3.1 Amaranthus Leaves

3.1.1 Materials

Leaves of different *A. hypochondriacus* lines namely PRA2007-1, PRA2007-2, PRA2008-1, PRA2008-2, PRA2009-1, PRA2009-2, PRA1, PRA2, IC42315-2, IC42328, IC540862, Annapurna and Durga and *A. caudatus* lines (IC107197 and ICR1711) were procured from Regional station of the National Bureau of Plant Genetic Resources (NBPGR), located in Phagli area of Shimla. Leaves from different amaranth lines were ground to pass through a sieve No. 72, by the British Sieve Standards (BIS), to obtain flour and were packed in airtight containers.

3.2 Methods

3.2.1 Leaves Characteristics

3.2.1.1 Proximate Composition

Amaranthus leaves were analyzed for their moisture, ash and protein (% N × 6.25) contents by employing standard methods of analysis (AOAC, 1990). The analysis were conducted in triplicates.

3.2.1.2 Hunter Color

Color measurements (*L*-*, *a*-*, & *b*-*) of the amaranthus leaves flour from different lines were carried out using a Ultra Scan VIS Hunter Lab (Hunter Associates Laboratory Inc., Reston, VA, U.S.A.). The ‘L*-’ is for lightness, the ‘a*-’ is for redness-greenness, and the ‘b*-’ is for yellowish-bluish. All the measurements were triplicated.

3.2.1.3 Antioxidant activity (DPPH free radical scavenging activity)

Antioxidant activity was measured using a modified version of the method described by Brand-Williams et al. (1995). Ground amaranthus leaves samples (100 mg)
were extracted with 1 ml methanol for 2 h and centrifuged at 3000 g for 10 min. The supernatant (100 μl) was reacted with 3.9 ml of a $6 \times 10^{-5}$ mol/l of DPPH solution. Absorbance (A) at 515 nm was read at 0 and 30 min using a methanol blank. Antioxidant activity was calculated as % discolouration.

$$\%\ \text{Antioxidant\ activity} = (1 - \frac{(A\ \text{of\ sample\ } t=30)}{(A\ \text{of\ control\ } t=0)}) \times 100$$

3.3 Grain Characteristics

3.3.1 Materials

Different lines of *A. hypochondriacus*, namely IC540807, IC540809, IC540812, IC540817, IC540835, IC540839, IC540842, IC540845, IC540864, IC540867, IC540872, IC540874, IC540879, IC540888, IC540892, IC540902, IC547370, IC547379, IC547381, IC547395, IC447680, IC447682, IC467884, IC467887, IC467888, IC467891, IC467893, IC467896, IC467899, IC467900, IC467911, IC042264-16, IC042265-2, IC042284-5, IC042311-7, IC042006, IC035407, IC037148, IC037153, IC038312, IC095253, IC095284, IC095301, IC095341, IC021810, PRA2, PRA3, Annapurna, and 11 lines of *A. caudatus*, viz., IC540805 and IC540869, IC258399, IC363742, IC038165, IC038181, IC547512 were procured from NBPGR, Shimla.

3.3.2 Methods

3.3.2.1 Seed Weight

Amaranthus grains were counted, weighed and weight expressed in gm. The thousand kernel weight (TKW) was determined by weighing thousand seeds. All the measurements were triplicated.

3.3.2.2 Bulk density

Amaranth seeds were filled in a 100 ml graduated cylinder and gently tapped until no diminution in the sample level was observed, after filling up to the 100 ml mark. The bulk density was calculated as weight of sample per unit volume of sample (g/cc). All the measurements were triplicated.
3.3.2.3 Hunter Color

Color measurements were taken as described above 3.2.1.2.

3.4 Flour Characteristics

3.4.1 Flour preparation

About 30 g of seeds from different amaranth lines were ground to pass through a sieve No. 72, by the British Sieve Standards (BIS), to obtain flour and were packed in airtight containers.

3.4.2 Proximate Composition

The flour samples were analyzed for their moisture, ash, fat, and protein (% N × 6.25) contents by employing standard methods of analysis (AOAC, 1990). The crude fibre content was determined using the AACC (2000) methods. The analysis was conducted in triplicate.

