture, and mouthfeel, among other sensory categories as shown in Fig.6.1. An intensity rating scale was used to rate each trait, and each result was presented separately. Higher scores imply higher preferences. Five-point scales were specified for the sensory evaluation, and each class was graded from Poor/Not good to Excellent. The preferences of the panellists for the pasta with various amounts of powdered leaf extract from *Clerodendrum glandulosum* Lindl. are shown in Table 6.5. In comparison to the control, pasta containing 2.5% leaf extract i.e., E-2.5(CP) was highly acceptable in terms of all sensory qualities assessed (appearance, colour, and taste). Extract inclusion at 2.5%, 5%, and 7.5% produced ratings that were similarly acceptable. In similar study, Spirulina (SP) Enriched Green pasta added with upto 12.5 % SP was rated as “Like very much” among all other formulations prepared with 2 to 15 % SP (Koli et al., 2022).





**Fig.6.1:** Images of uncooked and cooked pasta Left column - Uncooked pasta ; Right column - Cooked pasta



**Table 6.5:** Sensory evaluations of the formulated cooked pasta.

Sample	APPEARANCE					COLOUR					TASTE								
	P	F	M	G	E	T	P	F	M	G	E	T	P	F	M	G	E	T	
CNT(CP)	04	11	02	03	00	20	08	07	03	02	00	20	03	05	10	02	00	20	
R-2.5(CP)	00	08	02	06	04	20	00	00	03	11	06	20	00	06	08	06	00	20	
R-5.0(CP)	03	00	04	00	06	20	00	00	02	10	08	20	00	07	09	04	00	20	
R-7.5(CP)	04	06	03	06	03	20	05	05	07	03	00	20	07	10	03	00	00	20	
R-10.0(CP)	08	05	05	02	00	20	08	06	04	02	00	20	09	10	01	00	00	20	
E-2.5(CP)	00	00	00	03	17	20	00	00	00	02	18	20	00	03	09	08	00	20	
E-5.0(CP)	00	00	00	04	16	20	00	00	00	04	16	20	00	05	07	08	00	20	
E-7.5(CP)	00	00	04	08	08	20	00	00	04	07	09	20	00	07	09	04	00	20	
E-10.0(CP)	00	00	08	07	05	20	00	00	05	10	05	20	02	12	06	00	00	20	
	TEXTURE					MOUTHFEEL													
	P	F	M	G	E	T	P	F	M	G	E	T							
CNT(CP)	00	02	05	13	00	20	00	07	09	04	00	20							
R-2.5(CP)	00	05	05	10	00	20	00	04	10	06	00	20							
R-5.0(CP)	00	06	08	06	00	20	00	05	11	05	00	20							
R-7.5(CP)	05	10	05	00	00	20	05	13	02	00	00	20							
R-10.0(CP)	08	10	02	00	00	20	07	11	02	00	00	20							
E-2.5(CP)	00	04	04	12	00	20	00	06	11	03	00	20							
E-5.0(CP)	00	04	08	08	00	20	00	03	14	03	00	20							
E-7.5(CP)	00	08	07	05	00	20	00	08	12	00	00	20							
E-10.0(CP)	00	10	08	02	00	20	00	09	09	02	00	20							

P- Poor/ Not satisfactory; F-Fair; M-Medium; G-Good; E-Excellent; T-Total

### 6.3.6. Differential scanning calorimetry (DSC)

The various scanning calorimetry (DSC) thermograms of variously formed raw and cooked pasta are displayed in Table 6.6. Using DSC, the thermal characteristics (melting point) of various pasta samples were investigated. Heating was done from 10 to 150°C at a rate of 10°C/min for endothermic enthalpy.

