Optimization of storage conditions of Radish leaves at different maturity stage

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Research Article

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Abstract

Research on waste valorization and utilization of food by-products are increasing day-by-day. This trend not only protect the environment from pollution but also gives good value of food industries wastes and by-products. In this study, optimization of harvesting stage and storage conditions of radish leaves has been done by using response surface methodology. Different packaging materials such as paper, cling film and LDPE were tried for storage. Among all,

better retention of all quality parameters was observed in the leaves stored in LDPE. Maximum quality retention in terms of moisture content, ascorbic acid, DPPH scavenging activity, total phenols, total flavonoids and chlorophyll was observed with 9.55 cm length of leaves and 4°C storage temperature on 0th day. The applied model was found suitable for current study with 94% of anticipated values.

Keywords: Radish leaves, Response surface methodology, length of leaves, packaging material

Introduction

The compulsive spike in the world population is emphasizing the high demand for fruits and vegetables production and this drift is likely to continue in the future (Wadhwa *et al.*,2016). This in return is therefore, deriving the increased amounts of by-products and wastes for various applications. The green tops of many vegetables are not only edible but also delicious. Radish, beets, turnips, and carrots are often available in the supermarkets with no greens and that is a discomfiture.

Radish (*Raphanus sativus*) is an important edible root crop which is extensively grown and consume all over the globe for its potential nutritional profile and promising health benefits. It is indeed loaded with nutrients and bioactive components. It is a versatile vegetable domestically utilize for rating various ailments such as whooping cough, cancer, gastric discomfort, liver problems, constipation, dyspepsia, gallbladder problems, arthritis, gallstones, kidney stones, and so on (Singh and Singh 2013). The root part of the crop is usually enjoyed as raw, cooked, or pickled as well as in juice form whereas to a lesser extent the leaf part is eaten raw or cooked as a vegetable (Banihani, 2017). Radish leaves are important source of Vitamin C which contribute to enhance the antioxidant activity of radish leaves. Also, it is rich in iron, calcium, and dietary fibers which helps in the proper functioning of the body (Prasad, 2016). Radish leaves are also a good source of protein, about 22 proteins have been identified

and about 0.002% of essential oil is present. (Afsharypuor and Balam, 2005). However, these leaves are delicate and have a very short shelf life (1-2 days) at ambient temperature; and as such are categorized to be highly perishable products (Chandra, Kim, & Kim, 2012). Storage temperature, length of leaves and storage duration are some important factors which affect the quality of final product. Optimum storage temperature varies depending on the fruit or vegetable. For some chilling sensitive fruits and vegetables, the use of low temperature storage adversely affects quality attributes and causes deterioration more rapidly (Xiao et al., 2014). Thus, the selection of optimum storage temperature is crucial. Nutrient content of baby radish leaves get altered with the length of leaves, hence, appropriate length selection is of utmost importance. In order to maintain the longer shelf-life, modified atmosphere packaging (MAP) is an effective technology. This technique has been successfully applied in fresh and minimally processed produce, such as lettuce (*Lactuca sativa* L.), broccoli (*Brassica oleracea* L. cv. Acadi), spinach (*Spinacia oleracea* L.) and mushrooms (*Agaricus bisporus* cv. U3 Sylvan 381) (Sandhya, 2010). In this study, research has been carried out to optimize the storage temperature, length of leaves and storage duration to maintain high quality of baby radish leaves. Also, effect of different packaging materials has been studied to maintain quality and prolonging shelf-life of baby radish leaves.

