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Mehnaz Manzoor (✉ mehnazmanzoor321@gmail.com)

Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

Jagmohan Singh

Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

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Assessment of physical, microstructural, thermal, techno-functional, and rheological characteristics of apple (*Malus domestica*) seeds of Northern Himalayas

Mehnaza Manzoor^{1,*}, Jagmohan Singh¹

¹Division of Food Science and Technology, Sher-e-Kashmir University of Agriculture Science and Technology, Jammu, 180009, India

Corresponding author:

mehnazmanzoor321@gmail.com

Abstract

The study examined the physical, morphological, thermal, techno-functional, and rheological properties of two apple seed cultivars viz: red delicious (RD) and golden delicious (GD). Physical properties showed that red delicious seeds were significantly ($p \leq 0.05$) different in width, geometric mean diameter, arithmetic mean diameter, volume, and surface area than golden delicious seeds. The proximate composition of RD seed flour showed a higher amount of crude protein and fat content than GD seed flour. RD seed flour was significantly different in L^* , a^* , b^* values, bulk density, water/oil absorption capacity and the emulsifying ability than GD seed flour. From particle size analysis it was possible to found that GD was significantly ($p \leq 0.05$) lower than RD flour macromolecules. Scanning electron micrographs showed oval/spherical starch granules of small size embedded in a continuous protein matrix. Thermograph revealed exothermic transition enthalpy for both RD and GD seed flour, which indicates a high energy requirement for crystallite melting. The rheological assays revealed high elastic modulus (G'), of seed flours that will help in modifying the texture of foods. This study suggests the potential of apple seeds in the formulation of protein-enriched foods to combat malnutrition while contributing to the reduction in industrial wastage.

1 Introduction

Apple (*Malus domestica*) is one of the most important fruit crops cultivated throughout the temperate regions of the world. The fruit is consumed fresh or processed into products such as juice, concentrate, jam, cider, canned products. However, processing of apples generate huge quantum of pomace accounting for up to 20-35% share of fresh fruit weight and consists primarily of a mixture of skin/ pulp tissue (94.5%), with a significant amount of seeds (4.1%) and stems (1.1%)^{1,2}. Apple seeds are reported to be excellent sources of protein (38.85-49.55%) and lipids (20.69-24.32%) with linoleic and oleic acids as predominant amino acids³. The seeds also contain a considerably high amount of polyphenols which contribute to reducing the risk of obesity and diabetes mellitus⁴⁻⁶, help prevent bone loss, inhibit cancer cell growth, enhance memory, and life span⁶. They are also rich sources of natural antioxidants and can inhibit lipid peroxidation⁴. Currently, the seeds are gaining more interest

36 in food formulations and as potential delivery material for the administration of bioactive
37 compounds⁶.

38 Moreover, depending on the variety, apple seeds vary in shape size, and color. The knowledge of
39 the geometrical properties of seeds could be useful to separate seeds into different fractions for further
40 post-harvest processing and to further select the high-quality fractions in terms of nutritional and
41 functional value. Although many studies have determined physical and mechanical characteristics of
42 agricultural materials such as tung (*Aleurites fordii*) seeds⁷, melon (*Cucumis melo* L.) seeds⁸,
43 sunflower (*Helianthus annuus*) seeds⁹, Akee apple (*Bilphia sapida*) seed¹⁰, oats (*Avena sativa* L.)¹¹,
44 finger millet (*Eleusine coracana*)¹², no such study has been carried out for apple seeds. Besides, there
45 is no study on the thermal, morphological, and rheological properties of apple seed flours. Due to the
46 lack of such studies, apple seeds are rarely used at the commercial level and they generally goes
47 waste. The work was thus focused to determine the basic geometrical, morphological, thermal,
48 techno-functional, and rheological properties of underutilized apple seeds collected from two varieties
49 (red delicious and golden delicious) of Himalayan origin to feed the growing functional food industry.