**Table 6.6:** DSC parameters of Pasta samples

Sample	To (°C)	Tp (°C)	Tc (°C)	$\Delta T_r$ (°C)	$\Delta H$ (J/g)
<b>Uncooked Pasta (UP)</b>					
CNT(UP)	97.4	119.1	135.7	38.3	- 4718
R-2.5(UP)	101.7	122.0	131.7	30.0	- 4643
R-5.0(UP)	60.9	99.2	136.4	75.5	- 1788
R-7.5(UP)	88.0	110.9	124.7	36.7	- 2356
R-10.0(UP)	102.4	118.3	127.8	25.4	- 3212
E-2.5(UP)	98.3	120.6	133.3	35.0	- 4734
E-5.0(UP)	101.1	124.7	138.8	37.7	- 4949
E-7.5(UP)	101.6	120.6	136.1	34.5	- 5090
E-10.0(UP)	92.0	117.9	133.8	41.8	- 3565
<b>Cooked Pasta (CP)</b>					
CNT(CP)	99.1	119.4	136.2	37.1	- 4056
R-2.5(CP)	100.7	118.0	133.9	33.2	- 4909
R-5.0(CP)	102.9	122.9	136.8	33.9	- 6529
R-7.5(CP)	103.2	120.6	135.6	32.4	- 4929
R-10.0(CP)	98.8	117.3	134.2	35.4	- 3803
E-2.5(CP)	94.8	118.0	134.9	40.1	- 3638
E-5.0(CP)	70.3	106.5	135.9	65.6	- 2463
E-7.5(CP)	75.7	103.0	124.7	49.0	-1881
E-10.0(CP)	97.8	118.1	137.8	40.0	- 3951

The onset (To), peak (Tp), and endset (Tc) values of the uncooked pasta samples were highest in R-10 (UP) with 102.4°C, E-5 (UP) with 124.7°C, and E-5 (UP) with 138.8°C, respectively. The largest temperature range (Tr) was discovered in R-5 (UP) with 75.5°C in uncooked pasta. However, among the cooked pasta samples, R-7.5 (CP) with 103.2°C, R-5 (CP) with 122.9°C, E-10 (CP) with 137.8°C and E-5(CP) with 65.6 were shown to have the greatest values for onset (To), peak (Tp), endset (Tc) and temperature range (Tr). The thermal properties of the control sample were higher than those of the extracts-formulated samples, but they were lower than those of the raw powder-formulated samples. According to Kaur et al., (2016), this type of phenomenon in cereal flour may be ascribed to the extrusion method, which aids in the starch's pre-gelatinization. Since amylopectin is amorphous, higher transition temperatures have been recorded for starches with high crystallinity; this was also seen in the current investigation, where To, Tp, and Tc were negatively correlated with the amylose concentration of the starches. Starch's structural and granular characteristics, such as the presence of amylose and amylose-lipid complexes, presence of non-starch components (proteins and lipids), relative crystallinity, and granule-shape and size distribution, have all been reported to have an impact on how well starches gelatinize (Singh et al., 2010).

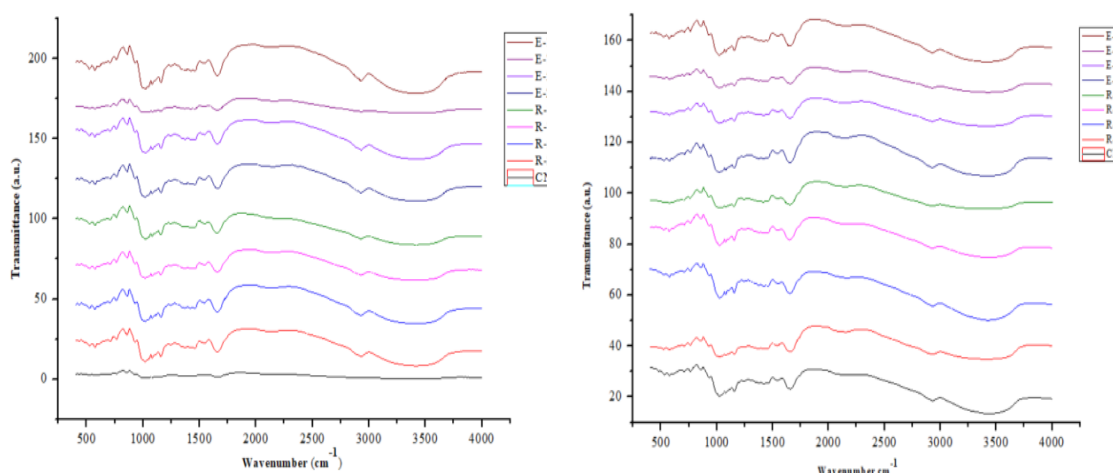
### 6.3.7. Thermogravimetric analysis (TGA)

Tools like thermogravimetry and derivative thermogravimetry can be used to examine how pasta is dried commercially. The TG curves of the thirteen pasta samples, both store-brand and name-brand, that were examined. Thermogravimetry was used to measure the drying of pasta at temperatures and relative humidity levels of 25–600 °C and 49.2–56.14%, respectively. There is one particularly fascinating temperature range where the samples exhibit a thermal profile, ranging from 180 to 340 °C. Compared to cooked pasta samples, the uncooked pasta samples had reduced relative humidity. Similar results have been reported by (Materazzi et al., 2005), who primarily concentrate on the temperature range of 180-230°C where the water release exhibits distinctive variances. They also noted thermal profiles of the research samples between 480 and 750 °C, which were not evident in the current investigation.

