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Exploring the post-harvest storage losses of phytonutrients in different aggregatum onion varieties of Tamil Nadu

S. Geethanjali^{a*}, T. UmaMaheshwari^a and STM Aravindharajan^b^aAnbil Dharmalingam Agricultural College & Research Institute, TNAU, Trichy- 620027, India^bResearch Scholar, Division of Agricultural Microbiology, IARI, New Delhi, India**ABSTRACT**

Post-storage deterioration of aggregatum onion under ambient conditions was investigated. Two indigenous [Perambalur local (PL) and Manachanallur Local (ML)] and two TNAU released varieties [Co (On) 5 & Co (On) 6] were taken up for this study. Determination of phenol, flavonoid, pyruvic acid, ascorbic acid and non-structural carbohydrates was carried out with an emphasis on different storage periods viz., 0th, 30th, 45th, and 60th days. As storage duration increased, the overall phenol content decreased. The mean total phenol concentration in PL was high (71.27 ± 0.64 mg GAE 100g-1 FW) on 60th day. The flavonoid concentration increased as the storage duration increased (0th to 60th day). The highest mean total flavonoid concentration was found in PL (69.07 ± 1.87 mg QE 100g-1 FW). The pyruvic acid content decreased with storage time, and the mean pyruvic acid content on the 60th day of storage was greater in PL 2.79 ± 0.05 mole g-1 FW. The ascorbic acid content was reduced as storage time increased. The Co (On) 6 has the highest ascorbic acid 8.52 ± 0.28 mg 100 g-1FW. As storage days varied, a fluctuating sugar profile pattern was seen among various onion cultivars. When germination first began, the concentrations of glucose and fructose increased while sucrose decreased. Thus, post-harvest storage studies confirm the nutrient loss during storage and the variety PL was regarded as one of the best cultivars that can withstand post-harvest nutrient loss than the other varieties.

Keywords: *Allium cepa*. var. *aggregatum*, Co (On) 5, Co (On) 6, Postharvest losses, Nonstructural carbohydrates

INTRODUCTION

The onion (*Allium cepa* L.) is a major vegetable crop that is grown and consumed all over the world. According to the most recent statistics, India produced $22,071 \times 10^3$ tonnes from an area of 1315×10^3 hectares, making India the world's second-largest producer of onion next to China [1]. Because of their distinct pungent flavor and sugar content, they can be consumed alone or in cooked form [2]. It has been cultivated for about 4000 years, with the largest producers being the United States, China, Turkey, Russia, and Egypt [3]. Onion has been shown to have anti-diabetic, antibacterial, antioxidant, antimicrobial, antifungal, antiasthmatic, anticancer, anti-inflammatory, hypolipidemic, and anticholesterolemic activities [4].

Onion is grown in three crop seasons in India: kharif, late kharif, and rabi. The main crop is harvested in rabi (60%) and kharif and late kharif (20% each). Kharif onions are accessible from October to December, and late kharif onions are available from January to March. Rabi onion is accessible from April to May. Rabi onion storage is used for both domestic and export markets

from June to December. As a result, rabi onion storage becomes essential for consistent supply [5]. Although onions are less perishable than many other vegetables, losses during storage are unavoidable. Due to postharvest losses, it is estimated that 40 to 50% of the yield never reaches customers. Physiological weight loss, sprouting, and rotting are the most common postharvest losses [6].

The quality of onion changes as a result of natural senescence and therefore pungent aroma and flavor decreases due to enzymatic degradation of S-alk(en)yl cysteine sulfoxide and formation of sulfurous compounds. Other quality changes are loss of firmness, top and root sprouting, and development of storage rots caused by organisms like *Botrytis allii* and *Botrytis aclada*, *Burkholderia cepacia* and *Fusarium oxysporum* [7].

Onions are generally stored in shelters at room temperature. Some bulb characteristics, including dry matter, total soluble solids, pungency, and dry scale number, are associated with onion shelf life [8]. The rate of development of the sprout inside the bulb varies depending on cultivar and storage temperature [9]. Storage losses can be minimized by following correct pre- and post-harvest management practices. Even if correct management practices are followed, if the variety has a limited storage life, all efforts to reduce losses will be pointless. Therefore, choosing a variety with a longer storage life is one of the greatest practices for decreasing storage losses [5]. Thus, the current study was carried out to assess storage losses in various aggregatum onion varieties and to identify varieties with a long storage life.