3.4.3 Pasting Properties

The pasting properties of the flours from the different amaranth lines were measured using a Rapid Visco Analyser (Newport Scientific Pvt Ltd., Warriewood NSW 2102, Australia). An aqueous dispersion of flour (3 g flour + 25 g distilled water; 28 g total weight) was heated from 50 to 95 °C at a heating rate of 6 °C/min (after an equilibration time of 1 min at 50°C), held for 2.5 min, cooled to 50°C at the same rate, and again held at 50°C for 2 min. The pasting properties were also determined by replacing distilled water with an amylase inhibitor (0.05 mM AgNO₃). The relative α-amylase activity was calculated as percent difference in the peak viscosities measured with and without AgNO₃ (Collado and Corke, 1999). The parameters recorded were the pasting temperature, peak viscosity (PV), final viscosity (FV), breakdown (BV), and setback viscosity (SB) and pasting temperature (PT). All the measurements were triplicated. A typical pasting curve is shown in Fig. 1.
3.4.4 Textural Properties of flour gels

The flour pastes prepared in the RVA were poured into small aluminum canisters and stored at 4 °C for 2 and 24 h. The gels formed in the canisters were evaluated for their textural properties by texture profile analysis (TPA) using a TA. XT. Plus Texture Analyser (Stable Micro Systems, Surrey, England). Each canister was placed upright on a metal plate and the gel was compressed at a speed of 0.5 mm/s to a distance of 10 mm with a cylindrical plunger (5 mm diameter). The textural parameters of hardness, cohesiveness, gumminess and adhesiveness were computed using TPA. Five repeated measurements were performed for each sample and their average was taken (Bourne, 1978). A typical texture profile curve is shown in Fig. 2.

3.5 Full fat and defatted flour characteristics

3.5.1 Materials

*A. hypochondriacus* lines namely, IC540860, IC540862, IC467901, IC467910, VL0344, RMA22, RMA30, IC95341, IC38312, IC42265-2, IC42284-5, IC42311-7, IC540839, PRA2, PRA3 and Annapurna were procured during 2007-08 from NBPGR, Shimla. *A. caudatus* lines namely, IC363742, IC38165, IC38181, IC423393, IC423448, IC467902, IC540828, IC547512, IC540805 and IC540869 were procured during 2008-09 from NBPGR, Shimla.

3.5.2 Methods

3.5.2.1 Defatting of Amaranthus flour

Amaranth flour was defatted with hexane (10% w/v suspension) at room temperature during 24 h, under continuous stirring during the first 5 h. The defatted flour was dried at room temperature and stored at 4 °C until used.
Fig. 1. Typical Rapid Visco Analyzer (RVA) Pasting Curve
Fig. 2. Typical Texture Profile Analysis (TPA) Curve
3.5.2.2 Composition

Ash, fat and Protein content of full fat flours and ash and Protein content of defatted flours from different amaranthus lines were determined in triplicate using AOAC methods (1990).

3.5.2.3 Color Characteristics

Color measurements ($L^*$, $a^*$ & $b^*$) of the full fat and defatted amaranthus flours from different cultivars were carried out as described in section 3.2.1.2

3.5.2.4 Pasting Properties

The pasting properties of the full fat and defatted amaranthus flour were measured as discussed earlier in section 3.4.3. A typical pasting curve is shown in Fig. 1.

3.6 Starch Properties

3.6.1 Materials

*A. hypochondriacus* lines namely (IC540860, IC540862, IC540901, IC540910, VL-0344, RMA22, RMA30, IC038312, IC042265-2, IC042284-5, IC042311-7, IC540839, IC095341) and *A. caudatus* lines namely (IC363742, IC038181, IC423393, IC423448, IC467902, IC547512, IC540805 and IC540869) was procured from National Bureau of Plant Genetic Resources Regional Station, Shimla, India.