### 6.3.8. FT-IR spectroscopy

Fig. 6.2 displays the FTIR spectrum of both raw and cooked pasta and spectral bending ranging from 576 to 2935  $\text{cm}^{-1}$  in cooked pasta as opposed to 764 to 2931  $\text{cm}^{-1}$  in raw pasta. Uncooked samples incorporated with *Clerodendrum glandulosum* Lindl. leaves extracts and leaves powder showed a specific absorption bands 764, 862, 1080, 1157, 1661, and 2931 whereas control sample showed no specific bands. While cooked samples showed absorption bands at 860, 1021, 1155, 1654, and 2935. Numerous bands that are more or less typical of food samples can be found in the spectra. The preparation steps, cooking techniques, and cooking time all have an impact on the spectra. The distinct bands that are unique to the dishes are highlighted and given the following names in the presented spectra. The "fingerprint" portion of the infrared spectrum, which spans the wavelengths of 1500 to 800  $\text{cm}^{-1}$ , has bands that correspond to the vibrations of the C-O, C-C, C-H, and C-N bonds (Smith, 1999). On the one hand, this area is incredibly information-rich, but on the other, because of its complexity, it is challenging to study. This section offers crucial details on the sample's organic constituents, including sugars, alcohols, and organic acids. 1080  $\text{cm}^{-1}$ , the widest band overall, was present in both cooked and uncooked samples. But whereas there wasn't a band in the uncooked control sample, there was in the cooked sample.

The O-H stretching band resulted in a band with stretching properties between 3695 and 3000  $\text{cm}^{-1}$ . The weak C-H stretch is indicated by the bands at 2931  $\text{cm}^{-1}$  and 2935  $\text{cm}^{-1}$ . In both cooked and uncooked samples, a faint C=C alkene band has been seen at 1661  $\text{cm}^{-1}$  and 1654  $\text{cm}^{-1}$ , respectively. The mild C-H bending results in the bands at 1661  $\text{cm}^{-1}$  and 1654  $\text{cm}^{-1}$  is mostly caused by the presence of aromatic compounds in the pasta sample. The C-O stretching of starch is connected to the peaks seen at 1080  $\text{cm}^{-1}$  in the uncooked samples. These three peaks were shown to have considerably enhanced gluten protein levels in cooked noodles (Li et al., 2006). This reflects any leftover starch that may have remained in the samples, and it may have been triggered by the increased connection that occurred between proteins and starch during cooking. However, no distinguishing characteristics were given for the band seen at 1021  $\text{cm}^{-1}$ . At 860  $\text{cm}^{-1}$  and 862  $\text{cm}^{-1}$ , respectively, there is a significant C-H bending in both cooked and uncooked pasta. The band at 764  $\text{cm}^{-1}$  in the cooked pasta is more intense than it is in the uncooked pasta due to the significant C-H bending. The findings are in line with the work of Bawa et al., (2022) for wheatgrass powder-enriched functional pasta.

