

Original Research Article

Utilization of edible Rhododendron (*Rhododendron arboreum* Sm.) flowers for development of spiced beverage (appetizer) and its shelf life evaluation during storage

ABSTRACT: In India, various types of unexplored edible flowers are being utilized traditionally as food and medicine by the rural communities since ancient time only during their flowering time due to short post harvest life. These flowers are rich in phytochemical (e.g., polyphenolics, anthocyanins~~-etc.~~) which possess numerous health benefits. So, the present investigations were conducted to develop a spiced beverage/appetizer (spiced squash) from rhododendron flower petals and its quality evaluation during storage. Different combinations of petals extract and total soluble solids (TSS) were tried to standardize proper combination for spiced beverage. Appetizer recipe (T₃) prepared with 35% extract, 40 °B TSS and 1.20 % acid was found to be best on the basis of quality characteristics of the product. The best selected appetizer recipe was packed in glass and PET bottles and stored for 6 months under ambient (15-25 °C) and refrigerated temperature conditions (4-7 °C). Overall effect of packaging and storage revealed that various quality characteristics like TSS, apparent viscosity, reducing and total sugars of appetizer increased slightly, whereas, other chemical characteristics like acidity, ascorbic acid, anthocyanins, total phenols and sensory characteristics scores of colour, body, taste, aroma, overall acceptability score decreased slightly during storage. The quality of beverage was retained better in glass bottles under refrigerated condition as compare to ambient storage condition.

Comment [RMdSPG1]: All abbreviations should be described when used for the first time.

KEYWORDS: —Edible flowers, Rhododendron (*Rhododendron arboreum* Sm.), Spiced squash, Antioxidant activity, Storage quality evaluation, Sensory acceptability

1 Introduction

—Rhododendron (*Rhododendron arboreum* Sm.) belongs to family Ericaceae is the largest genera of evergreen trees which is known for its highly nutritious edible flowers. The various species of rhododendron are concentrated in the temperate regions of Northern hemisphere especially in Sino-Himalayas including China, Japan, Myanmar, Thailand, Pakistan, Malaysia, India, Sri Lanka, Nepal, Tibet, Southern Europe and northern America (Purohit, 2014). In India, it is distributed in Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Manipur, Nagaland, Sikkim and Uttrakhand (Bhattacharya, 2011). It grows widely at an elevation of 1500-2400 m above mean sea level (Devi et al., 2017). In HP, Rhododendron arboreum Sm. commonly known as *Burans* is very popular for its attractive flowers. Its flowering period starts during March-April bearing deep red or crimson to pale pink flowers (Kashyap et al., 2017). Traditionally the flowers of *Rhododendron arboreum* Sm. are used for curing diarrhea, blood dysentery, high altitude sickness, headache, mental retardation, nasal bleeding, fever and stomach ache (Popesco & Kopp, 2013). Its flowers possess pharmacological and biological properties. The deep red to scarlet red flowers of *Rhododendron arboreum* Sm. are fairly sweet sour in taste which have been found to be rich source of anthocyanins, phenolics, vitamin C, carbohydrates, proteins, pectin, sugars, fibre, various amino acids and minerals beside flowers ethanolic extract exhibit antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*

(Saklani & Chandra, 2015). Traditionally the flowers are eaten raw, used for making chutneys etc. at home scale. But there are only a limited number of reports pertaining to utilization of rhododendron for the development of value added products and scattered information is available in the literature on the development of products from its flowers.- These flowers have abundant phytochemicals such as flavonoids, anthocyanins, phenolics and carotenoids; thus possess numerous health benefits (anti-oxidant ~~activity~~, anti-inflammatory ~~activity~~, anti-cancerous, anti-diabetic activity) and are successfully being utilized by tribal people in their daily diet (as food and medicine). A wide range of food processing techniques and food products should be developed using these flowers with the help of ethnic knowledge for effective delivery to the consumers (Pinakin et al., 2020). The utilization of *Rhododendron* flower petals for the development of variety of foods and nutraceutical products can help in the availability of these flower-based product during the year which can aid sustainability in development of rural tribal populations with great promise of employment (Kumar et al., 2019). Thus, the present studies were under taken with the objective to develop spiced beverage /appetizer from *Rhododendron arboreum* and to study its storage life.