3.6.2 Methods

3.6.2.1 Starch isolation

Starch was isolated from amaranthus by soaking the grains in 0.25% NaOH solution at 4°C for 24 hrs, and then ground with 6 volumes of 0.25% NaOH. The slurry was filtered through nylon cloth using small amount of water. The filtrate was then centrifuged at 3000 rpm for 15 minutes. Supernatant was discarded and the sediments were washed several times with distilled water. This process was repeated until clear supernatant was obtained with white sediments of pure starch. The starch cake was dried at 35°C for 24 h.
3.6.2.2 Starch characteristics

3.6.2.2.1 Swelling power (g/g) and Solubility (%)

Swelling power and solubility were determined in triplicate, using the method of Leach et al., (1959). A 2% aqueous starch suspension was heated in a water bath at 90 °C for 30 min with constant stirring. The gelatinized starch paste was then centrifuged at 3000 g rpm for 10 min. supernatant was collected in a pre-weighed aluminium dishes. The weight of the sediment was noted to determine water absorption. The pre-weighed aluminium dishes with supernatant were left in oven at 110 °C for 24 hours and finally, the weight of dry solids were noted to determine water solubility.

\[
\text{Swelling power (g/g)} = \frac{\text{Weight of swollen starch (g)}}{\text{Original weight of sample (g)}}
\]

\[
\text{Solubility (%) = } \frac{\text{Weight of supernatant (g)}}{\text{Original weight of sample (g)}} \times 100
\]

3.6.2.2.2 Amylose content and amyllopectin chain length distribution

Amylose content of isolated starches was determined by method as described by Williams et al., (1970). Amylopectin unit chains distribution between DP 6 and 30 were analyzed using fluorophore-assisted capillary electrophoresis as described previously (Srichuwong et al., 2005). Starch was debranched with isoamylase and labeled with 8-amino-1, 3, 6-pyrenetrisulfonic acid (APTS) (Edwards et al., 1999).

3.6.2.2.3 Particle size distribution

Particle size distribution of the starches was measured by laser scattering using a Malvern Mastersizer Hydro 2000S, (Malvern Instruments Ltd., UK). The sample was added to the sample port to reach an obscuration to ~10%. The size distribution was expressed in terms of the % volumes of equivalent spheres.

3.6.2.2.4 Transmittance

Transmittance of the starch samples was measured by method as described by Perera and Hoover (1999). A 2% aqueous suspension of starch from different amaranthus
lines was heated in a water bath at 90°C for 1 hour with constant stirring. The suspension was cooled for 1 hour at 30°C. The samples were stored for 5 days at 4°C in a refrigerator and transmittance was measured every 24 hours at 640 nm using a UV/VIS spectrometer Lambda 25 (Perkin-Elmer, Switzerland). All the measurements were duplicated.

3.6.2.2.5 Morphological Properties

Scanning electron micrographs were obtained with a scanning microscope (Carl Zeiss AG, Oberkochen, Germany). Starch samples were suspended in ethanol to obtain 1% suspension. One drop of the starch-ethanol solution was applied on an aluminum stub, and the starch was coated with gold–palladium (60:40). An acceleration potential of 25kV was used during micrography.

3.6.2.2.6 X-ray Diffractometry

X-ray diffractograms of fully moistened starch granules (exposed to 100% relative humidity for 3 days) were recorded by an X-ray diffractometer, using CuKα (Ni filtered); voltage 40 kV; electric current, 40 mA; and scanning speed of goniometer, 4º/min.

3.6.2.2.7 Pasting Properties

Pasting properties of starches were measured as described in 3.4.3. section.

3.6.2.2.8 Thermal Properties

Thermal properties were analyzed using differential scanning calorimeter DSC-822 (Mettler Toledo, Greifense, Switzerland) equipped with a thermal analysis data station. Starch sample (3 to3.5 mg) was weighed into a 40 μL capacity aluminium pan (Mettler, ME-27331) and distilled water was added with the help of Hamilton microsyringe to achieve a starch water suspension containing 70% water (w/w). Pans were sealed and allowed to stand for one hour at room temperature before heating in DSC. The analyser was calibrated using indium and empty aluminium pan was used as reference. Sample pans were heated at a rate of 10 °C/min from 30 to 100 °C. Onset temperature (To), peak temperature (Tp), conclusion temperature (Tc) and enthalpy of gelatinization (Δgel) were calculated for endotherms using Stare software for thermal analysis ver 8.10.
3.6.2.2.9 Dynamic Rheology

The storage modulus (G’) and loss modulus (G’’) of aqueous starch suspensions (20%, w/w) were measured using a Haake Rheostress-6000 (Thermo Electron, Germany) rheometer using parallel plate geometry (35 mm). The starch suspension after stirring for 30 min on a magnetic stirrer at room temperature was loaded on the ram of the rheometer and covered with a thin layer of low density silicone oil to minimize evaporation of losses. The gap size, strain and frequency were set to 1 mm, 1% and 1.0 rad, respectively. The starch samples were heated from 50 to 90 °C at a rate of 0.5 °C/min and held at 90 °C for 10 min and then cooled to 50 °C at the same rate. G’ peak, G’’ peak, G’ final, G’’ final, G’ breakdown, G’’ breakdown, G’ final, and G’’ final were measured.

