

Research Article

# Loss of seed viability in onion (*Allium cepa* L.) in relation to degradation of lipids during storage

### Vishwanath Rohidas Yalamalle\*

ICAR-Directorate of Onion and Garlic Research, Pune-412105 (Maharashtra), India Nilesh Nivrutti Gaikwad ICAR-National Research Center on Pomegranate, Solapur- 413 255 (Maharashtra), India Dinesh Manohar Ithape ICAR-Directorate of Onion and Garlic Research, Pune-412105 (Maharashtra), India Ashok Kumar ICAR-Directorate of Onion and Garlic Research, Pune-412105 (Maharashtra), India Kalyani Gorrepati ICAR-Directorate of Onion and Garlic Research, Pune-412105 (Maharashtra), India Major Singh ICAR-Directorate of Onion and Garlic Research, Pune-412105 (Maharashtra), India

\*Corresponding author. E-mail: vishwanath yal@yahoo.com

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#### Abstract

Onion seeds have one of the lowest longevity among the cultivated crops as it loses viability within a year. The unmarketable seeds have no reported food value or feed value. Onion seeds contain considerable oil content which has potential industrial utility. The present study reports the changes in the quality of seed oil extracted from seeds stored for one year (2019-2020), after which the seeds were unmarketable due to low germination. The physico-chemical properties of seed oil were studied in four onion (*Allium cepa* L.) varieties (Bhima Safed, Bhima Super, Bhima Dark Red and Bhima Shakti) by standard analytical test. The per cent oil content varied from 12.94% - 16.02% and storage resulted in 0.64% loss in seed oil content. The mean seed germination in freshly harvested seeds was 80.50%, which declined to 52.33% after one year of ambient storage. The electrical conductivity, which measures the membrane integrity increased by 108.60% in one-year-old seeds. The physico-chemical properties of the seed oil deteriorated with the age of the seed. In comparison to the control, the acid values and peroxide values of the seed oil increased by 51.11% and 12.90% respectively, while saponification value decreased by 36.18%. Among the varieties, Bhima Dark Red had the highest degradation in the seed storability and vigour, which also had the highest deterioration in the lipid quality. The present study reports for the first time the degradation of seed lipids during ageing in onion which might be contributing to the poor storability of onion seeds.

Keywords: Acid value, Onion seed oil, Peroxidase value, Saponification value, Seed viability

### INTRODUCTION

Onion (*Allium cepa* L.) is an important vegetable crop grown in almost all parts of the world as a flavouring agent in food preparation. The medical value of onion is well-documented, its regular consumption reduces the risk of cardiovascular diseases (Challier, *et al.*, 1998) and it has anti-inflammatory (Wilson and Demmig□Adams 2007) anti-diabetic (Wu and Xu 2014), anti-microbial (Sobolewska *et al.*, 2016) and hypolipidemic properties (Galavi and Hosseinzadeh, 2020). The onion seeds are also consumed in some of the worlds. The medicinal values of the onion seed oil are also documented. Onion seeds contain 20.4% lipids, 24.8% protein, cysteine derivatives, minerals etc. (Dini *et al.*, 2008). The onion seed oil contains a high proportion of polyunsaturated fatty acid content with linoleic acid being a major constituent (64%–65%). It contains high levels of  $\alpha$ -tocopherol (634 mg/L). The onion seed oil is reported to have anti-inflammatory, antioxidant and antimicrobial properties (Sim and Nyam, 2020). The quality of the seed oil extracted depends on the storages conditions and the storage period (Michael, 1998).

Seed ageing is related to the loses of the ability to scavenge the reactive oxygen species (ROS) and peroxides (Sau *et al.*, 2019; Yalamalle *et al.*, 2019) The seed deterioration process also induce qualitative and quantitative changes in nucleic acids, protein and li-

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pids (Kurek *et al.*, 2019). The ROS produced during ageing leads to peroxidation of the lipids in the membrane. The damages to the cellular membrane lead to loss of membrane integrity and solute leakage. Changes in the lipid quality parameters like acid value, peroxide value and saponification value can provide insight into seed ageing process (lqbal *et al.*, 2002).