2 Materials & methods

The flowers of *Rhododendron arboreum* Sm. procured from Rajgarh area of Sirmour district of HP during 2017-2018 were brought to the Department of Food Science and Technology, UHF, Nauni, Solan (HP), where they were used for the estimation of various physico-chemical characteristics, flower extract preparation and development of spiced beverage. Petals from flowers were separated and washed before preparation of extract. Its flower extract was successfully extracted/prepared by hot extraction (6 min. ~~ute~~ cooking with 15 % water) followed by enzyme treatment (0.08 % Pectinase at 50 °C for 60 min.), which was further used for the

development of beverages. According to food safety and standards act (2006), fruit squash should have minimum percentage of TSS as 40 °B and fruit juice as 25 per cent in final product which is diluted before consumption. So we tried to standardize the recipe (Figure 1) of rhododendron appetizer by mixing in different concentrations of its extract with sugar syrup as given in Table 1. A constant amount of spice extract (100 ml) as suggested by Thakur et al. (2016) was also added to all the treatment combinations. Spice extract was prepared by boiling a ground mixture of pre-determined quantities of spices like cardamom (1 g), cumin (2.5 g) black pepper (2.5 g), common salt (5 g), black salt (5 g) in 200 ml of water then straining and mixing the extract with mint extract (10 ml) and ginger extract (15 ml). To get the desirable concentration of acid (1.20 %) in appetizer, citric acid was added in different treatment combinations. Sodium benzoate (600 ppm) was added at the end of product preparation of appetizer in all the treatments.

2.1 Packaging and storage

-Spiced beverage prepared by best selected combination of petal extract, TSS and acid was packed in pre-sterilized glass and PET (polyethylene terephthalate) bottles (700 ml capacity). All the packed products were properly labelled and stored in ambient (15-25 °C) and low temperature (4-7 °C) conditions for six months. Changes in the quality characteristics of the products were estimated after every three months of storage intervals. The physico-chemical and sensory characteristics of all the products were estimated at zero, three and six months of storage.

2.2 Physico-chemical analysis and sensory evaluation

The colour (Red and Yellow) of appetizer in terms of different tintometer colour units (TCU) was observed with Tintometer (Lovibond Tintometer Model-E). Various chemical characteristics like moisture content, TSS (Total soluble solids), reducing sugars, total sugars, titratable acidity (% citric acid), anthocyanins content of prepared products were determined according to method described by Ranganna (2009). Total phenols content (mg/100 g) was determined by Folin-Ciocalteu procedure given by Singleton and Rossi (1965). DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity was measured as per the method of Brand-Williams et al. (1995). Nine points hedonic rating test was followed for conducting the sensory evaluation of rhododendron spiced beverage (Amerine et al., 1965). For sensory evaluation, the panel of ten judges were selected to evaluate the spiced beverage for sensorial parameters like colour, body, taste, aroma and overall acceptability.

2.3 Statistical analysis

Data on physico-chemical characteristics of appetizer was analysed by Completely Randomized Design (CRD) before and during storage by preparing one way and three way ANOVA table, whereas, data pertaining to the sensory studies were analyzed by using Randomized Block Design (RBD) as described by Mahony (1985). The experiments on recipe standardization and for storage studies were replicated three times.