50 **2. Results and Discussions**

51 **2.1 Physical characterization of apple seeds**

52 Physical dimensions measured in terms of length, width, thickness, arithmetic, and geometric
53 mean diameter, and shape provide valuable information for differentiating between varieties and/or
54 other grains and in designing material handling and processing machinery. Results of the physical
55 characterization of flour samples are presented in **Table 1**. The length, width, and thickness of apple
56 seeds varied significantly, with length ranging from 7.69 mm (GD) to 9.43 mm (RD), seed width in a
57 range of 4.02 mm (GD) to 4.87 mm (RD), and thickness in a range of 2.01 mm (RD) to 2.03 mm
58 (GD), respectively. The arithmetic mean diameter and geometric mean diameter of seeds measured
59 were also found to vary significantly and were 4.58 mm, 5.44 mm, and 3.97mm, 4.51 mm
60 respectively, for GD and RD cultivars. Similar results were obtained on Sabzar, SKO20, and SKO90
61 oats as reported by Shah *et al.*¹¹ with length ranging from 8.2 to 9.03, width 1.50 to 1.73, and
62 thickness 2.25 to 2.66, and geometric and arithmetic diameters varying from 3.12 to 3.40 and 4.07 to
63 4.33 mm respectively. Seed surface area, which determines the shape of seed and is considered
64 important in heat and mass transfer operations⁹ and ranged from 49.57 mm² to 64.19 mm². It was
65 found that the lowest volume was occupied by golden delicious variety (20.22 mm³) compared to red
66 delicious (29.08 mm³). Similarly, sphericity ranged from 48.18% (RD) to 51.72% (GD) indicating a
67 higher resemblance of golden delicious seeds towards a sphere. This can be attributed to the higher
68 moisture content of GD seeds compared to RD. The increase in sphericity with increased moisture
69 content was reported by Malik & Saini⁹ in sunflower seeds. Besides, sphericity and length are
70 inversely related, with round grains resembling more to a sphere than a cylinder. Similar relations
71 were depicted in our study with more spherical grains having the lowest length. However, both

72 varieties depicted non-significant variation in aspect ratio, which indicates the tendency of seeds to
73 roll on a flat surface. Also, the thousand-grain weight, which usually indicates the endosperm content
74 in the seeds, was found to be 44.83 g (GD) and 45.32g (RD) with no significant difference noticed
75 between cultivars. All such variations could be attributed to the genetic makeup of the seed cultivars
76 and will play an important role in the machine and other post-harvest equipment designing for
77 efficient handling, processing, and storage of materials.

78 **2.2 Chemical composition of seed flour**

79 Table 2 presents the proximate analysis such as moisture, crude fat, protein, and total ash
80 content of red delicious and golden delicious seed flour. Moisture content is an important parameter
81 that can help suggest the storage stability of dried products. Higher moisture content tends to shorten
82 the shelf life and result in loss of physical, chemical, biochemical properties, and as well as results in
83 rapid microbial growth¹². The results for moisture content were found to be 8.17% for RD and 8.57%
84 for GD seed flour with no significant variation observed among cultivars. These results were
85 agreeable to that reported for Akee apple seed flour (8.05%)¹⁰. It was also found that RD flour
86 contained significantly high ($p \leq 0.05$) crude protein and fat content. The crude protein content was
87 found to be 41.31% (GD) and 47.98% (RD) and was higher compared to other fruit seeds such as kiwi
88 fruit flour (4.75-8.74%)¹³, quinoa flour (13.46%)¹⁴, watermelon, guava, orange, apricot, paprika and
89 prickly-pear¹⁵ and also protein-rich flours, such as chickpea (20-24.3%)¹⁶, cowpea (24.1%) and horse
90 gram flour (22.5%)¹⁷. This high protein content might be substantial enough to have a decisive effect
91 for supplementing composite flours for the development of nutrient-enriched food products.
92 Regarding protein quality, apple seeds are reported to be rich in sulfur-containing amino acids and
93 appear to be reasonably balanced, with most of the essential amino acids above the FAO/WHO
94 pattern (FAO/WHO, 1993). Concerning lipid content, a significantly highest percentage was found in
95 RD cultivar 25.31% than GD cultivar 21.47%. These results are comparable to that reported for *L.*
96 *acidissima* (Wood apple) seed flour (24.9%)¹⁸ but superior to that reported for quinoa seed flour
97 which has only 5.47% oil¹⁴. However, total ash content, which is related to minerals, was not found to
98 vary significantly and was found to be 4.35% (RD) and 4.67% (GD). These results were found to be
99 higher than in *C. lanatus* (2.75%) and *L. acidissima* (2.84%) seed flour¹⁸. Overall, flours presented a
100 good nutritional value that can contribute to the formulation of protein-enriched functional foods to
101 combat malnutrition.