Onion seeds have one of the lowest viabilities among cultivated crops. It loses the seed viability withing a year (Yalamalle and Tomar, 2019). Poor germination is not just an issue in stored seeds, but many times freshly harvested seeds are unmarketable due to poor germination (Tajbakhsh *et al.*, 2004). Seed legislation bars the sale of seeds having germination below the minimum prescribed standards, in India, it is 70%. Such seeds are either discarded or admixture with seed lots with higher germination, causing disposal concerns or losses to the seed purchasers/ farmers (Gupta and Sharma, 2014).

Onion seeds are rich in lipids, and generally, seeds with high lipids content are poor storer (Yadav et al., 2013). Apart from use for propagation, there is a limited commercial utility of onion seeds, but have potential industrial uses like biodiesel, industrial lubricants, drying oils, cosmetics etc. Thus there is a need to find the changes in the changes in seed oil quality extracted from seeds after storage. The sale of oils and fats for consumption is governed by quality standards set by international agencies like Codex Standard for Named Vegetable Oils (Codex-standard, 1999) or local agencies like- FSSAI for India (FSSAI, 2015). The oil for consumption has to meet the standards set, if not such oils can be utilized for industrial uses. Some of the quality standards include- peroxidation value is an important method to estimate the oil quality; it indicates the extent of primary oxidation already occurred in the lipid. It is the most important quality parameter tested during production, storage and marketing of oils and fats. The acid value determines the free fatty acid present in the oil. Like peroxidase value, it also measures the relative rancidity as free fatty acid are produced during decomposition of triglycerides. Saponification value represents all the free fatty acid present in an oil (free and esterified). Lipids with high saponification value produce more glycerin and less biodiesel. Oils extracted from coconut, palm kernels, babassu etc. have high saponification value, and they also produce higher foam and are suitable for the soap industry. Physico-chemical characteristics values signify the quality of the oil and hence the economic value of the seed oil.

To best of our knowledge, there are no reports on the changes in the physico-chemical properties of onion seed oil and the quality changes with loss of seed viability. Particularly the changes in seed oil quality of seed lots stored beyond one year, when the seed lots have lost have viability or seeds which are unmarketable under Indian minimum seed certification standards, (2013). The main objective of the present study was to study the changes in seed lipids during storage and to investigate the reasons for poor storability of onion seeds.

# MATERIALS AND METHODS

**Plant material:** Seeds of onion (*Allium cepa* L.) varieties, Bhima Safed, Bhima Super, Bhima Dark Red and Bhima Shakti, was procured from the Seed Unit, ICAR -Directorate of Onion and Garlic Research, Pune, India. The seeds (approximately 1000 g per pack) were hermetically sealed and stored for 12 months at room temperature (12.20°C - 38.55°C). The seeds oil quality was estimated thrice, first one was on freshly harvested seed, the second was after 6 months of storage and third was after 12 months of storage.

Estimation of physico-chemical properties of seed oil: The seed oil was extracted mechanically by using a hydraulic press (Hanora A type, National Engineering, 541 DukEun Dong, Duk Yang Gu, Goyang City, Gyeonggi-Do, South Korea). Clean and dry seeds of each variety (approximately 1 Kg was fed into a hydraulic press, which was operated at 80 °C and 500 kg/cm<sup>2</sup> pressure. The seed oil was vacuum filtered and centrifuged at 8000 rpm for 10 minutes remove any debris. The samples were preserved in ambercoloured bottles, the headspace was purged with liquid nitrogen and stored at 4 <sup>o</sup>C until analysis. The per cent seed oil content in seeds was determined by Soxhlet method, using petroleum ether as solvent at 50 °C. The physico-chemical analysis of the lipids i.e peroxide value- which measures the extent of primary oxidation in the lipid, acid value- which measures free fatty acid present in the oil and saponification value which measures the average chain length of fatty acid was done as per Association of Official Analytical Chemists (AOCS 1990) (i) Peroxide value (method Cd 8-53) (ii) Acid value (method Ca 3a-63) (iii) and Saponification value (method Cd 3-25).

**Electrical conductivity:** In a 50 ml beaker, 100 mg seeds were weighed-in three replicates and to it, 25 mL distilled water was added. Then the beaker was placed at  $20 \pm 1^{\circ}$ C for 24 hours, the leachate was collected and the electrical conductivity (EC) was recorded with the help of a digital conductivity meter along with distilled water as control (Dadlani and Agrawal 1987).

