

## Original Research Article

### **Effect of **processing** on Nutritional, Organoleptic quality, and Storage Stability of Cowpea (**Vigna unguiculata** (L).Walp)**

#### **Abstract:-**

**Aim:** The aim of the study was to assess the changes that occur in the nutritional, organoleptic and storage stability of the cowpea variety Pant Lobia-5 in order to identify the best processing technique that may be taken up for commercialization

#### **Study Design: Experimental study design**

Place and Duration of the study: Department of Foods and Nutrition, College of Home Science, G.B. Pant University of Agriculture and Technology, Pantnagar, between July, 2016-June 2017.

**Methodology:** Cowpea (*Vigna unguiculata* (L). Walp) variety 'Pant lobia-5' was procured from the cowpea field of Breeder Seed Production Centre (BSPC), G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India). Whole Cowpea seeds were processed into Dehulled and Split forms. For the purpose of analysis the three forms of Cowpea were powdered and analyzed for Proximate principals, mineral composition (Calcium, Iron, zinc, and magnesium), amino acid profile (Methionine, Lysine and tryptophan), dietary fibre content and antioxidant activity ( total flavonoids, total phenolics and total antioxidant activity). "Dal"™ the common Indian pulse food was prepared from the three forms of cowpea and analyzed for sensory qualities. Storage stability of the three forms of the cowpea was also evaluated. In order to identify the differences in the three forms of the pulse the results were statistically analyzed through One way ANOVA (Analysis of variance technique).

Results: The results revealed that whole cowpea seeds of Pant lobia-5 were nutritionally superior to the processed forms. However among the two processed forms, dehulled seeds had the highest protein content, specifically the amino acid tryptophan. Organoleptically too dehulled seeds were found to be superior to the split and whole seeds. Storage studies showed that all forms of cowpea seeds can be stored safely up-to six months in air tight plastic containers.

**Key words:-** Whole cowpea seeds, Processed cowpea seeds, Nutritional quality, Organoleptic properties, Storage stability

## 1.INTRODUCTION

Cowpea can be processed into various different forms. Processing of cowpea involves the removal of hull. Cowpea husks were derived as a by-product of the processed pulse. The processing helps in eliminating tannins and trypsin inhibitor from the pulse [1]. Decortication and dehulling of cowpeas is done to improve appearance, texture, aroma and taste of the legume and reduce the cooking time [2,3]. Dehulling process decreases the stachyose and removes tannins. Therefore dehulling should be encouraged[4].After decortication of seeds there is very little tannin present in the cotyledons which indicates that almost all the tannins are usually present in the seed coat. The anti-nutritional problems in cowpea can be easily avoided with dehulling and heat treatments [5].

The process of dehulling increases the protein, starch, phytic acid, stachyose and verbascose content of pulses; at the same time it lowers the tannin, sucrose, raffinose and trypsin inhibitor activity [6]. Dehulled seeds have improved palatability and taste [7]. Studies have shown that dehulling reduced cooking time by removing the impermeable seed coat of bean, which hindered water uptake during cooking .It has been found that whole seeds are less desirable for cooking than dehulled seeds[8].

Cowpea (*Vigna unguiculata*(L).Walp) is a warm season crop commonly referred as *Lobia* in India.It has other local names viz Black eye pea, Crowder Pea, Neibe and Frijole, Southern pea [9].Cow Pea has a high protein content (18-35%);along with a good amino acid profile which can supplement cereal grains deficient in lysine [2]. The carbohydrate and vitamin content of the pulse is also good.[10].Cowpea contains good amounts of protein, dietary fibre, a variety of micronutrients e.g.-

minerals (calcium and iron) and vitamins and phytochemicals[11]. Whole of the cowpea crop is edible, as whole of the plant is rich in terms of its nutrient quotient and fibre. Cowpea is rich in protein, vitamins, minerals, unsaturated fatty acids, antioxidants, phenolic compounds, and soluble and insoluble fiber[12].