102 **2.3 Flour flowability properties**

103 **2.3.1 Bulk density, True density, Carr's index, and Hausner ratio**

104 The bulk density, which indicates the load flour samples can withstand if allowed to rest directly on
105 one another¹² was recorded to be 0.38 g/ml and 0.60 g/ml for RD and GD cultivars (Table 3). This
106 variation in bulk density can be associated with particle size, with finer particles resulting in higher
107 bulk density since particles orient in a manner to decrease inter practical space to occupy less space
108 compared to coarse particles. These results can also be validated from particle size distribution and

109 SEM results which depict a larger size of RD cultivar resulting in reduced mass per unit volume
110 occupied by flour sample compared to GD flour. The higher bulk density value of GD flour can also
111 be attributed to its high moisture content that increases total mass per unit volume occupied by flour
112 sample. The true density was found higher than bulk density with mean measured values of 0.58 g/mL
113 for RD and 0.69 g/mL for GD. For good flowability of powdered samples, bulk and true density
114 values should be close enough to have a lower Carr index. The Carr compressibility index of RD and
115 GD flour was found to be 34.51% and 37.70% while the Hausner ratio was found to be 1.52% and
116 1.6% respectively (Table 3). Carr index > 25% is considered to be a poor flowability indicator, and <
117 15% a good flowability indicator¹⁸. These results are in line with the findings of Sonawane et al.¹⁸
118 where bulk and true densities measured were 0.26, 0.28 g/ml and 0.39, 0.47 g/ml, which resulted in
119 compressibility index of 34.17% and 41.27% for *C. lanatus* and *L. acidissima* flour respectively.

120 **2.4 Particle size distribution and polydispersity index**

121 Particle-size distribution is a quality parameter influencing the processing performance of
122 flour samples. Table 3 shows the average hydrodynamic diameter and particle size distribution of
123 flour samples in aqueous suspension. The particle sizes of GD at 10% (Dv10), 50% (Dv50), and 90%
124 (Dv 90) volume distribution was found to be 2.603, 4.419, and 6.122 μm respectively, which was
125 lower than corresponding values for RD cultivar (6.269, 7.580 and 9.060 μm). Moreover, the average
126 hydrodynamic diameter of GD and RD flour fractions was found to be 2.97 μm and 8.73 μm
127 respectively which indicates that these macromolecules were finely ground with varying particle size
128 depended on seed cultivar. It has already been stated that difference in particle size distribution among
129 different flours might depend on endosperm structure (hard or soft), the grade of material, endosperm
130 cells, and grinding equipment used¹⁹. Besides, the polydispersity index which reflects homogeneity of
131 sample based on size was found to be 0.12 and 0.43 for GD and RD seed flours respectively, and
132 around 0.4 indicates narrow size distribution of sample particles with good stability in solution²⁰.

133 **2.5 Color**

134 Color, an important quality attribute, immensely influences the consumers' preference and
135 choice of food. The results of the color characteristics (table 4) of both flours presented satisfactory
136 whiter color with higher brightness values (L^*) > 75. The significantly ($p \leq 0.05$) higher L^* value of
137 GD flour may be attributed to its reduced particle size (as also reported from SEM results) as
138 compared to RD flour, increasing surface area and thereby allowing greater reflection of light. The
139 lower a^* values in both flour samples which is an indication of greenness also displayed a significant
140 ($p \leq 0.05$) difference among varieties with red delicious presenting more greenness than golden
141 delicious. Similarly, b^* values which indicate blueness and yellowness ranged from 9.18 to 10.64
142 with a lower value of yellowness for RD seed flour which may be due to a reduction in pigments
143 during processing. Lightness and yellowness are important color attributes that influence consumers'
144 preference and acceptability of products¹². These results are comparable to those reported by Kaur &
145 Singh ¹⁶ who studied the variation in color in different varieties of chickpea flour. Such variations in

146 color characteristics may also result from differences in inherent colored pigment among the flour
147 varieties, resulting from genetic variations of the seeds.

148 **2.6 Scanning electron microscopy (SEM)**

149 The morphological structures of both apple seed flour are depicted in Fig 1a and 1b. The SEM
150 morphograph revealed the presence of small and as well as large starch particles of varying shapes
151 and dimensions with some dents/fissures. The starch macromolecules were embedded in a continuous
152 matrix formed by proteins and other non-protein components. The micrograph indicated that starch
153 granules in red delicious seed flour form clusters and were semi-spherical, oval-shaped, and polygonal
154 with a rough surface and size ranging from 4.56-15.27 μm (Fig 1b). However, golden delicious (Fig
155 1a.) depicted spherical, oval-shaped, and somewhat irregular but smooth-surfaced starch granules
156 with sizes ranging from 2.04-14.32 μm very similar to results for oat flour¹¹. These changes in
157 conformation, as well as shape and size distribution of starch granules, could influence the
158 functionality of seed flours for their further use in functional food development.