Novelty of the study

This research aimed for the future utilization of radish leaves as a sustainable source of nutrients and health benefiting bioactive compounds, which will help in increasing nutritional value of the food and will provide a healthier option for the consumer in the market. Leaves of radish are regarded as waste globally except for the fewer regions of the world, despite its high nutritive value and health benefiting property. The increment of bio-waste after processing is imposing a serious threat not only mankind but also to the environment. So there is a desperate need for appropriate measures to reduce the waste which will also benefit society. Various

practical approaches are being investigated for the processing of bio-waste which are generated from fruits and vegetables. so the leaves of radish which are considered as waste can be used as the sustainable source of nutrient like vitamin (vitamin C, B complex, vitamin K etc.,), minerals (conation iron, potassium, calcium, phosphorous and magnesium) and bioactive compounds (isothiocinides, glucosinolates, phenolic compounds, organic acids etc.,). The content of these metabolites is higher in leaves as compared to roots which make it a more reliable source of nutrient for maintaining good health. These compounds are highly effective against various disease like cancer, cardiovascular disease, kidney and liver problems, ulcer etc. In this study, work has been done for the shelf-life enhancement of raddish leaves for their effective utilization.

Materials and methods

Sample preparation

Radish (*R. sativus* var. Ivory white) seeds were purchased from local market of Jalandhar, Punjab. Seeds were sown in agriculture farm of Lovely Professional University by dibbling method. Sowing of seeds was done in the 3rd week of November in which temperature varies between 18 -25°C. The soil texture was sandy loam with pH-7.2. Harvesting of leaves was done on the basis of length of leaves. Young leaves (4 cm), mature (8 cm) and over mature (12 cm) leaves were harvested by cutting stem ends with scissors sterilized with 75 mL/100 mL alcohol. After harvest, radish leaves were inspected prior to any treatment and plants with defects were discarded.

Selection of packaging material

Baby leaves (4 cm), mid mature leaves (8 cm) and over mature leaves (12cm) were packaged in cling wrap, low density poly ethylene (LDPE) and paper bags. All samples were stored at refrigerated temperature (4°C) and at ambient temperature (22°C) and were analysed for quality attributes up to 4 days.

84

85 **Optimization of storage conditions**

86 LDPE was observed to maintain the quality of radish leaves for longer duration, hence, selected
87 for further studies. Optimization of storage conditions of radish leaves to maintain quality for
88 longer duration were carried out by using RSM (Response surface methodology). Response
89 surface methodology (RSM) is a collection of mathematical and statistical techniques for
90 empirical model building. By careful design of experiments, the objective is to optimize a
91 response (output variable) which is influenced by several independent variables (input
92 variables). An experiment is a series of tests, called runs, in which changes are made in the
93 input variables in order to identify the reasons for changes in the output response. Design
94 Expert 9.0 (Stat-Ease, Inc. Minneapolis, MN, USA) statistical software with Box-behnken
95 design was used to demonstrate the effect of factors (length of leaves, storage temperature,
96 storage duration) on moisture content, chlorophyll, ascorbic acid, DPPH scavenging activity,
97 total phenols and flavonoids. The parameters and responses were selected on the basis of
98 literature available and low and high values were decided on the basis of preliminary studies.

99 **Moisture content**

100 The moisture content (%) of the leaves was calculated as per the standard oven dry method by
101 measuring the mass before and after drying the powder in an oven at 105°C until it reached
102 constant weight (Saikia et al., 2015).

103 **Chlorophyll content**

104 Chlorophyll (Chl) content was measured non-destructively with the portable SPAD 502
105 Chlorophyll Meter (Minolta Co., Ltd.).

106 **Ascorbic acid**

107 Ascorbic acid content of radish leaves was calculated by as per the method given by Ranganna,
108 1986 by using the following formula

$$\text{Ascorbic acid } \left(\frac{\text{mg}}{100\text{gm}} \right) = \frac{\text{Titre}(\text{ml}) \times \text{Dyefactor} \times \text{Volume made up}(\text{ml})}{\text{aliquot taken for estimation}(\text{ml}) \times \text{weight of sample}(\text{gm})} \times 100$$