3.7 Amaranthus protein Isolate (PIs)

3.7.1 Materials

Lines of *A. hypochondriacus* (IC540860, IC540862, IC540901, VL0344, RMA22, RMA30, IC038312, IC042265-2, IC042284-5, IC042311-7, IC540839, IC095341, PRA-2, PRA-3 and Annapurna) and *A. caudatus* lines (IC363742, IC038181, IC38165, IC258399, IC423393, IC423448, IC467902, IC547512, IC540828 and IC540869) were procured NBPGR, Shimla.

3.7.2 Methods

3.7.2.1 Preparation of protein isolates

Amaranth protein isolate was prepared from defatted amaranth flour (Martínez and Anón, 1996). Flour was obtained by grinding the whole seeds. Lipids were extracted during 24 h with hexane (10%, w/v) at 48°C under continuous stirring. Flour was air dried at room temperature and stored at 48 °C until used. Amaranth flour was dispersed in distilled water (1:10, w/v). The dispersion was adjusted to pH 9.0 with 2 N NaOH, stirred at room temperature for 30 min and centrifuged at 9000 g for 20 min. The supernatant was adjusted to pH 5 with 2 N HCl and centrifuged at 9000 g for 20 min. The pellet was suspended with distilled water, adjusted to pH 7 with 0.1 N NaOH and freeze-dried.
Fig. 3. Typical uni-, bi- and tri-modal granule distribution
3.7.2.2 Color and protein content

Color parameters ($L^*$-, $a^*$- and $b^*$-) of protein isolates were measured using Ultra Scan VIS Hunter Lab as described earlier in section 3.2.1.2. The protein content of the protein isolates was determined using standard methods (AOAC, 1990).

3.7.2.3 Functional Properties

3.7.2.3.1 Foaming capacity (FC) and Foaming stability (FS)

The capacity and stability of foams were determined by the method of Lin et al. (1974). 100 ml of 1% (w/v) dispersions of sample in distilled water were homogenized using homogenizer (IKA-T25 digital ultra turrax) at high setting (8000 rpm), for 5 min. The blend was immediately transferred into a graduated cylinder and the homogenizer cup was rinsed with 10 ml distilled water, which was then added to the graduated cylinder. The volume was recorded before and after whipping. FC was expressed as the volume (%) increase due to whipping. For the determination of FS, foam volume changes in the graduated cylinder were recorded at intervals of 45 min and 90 min give foam stability.

3.7.2.3.2 Oil and Water absorption capacity

Oil absorption capacity (OAC) was determined using the method described by Lin and Zayas (1987). 100 mg of protein isolate samples was vortex-mixed with 1000 microlitres of sunflower oil using a Labotech vortex shaker (B.D. Instrumentation (India). After the mixture was thoroughly wetted, samples were allowed to stand at room temperature for 30 minutes. The protein suspension was then centrifuged at 3000g for 10 min. The supernatant was decanted, and the sediments were weighed.

Water absorption capacity (WAC) of PIs from different lines/cultivars were determined following the methods described by Ogunwolu et al. (2009) The sample (100 mg) was dispersed in 1.2 ml of distilled water and placed in preweighed centrifuge tubes. The dispersions were stirred using a Labotech vortex shaker after interval of 5 min, held for 30 min, followed by centrifugation for 10 min at 3000g. The supernatant was decanted, and the sediments were weighed.
3.7.2.3 Dynamic Rheology