*Corresponding Author: : S. Geethanjali
Email Address: geethanjali.s@tnau.ac.in

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MATERIALS AND METHOD

Collection and preparation of samples

Two indigenous [Perambalur local (PL) and Manachanalur Local (ML)] and two Tamil Nadu Agricultural University released [Co (On) 5, Co (On) 6] aggregatum onion varieties were collected from a farmer's field in Perambalur district, Tamil Nadu, using proper sampling procedures. The collected samples were cleaned before being cut into 8 mm cubes and dried in a static oven at 40°C for 72 h. The dried onions were deep frozen with liquid nitrogen and immediately powdered with mortar and pestle, until a fine and homogenous powder was obtained. The powder was used for biochemical assessment [4]

Estimation of total phenol content (TPC)

100 mg of onion powdered samples were extracted overnight in 10 ml of 70% ethanol. It was centrifuged for 20 minutes at 10000 rpm. The supernatant was collected and used to determine the phenolic content using the Folin-Ciocalteus assay.

Folin-Ciocalteus assay

The total phenol content (TPC) was determined using a modified Folin-(FC) Ciocalteu's method. 0.125 mL of onion extract was mixed with 0.125 mL of FC reagent and 3.5 mL of distilled water. The test tube contents were thoroughly mixed and incubated for 6 minutes prior to the addition of 1.25 mL of 7% sodium carbonate solution to all tubes and incubated at room temperature for 90 minutes. The absorbance at 660 nm was measured with a Perkin Elmer UV/VIS Lambda 365 spectrophotometer. Gallic acid was used as the standard, and the concentration was expressed in mg gallic acid equivalents per 100 g (mg GAE /100 g) [10,11].ments, each with three replications, were implemented.

Estimation of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using Zhishen et al. [12], with minor modifications. 75 µl of 5% sodium nitrite and 3 mL of distilled water were added to 0.2 mL of onion extract. The mixture was then incubated at room temperature for 6 minutes with 150 µl of 10% aluminum chloride and 0.5 ml of 1 M sodium hydroxide. The absorbance was measured using a Perkin Elmer UV/VIS Lambda 365 spectrophotometer at 510 nm. The standard was quercetin, and the results were expressed as quercetin equivalence (mg QE/100 g).

Estimation of pyruvic acid

The Di Nitro Phenyl Hydrazine (DNPH) method was used to analyze pyruvic acid with minor modifications [13]. 10 g of aggregatum onion was chopped and homogenized with 10 ml of phosphate buffer. The homogenate was centrifuged at 25000 rpm for 15 minutes, and the supernatant was collected for pyruvate analysis. After that, 1.5 mL of supernatant was diluted 10-fold in phosphate buffer. In a boiling tube, an aliquot of 0.5 mL was added to 0.5 mL of 2, 4-dinitrophenyl hydrazine (0.0198g L-1DNPH in 2 M HCl) and 1.0 mL deionized water. The reaction mixture was vortexed and kept at 37 °C for 20 - 30 minutes. After cooling, 5 mL of 0.8 N NaOH was added, and the absorbance at 610 nm was measured with a Perkin Elmer UV/VIS Lambda 365 spectrophotometer. The calibration curve was generated by preparing concentrations of pyruvic acid solutions. The calibration curve was created by making pyruvic acid solutions in water at concentrations ranging from 0.04-0.4

mmol/L, and the pyruvic acid concentration was expressed in terms of (µmol/g fresh weight (FW) aliquot. The results were given in µmol of PA/g FW.