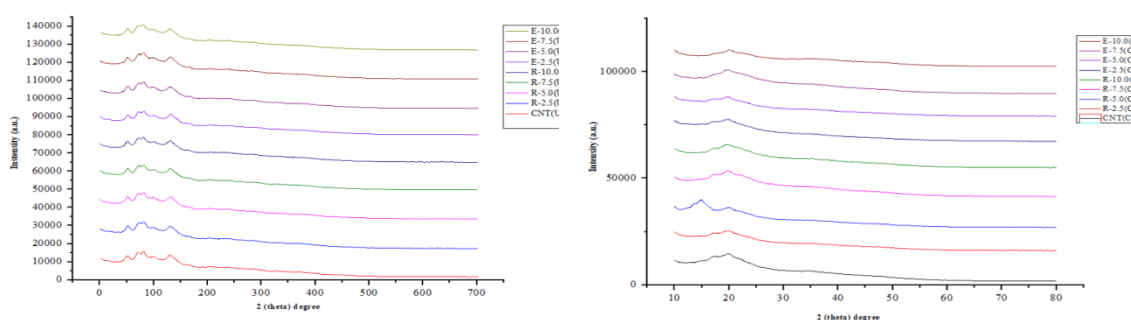


**Figure 6.2:** FTIR spectra of the uncooked and cooked pasta samples

### 6.3.9. XRD and its crystallinity

A strong instrument that is frequently used in both research and industry is X-ray diffraction (XRD). While XRD is frequently used for qualitative and quantitative analyses of the crystalline phases in materials, much more information can be gleaned from a careful examination of the diffraction patterns or by using particular XRD settings, such as the characterization of solid solutions, crystallite size and shape, crystal orientation, internal elastic strains/stresses at different levels, effect of temperature, close surface characterization, etc. According to reports, starches with high amylose/low amylopectin concentrations typically have a type B structure, whereas those with low amylose/high amylopectin contents typically have either a type-A or an intermediate type-C shape (Padmanabhan and Lonsane, 1992). Since all the starches under study were of type B and had a high amylose concentration, the results are consistent with these findings (Fig.6.3). The main aim of carrying out this XRD analysis in the current study is that the base ingredient of the pasta i.e., semolina is starch based product and this would provide more information on what type of starch is present in it. XRD patterns for raw and cooked control pasta as well as synthetic pasta are displayed in Fig. 2. Using a technique developed by (Lopez-Rubio et al., 2008) the diffractograms were all peak-fitted. The graph makes it evident that the samples' XRD examination indicated high peaks at  $2\theta$ , specifically 14.99, 17.09, and 22.98, as well as a smaller peak at 18.09 and 19.78. Moorthy (2002) claimed, however, that the peaks at  $2\theta = 15$  and  $23$  displayed mixed patterns, while  $18$  had a C type pattern. Previous studies have demonstrated that type-C and type-A starches are more

easily digested than type-B starches (Riley et al., 2004). After cooking, the pasta's wheat starch was completely gelatinized, as evidenced by the loss of the original A-type crystalline arrangement and replacement plant powder reflections. In cooked pasta, weak B-type (diffraction peak at  $17.19^\circ 2\theta$  and a greater diffraction pattern at  $19.78^\circ 2\theta$ , corresponding to single helical amylose and retrograded starch, respectively and reflections were visible (Godet et al., 1993). Although the cooked samples' crystallinity was not significantly altered by the peak fitting technique, increasing the plant powder substitution appeared to result in more clearly defined diffraction peaks.