110

111

112 **DPPH scavenging activity**

113 DPPH scavenging activity was checked as per the method suggested by Sharma et al., 2020 by
114 using the formula

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_s)/A_0 \times 100$$

116 - A_0 - Absorbance of control at 517nm

117 - A_s - Absorbance of sample at 517nm

118

119 **Total phenols**

120 Total phenolic content was determined by as per the method given by Ranganna, 2009. Briefly,
121 One gram of sample was taken and grounded with 10 ml of 80% ethanol in mortar pestle and
122 centrifuged for 20 min at 1000 rpm. After that, supernatant was evaporated in oven up to
123 dryness and dried extract was dissolved in 5 ml distilled water. Different aliquots within range
124 (0.2-2.0 mL) were taken in separate test tubes and volume was made up to
125 3 ml. Then 0.5 ml Folin-ciocalteu reagent was added in each test tube. After 3 min 2 ml
126 Na_2CO_3 (20%) as added and mixed. Test tubes were placed in boiling water bath for one min
127 and then cooled. Optical density of the sample was recorded at 650 nm with the help of
128 spectrophotometer (Spectronic 20D). The concentration was determined as per the standard
129 procedure from the standard curve. The standard curve was prepared using different
130 concentrations of gallic acid using the above procedure. The results were expressed as mg GAE
131 per 100 gm.

132

Flavonoids

The concentrations of flavonoids were quantified based on a colorimetric assay as per the method given by Zhao et al., 2018. Briefly, quercetin was used as a standard. 1.00 g sample was weighted, and 10 mL 60% ethanol aqueous was used to extract flavonoids from these samples. These samples were further centrifuged at 3000g. All the supernatant was transferred to 25 mL volumetric flask and then was fixed to 25 mL with 60% ethanol aqueous. 1.5 mL of extract and 4.5 mL of distilled water were pipetted into a 25 mL tube and then mixed with 1 mL 5% (w v⁻¹) NaNO₂ solutions. After incubation for 6 min, 1 mL of the 10% (w v⁻¹) Al (NO₃)₃ solutions was added to the mixture. The mixture was kept for 6 min before adding 10 mL 4% (w v⁻¹) NaOH solutions and fixed to 25 mL with 60% ethanol aqueous. Finally, the mixture was reacted for 15 min and the absorbance of the mixture solution was measured with a spectrophotometer at 510 nm against a blank containing 5 mL of extraction solvent. Samples were independently analyzed in triplicate times, and the mean of three tests were used and the total flavonoid content was expressed as mg quercetin equivalent per g dry weight (DW).

Statistical analysis

Analysis of variance test was carried out using commercial statistical package, SPSS ver. 11.5 (SPSS Inc., Chicago, IL, USA). All results of quality parameters were recorded as mean ± SD of three replicates. Mean values were compared and significant differences were given using Duncan's and LSD test ($p \leq 0.05$).

Results and Discussion

155 The changes in moisture, chlorophyll, ascorbic acid, DPPH scavenging activity, total phenols
156 and flavonoid of different maturity stage leaves during storage in different packaging materials
157 are presented in the Table 1, 2 and 3.

158 Moisture content of all samples was found to decline during storage. The moisture content of
159 all samples, fresh baby leaves (4 cm), mature leaves (8 cm) and over mature leaves (12 cm)
160 was decreased gradually with increase in storage period. The rate of decrease in moisture was
161 low in samples packed in LDPE followed by cling and paper for a period of 4 days.

162 Chlorophyll content of all samples (fresh baby leaves (4 cm), mature leaves (8 cm) and over
163 mature leaves (12 cm)) packed in different packaging materials (Paper, cling, LDPE) was
164 observed to decrease during storage period. The rate of decrease in moisture was low in samples
165 packed in LDPE followed by cling and paper for a period of 4 days.