Dynamic rheological measurements of 15% PI suspensions in deionised water were taken using dynamic rheometer (Haake RS 6000, Thermo Electron, Karlsruhe, Germany) equipped with cone and plate geometry (35 mm x 2°). The suspensions were allowed to equilibrate overnight at 4 °C and magnetically stirred for 30 min at room temperature before analysis. A thin layer of low-density silicon oil was applied to minimise evaporation. The gap between cone and plate was 0.105 mm. Stress and frequency was set at 1.0 Pa, 0.1 Hz, respectively. These values were within the linear viscoelastic region. PIs suspensions were heated from 40 to 95 °C at a scan rate of 2 °C min\(^{-1}\) and then held at 95 °C for 10 min followed by cooling from 95 to 40 °C at same scan rate. Storage modulus (\(G'\)), loss modulus (\(G''\)) and phase angle (tan δ) were continuously recorded as function of temperature.

3.7.2.4 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Proteins isolates (PIs) (20 mg) were transferred to autoclaved eppendorff tubes (ET) and 1.0 mL of extraction buffer [66 mM Tris buffer (pH 6.8), sodium dodecyl sulfate (SDS) (2.0 %), 2% β-mercaptoethanol, 10% glycerol, and 15 µl/mL protease inhibitor cocktail (Sigma-Aldrich, USA)] was added. ETs were incubated for overnight at 25 °C followed by heat treatment at 95 °C for 10 min in a boiling water bath. ETs were centrifuged (15,000 rpm/ 10 min) at 25 °C to remove undissolved materials. Supernatant was transferred on to fresh sterile 1.5 mL tubes. SDS-PAGE analysis of PIs was carried out according to modified method of Laemmli (1970). Total PIs proteins solution (15 µl) was mixed in equal volume of 2X Laemmli buffer [100 mM Tris buffer (pH 6.8); 4% SDS; 2% β-mercaptoethanol; 20% glycerol; 0.04% bromophenol blue] and mixed by gentle vortexing followed by brief spin and loaded on to the wells. The electrophoresis was carried out at 35 mA constant current followed by staining of proteins with coomassie brilliant blue R250 (CBB-R250) dye (50% methanol; 10% glacial acetic acid; 0.2% w/v CBB-R250). Stained gels were destained by using destaining solution (20% methanol and 12 % glacial acetic acid) followed documentation by using HP Scanjet 4010 scanner at 600 dots per inch resolution.
3.7.2.5 Fourier Transform Infrared Spectroscopy

Fourier-transform infrared (FTIR) spectra of PIs from different lines/cultivars were recorded using FTIR spectrometer (Vertex 70, Bruker Optics GmbH, Ettlingen, Germany) with a Attenuated Total Reflectance cell (MIRacle ATR Accessory, PIKE Technologies, Inc., Madison, WI, USA). PIs were stored in desiccators over P₂O₅ for 2 week to remove moisture. The moisture-free isolates were placed on the ATR crystal and pressed down to ensure good contact. The spectra in the range of 4000–600 cm⁻¹ were recorded (average of 120 spectra at 4 cm⁻¹ resolution). The amide I region (1700–1600 cm⁻¹) in the spectra of proteins consists of many overlapping peaks that represent different structural elements. Thus, the spectra were subjected to Fourier self-deconvolution (FSD) and second derivative analysis to enhance overlapping peaks in amide 1 region using Omnic software (Thermo Nicolet Cooperation, Madison, WI, USA) at a bandwidth of 30 and enhancement factor of 1.3 (Georget and Belton, 2006).

3.8 Flour films

3.8.1 Material

Lines of A. hypochondriacus namely, IC540839 and IC42284-7 and A. caudatus lines namely, IC423393 and IC540869 were procured from NBPGR, Shimla.

3.8.2 Methods

3.8.2.1 Film preparation

The amaranth flour films were prepared from different A. hypochondriacus and A. caudatus lines by the methodology proposed by Tapia-Blácido et al. (2005). Flour suspension (4 g/100 g) in water was homogenized in a mixer for 25 min, and the pH was regulated to 10 using NaOH (0.1 mol equi/L) to dissolve the protein. This suspension was then heated 90°C for 15 min, and varying amount of glycerol (20, 25, 28 and 30 g glycerol/100 g flour) was finally added as plasticizer and varying amount of film forming solution (8.6, 9.6, 10.6 and 15.6 g) was poured in plastic petri dishes to produce the films of varying thickness. The films were dried at 40°C and 55% RH in an hot air oven with controlled temperature and relative humidity system. Prior to characterization,
all the films were preconditioned for at least 48 h in desiccators containing a saturated NaBr solution (58% RH).