For the present study the cowpea variety, *Pant lobia-5* released by G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India was selected. *Pant lobia -5* is a popular early maturing variety. It has oval light brown colour seed with 1000 seed weight of 170-180g. The yield of *Pant lobia-5* is 2161 kg/ ha under good management and favourable environment conditions. Its growth habits are erect bush type with early maturity. Also the seeds have high level of resistance to CYMV (Cowpea yellow mosaic virus) and bacterial blight. It also has high level of tolerance to aphid, thrips and bruchid.

Little or no information is available on quality characters of processed cowpea seeds. Therefore, the study was undertaken to analyze the nutritional quality, mineral composition, amino acid content, total dietary fibre content, antioxidant properties of two processed forms of cowpea along with the unprocessed or whole seed. In addition consumer acceptability and storage stability of the three forms of cowpea seeds were evaluated through organoleptic studies

## 2.MATERIAL AND METHODS:

**2.1 Procurement and processing of sample:** Samples of indigenous variety of cowpea (*Vigna unguiculata* (L). Walp) '*Pant lobia-5*' was procured from the cowpea field of Breeder Seed Production Centre (BSPC), G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India). Procured cowpea seeds were cleaned and sorted for blemishes. Cowpea sample was divided into two parts, one that was taken for dehulling and splitting and other was left with hull. Processing viz., dehulling and splitting of *Pant lobia -5* was done in Mini Dal Mill present in the Department of Post-Harvest Processing and Food Engineering, College of Technology, G.B.P.U.A.T, Pantnagar. After processing, cowpea seeds were sorted and dehulled, split and whole seeds were separated. The samples of cowpea seeds were then ground in an electric grinder (Bajaj electric grinder). The prepared powder was used for nutritional analysis.

**2.2 Percent recovery of cowpea seeds:** Percent recovery was calculated by dividing the amount of cowpea seeds obtained after processing from the amount of initial sample taken.

$$\text{Percent recovery of grain} = \frac{\text{Weight of processed grain}}{\text{Initial weight of grain}} \times 100$$

**2.3 Proximate composition:** Processed cowpea seeds were analysed in triplicate for proximate composition such as per cent moisture, crude protein, total ash, crude fat and crude fibre, carbohydrate by difference and physiological energy. Proximate composition was analysed by AOAC 2000 method[13]. The physiological energy (Kcal/100g) of sample was calculated by summing up the product of multiplication of per cent carbohydrate, crude protein and crude fat in the sample by 4, 4 and 9 respectively[14].

**2.4 Mineral composition:** Mineral content of processed cowpea seeds (Calcium, iron, zinc, and magnesium) were estimated using Atomic Absorption Spectrophotometer (AAS) by standard method[13]. Ash solutions were prepared using wet ash procedure using tri-acids [15].

**2.5 Amino acids composition:** The amino acids composition of processed cowpea seeds (Methionine, Lysine and tryptophan) were analyzed by standard method[16].

**2.6 Total dietary fibre:** Total dietary fibre content of processed cowpea seeds was estimated by the standard method[17].

**2.7 Antioxidant activity:** Antioxidant activity such as total flavonoids, total phenols and total antioxidant activity was analysed by standard method[18,19,20].

**2.8 Sensory evaluation:** Processed cowpea dal (whole, dehulled and split) was evaluated for sensory characteristics using Nine Point Hedonic Scale and Sensory Score Card method[21]. Sensory evaluation was done by semi trained panel consisting of 30 members.

**2.9 Storage stability of cowpea seeds:** The storage stability of cowpea seeds was done by storing the three forms (whole, dehulled and split) in air tight plastic containers for a period of 6 months. The cowpea seeds were checked for spoilage after one month. The moisture content and per cent weevil seeds were the parameters used for assessing the storage life of cowpea seeds.

**2.10 Statistical analysis:** The data was analysed for Mean  $\pm$  S.D and One way ANOVA using FORTRAN 95 software[22].

### 3. RESULTS AND DISCUSSION:

#### 3.1 RESULTS

**3.1.1 Percent recovery of cowpea seeds:** The percent recovery of cowpea seeds (*Pant lobia-5*) was found to be 88.28 per cent. The broken grains and husk of cowpea seeds accounted for 0.857 per cent and 10.85 per cent recovery respectively.