166 Ascorbic acid content of all samples (fresh baby leaves (4 cm), mature leaves (8 cm) and over
167 mature leaves (12 cm)) packed in different packaging materials (Paper, cling, LDPE) was
168 observed to decrease during storage period. However, there was no significant ($P < 0.05$) change
169 was observed in baby leaves stored at refrigerated condition. This decrease might have been
170 due to the oxidation of ascorbic acid during storage. The rate of loss in ascorbic acid content
171 was significantly ($p < 0.05$) higher in the samples stored in paper and cling wrap as compare to
172 samples stored in LDPE. The decrease in ascorbic acid with storage has also been noted in
173 dehydrated fenugreek and savoy beet leaves (Negi et al., 2001a, Negi et al., 2001b) and
174 dehydrated curry and drumstick leaves (Singh et al., 2010) and dehydrated spinach, mint,
175 coriander, amaranthus and benghal gram leaves (Singh et al., 2003).

176 Flavonoids possess a wide spectrum of chemical and biological activities, including free radical
177 scavenging properties. Flavonoids exist widely in the plant kingdom and are common in leaves,

flowering tissues, and pollens. Among the all samples analyzed in the present study, the total flavonoids content was significantly ($P<0.05$) decline in all treatments. Nonetheless, less changes were observed in samples packed in LDPE than packed in paper and cling wrap.

Total phenolic content of all samples was found to decline during storage. The total phenolic content of all samples; fresh baby leaves (4 cm), mature leaves (8 cm) and over mature leaves (12 cm) was decreased gradually with increase in storage period. The rate of decrease in total phenols was low in samples packed in LDPE followed by cling and paper for a period of 4 days.

Similarly, retention of antioxidant compounds was found more in the leaves store in LDPE in all samples. This trend can be associated with retention of total phenols and flavonoid more in the samples packed in LDPE.

Out of all three packaging materials, better retention of quality was observed in LDPE. Therefore, it was used for packaging and further storage conditions were optimized by RSM.

Optimization of storage conditions

Moisture/Weight loss

Moisture content is one of the major parameters to maintain the freshness of fruits and vegetables especially in leafy vegetables. Fresh fruits and vegetables are highly susceptible to moisture loss which leads to weight loss. Therefore, evaluating the weight loss percentage is very important for fruits and vegetables during storage. Weight loss varies depending on the vegetable, the temperature, the packaging and the humidity of the storage chamber. (Watada et al,1987). The results for the moisture content are shown in figure 1. Moisture ranges between 72.75-94.45% ANOVA for the model of moisture as fitted shows significance ($P<0.0001$) and the lack of fit is non-significant (P) (Table 5). The response surface regression model on moisture excellently fits with coefficient of determination ($R^2=0.9938$)

Length of leaves has a significant positive quadratic effect ($P < 0.0001$) which represents lower response at the centre value of length of leaves. Furthermore, storage temperature has a significant negative linear effect ($p < 0.05$) constituting decrease in moisture content with increase in storage temperature. This trend can be related to the water loss through transpiration. However, significant positive quadratic effect ($P < 0.0001$) which represents lower response at the centre value of length of leaves. These results are in association with Pandrangi et al., 2006 who reported the weight loss in spinach leaves with increase in temperature. Effect of storage duration was also observed on moisture content and negative significant linear effect ($P < 0.0001$) was observed which demonstrate that moisture content of leaves get decreases with increase in storage time. However, significant positive quadratic effect ($P < 0.001$) was observed which represents lower response at the centre value of storage duration. Moisture leaves from the leaves during storage duration leads to wilting in leaves. Loss of water from leaves occur due to transpiration through stomata. (Ben – Yehoshua and Rodov, 2003). Similar results were observed by Managa, 2018 in which weight loss in lettuce leaves increased with storage duration due to moisture loss. When the effect of length of leaves and storage temperature was observed on the moisture content at the interaction, it was found to show significant ($P < 0.5$) negative interaction. Similarly, storage temperature and storage time showed significant ($P < 0.01$) negative relation at interaction point.