Estimation of ascorbic acid

The 2, 6-dichlorophenol indophenol (DCPIP) titration procedure was used to determine the ascorbic acid content [10]. The ascorbic acid content (mg 100g-1) was calculated using the formula:

$$\text{Amount of ascorbic acid (mg 100g-1)} = \frac{0.5 \text{ mg}}{V_1 \text{ mL}} \times \frac{V_2}{5 \text{ mL}} \times \frac{100 \text{ mL}}{\text{Wt. of the sample}} \times 100$$

V1 = dye consumed by 0.5 mg ascorbic acid; V2 = dye consumed by 5 ml of test solution.

HPLC Conditions for Non-Structural Carbohydrates (NSC) quantification

One g of onion samples was homogenized in 5 ml of 100% methanol. The samples were vortexed for 5 minutes and then placed in a sonicator for 30 minutes before being centrifuged at 4,500 rpm for 10 minutes. Using a 0.45 µm syringe filter, filter the supernatant. The samples were then injected into an HPLC system for NSC quantification.

For the quantification and identification of NSC in onion samples, an HPLC system (Agilent 1260 Infinity II series, USA) equipped with a UV-RID detector was used. NSC was separated using a Zorbax NH2 Analytical (4.6 x 250mm x 5 mm) column kept at 25°C. Acetonitrile/Water (65:35) was the binary mobile phase (Isocratic). Prior to analysis, all solvents were thoroughly filtered through a 0.45 µm membrane filter. The flow rate was held constant at 1.0 ml/min, and the injection volume of various onion samples was 5 µl. The NSC was monitored using the Refractive Index-1260 Infinity II detector. The sample was run for 6 minutes. The resulting chromatogram was evaluated using the samples' retention time in comparison to the standard [14].

Statistical analysis

All analyses were performed in triplicate, and the results are presented as the mean standard deviation on a fresh weight basis. Using SPSS statistics, the data is subjected to a one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) (version 26).

Results and discussion

During onion bulb storage, many physiological and biochemical characteristics change, including water content and the concentration of flavor compounds, carbohydrates, minerals, and plant growth regulators. Changes in these characteristics are most probably connected to respiration and carbohydrate remobilization to provide energy for the growing sprout. Because all nutrients required for sprout growth must come from within the bulb, changes in the concentrations of key metabolites could be used to predict the onset of sprouting. To evaluate onion bulb quality, dry weight, NSCs, pyruvate, and flavonols can be used. These characteristics differ between cultivars as well as during storage [9]

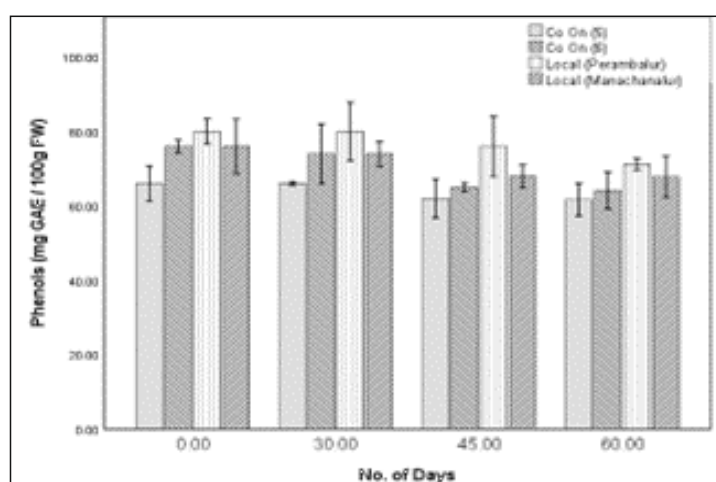
Changes in total phenol content (TPC)

TPC was found to decrease as storage time increased (0th day to 60th day). The mean total phenol content was found to be high in PL at the beginning of the study (80.04±23.08 mg GAE/100g FW), followed by ML and Co (on) 6 (76.04±2.95 and 76.05 ± 0.69 mg GAE/100 g FW). TPC in Co (on) 5 was also on par on the

0th and 30th day of storage, whereas ML and Co (On) 6 were on par on the 30th day of storage.