159 **2.7 Thermal characterization of apple seed flours**

160 The thermal characteristics of two seed flours as determined by DSC differed significantly (p
161 ≤ 0.05). The gelatinization transition temperatures (onset temperature, T_o , endothermic peak
162 temperature, T_p and conclusion temperature, T_c) and enthalpy of gelatinization (ΔH) of two seed
163 flour samples were determined by DSC. The values obtained for Golden and red delicious seed flours
164 were $T_p = 98.37, 102.17 \text{ }^\circ\text{C}$, $T_o = 78.95, 89.07 \text{ }^\circ\text{C}$, $T_c = 108.71, 117.53 \text{ }^\circ\text{C}$ and $\Delta H = 18.41, 9.78 \text{ J/g}$
165 respectively (Table 4). Results revealed that gelatinization temperatures of red delicious were
166 significantly ($p \leq 0.05$) higher than golden delicious variety. As already stated, gelatinization
167 transition temperatures vary with botanical species and usually differ with shape, size, and the internal
168 arrangement of starch fractions within granule¹⁶. At the same time, the higher endothermic peak
169 temperature of red delicious flour as compared to golden delicious variety ($p \leq 0.05$) might be
170 attributed to its high proteins, lipids, and fiber content that gets degraded at high temperature²¹.
171 Further, a significant ($p \leq 0.05$) difference in the gelatinization enthalpy was also recorded among the
172 two cultivars with red delicious flour having generally lower ΔH value ($9.78 \pm 1.33 \text{ J/g}$) compared to
173 golden delicious seed flour ($18.41 \pm 0.63 \text{ J/g}$) indicating its low energy requirement for crystallite
174 melting. It may be due to a greater number of double-helical regions within golden delicious granules
175 which require more energy for breaking hydrogen bond between glucan chains for complete starch
176 gelatinization. Further Ren²², reported that particle size also affects ΔH value suggesting that flour
177 with small particles result in complete protein denaturation and starch gelatinization which is
178 consistent with the SEM results of this study.

179 **2.8 Techno-functional properties of flour samples**

180 The techno-functional properties that have been categorized as non-nutritive food characteristics
181 with a critical contribution in improving the existing functionalities and help in new product
182 development were also investigated with results depicted in Table 4.

183 **2.8.1 Water holding capacity**

184 Water holding capacity determines the ability of a product to hold water depending on the
185 presence of polar/ hydrophilic proteins and polysaccharides^{16,23}. The results depicted a significantly (p
186 ≤ 0.05) higher water holding capacity (WHC) of RD (327.65 g/100g) compared to GD (312.58
187 g/100g). Earlier, El-safy *et al.*¹⁵ reported comparable results for the Egyptian variety of apple seed
188 flour (3.58 g/g), whereas lower values were found for watermelon, guava, orange, apricot, paprika,
189 and prickly-pear. The existence of higher protein content in flour samples (Table 1) might contribute
190 to their higher WHC. Since it has been reported that the presence of several hydrophilic proteins
191 especially polar amino acid residues bind subsequently with more water molecules resulting in higher
192 water absorption¹⁷.

193 **2.8.2 Oil holding capacity**

194 Regarding oil absorption capacity (OHC), the values observed in the present work (92.97 g/100g
195 and 86.76 g/100g for RD and GD respectively) are low compared to values given for the Egyptian
196 variety of apple seeds flour¹⁵. This variation in fat absorption among two cultivars can be attributed to
197 variation in protein concentration, hydrophobicity of proteins that determine its degree of interaction
198 with oil and water²⁴.

199 **2.8.3 Emulsion activity and stability**

200 Emulsification activity (EA) characterizes the capacity of flour samples to form stable emulsion
201 via interaction of polar and non-polar proteins with oil droplets at the oil-water interface was recorded
202 to be 79.61% for RD and 72.01% for GD with significant (p ≤ 0.05) difference observed among
203 cultivars. Therefore, more is protein content more will be adsorption ability towards oil. Also,
204 emulsification stability (ES), which indicates if the globular proteins present in the flour sample can
205 prevent deformation of the emulsion system under shear stress conditions¹⁷ was found to have higher
206 values for RD flour (51.81%) than GD seed flour 46.56% after 60 minutes (**Table 4**). A
207 comparatively lower EA of 47.58% and 40.60% and ES of 45.22% and 11.48% were reported for *C.*
208 *lanatus* and *L. acidissima* seed flours respectively¹⁸. Therefore high EA and ES will render these
209 flours more suitable for surface adsorption for use in stabilizing colloidal food systems and in
210 processed meat products such as sausages¹⁸.