Moisture loss (%)

$$= 77.4 - 1.25 B - 7.72C - 0.68 AB - 1.4 AC - 2.28 BC + 11.12A^2 \\ + 4.52 B^2 + 3.22 C^2$$

Chlorophyll

Chlorophyll is the pigment that colour green the leaves of vegetables. Vegetables rich in chlorophyll include spinach, beet tops, broccoli, collard greens, Tuscan kale, dandelion, chicory, turnip greens, catalogna chicory, agretto and spirulina algae. After the harvesting of

green vegetables, loss of chlorophyll occur. Decrease in chlorophyll content is associated with cellular degradation and/or senescence which is often used to estimate quality loss of green vegetables. (Hodges, Forney and Wismer, 2000). In this study chlorophyll range for all the designs was observed between 25.25 – 41.26% ANOVA for the model of chlorophyll content as fitted showed significant ($P < 0.0001$) and the lack of fit is non-significant ($P < 0.05$) (Table5). The response surface regression model on chlorophyll content excellently fits with coefficient of determination ($R^2 = 0.9878$). Length of leaves has a significant positive linear effect ($P < 0.0001$) depicting increase in chlorophyll content with increase in the length of leaves (Figure 1). However, significant negative quadratic effect ($P < 0.0001$) was observed which represents higher response at the centre value of length of leaves. These results are in association with Albornoz and Cantwell (2016) in which they reported the highest chlorophyll content in mid mature leaves. However, lower content was observed in over-mature leaves of kale. Furthermore, storage temperature has a significant negative linear effect ($p < 0.5$) constituting decrease in chlorophyll content with increase in storage temperature. Also significant negative quadratic effect ($P < 0.0001$) was observed which represents higher response at the centre value of storage temperature. The chlorophyll content decreases with the increase in dehydration temperature. A significant reduction in chlorophyll content over time has also been reported by Albornoz and Cantwell (2016).

Effect of storage duration was also observed on chlorophyll content and negative significant linear effect ($P < 0.001$) was observed which demonstrate that chlorophyll content of leaves will decrease with increase in storage time. This trend can be related as with time due to the transpiration water content get decreases which promotes the decomposition of chlorophyll and accelerate leaf yellowing. Similar observations were noted by Ying et al. (2018) during the storage of green leaves. The similar results were found by Raya et al., (2015) in their experiment they found a decrease in chlorophyll content in both young and matured leaves of

plant *Clinacanthus nutans*. The decrement in chlorophyll was also found by Yamauchi & Watada, (1993) they observed that the decrement in chlorophyll was 40% during first 3 days of storage which get increased to 64% after 5 days of storage at 20°C in Parsley (Yamauchi & Watada., 1993). Effect of length of leaves and storage temperature was observed on the chlorophyll content at the interaction, it was found to show significant (P<0.5) negative interaction. Similarly, length of leaves and storage time also showed significant (P<0.05) negative relation at interaction point. Similar results were obtained by Alborno and Cantwell et al. (2015) where they found that the chlorophyll content of leaves of plant *Brassica oleracea* var *acephala* decreased with time at storage temperature of 5°C on day 28 while there were no significant changes on chlorophyll content at 0°C. According to them, this change in chlorophyll content may be due to change in ammonia content which got increased more at 5°C as compared to 0°C.

chlorophyll content (mg/100gm)

$$= 32.83 + 4.06A - 0.3212B - 6.39C - 0.3925AB - 0.645AC - 0.025BC \\ - 6.06A^2 + 4.93B^2 + 3.27C^2$$

Ascorbic acid:

Ascorbic acid is most heat labile of all the vitamins in food. It is very sensitive to heat and rapidly destroyed during dehydration. The nutrient gets oxidized rapidly on exposure to heat and air. In this study, ascorbic acid range between (21.36-35.43%). ANOVA for the model of ascorbic acid fitted as significant (P< 0.0001) and lack of fit is non-significant(P<0.05) (Table 5). The response surface regression model on ascorbic acid excellently fits with coefficient of determination (R²=0.99913). Length of leaves has a significant negative linear effect (P<0.01) depicting decrease in ascorbic acid content with increase in the length of leaves (Figure 2). However, significant negative quadratic effect (P<0.0001) was observed which represents higher response at the centre value of length of leaves. Furthermore, storage temperature has a

significant negative linear effect ($p < 0.1$) constituting decrease in ascorbic acid with increase in storage temperature. This is due to the heat sensitive behaviour of ascorbic acid. However, significant positive quadratic effect ($P < 0.01$) was observed which represents lower response at the centre value of storage temperature. These results are in association with by Bergquist et al., 2006. In their study they found that the leaves of baby spinach (*Spinacea Oleracea*) stored at 2°C showed smaller reduction in ascorbic acid content when compared to the leaves stored at 10°C .

Effect of storage duration was also observed on ascorbic acid content and negative significant linear effect ($P < 0.001$) was observed which demonstrate that ascorbic acid content of leaves will decrease with increase in storage time. However, significant positive quadratic effect ($P < 0.5$) was observed which represents lower response at the centre value of storage duration. A significant reduction in ascorbic acid content of vegetable leaves during storage has been reported in literature (Gupta et al, 2013, Joshi and Mehta, 2010, Vyankatrao, 2015). Effect of length of leaves and storage duration was observed on the ascorbic acid content at the interaction, it was found to show significant ($P < 0.05$) negative interaction. Similarly, storage temperature and storage time also showed significant ($P < 0.05$) negative relation at interaction point. The same results were found by Raya et al. (2015). In their study on plant *Clinacanthus nutansa* the ascorbic acid in both the young (mean effect of 0.38250a mg/100g) and matured leaves (mean effect of 0.35667amg/100g) decreased when exposed to prolonged storage. However, the decrement was gradual in matured leaves when compared to younger leaves which on other hand showed rapid decrement.

Ascorbic acid mg/100gm

$$= 32.29 - 1.39A - 0.6225B - 2.84C - 0.825AC - 0.54BC - 8.35A^2 + 1.21B^2 + 0.598C^2$$

DPPH scavenging activity

DPPH scavenging activity is related to the antioxidant potential of food products. Antioxidants are required to combat free radicals that are generated in the body as these free radicals accelerate hyper pigmentation syndrome, heart disease, stroke, atherosclerosis, diabetes and cancer. The results for the DPPH scavenging activity are shown in figure 2. DPPH scavenging activity range between 33.04-42.66% ANOVA for the model of DPPH inhibition as fitted significance ($P < 0.0001$) and the lack of fit is non-significant ($P < 0.05$) (Table 5). The response surface regression model on DPPH inhibition excellently fits with coefficient of determination ($R^2 = 0.9764$). Length of leaves has a significant positive linear effect ($P < 0.001$) depicting increase in DPPH scavenging activity with increase in the length of leaves. However, significant negative quadratic effect ($P < 0.001$) was observed which represents higher response at the centre value of length of leaves. According to Pandajaitan et.al, (2005), mid leaves had much higher levels of total phenolics and total flavonoids than immature and mature leaves. These compounds are responsible for scavenging activity hence, DPPH scavenging activity is higher in midmature stage. Similar trend was reported by Pandajaitan et.al, (2005) where mid maturity spinach leaves showed higher levels of antioxidant potential than immature and mature leaves. Furthermore, storage temperature has a significant negative linear effect ($p < 0.1$) constituting decrease in DPPH scavenging activity with increase in storage temperature. The possible reason for the low free radical scavenging activity may be the breakdown of the structure of phenolics because of the exposure to higher temperatures. However, significant positive quadratic effect ($P < 0.01$) was observed which represents lower response at the centre value of storage temperature. These results are in association with Madau et al. (2015). Effect of storage duration was also observed on DPPH scavenging activity and negative significant linear effect ($P < 0.001$) was observed which demonstrate that DPPH scavenging activity of leaves get decreases with increase in storage time. These results are similar to Raya et al.,

(2015). In their study, they found that there was gradual decrease in DPPH scavenging activity in leaves of plant *C.nutans* due to storage and just within 4 days of storage the DPPH activity decreases to almost 69% . When the effect of length of leaves and storage temperature was observed on the DPPH activity at the interaction, it was found to show significant ($P<0.5$) negative interaction. Similarly, length of leaves and storage time and storage temperature and storage time also showed significant ($P<0.05$) negative relation at interaction point.