When compared to other onion cultivars, the PL cultivar was shown to be higher (76.02 ± 3.29 and 71.27 ± 0.64 mg GAE/100 g) on the 45th and 60th day. Following PL, the 45th and 60th days of TPC in ML (68.03 ± 1.23 and 67.84 ± 2.26 mg GAE/100 g), Co (On) 6 (65.02 ± 0.47 and 64.14 ± 2.19), and Co (On) 5 (62.03 ± 2.09 and 61.74 ± 1.77 mg GAE/100 g). It is apparent that the drop in TPC followed the pattern (PL > ML > Co (On) 6 > Co (On) 5) during the storage time. The TPC changes during post-storage under ambient conditions are described in Fig 1.

Fig 1 - Changes in total phenols in aggregatum onion during post-storage (mg GAE/100g FW)



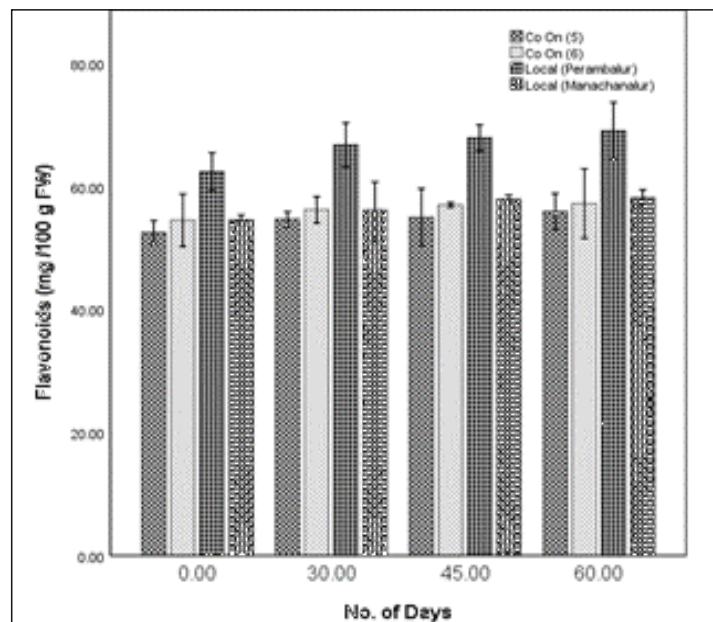
Benkeblia and Shiomi [15] found that total phenolics in onions increased and decreased during a 10-week storage period under controlled conditions. However, Sharma et al. [16] reported that post-storage of onions under ambient conditions results in a regular increase of total phenolic content until the 8th week, after which a decrease was observed and the onion was completely decayed. According to Gorrepati et al. [5], the total phenol content of Raj (186.33 mg GAE/100g) was significantly higher than that of B. Kiran (138.64 mg GAE/100g), B. Shakti (137.09 mg GAE/100g), B. Shweta (114.29 mg GAE/100g), and B. Shubra (112.04 mg GAE/100g). TPC increased up to 60 days of storage and then decreased up to 90 days. Similarly, another study by Kaur et al. [17] found that the phenolic content of onions varied widely between cultivars, ranging from 41.74 to 146.90 mg GAE/100 g.

Changes in total flavonoids

The total flavonoid content was found to increase with storage time (0th day to 60th day). Regardless of the variety, the 60th day had significantly higher flavonoid content than the 45th day. PL had the highest mean total flavonoid content of the varieties (62.42 ± 1.24 mg QE /kg FW of onion). During the initial period, Co (on) 6 and ML were discovered to be similar (54.52 ± 1.72 and 52.63 ± 0.30 mg QE /kg FW of onion). Co (on) 5 had the lowest mean total flavonoid content, measuring 52.53 ± 0.78 mg QE /kg FW. However, the TFC content was found to gradually increase in all varieties. TFC was found to increase for PL (69.07 ± 1.87 mg QE/kg FW) during the last storage period, while the remaining

varieties were on par, viz., Co (On) 5, Co (On) 6, and ML were 55.93 ± 1.18 , 57.23 ± 2.27 and 58.17 ± 0.53 respectively. Fig. 2 depicts the gradual increase of TFC in terms of quercetin over time.