211 **2.9 Dynamic rheology**

212 The difference in rheological behaviour (visco-elastic properties) of two flour samples was
213 expressed in terms of loss moduli G'' and storage moduli G'. Variation curves of G'' and G' as a
214 function of oscillatory frequency are depicted in Figure 2. The mechanical spectra of both samples
215 obtained in this study displayed consistently higher G' than G'' throughout the frequency range
216 studied, which indicated typical viscoelastic behaviour with a predominant solid or elastic (gel-like)
217 character. Generally, higher G' is an indication of weak gel behaviour. Lower viscous modulus G''
218 values compared to elastic modulus G' indicates that flour samples are of good quality which could
219 lead to higher bread loaf volume. Interestingly, Jhan *et al.*²⁴ reported a similar trend of dynamic

moduli of pearl millet used in their studies. Moreover, red delicious seed flour exhibited a pronounced increase in visco-elastic behavior compared to golden delicious variety. This could be due to the higher protein percentage in red delicious seed flour which favours protein aggregations resulting from increased disulfide linkage and enhanced interaction of gluten network within the matrix favouring visco-elasticity of the continuous phase. The effect of protein in increasing the G' and G'' was reported by Marco & Rosell²⁵. The results were also consistent with the proximate composition wherein RD and GD seed flours showed higher protein concentration.

Conclusion

The study provided a basic understanding of various geometrical, physiochemical, functional, structural, thermal, and rheological properties of two underutilized apple seed cultivars. The seeds showed considerable difference in dimensional properties which will enable the sorting of apple seeds into different fractions for post-harvest operations. Likewise, a significant difference was observed in chemical composition and techno-functional properties with red delicious seed flour having significantly higher water/oil holding capacity, emulsification capacity, and emulsification stability. A considerable good amount of protein and lipid content was observed in seed flours that suggest their use in the development of protein-enriched food products with different end uses. Further, flour prepared from red delicious seeds depicted smaller particle size distribution with higher stability to thermal degradation mostly suitable for various food processing operations. Rheological results revealed predominant visco-elastic behaviour of seed flour which could help modify the texture of foods. These findings will be useful to measure the quality of seeds in terms of nutritional and functional value. Further, the exploitation of apple seeds can be a boon for food security while minimizing industrial food wastage and as well contribute in development of protein-enriched foods to combat malnutrition.

3. Materials and methods

3.1 Materials

Apple seeds (Golden delicious and Red delicious) were procured from Juice processing industry (FIL Industries Pvt Ltd, Srinagar, J&K, India). The samples were cleaned, dried, dehulled and then stored at 4 °C in air tight containers. All chemicals and reagents used were of analytical grade and purchased from High media Laboratories Pvt Ltd and Sigma Aldrich (USA).

3.2 Sample preparation

The seed flours were prepared following method described in our previous work ⁴.

3.3. Physical characterization of apple seeds

3.3.1. Geometric properties

The three principal axial dimensions (mm) including length (L), width (W), and thickness (T) of 100 randomly selected seeds of each variety were measured by digital vernier caliper reading to an accuracy of 0.01mm.

3.3.1.1 Geometric and arithmetic mean diameter

Geometric mean diameter, D_g (mm), and arithmetic mean diameter, D_a (mm) of seeds were calculated using equations (1) and (2) as described by Shah *et al.*¹¹.

$$260 \quad D_g = \sqrt[3]{LWT} \quad (1)$$

261

$$262 \quad D_a = \frac{LWT}{3} \quad (2)$$

263 3.3.1.2 Sphericity

264 The shape of seeds can be determined in terms of sphericity (ϕ), which is index of roundness and
265 was calculated by the formula used by Ramashia *et al.*¹².

$$266 \quad \Phi = \frac{\sqrt[3]{LWT}}{l} \quad (3)$$

267 Where; L, W, and T are length, width, and thickness of seeds respectively.

268 3.3.1.3 Aspect ratio

269 The aspect ratio (R_a) was calculated by the equation used by Shah *et al.*¹¹ as:

$$270 \quad R_a = \frac{W}{L} \times 100 \quad (4)$$

271 3.3.1.4 Volume and surface area

272 Sample volume, V (mm^3), and surface area S , (mm^2) was calculated from geometric mean
273 diameter by following equation:

$$274 \quad S = \pi \times D_g^2 \quad (5)$$

$$275 \quad V = \frac{\pi}{6} D^3 g \quad (6)$$

276 3.3.1.5 1000-grain weight

50 seeds were selected randomly and weighed in digital electronic balance (Shimadzu, unic Bloc) of 0.001 g accuracy. The value was then multiplied by 20 to get the weight of 1000 seeds.