3.8.2.2 Film characterization

3.8.2.2.1 Thickness

Film thickness was measured by a screw gauge. Measurements were done in triplicate.

3.8.2.2.2 Color

Color parameters \(L^*\), \(a^*\) and \(b^*\) of flour film samples were carried out in triplicate, as described in section 3.2.1.2.

3.8.2.2.3 Opacity

Each film specimen was cut into a rectangular piece and placed directly in a spectrophotometer test cell, and measurements were performed using air as the reference. A spectrum of each film was obtained in an UV–Vis spectrophotometer Lambda 25 (Perkin-Elmer, Switzerland). The opacity of the film (UA/mm) was calculated by dividing the absorbance at 500 nm by the film thickness (mm) (Cao et al., 2007). All determinations were performed in triplicate.

3.8.2.2.4 Moisture Content

MC was determined after drying in an oven at 105 °C for 2 h. Small specimens of films collected after conditioning, were cut and placed on Petri dishes that were weighed before and after oven drying. MC values were determined in triplicate for each film, and calculated as the percentage of weight loss based on the original weight (ASTM D644-94, 1994).

3.8.2.2.5 Water solubility

Solubility of flour films was determined as described by (Gontard et al., 1992). Discs of film (2 cm diameter) were cut, weighed, immersed in 50 mL of distilled water, and slowly and periodically agitated for a period of 4 h. The amount of dry matter in the
initial and final samples was determined by drying the samples at 105 °C for 24 h. All determinations were performed in triplicate.

3.8.2.2.6 Mechanical properties

Sample films were cut into 0.5 cm wide strips at least 8 cm in length. The initial grip separation was set at 100 mm and the crosshead speed at 1.0 mm/s. The puncture force and tensile strength (force/initial cross-sectional area) were calculated using Texture Profile Analysis (TPA) using a TA. XT. Plus Texture Analyser (Stable Micro Systems, Surrey, England). The results of puncture force and tensile strength were expressed by newtons (N) and mega pascals (MPa), respectively. At least three samples from each film were evaluated.

3.9 Amaranth Leaves powder and corn grit Extrudates

3.9.1 Materials

Thirty kg of Amaranthus leaves were procured from market of Amritsar. Twenty five kg of corn grit was procured from B.N.F mills (Batala).

3.9.2 Sample preparation

Amaranthus leaves were cleaned under running tap water and excessive water was drained off. Amaranthus (30 kg) was chopped into small pieces. Blanching was done by simmering the vegetables in boiling water, draining the sample and leaving it to cool at room temperature. All the blanched samples were dried in cabinet dryer then transferred in air tight containers. Dried samples of Amaranthus leaves were ground to pass through sieve No.72 (BIS) to obtain powder, which was packed in airtight containers and then mixed with corn grit at different levels (0, 2, 4 and 6%).

3.9.3 Extrusion Cooking

The extrusion cooking of corn grit blended with amaranthus leaves flour was carried out in a twin screw extruder (Clextral, BC 21, Firminy, France). The screw diameter, L/D ratio and die diameter was 25 mm 16 and 5 mm, respectively. The extruder
was equipped with a torque indicator, which showed percent of torque in proportion to the current drawn by the drive motor. The effect of screw speed (300, 400 and 500 rpm) barrel temperature (130 °C and 160 °C) and feed moisture content (15% and 17%) was evaluated.

3.9.4 Extrudates characteristics

3.9.4.1 Color Properties

Color parameters ($L^*$-, $a^*$- and $b^*$-) of 48 extruded samples were carried out in triplicate, described in section 3.2.1.2.

3.9.4.2 Expansion/Diameter

The expansion (diameter) of extrudates was measured by using digital vernier caliper taking 10 measurements at random and then mean for each sample was calculated.

3.9.4.3 Preparation of extrudates flour

The extrudates procured from twin screw extruder were ground to pass through 60 mm sieve.