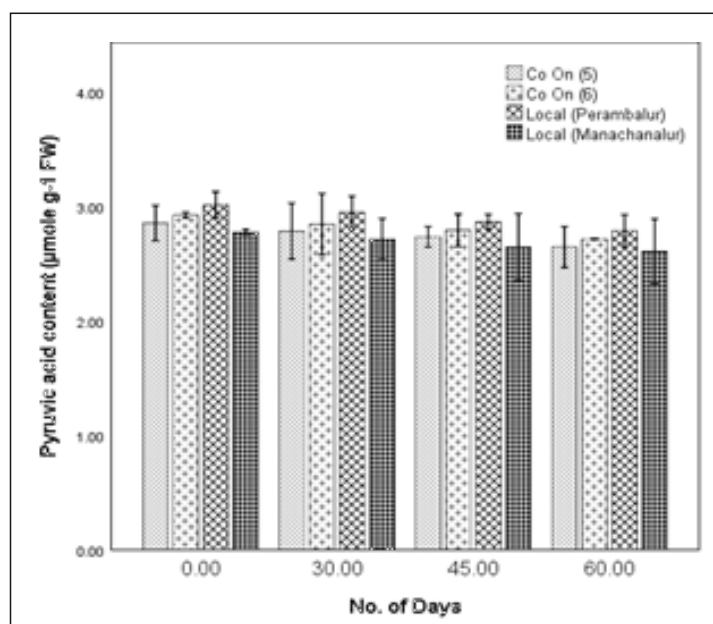
Fig 2 - Changes in total flavonoids in aggregatum onion during post-storage (mg QE/100g FW)



Bibi et al. [18] showed that the TFC content of onion bulbs ranged from 53.29 ± 1.42 to 303.0 ± 6.67 mg QE/100 g at Kalar Kahar, 31.86 ± 1.43 to 230.3 ± 3.78 mg QE/100 g at Lahore, and 28.95 ± 1.40 to 157.7 ± 0.00 mg QE/100 g at Swabi. TFC levels were highest in the bulbs of V1 and V6 types planted in Kalar Kahar, and lowest in the bulbs of V7 cultivars planted in Lahore and Swabi. TFC levels were highest in the outside scales, followed by the middle scales, and lowest in the interior scales (Sharma et al. 2015). According to Duan et al. [19], the waste onion brown peel contains more flavonoids than the inside edible flesh.

Changes in pyruvic acid

Onion pyruvic acid content is affected by various parameters, including dry matter, sugar content, cultivars, maturity, and sulfur nutrition [20]. As the storage period extended, the flavor and taste of onions deteriorated, and the fresh weight of onion bulbs steadily reduced because the soluble solids content increased as the water content of onion bulbs declined [21]. The pyruvic acid level was shown to decrease as storage time increased (0th to 60th day). During the 0th day of storage, the mean pyruvic acid content was higher in PL, followed by Co (On) 6 (3.02 ± 0.04 & 2.93 ± 0.01 $\mu\text{mole g}^{-1}$, respectively). The pyruvic acid level in Co (On) 5 and Co (On) 6 was identical to the 30th and 45th days. Amongst every type, the 60th day had the lowest mean pyruvic acid level. It should also be noted that the pyruvic acid content of Co (On) 5, Co (On) 6, and PL was on par. Fig.3 illustrates the results of the change in pyruvic acid during the storage period.