Seed germination and seed vigour: In 150 mmdiameter Petri plates, three replicates of 50 seeds were placed on 150 mm-diameter moistened filter paper discs (Axiva, India catalogue No. 100150R). The Petri plates were placed in a BOD incubator set at 20  $\pm$  1°C for 12 days (ISTA, 2015). The seedling dry weight and the seedling length were recorded on the 12<sup>th</sup> day on 10 randomly selected seedlings. The seed vigour indices were calculated according to Abdul Baki and Anderson (1973): vigour index-I (VI-I) = germination % X average length of 10 seedlings in cm.

#### **RESULTS AND DISCUSSION**

**Seed oil content:** The average oil content in freshly harvested seeds was 14.39%, which was also statistically similar to the seeds stored at 6 months (Table 1). At the end of 12-month storage, there was 0.64% reduction in the oil content. Among the varieties highest oil content was recorded in variety Bhima Shakti (16.65%) and lowest in Bhima Super (12.51%). The results are in agreement with Amalfitano et al., (2020), wherein the average onion oil in Italian variety Ramata di Montoro ranged from 14.9% - 16.5%. The reduction in seed oil content during storage has been previously reported by Neme et al, (2020) in sesame, which may be attributed to enzymatic breakdown of lipids for seed metabolism during storage.

**Germination and seed vigour:** Storage duration had a significant effect on the germination. The average germination in freshly harvested seeds was 80.50%. After 6 months of storage, all varieties except Bhima Super maintained germination above Indian minimum seed standards, (2013) of (70% for germination) (Table 2). At the end of 12-month storge, the average germination was 52.33% and none of the varieties maintained germination above the statutory requirement of 70%. Among the varieties highest reduction in germination was recorded in Bhima Dark Red variety. The storage potential of different varieties differed which might be due to different maternal environments during seed development and/or varietal differences (Thirusendura *et al.*, 2018, Yalamalle *et al.*, 2020).

As expected the vigour of onion seeds decreased with the seed age. The average seedling length in freshly harvest seeds was 10.62 cm. There was 69.64% and 115.41% reduction in seedling length in 6 months and 12-month-old seedlings respectively. By the end of 6 months nearly 100% reduction in the vigour was recorded and by the end of one year there was 218.75% reduction. The electrical conductivity measures the membrane integrity of the seeds. Seed ageing leads to accumulation of ROS, which damages the cellular membrane. The degraded membranes leach the solutes leading to loss of seed viability and vigour (Kurek *et al.*, 2019; Yalamalle *et al.*, 2020). In the present study, there was an increase in the EC with the seed age. In comparison to the freshly harvested seeds, there was 40.83% and 108.60% higher EC in 6 months and 12 months stored seeds, respectively (Table 3). Among the varieties highest mean EC was recorded in variety Bhima Dark Red, signifying higher deterioration. The highest degradation in seed quality parameters was also recorded in Bhima Dark Red variety. The rapid reduction in seed quality in onion, reported previously (Mollah *et al.*, 2016, Yalamalle and Kuchlan 2016) were in there was up to 40- 60% loss in seed viability within a short period under ambient conditions.

**Changes in seed oil quality:** Peroxide value signifies the extent of auto-oxidation of oils and fats. The peroxide value increased with the seed storage period and the highest value (1.36 meq/Kg) was recorded in 12 months stored seeds. After 12 months of storage among varieties, the highest peroxide value was recorded in Bhima Dark Red which also had the lowest seed quality after storage. The results are in agreement with previous studies by (Iqbal *et al.*, 2002) in cotton seeds and Suresh et al., (2018) in *Jatropha curcas* L were in seed ageing increased the peroxida-tion value.