3.9.4.4 Water solubility Index (WSI) and Water absorption index (WAI)

Water solubility index (WSI) and Water absorption index (WAI) were determined for each sample by method of Andernson et al., (1969). For measuring WSI and WAI, 2.5 g of grind sample was taken into 50 ml beaker and then 25 ml of distilled water was added. Sample was thoroughly mixed on magnetic separator. The mixture as then transferred to tarred centrifuge tube. The beaker was then washed using 5 ml of distilled water and wash water was included in the solution making total volume to 32.5ml. Then centrifugal tubes were centrifuged at 3000 rpm for 10 minutes. The supernatant layer was decanted for determination of its solids. Supernatant was put in pre-weighed aluminum dishes and kept in oven at 90 °C for 24 hours. Then the weighed of solids were calculated. The sediment was weighed directly.
WSI and WAI were estimated using this formula:

\[
\text{WAI (g/g)} = \frac{\text{Weight of sediment}}{\text{Weight of the sample}}
\]

\[
\text{WSI (\%)} = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Weight of dry solids}} \times 100
\]

3.9.4.5 Frying of extrudates

3.9.4.5.1 Frying conditions

Frying of extrudates was carried out in an electrically heated fryer (model PFE8, IME, Austria), which was thermostatically controlled to maintain the set frying temperature 190 ±2 °C using an electrical control system. Groundnut oil was used as frying medium and corn-amaranthus extrudates were deep-fried at 190 °C for 60 seconds. The fried samples were sealed in plastic bags after the temperature of the samples decrease to room temperature for further analysis.

3.9.4.5.2 Hunter Color

Color parameters (L*-, a*-, and b*-) of 48 fried extruded samples were carried out in triplicate, as described in section 3.2.1.2.

3.9.4.5.3 Expansion (diameter)

The expansion of fried extrudates was measured as described in section 3.9.4.2.

3.9.4.5.4 Oil uptake

Oil uptake was determined by employing standard methods of analysis (AOAC, 1990).

3.9.4.5.5 Textural profile analysis (TPA)

TPA of fried extrudates of corn and amaranth was performed using a single extrudate of each sample by texture analyzer for testing. The extrudates were subjected to 75% compression with a cylindrical probe (P/75) at a crosshead speed of 1mm/s twice in two cycles using a 50 kg load cell. Hardness was determined as the maximum peak force.
3.9.4.5.6 Sensory evaluation

The sensory assessments were conducted in a purpose-built, ten-booth sensory evaluation laboratory. The panel of 10 members consisted of staff and graduate students. The panelists were naïve to project objectives. Selected samples (0%, 2%, 4% and 6% amaranthus leaves flour blended with corn grit extrudates were used in the evaluation. Panelists were provided with a glass of water and, instructed to rinse and swallow water between samples. They were given written instructions and asked to evaluate the products for acceptability based on its flavor, texture, color and overall acceptability using nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) as stated by Meilgaard et al., (1999).

3.10 Amaranth Flour-corn grit extrusion cooking

3.10.1 Materials

5kg of Amaranthus (family Amaranthaceae) grains were procured from market of Amritsar. Ten kg of corn grit was procured from B.N.F mills (Batala).

3.10.2 Preparation of samples before extrusion

Amaranthus grains were ground to pass through sieve No.72 (BIS) to obtain flour, which was packed in airtight containers and then mixed with corn grit 0%, 10%, 20%, 30%, 40% and 50%, respectively.

3.10.3 Extrusion cooking

The extrusion cooking of various samples was carried out in a twin screw extruder fitted with die nozzle having 5mm diameter, barrel length of 400mm and screw diameter 2.5mm. The extruder was equipped with a torque indicator, which showed percent of torque in proportion to the current drawn by the drive motor. The extruder screw was run at three temperature 125, 150 and 175 °C and moisture 15%, respectively. 18 samples were obtained from twin screw extruder.
3.10.4 Extrudates evaluation

3.10.4.1 Color properties

Color parameters ($L^*$, $a^*$ and $b^*$) of 18 extruded samples were carried out in triplicate, described in section 3.2.1.2.

3.10.4.2 Expansion and bulk density

The expansion (Diameter) of extrudates was measured as described in section 3.9.4.2. The extrudates were filled in a 100 ml graduated cylinder and gently tapped until no diminution in the sample level was observed, after filling up to the 100 ml mark. The bulk density was calculated as weight of sample per unit volume of sample (g/cc). All the measurements were triplicated.