Fig 3 Changes in pyruvic acid content ($\mu\text{mole g}^{-1}$) during storage

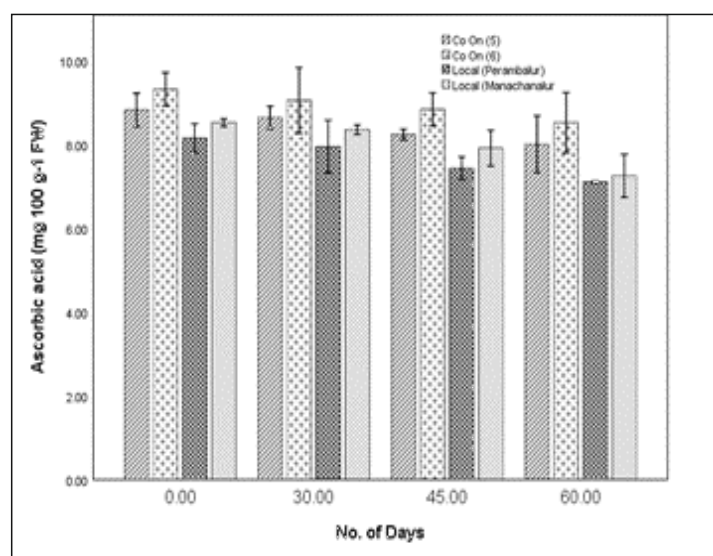
Pyruvic acid, a byproduct of the hydrolysis of S-alk(en)yl-l-cysteinesulphoxides (ACSOs), is a good indication of pungency. When onion cells are sliced or chopped, the enzyme alliinase hydrolyzes the ACSOs to pyruvate and other stable compounds [22].

According to Sharma and Lee [23], the concentration of pyruvic acid rises in a fresh sample. The pyruvic acid content of onion was measured at different temperatures (4°C and 10°C), which scaled to 32.5 and $42.5 \mu\text{mol/g}$ of FW for the first 7 months, followed by a reduction of 22.5 and $32.5 \mu\text{mol/g}$ FW, respectively. In another investigation, the pyruvic acid content fluctuated with increase and decline until 6 months and then increased by $22.2 \mu\text{mol/g}$ FW after 9 months. Lee et al. [24] discovered that pyruvic acid concentration steadily dropped until 75 days of storage, then gradually increased until 135 days of storage. Pyruvic acid concentration increased significantly after 135 days.

Changes in Ascorbic acid

The ascorbic acid content was reduced as storage time increased. At the initial period Co (on) 6 has the highest ascorbic acid level, followed by Co (on) 5 and ML, with mean values of 9.32 ± 0.16 , 8.82 ± 0.16 and $8.52 \pm 0.04 \text{ mg } 100 \text{ g}^{-1}$, respectively. PL samples had the lowest ascorbic acid content, with a mean value of $8.16 \pm 0.14 \text{ mg } 100 \text{ g}^{-1}$. The ascorbic acid concentration was measured in the same manner until the last storage period. On the 60th day, Co (On) 6 presents 8.52 ± 0.29 , followed by Co (On) 5 with 8.01 ± 0.27 , ML with 7.25 ± 0.27 , and finally the PL cultivar with $7.10 \pm 0.01 \text{ mg } 100 \text{ g}^{-1}$.

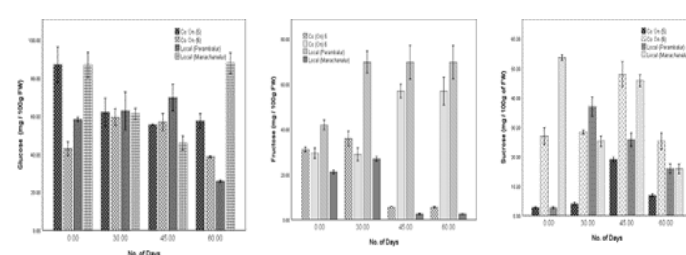
Fig. 4 indicates the ascorbic acid content in different onion varieties. According to Hira Singh et al. [25], the ascorbic acid concentration in raw bulbs ranged from 4.94 to $45.05 \text{ mg}/100 \text{ g}$ on a fresh weight (FW) basis, and from 59.51 to $173.56 \text{ mg}/100 \text{ g}$ on a dry weight (DW). On the basis of fresh weight, the red variety 'NHRDF-Red L-28' had the highest concentration of vitamin C ($45.05 \text{ mg}/100 \text{ g}$ of FW), followed by 'RO-59' (31.01) and Bhima Dark Red (20.48). Sami et al. (2021) found that the Red variety had the highest vitamin C value ($45.07 \text{ mg}/100 \text{ g}$ FW), followed by the Baby variety ($38.12 \text{ mg}/100 \text{ g}$ FW), while the Green and Leek types had the lowest (10.10 and $12.03 \text{ mg}/100 \text{ g}$ FW, respectively).