The acid value is the amount of the KOH required to neutralize the free fatty acids present in the oils. Like peroxidase value, it also measures the relative rancidity as free fatty acid are produced during decomposition of triglycerides. Even though there was a considerable reduction in the seed vigour after six months of storage the acid value did not differ significantly and the highest value was recorded after 12 months of storage (0.70 mg KOH g<sup>-1</sup>) (Table 5). Among the varieties, the lowest mean acid value was recorded in Bhima Super and Bhima Safed, which also had a lower reduction in the seed quality during storage. Previous studies by de Oliveira et al. (2016) in Babassu palm seeds and Abduh et al. (2019) in Reutealis trisperma seeds also report an increase in the acid value with the seed age.

|             |                     | Moon               |                    |                    |  |
|-------------|---------------------|--------------------|--------------------|--------------------|--|
| Variety (V) |                     | Storage period     | (D)                | iniean             |  |
|             | Initial             | 6 m                | 12 m               |                    |  |
| BDR         | 14.36               | 16.10              | 13.45              | 14.66 <sup>b</sup> |  |
| BST         | 16.02               | 17.85              | 16.09              | 16.65 <sup>a</sup> |  |
| BSR         | 12.94               | 12.34              | 12.25              | 12.51 <sup>d</sup> |  |
| BSD         | 14.25               | 13.16              | 13.22              | 13.54 <sup>°</sup> |  |
| Mean        | 14.39 <sup>a^</sup> | 14.85 <sup>ª</sup> | 13.75 <sup>b</sup> |                    |  |
| Interaction | S.Em±               | LSD at 5%          |                    |                    |  |
| D           | 0.86                | 0.44**             |                    |                    |  |
| V           | 0.99                | 0.51**             |                    |                    |  |
| DxV         | 1.72                | 0.88**             |                    |                    |  |

Table 1. Change in per cent oil content in onion varieties stored for different durations.

\*\* Significant at *P*≤0.01; BDR - Bhima Dark Red, BST-Bhima Shakti, BSR-Bhima Super, BSD- Bhima Safed. ^ values with same letters in a column/ row are not statistically significant. m- months. The values represent the means of three samples (n=3).

| Variety (V) |                     | Germir             | lation             |                    |                    | Seedling le       | ingth (cm)        |      |                     | Vigor ii            | ndex-l              |                      |
|-------------|---------------------|--------------------|--------------------|--------------------|--------------------|-------------------|-------------------|------|---------------------|---------------------|---------------------|----------------------|
|             |                     | Storage p          | eriod (D)          |                    |                    | Storage           | period            |      |                     | Storage             | period              |                      |
|             | Initial             | 6 m                | 12 m               | Mean               | Initial            | 6 m               | 12 m              | Mean | Initial             | 6 m                 | 12 m                | Mean                 |
| BDR         | 80.00               | 73.33              | 26.67              | 60.00 <sup>c</sup> | 10.8               | 6.8               | 4.0               | 7.20 | 865.7               | 500.8               | 107.4               | 491.32 <sup>c</sup>  |
| BST         | 82.00               | 74.00              | 69.33              | 75.11 <sup>ª</sup> | 11.2               | 5.6               | 5.3               | 7.36 | 917.9               | 412.1               | 368.6               | 566.17 <sup>a</sup>  |
| BSR         | 80.67               | 66.67              | 47.33              | 64.89 <sup>b</sup> | 10.3               | 6.6               | 5.0               | 7.28 | 830.7               | 436.5               | 238.1               | 501.73 <sup>bc</sup> |
| BSD         | 79.33               | 72.00              | 66.00              | 72.44 <sup>ª</sup> | 10.2               | 6.1               | 5.4               | 7.24 | 806.6               | 439.8               | 359.2               | 535.19 <sup>ab</sup> |
| Mean        | 80.50 <sup>a∧</sup> | 71.50 <sup>b</sup> | 52.33 <sup>c</sup> |                    | 10.62 <sup>a</sup> | 6.26 <sup>b</sup> | 4.93 <sup>c</sup> |      | 855.21 <sup>a</sup> | 447.30 <sup>b</sup> | 268.30 <sup>c</sup> |                      |
| Interaction | S.Em±               | LSD at<br>5%       |                    |                    | S.Em±              | LSD at<br>5%      |                   |      | S.Em±               | LSD at<br>5%        |                     |                      |
| D           | 0.99                | 2.91**             |                    |                    | 0.13               | 0.27**            |                   |      | 11.85               | 34.76**             |                     |                      |
| >           | 1.15                | 3.36*              |                    |                    | 0.15               | NS                |                   |      | 13.68               | 40.13**             |                     |                      |
| D×V         | 1.98                | 5.82*              |                    |                    | 0.27               | 0.78*             |                   |      | 23.70               | 69.52**             |                     |                      |