DPPH scavenging activity (%)

$$= 39.73 + 2.11A - 0.6287B - 3.03C - 0.4975AB - 1.11AC - 0.9225BC - 2.45 A^2 + 1.53B^2$$

Phenols:

Phenols are the plant constituents that offer various health benefits from human physiological standpoint such as anti-inflammatory, antioxidant, antiproliferative activities. The results for the total phenolics are shown in figure 3. In this study, the total phenols of radish leaves range between 532-650mg GAE/100gm. ANOVA for the model of phenols as fitted shows significant ($P<0.05$) and the lack of fit is non-significant ($P<0.05$). The response surface regression model on phenol excellently fits with coefficient of determination ($R^2=0.8265$). Length of leaves has a significant negative linear effect ($P<0.5$) depicting decrease in total phenols with increase in the length of leaves. Similarly, significant negative quadratic effect ($P<0.001$) was observed which represents higher response at the centre value of length of leaves. Maturity stage of leaves affect the total phenolic content. According to M'rabel et al., (2017) mid maturity stage leaves showed the highest total phenols as compared to immature and over mature. This is due to the production of secondary metabolites in mid mature leaves which are not present in baby leaves and present in less amount in older leaves. Furthermore, storage temperature has a significant negative linear effect ($p<0.5$) constituting decrease in total

phenols with increase in storage temperature. The possible reason for this can be related with the breakdown of the structure of phenolics because of the exposure to higher temperatures. Similarly, significant negative quadratic effect ($P < 0.5$) was observed which represents higher response at the centre value of storage temperature. These results are in association with Rickman et al., (2007) who reported the rapid degradation in phenolic content of spinach leaves with increase in temperature. Effect of storage duration was also observed on total phenols and negative significant linear effect ($P < 0.05$) was observed which demonstrate that total phenols of leaves get decreases with increase in storage time. However, significant positive quadratic effect ($P < 0.5$) was observed which represents lower response at the centre value of storage time. It has been suggested that degradation of total phenolic content during storage may possibly be attributed to sensitiveness toward oxidation. These studies are in agreement with Rickman et al. (2007) observations. During their study, the phenolic content of spinach leaves was found to decline during storage period. When the effect of length of leaves and storage temperature was observed on the total phenols at the interaction, it was found to show significant ($P < 0.5$) negative interaction.

$$\begin{aligned} \text{Total Phenolics} \left(\text{mg} \frac{\text{GAE}}{100\text{gm}} \right) \\ = 647.12 - 15.62A - 11.17B - 27.69C - 9.17AB - 37.33A^2 - 10.24B^2 \\ + 10.57C^2 \\ = \end{aligned}$$

Flavonoids

Flavonoids have been reported to exert a wide range of biological activities be like antioxidant property, cardioprotective effects, anti-inflammatory and anticarcinogenic effects. Indeed, flavonoids serve as a source of potential bioactive molecules that posses promising health