Fig 4 Changes in ascorbic acid content ($\text{mg } 100 \text{ g}^{-1}$) during storage

Changes in Nonstructural carbohydrates

Fresh onions contain 80 to 85% moisture, with non-structural carbohydrates accounting for up to 80% of the dry matter. Onion non-structural carbohydrates include glucose, fructose, sucrose, and low molecular weight fructans [26]. Fructans are oligo- and polysaccharides derived from fructose, which are the primary reserve carbohydrates in onions [27]. They gather in large quantities in the onion bulb and give energy for sprouting.

The sugar profile (Fructose, Glucose, and Sucrose) of chosen onion cultivars with significance to different storage periods is shown in Fig 5. The sugar content fluctuated continually during the 60 days of storage at room temperature. This change shows that sugar concentration is largely dependent on the onion's physiological and metabolic activity. Initially, during the harvest period, large quantities of glucose were reported for Co (On) 5 and ML viz., 87.11 ± 3.74 and $86.96 \pm 2.67 \text{ mg } 100 \text{ g}^{-1}$ respectively. The fructose levels in all of the aggregatum onion varieties were moderate (31.12 ± 40 , 29.45 ± 0.93 , 41.98 ± 0.87 , $21.14 \pm 0.32 \text{ mg } 100^{-1}$). On a fresh weight basis, all onion types had low sugar content. On the 30th day of storage, glucose was found in high concentrations in all onion varieties, but the concentration was lower than in the initial phase. Fructose and sucrose have been identified in modest amounts ranging from 26.96 ± 39 to 69.81 ± 1.96 and 25.25 ± 1.11 to $47.85 \pm 1.73 \text{ mg}/100 \text{ g}$. The concentrations of glucose, fructose, and sucrose in Co (On) 5 and ML types suddenly decrease on the 45th day. Moderate fructose and glucose levels were reported in Co (On) 6 and PL.

Fig 5. Changes in NSC ($\text{mg } 100 \text{ g}^{-1}$) during storage

Finally, sucrose and glucose were detected in good concentrations in Co (On) 5 at the end of the storage period (60th day), however, fructose was determined to be very low in the same variety. Fructose, on the other hand, was observed to be high, with extremely low levels of glucose and moderate levels of sucrose in Co (On) 6 and PL, respectively. However, the ML variety has a high concentration of glucose and has chosen to have relatively low quantities of fructose and sucrose.

When the storage period is extended and sprouting begins, the amount of carbohydrates like fructose and glucose increases significantly. There are conflicting findings on the change of glucose and fructose in onions during storage [26]. In the current investigation, it could be the cause of the variation that, with the onset of sprouting glucose, fructose concentration increases, and sucrose concentration is moderate. When emergence began, glucose, fructose, and sucrose levels began to fall. Later on, sucrose-starch interconversion may occur, resulting in increasing sucrose content. The enzyme invertase catalyzes the conversion of sucrose to glucose and fructose, which is temperature independent and dependent on onion physiology [15]. Chope et al. [28] observed a similar pattern, with fructose and glucose trends overlapping and sucrose levels varying independently.

Thus, during the storage period of onions, metabolic activity occurs, resulting in a change in sugar concentration [29]. Carbohydrates are also crucial in onion physiology due to the source-to-sink transition. The source-to-sink transition is in charge of onion metabolic activity. Fructans can be delivered directly to sink tissues via the phloem or hydrolyzed first before being transferred and resynthesized [30, 31].

Conclusion

This study reveals that during storage under ambient conditions, onions undergo dormancy breaking and eventual bulb degeneration. As a result, the aggregatum onion's quality gradually deteriorates. It was discovered that the total phenol content, pyruvic acid content, and ascorbic acid content declined as storage periods increased. However, Flavonoid content, increased as storage time increased (0th to 60th day). In the different onion cultivars, a diverse sugar profile pattern was identified. In all the storage analyses, PL had a good shelf life, retaining most of the nutrients at the end of the storage period. These cultivars can also be investigated in the breeding process for improved productivity and storability.

Declarations

Conflict of Interest: The author declares that they have no conflict of interest

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