279 3.4. Proximate composition of flour

The moisture (925.10), fat (920.85), ash (923.03), and protein (920.87) contents were determined by official AOAC method²⁶.

282 3.5 Flour flowability properties

283 3.5.1 Bulk density and tapped density

Bulk density was measured according to the method described by Gani *et al.*²⁷. The tapped density was determined by the same procedure, but the sample in the measuring cylinder was tapped on the bench very carefully until a constant flour volume was observed.

287 3.5.2 Compressibility index and Hausner ratio

The Compressibility index (CI) and Hausner ratio (HR) were calculated as per the formula given by Carr²⁸.

$$290 \quad CI = 100 \times \left[1 - \frac{\rho_b}{\rho_t} \right] \quad (7)$$

291
$$HR = \frac{\rho_t}{\rho_b} \quad (8)$$

292 Where ρ_b is flour bulk density and ρ_t is flour tapered density.

293 **3.6 Particle size distribution and polydispersity index**

294 The particle size distribution of flour samples was measured using a Litesizer 500 laser light
295 scattering instrument (Anton Paar, Australia). 0.01% flour samples were suspended in Milli-Q water
296 (Elix-10, Millipore, Mosheim, France) and sonicated at 40 kHz (15-30 min) for complete dispersion
297 of flour fractions. The measurements were performed at 20°C at neutral pH.

298 **3.7 Color**

299 The colour of the flour samples were determined by Color Flex Spectrocolorimeter (Hunter
300 Lab D-25, Ruston, USA) which was calibrated using a white reference tile. The results were
301 expressed in terms of L* (lightness/brightness), a* (redness/greenness), and b* (yellowness/blueness)
302 values.

303 **3.8 Scanning electron microscopy (SEM)**

304 The microscopic structure of flour samples were analyzed by scanning electron microscope
305 (Zeiss, EVO 50) under a high vacuum. The samples were mounted on a circular aluminum specimen
306 stub using double-sided adhesive carbon tape. After coating vertically with gold-palladium, the
307 samples were photographed at an accelerator potential of 10.00 kV.

308 **3.9 Thermal characterization of apple seed flours**

309 The thermal characteristics of flour samples were studied using Mettler Toledo DSC-1 STAR[®]
310 System. Samples (3.5 mg) were weighed into aluminum pans and mixed with Milli Q water (8 µL).
311 The pans were sealed hermetically and allowed to equilibrate for 1 h before analysis. The heat rate
312 was 10 °C/min over a temperature range of 20 to 180 °C in a nitrogen atmosphere. An empty
313 platinum pan was used as the reference. From the curve, enthalpy of gelatinization (ΔH), the onset
314 (To), peak (Tp), and end (Tc) temperatures were obtained using the data processing software supplied
315 with the DSC instrument.

316 **3.10. Techno-functional properties**

317 **3.10.1 Water/oil absorption capacity (WAC/OAC)**

318 The samples (1 g) were poured in a pre-weighed centrifuge tube and mixed with 10 mL of
319 water/oil. The tubes were vortexed for 5 min and then allowed to stand for 20 min at room
320 temperature. Thereafter tubes were centrifuged (5810R, Eppendorf, Germany) at 3000 rpm for 30 min
321 and supernatant (distilled water/oil) decanted and wet residue weighed for determination WAC and
322 OAC.

323 **3.10.2 Emulsification properties**

324 Emulsion capacity (EC) and stability (ES) were determined according to the method described by
325 Hussain *et al.*²³. Flour samples (0.5 g) were mixed with 5 ml distilled water in centrifuge tubes and
326 vortexed for 30 s. 5 ml oil was added to the suspension, homogenized, and then centrifuged at 1100g

327 for 5 min (Eppendorf, Germany). The emulsification activity expressed in percentage was calculated
328 as:

329
$$\text{Emulsion capacity (\%)} = \frac{H_2 - H_1}{H_1} \times 100 \quad (9)$$

330 Where H_2 is the height of the emulsified layer in the tube and H_1 is the height of total contents before
331 centrifugation

332 The emulsification stability was estimated by further heating the emulsion for 30 min at 80 °C
333 and centrifuged at 1100g for 5 min and expressed as:

334
$$\text{Emulsion stability (\%)} = \frac{H_t}{H_2} \times 100 \quad (10)$$

335 Where H_t is the height of the emulsified layer after heating and H_2 is the height of the emulsified layer
336 before heating.