Saponification number represents all the free fatty acid present in an oil (free and esterified). The saponification value decreased with storage duration (Table 6). Among the varieties, highest mean saponification value during the storage was recorded in Bhima Dark Red (143.57 mg KOH gram<sup>-1</sup>). The reduction is saponification value during accelerated ageing has also been reported by lqbal *et al.*, (2002) in cotton seeds which may be due to the reduction of average molecular weight of fatty acid due to bond breakage during seed ageing. The saponification value has industrial significance and oils with higher saponification value are best suitable for biodiesel.

The ROS accumulated during seed ageing, combine with oxygen to produce hydroperoxides. These hydroperoxides attack the lipids present in the seeds leading to lipid peroxidation. The stability of lipids in the seed is very crucial for the survival of the seeds since unsaturated fatty acids form a major component of the cell membranes and its degradation leads to loss of vigour and viability in onion seeds. The increase in the peroxide value and acid value in the onion seed with the seed ageing signifies that the seed ageing in onion is related to the breakdown of the fatty acids. Among the varieties, highest degradation of seed quality as well lipids was in seeds of Bhima Dark Red which indicates that Bhima Dark Red variety is a poor storer variety.

## Conclusion

The present study concluded that the poor storability of onion (*Allium cepa* L.) seed shown to be related to the loss of membrane integrity and degradation of the fatty acids. Although the increased peroxide value (1.36 meq/Kg) and acid value (0.70 mg KOH  $g^{-1}$ ) in stored seeds indicate the degradation of fatty acid attributing to the poor storability of the onion seeds, but the quality of the seed oil was within the maximum permissible limits of CODEX, (1999) standards for edible oil i.e. peroxide value (15 meq/kg oil) and acid value (4.0 mg KOH/g Oil). The utility of oil extracted from non-viable seeds could be explored for food and industrial application.

# **Conflict of interests**

The authors declare that they have no conflict of interests.

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|-----------------|------------------|---------------|--------------|------------|
|-----------------|------------------|---------------|--------------|------------|

|             |                     | <b>Electrical conductivit</b> | Moan                |                     |  |
|-------------|---------------------|-------------------------------|---------------------|---------------------|--|
| Variety (V) | /ariety (V)         |                               | od (D)              |                     |  |
|             | Initial             | 6 m                           | 12 m                |                     |  |
| BDR         | 95.15               | 128.40                        | 190.68              | 138.08 <sup>ª</sup> |  |
| BST         | 76.83               | 111.34                        | 185.88              | 124.68 <sup>b</sup> |  |
| BSR         | 73.00               | 94.10                         | 157.95              | 108.35 <sup>c</sup> |  |
| BSD         | 79.65               | 123.36                        | 142.70              | 115.24 <sup>b</sup> |  |
| Mean        | 81.16 <sup>c^</sup> | 114.30 <sup>b</sup>           | 169.30 <sup>a</sup> |                     |  |
| Interaction | S.Em±               | LSD at 5%                     |                     |                     |  |
| D           | 3.59                | 9.11**                        |                     |                     |  |
| V           | 3.11                | 10.52**                       |                     |                     |  |
| DxV         | 6.22                | 18.23**                       |                     |                     |  |

\*\* Significant at *P*≤0.01; BDR - Bhima Dark Red, BST-Bhima Shakti, BSR-Bhima Super, BSD- Bhima Safed. ^ values with same letters in a column/ row are not statistically significant. m- months. The values represent the means of three samples (n=3).