**3.1.2 Proximate composition:** The results of proximate composition are presented in Table 1. The whole *Pant lobia-5* seeds were found to have 13.86% moisture, 22.62% crude protein, 3.48% total ash, 1.15% fat, 4.85% fibre, 54.04% carbohydrate and 316.99 Kcal physiological energy. On the other hand, dehulled and split *Pant lobia-5* seeds were found to have 12.96% and 12.82% moisture, 23.49% and 21.09% crude protein, 3.20% and 3.31% total ash, 1.00% and 1.09% fat, 3.00% and 4.30% fibre, 56.35% and 57.39% carbohydrate and 328.36 Kcal/100g and 323.73Kcal/100g physiological energy respectively.

Among all the proximate principles evaluated, whole *Pant lobia-5* seeds were found to have higher moisture, total ash, crude fibre and crude fat content as compared to other processed forms. Dehulled *Pant lobia-5* seeds were found to have higher protein (23.49%) and physiological energy (328.36 kcal/100g) as compared to other processed forms. The value for carbohydrate content was found to higher in split *Pant lobia-5* seeds.

**3.1.3 Minerals composition:** The results of mineral composition are presented in Table 2. Whole *Pant lobia-5* seeds were found to be a rich source of minerals. It had 7.81 mg/100g iron, 154.2 mg/100g magnesium, 76.03mg/100g calcium and 4.50 mg/100g zinc. There was no significant change in the calcium; magnesium and zinc content of cowpea seeds.

**3.1.4 Amino acids composition:** The results of amino acids composition are presented in Table 3. Methionine (80.03 mg/g Nitrogen) and lysine (5.47 g/16g Nitrogen) content of whole *Pant lobia-5* seeds was found to be higher than other processed forms. Tryptophan content of dehulled *Pant lobia-5* seeds was significantly higher i.e. 0.990 g/16g Nitrogen) than other processed forms.

**3.1.5 Total dietary fibre content:** The results of total dietary fibre composition are presented in Table 4. The total dietary fibre content of whole *Pant lobia-5* was found to be 30.35mg/100g which was significantly higher than the other processed forms.

**3.1.6 Antioxidant activity:** The results of antioxidant activity are presented in Table 5. All three antioxidant principles evaluated were found to be higher in whole *Pant lobia-5* seeds. The whole *Pant lobia-5* seeds was found to have total phenolic content of 79.43mg GAE/ 100g, total flavonoids of 164.6mg RE/100g and total antioxidant activity (DPPH Scavenging activity) of 91.40 mg T.E./100 gm respectively.

**3.1.7 Sensory evaluation:** The result of sensory evaluation of cowpea dal using score card method is presented in Fig 1, 2& 3. It was observed that among the three processed forms of cowpea (whole, dehulled and split form), dehulled cowpea dal was liked very much by 56.6% respondents whereas whole cowpea dal was liked by 20% and split cowpea dal was liked by 13.32%. The data for sensory evaluation through score card method was presented in table 6 showed that the overall acceptability of dehulled cowpea seeds was found to be higher than the other processed forms i.e. 7.65.

**3.1.8 Storage stability:** The data on moisture content for storage stability is presented in table 7. The results of storage stability of different forms of cowpea seeds showed that the moisture content increases progressively from first month to the sixth month. The average moisture content of whole cowpea seeds was highest in the beginning i.e. 11.46 per cent whereas the moisture content of dehulled cowpea seeds was low in the beginning of shelf life study i.e. 10.54 Per cent. At the end of study period of 6 months whole cowpea seeds showed moisture content of 13.68 per cent followed by split dal (13.38 per cent) and dehulled seeds showed minimum moisture content at end of the study period i.e. 13.31 per cent.

The cowpea seeds were checked for spoilage by weevil after one month. The study found that there were no weevil seeds during the initial periods of storage study as well as after the end of the study period. The storage stability revealed that the processed cowpea seeds can be stored for a period of 6 months in plastic containers. As the codex standards for storage of cowpea seeds showed moisture content of 15-18 per cent which is based on different climatic conditions.