337 **3.11 Dynamic rheology**

338 The rheological analysis was carried out using MCR 102 rheometer (ANTON Par, Austria).
339 The flour suspensions (1% w/v) were prepared with Milli-Q water. The suspensions were
340 homogenized at 1000 × g for 20 min and thereafter equilibrated at room temperature for 5 min. The
341 homogenized samples were subjected to frequency sweep testing from 0.1- 100 rad/s performed at 25
342 °C for determining the changes in storage modulus (G') and loss modulus (G'') occurring during the
343 test.

344 **3.12 Statistical analysis**

345 All experiments have been carried out in triplicates and data were statistically analyzed by one-
346 way analysis of variance test (ANOVA) using commercial statistical package SPSS version 16.0
347 (USA) to establish a significant difference between samples ($p \leq 0.05$ level). The results reported are
348 expressed as mean ± standard deviation.

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- 418

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424 **Author contributions:**

425 **Mehnaza Manzoor** planned this research work, conducted all the experimental work and also
426 wrote original draft **Dr. Jagmohan Singh** revised the manuscript.

427 **Competing interests statement**

428 The authors declare no competing interests.

429 **Additional information**

430 Correspondence and requests for material should be addressed to M.M

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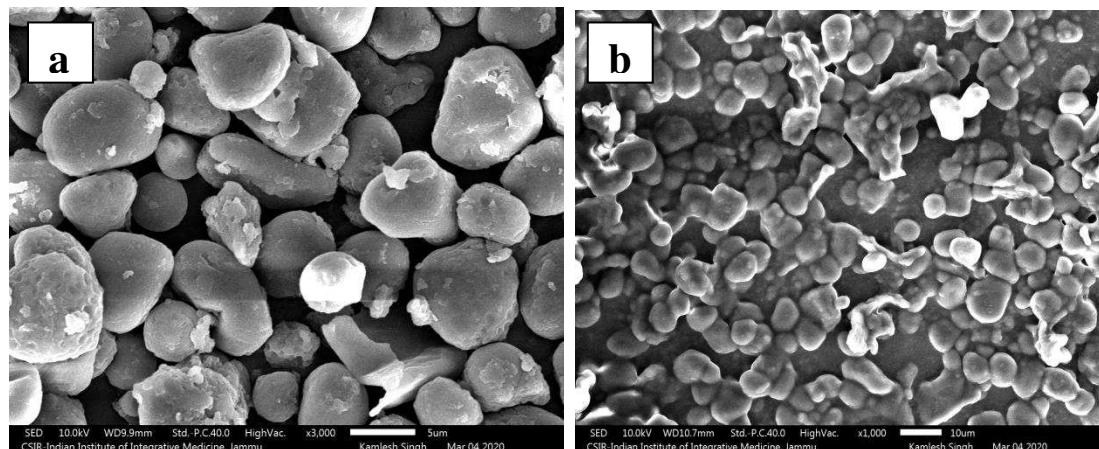
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437 **Figures:**

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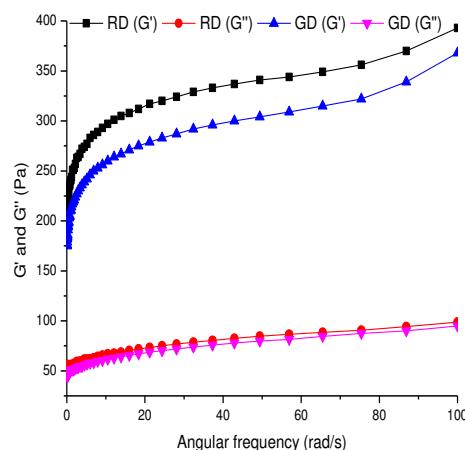


440 Fig. 1 Micrograph of (a) golden delicious seed flour (b) red delicious seed flour at x 1k and x

441 3k

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445 Fig. 2 Loss (G'') and storage (G') modulus of red (RD) and golden delicious (GD) seed flour