|  | Table 4. | Changes in | Peroxide | value of | seed oil | extracted | from onic | on varieties | stored fo | r different | durations. |
|--|----------|------------|----------|----------|----------|-----------|-----------|--------------|-----------|-------------|------------|
|--|----------|------------|----------|----------|----------|-----------|-----------|--------------|-----------|-------------|------------|

|             |                    | Peroxide value    | Maan              |                          |  |
|-------------|--------------------|-------------------|-------------------|--------------------------|--|
| Variety (V) |                    | Storage perio     | od (D)            | Weall                    |  |
|             | Initial            | 6 m               | 12 m              |                          |  |
| BDR         | 1.22               | 0.98              | 1.38              | 1.18 <sup>a</sup>        |  |
| BST         | 0.72               | 0.72              | 1.33              | <b>0.94</b> <sup>b</sup> |  |
| BSR         | 0.83               | 1.12              | 1.36              | 1.10 <sup>a</sup>        |  |
| BSD         | 0.82               | 1.27              | 1.36              | 1.15ª                    |  |
| Mean        | 0.90 <sup>c^</sup> | 1.02 <sup>b</sup> | 1.36 <sup>ª</sup> |                          |  |
| Interaction | S.Em±              | LSD at 5%         |                   |                          |  |
| D           | 0.03               | 0.07*             |                   |                          |  |
| V           | 0.03               | 0.09*             |                   |                          |  |
| DxV         | 0.05               | 0.15*             |                   |                          |  |
|             |                    |                   |                   |                          |  |

\*Significant at  $P \le 0.05$ ; BDR - Bhima Dark Red, BST-Bhima Shakti, BSR-Bhima Super, BSD- Bhima Safed. ^ values with same letters in a column/ row are not statistically significant. m- months. The values represent the means of three samples (n=3).

Table 5. Changes in the Acid value of seed oil extracted from onion varieties stored for different durations.

| Variety (V) |                    | Storage period (D) |                   |                    |  |  |
|-------------|--------------------|--------------------|-------------------|--------------------|--|--|
|             | Initial            | 6 m                | 12 m              |                    |  |  |
| BDR         | 0.57               | 0.64               | 0.75              | 0.65 <sup>ab</sup> |  |  |
| BST         | 0.63               | 0.56               | 0.62              | 0.60 <sup>c</sup>  |  |  |
| BSR         | 0.70               | 0.62               | 0.80              | 0.71 <sup>a</sup>  |  |  |
| BSD         | 0.60               | 0.60               | 0.62              | 0.60 <sup>c</sup>  |  |  |
| Mean        | 0.62 <sup>a^</sup> | 0.60 <sup>a</sup>  | 0.70 <sup>b</sup> |                    |  |  |
| Interaction | S.Em±              | LSD at 5%          |                   |                    |  |  |
| D           | 0.02               | 0.05**             |                   |                    |  |  |
| V           | 0.02               | 0.06**             |                   |                    |  |  |
| DxV         | 0.03               | 0.10*              |                   |                    |  |  |

\*\* Significant at *P*≤0.01; \*Significant at *P*≤0.05; BDR - Bhima Dark Red, BST-Bhima Shakti, BSR-Bhima Super, BSD- Bhima Safed. ^ values with same letters in a column/ row are not statistically significant. m- months. The values represent the means of three samples (n=3).

Table 6. Changes in Saponification value of seed oil extracted from onion varieties stored for different durations.

|             |                     | Saponification value (m | ng KOH g <sup>-1</sup> of oil) |                      |
|-------------|---------------------|-------------------------|--------------------------------|----------------------|
| Variety (V) |                     | Storage peri            | od (D)                         | Mean                 |
|             | Initial             | 6 m                     | 12 m                           |                      |
| BDR         | 173.91              | 132.66                  | 124.13                         | 143.57 <sup>a^</sup> |
| BST         | 176.25              | 116.18                  | 104.48                         | 132.30 <sup>b</sup>  |
| BSR         | 163.62              | 121.13                  | 99.02                          | 127.93 <sup>bc</sup> |
| BSD         | 158.95              | 117.36                  | 101.68                         | 126.00 <sup>c</sup>  |
| Mean        | 168.18 <sup>ª</sup> | 121.83 <sup>b</sup>     | 107.33 <sup>°</sup>            |                      |
| Interaction | S.Em±               | LSD at 5%               |                                |                      |
| D           | 1.61                | 4.73**                  |                                |                      |
| V           | 1.86                | 5.47**                  |                                |                      |
| DxV         | 3.23                | 9.47**                  |                                |                      |

\*\* Significant at *P*≤0.01; BDR - Bhima Dark Red, BST-Bhima Shakti, BSR-Bhima Super, BSD- Bhima Safed. ^ values with same letters in a column/ row are not statistically significant. m- months. The values represent the means of three samples (n=3).

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