159 **Table 1: Proximate composition of cowpea seeds**

| Proximate compositions                 | Whole <i>Pant lobia-5</i> | Dehulled <i>Pant lobia-5</i> | Split <i>Pant lobia-5</i> |
|----------------------------------------|---------------------------|------------------------------|---------------------------|
| Moisture %                             | 13.86 <sup>a</sup> ±0.30  | 12.96 <sup>b</sup> ±0.41     | 12.82 <sup>b</sup> ±0.32  |
| Total ash %                            | 3.48 <sup>a</sup> ±0.02   | 3.20 <sup>a</sup> ±0.03      | 3.31 <sup>a</sup> ±0.05   |
| Crude fibre%                           | 4.85 <sup>a</sup> ±0.02   | 3.00 <sup>b</sup> ±0.10      | 4.30 <sup>c</sup> ±0.03   |
| Crude fat%                             | 1.15 <sup>a</sup> ±0.01   | 1.00 <sup>b</sup> ±0.01      | 1.09 <sup>b</sup> ±0.02   |
| Crude protein%                         | 22.62 <sup>a</sup> ±0.01  | 23.49 <sup>b</sup> ±0.02     | 21.09 <sup>c</sup> ±0.05  |
| Carbohydrate by difference %           | 54.04 <sup>a</sup> ± 0.15 | 56.35 <sup>a</sup> ±0.95     | 57.39 <sup>a</sup> ±0.05  |
| Physiological energy value (Kcal/100g) | 316.99 <sup>a</sup> ±2.02 | 328.36 <sup>b</sup> ±4.32    | 323.73 <sup>b</sup> ±4.05 |

160 \*Values are on as is basis

161 Means in each column for each form of cowpea followed by the different letter (a, b & c) are  
 162 significantly different ( $P \leq 0.05$ )

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164 **Table 2: Mineral composition of cowpea seeds**

| Minerals                   | Whole <i>Pant lobia-5</i> | Dehulled <i>Pant lobia-5</i> | Split <i>Pant lobia-5</i> |
|----------------------------|---------------------------|------------------------------|---------------------------|
| Calcium Content (mg/100g)  | 76.03 <sup>a</sup> ±0.05  | 70.00 <sup>b</sup> ±0.15     | 76.00 <sup>a</sup> ±0.02  |
| Iron Content(mg/100g)      | 7.81 <sup>a</sup> ±0.07   | 7.20 <sup>b</sup> ±0.02      | 7.65 <sup>c</sup> ±0.05   |
| Magnesium Content(mg/100g) | 154.2 <sup>a</sup> ±0.05  | 147.2 <sup>b</sup> ±0.5      | 154.0 <sup>a</sup> ±0.1   |
| Zinc content(mg/100g)      | 4.5 <sup>a</sup> ±0.03    | 4.05 <sup>a</sup> ±0.02      | 4.45 <sup>a</sup> ±0.01   |

165 \*Means in each column for each form of cowpea followed by the different letter (a & b) are  
 166 significantly different ( $P \leq 0.05$ )

167 **Table 3: Amino acids composition of cowpea seeds**

| Amino acids                 | Whole <i>Pant lobia-5</i> | Dehulled <i>Pant lobia-5</i> | Split <i>Pant lobia-5</i> |
|-----------------------------|---------------------------|------------------------------|---------------------------|
| Methionine (mg/g Nitrogen)  | 80.03 <sup>a</sup> ±0.02  | 78.94 <sup>b</sup> ±0.01     | 80.00 <sup>a</sup> ±0.01  |
| Tryptophan (g/16g Nitrogen) | 0.950 <sup>a</sup> ±0.20  | 0.990 <sup>b</sup> ±0.03     | 0.849 <sup>c</sup> ±0.08  |
| Lysine (g/16g Nitrogen)     | 5.47 <sup>a</sup> ±0.2    | 5.20 <sup>b</sup> ±0.1       | 5.13 <sup>c</sup> ±0.52   |

168 \*Means in each column for each form of cowpea followed by the different letter (a & b) are  
 169 significantly different ( $P \leq 0.05$ )