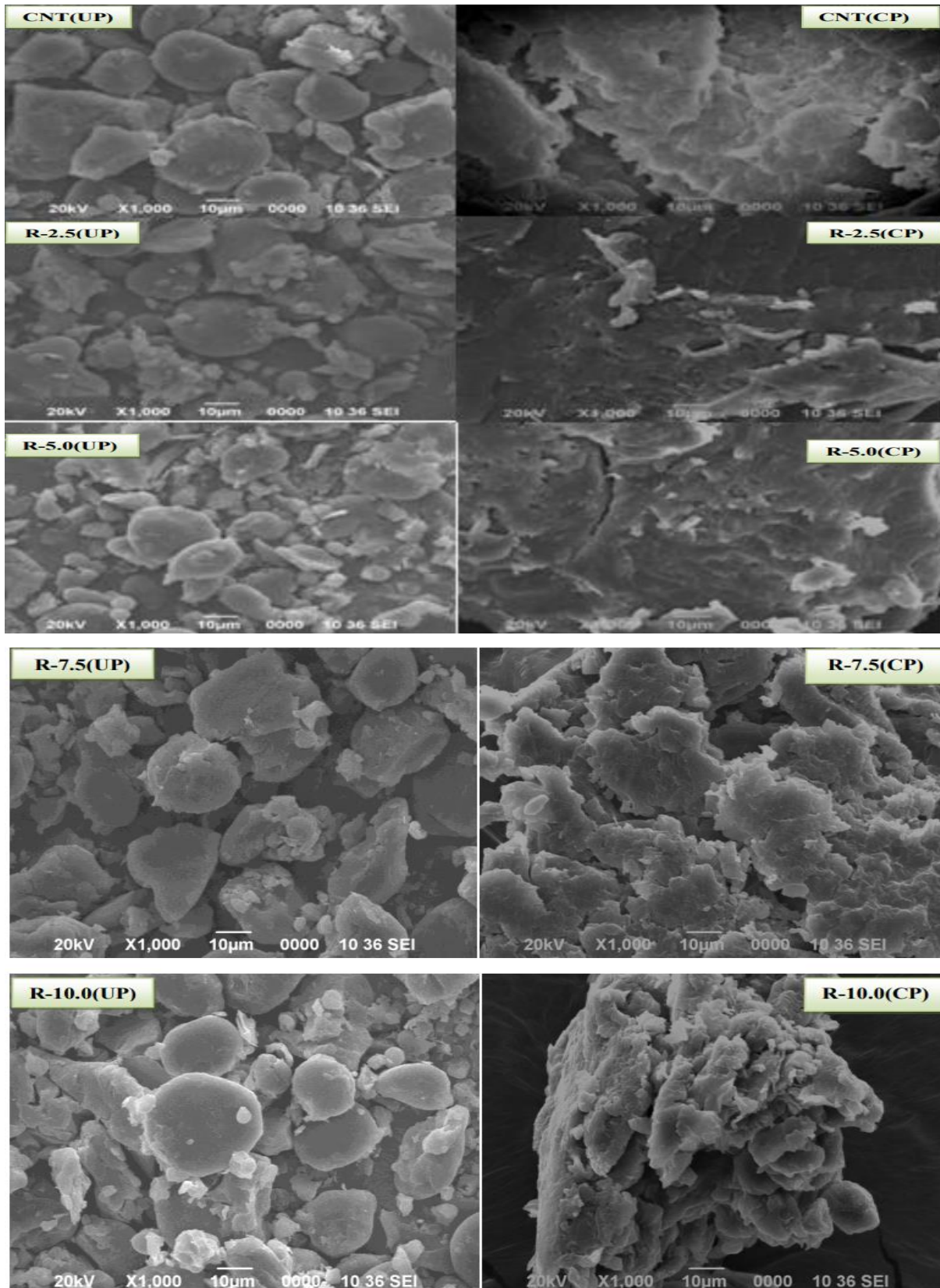


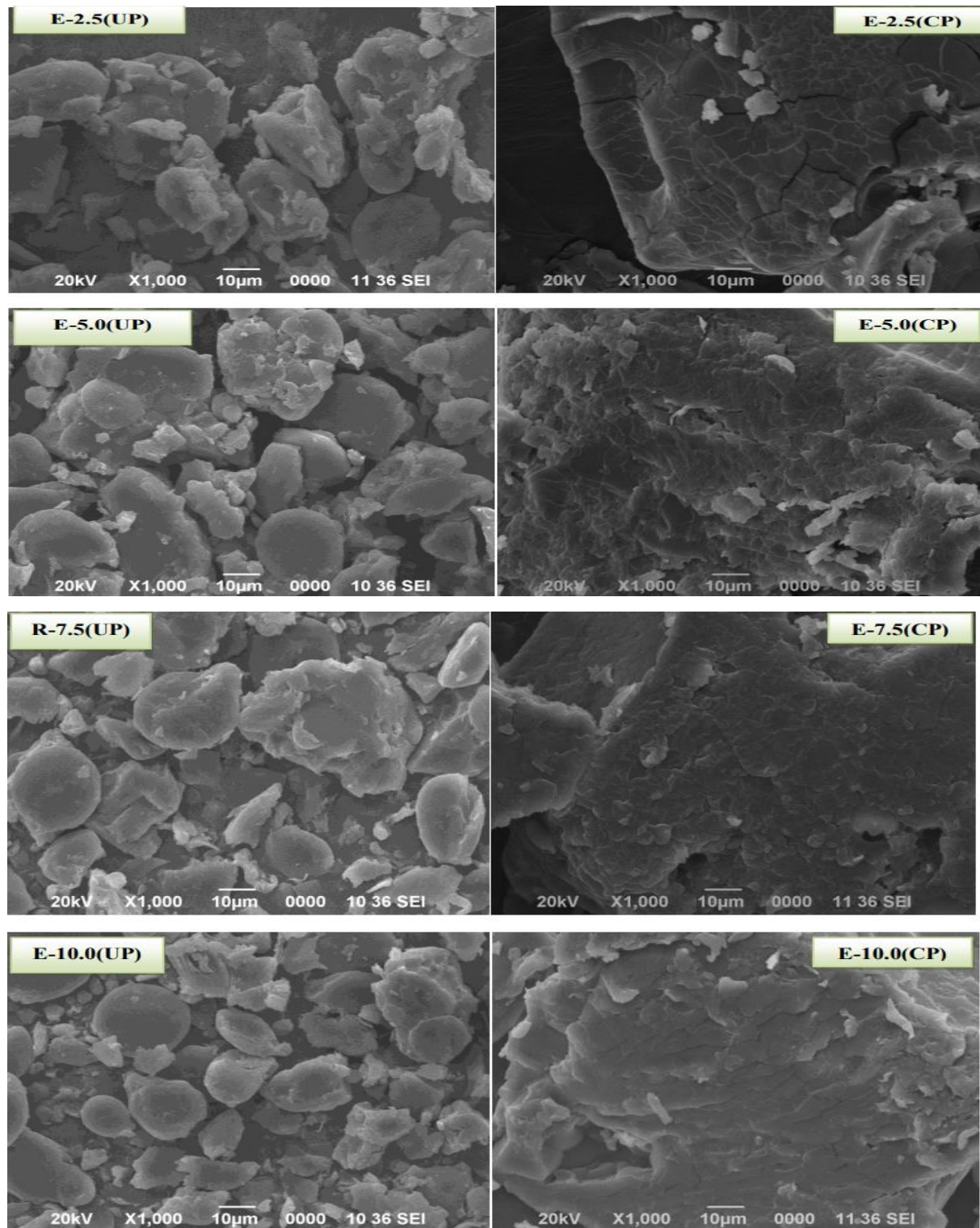
**Fig. 6.3:** X-ray diffraction pattern of uncooked and cooked Pasta samples