3.10.4.3 Sample preparation after extrusion

Sample preparation after extrusion was done as described in section 3.9.4.3.

3.10.4.4 Water solubility Index (WSI) and Water absorption index (WAI)

Water solubility index (WSI) and Water absorption index (WAI) were described in section 3.9.4.4.

3.10.5 Frying of extrudates

3.10.5.1 Frying conditions

Frying conditions of extrudates were earlier described in section 3.9.4.5.1.

3.10.5.2 Hunter Color

Color parameters ($L^*$, $a^*$ and $b^*$) of fried 18 extruded samples were carried out in triplicate, described in section 3.2.1.2.

3.10.5.3 Expansion (Diameter) and Oil uptake

The expansion (Diameter) of fried extrudates was measured as described in section 3.9.4.2. Oil uptake of fried extrudates was described in section 3.9.4.5.4.
3.10.5.4 Textural profile analysis (TPA)

Textural profile analysis (TPA) of fried extrudates was measured as described in section 3.9.4.5.5.

3.10.5.5 Sensory evaluation

Sensory evaluation of fried extrudates were described in section 3.9.4.5.6.

3.11 Popping Characteristics

3.11.1 Material

Lines of *A. hypochondriacus* namely IC38312, IC42265-2, IC42284-5, IC42284-7, IC467901, IC467910, IC540839, IC540860, IC540862, PRA-2, PRA-3 and Annapurna grown at the Regional Station of the National Bureau of Plant Genetic Resources (NBPGR), located in the Phagli area of Shimla. The grain was cleaned and stored for further evaluation.

3.11.2 Popping of Amaranthus

*A. hypochondriacus* grains (50g), uniformly mixed. Thereafter, it was popped in hot pan at 190°C and note the popping time. The popping pan consisted of an iron having a diameter of 915 mm and depth of 610 mm. The cooking pan was heated by using LPG (liquid petroleum gas) burner. Gas control knob of the burner was set at the same fixed level to keep surface temperature of the cooking pan constant during popping. The grain was vigorously stirred to ensure uniform heating. It was immediately removed from the hot pan and spread on a marble slab for cooling. Popping process was carefully optimized in such a way that it resulted in grain with maximum expansion and no burning.

3.11.3 Popped characteristics

Seed volume, puffing capacity and puffing index were determined after roasting using the method of Singh et al. (1992). Bulk density of popped *A. hypochondriacus* grains was calculated as mass per unit volume. Expansion index of roasted seeds was determined using expression:
Expansion index = \( \frac{\text{Volume after puffing (ml)}}{\text{Volume before puffing (ml)}} \)

### 3.11.4 Color characteristics

Color characteristics \((L^*, a^*\text{-} and b^*)\) of raw and popped grains were carried out in triplicate, described in section 3.2.1.2.

### 3.12 Statistical Analysis

The data of Amaranth leaves flour/powder were subjected to analysis of variance (ANOVA) by Duncan's test \(p \leq 0.05\) using Minitab Statistical Software (MINITAB® v 14.12.0, State College, PA). The data reported were average of triplicate observations. Pearson correlation coefficients \((r)\) and principle component analysis were calculated for determining the relationship between different grain and flour properties using Minitab Statistical Software (MINITAB® v 14.12.0, State College, PA). The data of full fat and defatted flours from Amaranth lines were subjected to analysis of variance (ANOVA) by Duncan's test \(p \leq 0.05\) using Minitab Statistical Software (MINITAB® v 14.12.0, State College, PA). The data of starches form different Amaranth lines were subjected to analysis of variance (ANOVA) by Duncan's test \(p \leq 0.05\) using Minitab Statistical Software (MINITAB® v 14.12.0, State College, PA). The data reported were average of triplicate observations. Pearson correlation coefficients \((r)\) and principle component analysis were calculated for determining the relationship between starch properties. The data of film forming properties were subjected to two way analysis of variance (ANOVA) and Duncan's test \(p \leq 0.05\) by using Minitab Statistical Software (MINITAB® v 14.12.0, State College, PA). The data of extrudates was subjected to regression analysis using Minitab Statistical Software (MINITAB® v 14.12.0, State College, PA). Popping characteristics data were subjected to Duncan's test \(p \leq 0.05\).