170 **Table 4: Total dietary fibre content (mg/100g) of cowpea seeds**

| Dietary fibre           | Whole <i>Pant lobia-5</i> | Dehulled <i>Pant lobia-5</i> | Split <i>Pant lobia-5</i> |
|-------------------------|---------------------------|------------------------------|---------------------------|
| Soluble dietary fibre   | 8.62 <sup>a</sup> ±0.03   | 3.20 <sup>b</sup> ±0.01      | 8.15 <sup>c</sup> ±0.03   |
| Insoluble dietary fibre | 21.73 <sup>a</sup> ±0.04  | 24.15 <sup>b</sup> ±0.05     | 20.38 <sup>a</sup> ±0.02  |
| Total dietary fibre     | 30.35 <sup>a</sup> ±0.02  | 27.35 <sup>b</sup> ±0.05     | 28.53 <sup>b</sup> ±0.08  |

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172 \*Means in each column for each form of cowpea followed by the different letter (a & b) are  
 173 significantly different ( $P \leq 0.05$ )

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175 **Table 5: Antioxidant activity of cowpea seeds**

| Antioxidant activity                                        | Whole <i>Pant lobia-5</i> | Dehulled <i>Pant lobia-5</i> | Split <i>Pant lobia-5</i> |
|-------------------------------------------------------------|---------------------------|------------------------------|---------------------------|
| Total phenolic content (mg Gallic acid equiv./100g)         | 79.43 <sup>a</sup> ±0.7   | 47.83 <sup>b</sup> ±4.2      | 75.25 <sup>b</sup> ±5.0   |
| Total flavonoid content (mg Rutin equiv./100g)              | 164.6 <sup>a</sup> ±2.00  | 150.2 <sup>b</sup> ±3.2      | 163.8 <sup>a</sup> ±2.5   |
| Total antioxidant activity (mg Trolox equivalent per 100 g) | 91.4 <sup>a</sup> ±0.02   | 58.52 <sup>b</sup> ±0.50     | 90.2 <sup>c</sup> ±0.03   |

176 \*Means in each column for each form of cowpea followed by the different letter (a & b) are  
 177 significantly different ( $P \leq 0.05$ )

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185 **Table 6: Sensory evaluation of cowpea dal using Score card method**

| Cowpea dal                   | Taste             | Colour            | Consistency       | Flavour           | Overall acceptability |
|------------------------------|-------------------|-------------------|-------------------|-------------------|-----------------------|
| Whole <i>Pant lobia-5</i>    | 7.55 <sup>a</sup> | 7.75 <sup>a</sup> | 6.90 <sup>a</sup> | 7.30 <sup>a</sup> | 7.25 <sup>a</sup>     |
| Dehulled <i>Pant lobia-5</i> | 7.87 <sup>a</sup> | 7.05 <sup>b</sup> | 7.75 <sup>b</sup> | 7.70 <sup>b</sup> | 7.65 <sup>b</sup>     |
| Split <i>Pant lobia-5</i>    | 6.95 <sup>b</sup> | 6.40 <sup>c</sup> | 6.85 <sup>a</sup> | 6.90 <sup>c</sup> | 6.87 <sup>c</sup>     |
| SEM±                         | 0.147             | 0.138             | 0.121             | 0.143             | 0.141                 |
| C.D. at 5%                   | 0.030             | 0.0001            | 0.002             | 0.072             | 0.081                 |

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187 \*Means in each row for each processed form of cowpea followed by the different letter (a,b,c) are  
 188 significantly different ( $P \leq 0.05$ ). Values are mean of 30 observations.

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192 **Table 7: Moisture content of cowpea seeds for storage stability**

| Study period | Whole <i>Pant lobia-5</i> | Dehulled <i>Pant lobia-5</i> | Split <i>Pant lobia-5</i> |
|--------------|---------------------------|------------------------------|---------------------------|
| 1st month    | 11.46                     | 10.54                        | 11.44                     |
| 2nd month    | 11.76                     | 10.65                        | 12.01                     |
| 3rd month    | 12.06                     | 11.02                        | 12.58                     |
| 4th month    | 12.21                     | 12.55                        | 12.81                     |
| 5th month    | 12.28                     | 13.19                        | 13.15                     |
| 6th month    | 13.68                     | 13.31                        | 13.38                     |
| SEM±         | 0.313                     | 0.303                        | 0.154                     |