### 6.3.10. SEM micrographs

The digestibility of nutrients is significantly influenced by the composition of foods (Dona et al., 2010). The graphic displays SEM images of all the raw and cooked goods' surfaces and cross sections at a magnification of 1000. The pasta's gluten network and starch granule distribution are shown in Fig. 6.3 Numerous starch granules could be seen on the surface of the uncooked pasta samples, as illustrated in the figures. The control sample (CNT) had a smoother surface than the formed samples, which can be seen clearly in the figure 3. The SEM images revealed that the powdered *Clerorodendrum glandulosum* Lindl. had an impact on the product's interior architecture. The continuous structure of the control sample was described as looking smoother and aggregated. By adding powdered plant leaves, noticeable changes were seen in the surface, which become stretched, cracked, and harsher. This may be due to modifications in the structural and morphological characteristics of pasta (Raina et al., 2022). Compared to the control sample, the formed samples had significantly more flattened and sheared granules. Additionally, while comparing the cooked and uncooked samples, it was found that the uncooked pasta's

surface appeared to have microscopic pores that could have allowed water to seep into the pasta during cooking. When water is introduced during the mixing process, the durum





**Fig. 6.4:** SEM micrographs of cooked pasta with different levels of raw plant powder (RS) and plant extracted powder (ES). Left row of micrographs shows surface of uncooked pasta and right row shows surface of cooked pasta. Both the rows represent pasta with 0%, 2.5%, 5%, 7.5% and 10% respectively.



wheat semolina's tight, compact structural properties open up (Matsuo et al., 1978). As is evident in the SEM images, leaching during cooking led to increased cooking loss. Another homogenous and porous structure with certain carbohydrates and granules firmly embedded in a protein matrix was described by (Cunin et al., 1995). However, all cooked pasta samples showed a smooth outer surface with entirely integrated starch granules in the protein matrix. This might be due to cooking, as the volume of the pasta strands increases, placing a large lot of stress on the surrounding protein layer, which causes the surface of the pasta samples to smooth out (Gull et al., 2016). Furthermore, it is clear from cooked pasta micrograph images that display an improved protein starch network. Similar to this, (Rajeshwari et al., 2013) observed that the addition of gum Arabica to onion-based pasta caused the starch granules to become strongly bonded in the gluten network.

### 6.3.11. Conclusions

In recent years, customer purchase decisions have become more influenced by the items' health advantages than by their nutritional value. One of the most significant groups of bioactive chemicals that can guarantee the reduction of the risk of chronic inflammations is antioxidants. Due to its widespread usage around the world, pasta is one of the healthiest foods. In light of this, the current study pasta was prepared by incorporating *Clerodendrum glandulosum* Lindl. leaf powder and leaf extract powder which is well-known in the north-eastern region of India as a hypotensive plant. Results reveal that functional pasta showed significantly higher in antioxidant activity, total phenolic content and lower swelling index and water absorption over the control pasta. Therefore, pasta enriched with *Clerodendrum glandulosum* Lindl. shall surely have a positive impact on the consumer and be considered as a feasible and attractive option for improving the nutritional health of the population besides providing the industry with a diversified functional product. Furthermore, a more thorough investigation is required to assess the potential marketability of the formulated pasta due to the extremely restricted availability and participation in the sensory survey. To address the need of health-conscious consumers, several research projects have been carried out globally to create pasta products with unconventional ingredients and added useful features.