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454 **Tables:**

455 **Table 1 Geometrical properties of apple seeds (n = 3)**

Parameter	RD	GD
Length (mm)	9.43 ± 1.07 ^a	7.69 ± 0.43 ^a
Width (mm)	4.87 ± 0.15 ^a	4.02 ± 0.01 ^b
Thickness (mm)	2.01 ± 0.14 ^a	2.03 ± 0.13 ^a
Geometric mean diameter (mm)	4.51 ± 0.20 ^a	3.97 ± 0.11 ^b
Arithmetic mean diameter (mm)	5.44 ± 0.39 ^a	4.58 ± 0.15 ^b
Seed volume (mm ³)	29.08 ± 3.63 ^a	20.22 ± 1.65 ^b
Sphericity (%)	48.18 ± 4.06 ^a	51.72 ± 2.31 ^a
Surface area (mm ²)	64.19 ± 5.95 ^a	49.57 ± 2.96 ^b
Aspect ratio (%)	52.05 ± 4.73 ^a	52.38 ± 2.91 ^a
Thousand-grain weight (wt.g)	45.32 ± 0.47 ^a	44.83 ± 0.22 ^a

456 Different superscript on mean values with standard deviation (±) in same row indicates
457 statistical difference (p ≤ 0.05).

458 RD and GD represent red delicious seed flour and golden delicious seeds.

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462 **Table 2 Physiochemical properties of flour samples (n = 3)**

Cultivar	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)
RDF	8.17 ± 0.17 ^a	47.98 ± 2.04 ^a	25.31 ± 0.99 ^a	4.35 ± 0.73 ^a
GDF	8.57 ± 0.25 ^a	41.31 ± 2.52 ^b	21.47 ± 1.12 ^b	4.67 ± 0.60 ^a

463 Different superscript on mean values with standard deviation (±) in same column indicates
464 statistical difference (p ≤ 0.05).

465 RDF and GDF represent red delicious seed flour and golden delicious seeds.

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469 **Table 3 Particle size distribution and average hydrodynamic particle size and
470 flowability properties of flour samples (n = 3)**

Parameter	RDF	GDF
Particle size distribution (μm)		
Dv(10)	6.26 ± 2.20 ^a	2.60 ± 3.910 ^b
Dv(50)	7.58 ± 3.80 ^a	4.41 ± 2.90 ^b
Dv(90)	9.06 ± 1.10 ^a	6.12 ± 2.30 ^b
Average hydrodynamic particle size (μm)		
Polydispersity index	8.73 ± 4.10 ^a	2.97 ± 2.10 ^b
Flowability properties		
Bulk density (g/mL)	0.38 ± 0.10 ^a	0.604 ± 0.09 ^b
Tapped density (g/mL)	0.58 ± 0.17 ^a	0.69 ± 0.13 ^b
Carr's index (%)	34.51 ± 2.99 ^a	37.70 ± 1.62 ^a
Hausner ratio (%)	1.52 ± 0.06 ^a	1.6 ± 0.04 ^a

471 Different superscripts on mean values with standard deviation (\pm) in same row indicate
 472 statistical difference ($p \leq 0.05$).
 473 Dv (10), Dv (50) and Dv (90) symbolize the points in the size distribution upto which 10%,
 474 50% and 90% of total volume of material in sample is contained.
 475 RDF and GDF represent red delicious seed flour and golden delicious seed flour.
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 478

479 **Table 4 Color, thermal properties and techno-functional properties of flour samples (n
 480 =3)**

Parameter	RDF	GDF
Color		
L*	76.87 \pm 0.52 ^b	78.93 \pm 1.02 ^a
a*	0.97 \pm 0.01 ^b	1.31 \pm 0.12 ^a
b*	9.18 \pm 0.41 ^b	10.64 \pm 0.37 ^a
Thermal properties		
Onset temperature (°C)	89.07 \pm 2.94 ^a	78.95 \pm 1.69 ^b
Peak temperature (°C)	102.17 \pm 2.15 ^a	98.37 \pm 1.25 ^b
Conclusion temperature (°C)	117.53 \pm 1.66 ^a	108.71 \pm 2.49 ^b
Enthalpy of gelatinization (J/g)	18.41 \pm 0.63 ^a	9.78 \pm 1.33 ^b
Technofunctional properties		
WHC (g/100g)	327.65 \pm 1.43 ^a	312.58 \pm 1.57 ^b
OHC (g/100g)	92.97 \pm 2.04 ^a	86.76 \pm 1.74 ^b
EA (%)	79.61 \pm 1.43 ^a	72.01 \pm 0.62 ^b
ES (%)	51.81 \pm 1.07 ^a	46.56 \pm 1.17 ^b

481 Results are mean \pm standard deviation.
 482 Mean values with different letters in the same row indicate a statistical difference ($p \leq 0.05$).
 483 RDF, GDF, WHC, OHC, EA, and ES represent red delicious seed flour, golden delicious
 484 seed flour, water holding capacity, oil holding capacity, emulsion activity, and emulsion
 485 stability, respectively.
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