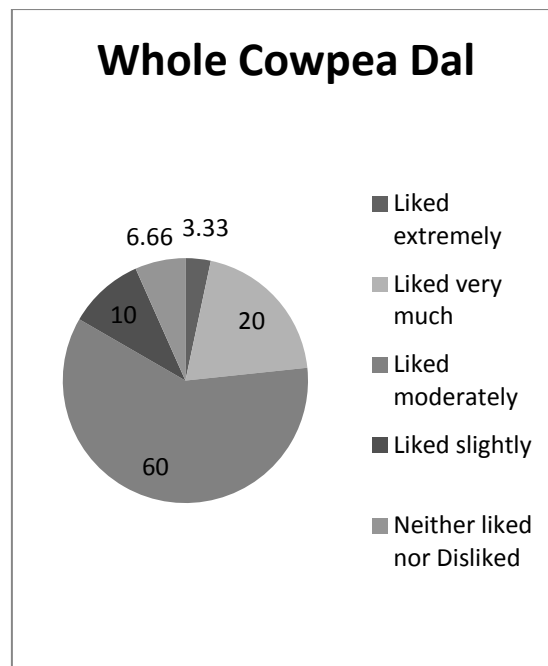
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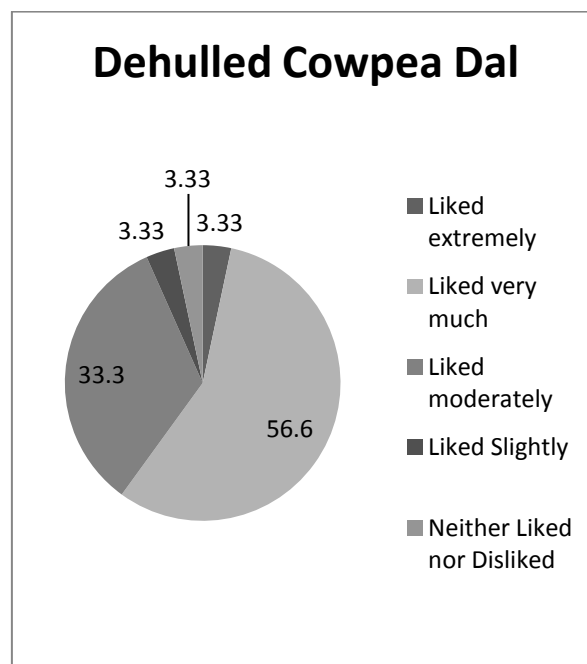
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199 **Fig 1: Sensory evaluation of Whole cowpea dal using Nine Point Hedonic Scale**



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201 **Fig 2: Sensory evaluation of Dehulled cowpea dal using Nine Point Hedonic Scale**

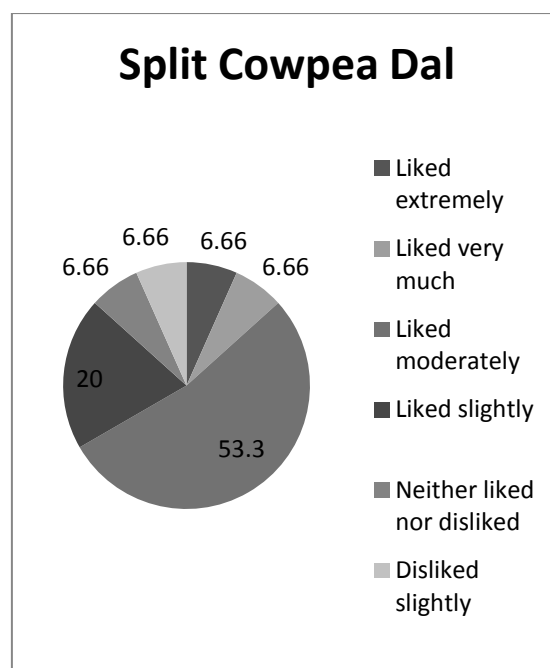


**Fig 3: Sensory evaluation of Split cowpea dal using Nine Point Hedonic Scale**

**3.2 DISCUSSION:** The study reveals that dehulling increases the protein content of the test variety (Pant Lobia-5) from 22.62 Per cent in whole seeds to 23.49 Per cent. Other studies show similar results where dehulled cowpea flour was found to have 23.12 per cent protein whereas whole cowpea flour contained 22.85 per cent protein. Therefore processing brings incremental change in protein content of cowpea [1].