benefits. The results for the flavonoids are shown in figure. Flavonoids range between (958-1026) (mg quercetin/100gm) ANOVA for the model of flavonoids as fitted shows significance ($P<0.001$) and the lack of fit is non-significant ($P<0.05$) Table 5 . The response surface regression model on flavonoids excellently fits with coefficient of determination ($R^2=0.7553$). Length of leaves has a significant negative linear effect ($P<0.1$) depicting decrease in total flavonoids with increase in the length of leaves. Similarly, significant negative quadratic effect ($P<0.5$) was observed which represents higher response at the centre value of length of leaves. Obtained results are similar to the result of Pandajaitan et.al, (2005) who reported that leaves should be harvested at mid maturity stage for consumes to benefit from the elevated levels of health promoting flavonoids. Similar studies are reported by Wongsen et al. (2013). Furthermore, storage temperature has a significant negative linear effect ($p<0.5$) constituting decrease in flavonoids with increase in storage temperature. Effect of storage duration was also observed on flavonoids and negative significant linear effect ($P<0.1$) was observed which demonstrate that total phenols of leaves get decreases with increase in storage time. However, significant positive quadratic effect ($P<0.5$) was observed which represents lower response at the centre value of storage duration. When the effect of length of leaves and storage temperature was observed on the flavonoids at the interaction, it was found to show significant ($P<0.5$) positive interaction. However, storage temperature and storage time showed significant ($P<0.5$) negative relation at interaction point.

$$\begin{aligned}
 \text{Flavonoids} \left(\text{mg} \frac{\text{quercetin}}{100\text{gm}} \right) &= 1014.62 - 0.9575A - 9.5B - 23.13C + 14AB - 10.5 BC - 11.38 A^2 \\
 &+ 7.11 C^2
 \end{aligned}$$

Selection of optimum condition

Design expert software version 7.0 was used to optimize the storage conditions for the radish leaves. The criteria applied for the numerical optimization was the independent variables (length of leaves (4-12 cm), storage temperature (4- 25) and storage duration (24-120 hrs) were kept within range and responses (Moisture content, DPPH scavenging activity, Ascorbic acid, Chlorophyll content, total phenols and total flavonoids) were kept at maximum (++++). By applying the methodology of desired function, the optimized level of different variables was obtained. It indicated that at length of leaves 9.55 cm and storage temperature 4°C give the maximum value for moisture content (94.44%), DPPH scavenging activity (45.32%), Ascorbic acid (35.37 mg/100gm), chlorophyll content (48.795 mg/100 gm), total phenols (684.025 mg GAE/100gm) and total flavonoid (1040.066 mg quercetin/100gm) with overall desirability of 0.99.

Verification of optimized value

The fitness of model was investigated by setting up the experiment at the optimum conditions (length of leaves 9.55 cm, storage temperature 4°C and storage time, 0th day) generated by the software. The values for different responses given by software are; Moisture content (94.44%), chlorophyll (48.795 mg/100gm), total phenols (684.025 mg GAE/100gm), Flavonoids (1040.066 mg quercetin/100gm), ascorbic acid (35.37 mg/100gm) and DPPH scavenging activity (45.32%) . The experimental values obtained are given in Table 4 which are within 94% of anticipated values. This shows the suitability of model.

Conclusion and future trends

This paper is aimed for the future utilization of radish leaves as a sustainable source of nutrients and health benefiting bioactive compounds, which will help in increasing the nutritional value of the food and will provide a healthier option for the consumer in the market. Leaves of radish are regarded as waste globally except for the fewer regions of the world, despite its high nutritive value and health-benefiting property. The increment of bio-waste after processing is imposing a serious threat not only to mankind but also to the environment. So there is a desperate need for appropriate measures to reduce the waste which will also benefit society. In this paper, work has been done to maintain the freshness of radish leaves for longer duration without affecting its quality parameters. Box- behnken design was used to optimize the storage conditions. Length of leaves, 9.5 cm stored at 4 degree C were observed to show better quality retention. In this direction, further work can be carried out for longer duration storage without hampering its quality.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Poorva Sharma and Jasleen Kaur. The first draft of the manuscript was written by Jasleen Kaur and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

Figure 1

Response surface on 3 D plot for Moisture content and Chlorophyll content

Figure 2

Response surface on 3 D plot for Ascorbic acid and DPPH scavenging activity

Figure 3

Response surface on 3 D plot for total phenols and flavonoid content

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