The storage study revealed that the processed cowpea seeds can be stored for a period of 6 months in plastic containers fulfilling all the codex standards. It emerges from the sensory evaluation study that dehulled cowpea seeds are organoleptically superior to split and whole cowpea seeds.

On the basis of other nutritional parameters though whole Cowpea seeds are superior as compared to the processed forms. Various researchers reported that the whole cowpea seeds contain 13.4 per cent moisture, 3.2 per cent total ash, 24.1 per cent protein, 1 per cent fat and 3.8 per cent crude fibre [23].

Mineral composition and amino acid composition of whole cowpea seeds were better in addition to different antioxidant properties. The findings of the present study are in line with a study that reported that the hull shows the highest antioxidant activity and therefore the whole seeds containing hull have the highest antioxidant values [24].

#### 4. CONCLUSION:

The present study evaluated the nutritional, organoleptic and storage stability of different forms of cowpea (whole, dehulled and split) seeds. It was found that whole *Pant lobia-5* seeds have a higher nutritional value. The study establishes that cowpea seeds can also be utilized in dehulled and split forms besides the traditional use as whole cowpea seed. Processing of cowpea seeds may be promoted because the above study reveals that there are no statistically significant change in the proximate composition of the whole, split and dehulled cowpea seeds. In fact the protein content of dehulled cowpea seeds shows significant increase. Processing reduces the crude fat content which may prove beneficial for health. Though there is a significant decrease in crude fibre content, there is no change in the total dietary fibre. Regarding minerals, it was found that except for calcium content; the zinc, magnesium and iron levels do not change significantly on processing. There is no change in the amino acid content. Processing reduces the antioxidants which are seen in a concentrated form in the husk. Dehulling of cowpea seeds has emerged as a better processing technique as compared to splitting because the protein and specifically the tryptophan content increases due to this process.

The storage studies of processed cowpea seeds further showed that processing does not bring about a significant change in the storage stability of the processed cowpea seeds upto six months when stored in air tight plastic container. Sensory evaluation of the different forms of cowpea showed that dehulled cowpea dal had the highest acceptance. This might be because dehulled dal gave a new taste to the consumers; as mostly cowpea dal is consumed in whole form.

Looking onto its health properties, processed cowpea seeds can be proposed for commercial production in order to increase its consumption thereby increasing the protein intake of the vulnerable population.

#### COMPETING INTERESTS

“Authors have declared that no competing interests exist.”

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## 307 APPENDICES

### 308 Appendix I: NINE POINT HEDONIC SCALE

309

310 Name:

Date:

311 Product:

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313 **Hedonic scale:**

314 Taste the following products and check how much you liked or disliked them.

315

| Hedonic Scale              | Product code |   |   |   |
|----------------------------|--------------|---|---|---|
|                            | A            | B | C | D |
| Liked extremely            |              |   |   |   |
| Liked very much            |              |   |   |   |
| Liked moderately           |              |   |   |   |
| Liked slightly             |              |   |   |   |
| Neither liked nor disliked |              |   |   |   |
| Disliked slightly          |              |   |   |   |
| Disliked moderately        |              |   |   |   |
| Disliked very much         |              |   |   |   |
| Disliked extremely         |              |   |   |   |

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319 **Comments:**

**Signature:**

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## 326 **Appendix II: SENSORY SCORE CARD**

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328 Name:

Date:

329 Product:

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### 331 **Score card:**

332 Taste the following products and check how much you liked or disliked them.

333 Scoring scale: 1-2 Very poor, 3-4 Poor, 5-6 Fair, 7-8 Good, 9-10 Very good

334

| Product code  | Taste | Colour&Appearance | Consistency | Flavour | Overall Acceptability |
|---------------|-------|-------------------|-------------|---------|-----------------------|
| Maximum score | 10    | 10                | 10          | 10      | 10                    |
| <b>A</b>      |       |                   |             |         |                       |
| <b>B</b>      |       |                   |             |         |                       |
| <b>C</b>      |       |                   |             |         |                       |
| <b>D</b>      |       |                   |             |         |                       |

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338 **Comments:**

**Signature:**

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