3 Results and discussion

3.1 Standardization of recipe for the preparation of Rhododendron appetizer

Data on physico-chemical characteristics of Rhododendron appetizer was given in Table 2. With the increase in extract content in different recipes a significant effect on physico-chemical characteristics of rhododendron appetizer recipes has been observed. Data presented in Table 2 show that recipe T₈ and T₄ contain higher values of anthocyanins, total sugars, reducing sugars, pH, apparent viscosity, total phenols, ascorbic acid and antioxidant activity. This might be due to the use of high amount of flower extract used as compared to other recipes like T₁ and T₅. The variation in extract content has also affected the colour units of different recipes of appetizer. Data on sensory characteristics of different recipes of rhododendron appetizer given in Table 3 indicate that the mean colour score was obtained highest (8.40) in recipe T₃ and lowest (7.90) was awarded to T₁. The highest score (8.50) of taste was again awarded to T₃ while T₁ (8.00) got the lowest score. The maximum (7.52) score for aroma was also recorded in recipe T₃ and minimum (7.17) was recorded in T₁. The highest score (8.30) of overall acceptability was also obtained in T₃ and lowest (7.73) in T₁. Data given in Table 3 show that there was a significant effect of extract-acid-syrup blend on sensory scores of different recipes of rhododendron appetizer. The higher colour and body scores obtained in recipe T₃ might be due to best combination of flower extract-sugar syrup. The higher taste and aroma scores of appetizer obtained in the recipe T₃ might be due to the best combination of sugar- acid-spices-extract blend in this recipe. The higher overall acceptability scores for recipe T₃ might be due to better combination of extract-acid-spices syrup blend coupled with attractive colour and body of the product.

3.2 Shelf life evaluation

3.2.1 Physico-chemical characteristics

There was a significant decrease in colour (Fig. 2a and 2b) during storage of rhododendron appetizer. More decrease in red and yellow colour units of appetizer was recorded under ambient storage conditions as compared to refrigerated conditions. Decrease in red and yellow colour units during storage might be due to degradation of anthocyanin pigments (Thakur et al., 2016). Similar trend of decrease in red and yellow TCU has been observed by Suryawanshi et al. (2008) in pomegranate extract, Thakur et al. (2017) in wild pomegranate appetizer, Hamid and Thakur (2017) in mulberry appetizer. Significant increase (Fig. 2c) in apparent viscosity of rhododendron appetizer may be due to increase in TSS and soluble sugar which increased strain and shearing rate. As the flow index decreases it helps to develop pseudo plasticity and increased the apparent viscosity of the product (Bal et al., 2014). This increase in apparent viscosity was observed more in appetizer stored under ambient temperature conditions as compare to refrigerated storage conditions. Similar results have been reported by Khurdiya & Lotha (1994) for kinnow mandarin juices and Thakur et al. (2018) in wild aonla appetizer.

The TSS content of appetizer increased slightly during storage (Fig. 2d) and this slight increase in total soluble solids during storage might be due to hydrolysis of polysaccharides into simple sugars (Kesharwani et al., 2015). More increase in TSS was found in appetizer stored under ambient conditions (Increased from 40 to 40.54 and 40.66 °B in both glass and PET) as compared to refrigerated storage conditions (Increased from 40 to 40.29 and 40.41 °B in both glass and PET) and this might be due to the faster rate of reaction because of high temperature in ambient conditions. Reducing and total sugars of appetizer showed (Fig. 2e,f) a significant increase in storage which was comparatively less in refrigerated storage conditions than in ambient conditions. More increase in sugars was found in appetizer stored under ambient

conditions. Increase in sugars during storage might be attributed to the hydrolysis of starch into sugars (Heikal et al., 1964) and higher increase might be due to the faster rate of reactions because of high temperature in ambient conditions. Our results were in accordance with Selvamuthukumar & Khanum (2013) in spiced seabuckthorn mixed fruit appetizer, Thakur et al. (2016) in box myrtle appetizer, Hamid & Thakur (2017) in mulberry appetizer and Thakur et al. (2018) in wild aonla appetizer.

The decrease in titratable acidity (Fig. 2g) of appetizer could be attributed to copolymerization of organic acids (Selvamuthukumar & Khanum, 2013). Decrease in this parameter of beverage could be attributed to the chemical interactions of organic acids of appetizer with sugars and amino acids. Slower rate of reactions of these constituents in refrigerated conditions might have contributed to the less loss of acid during storage as compared to ambient condition. These are in accordance with the results of Thakur et al. (2017) in wild pomegranate appetizer. With the progress of storage period (Fig. 2h), there was a continuous decrease in ascorbic acid content of spiced beverage, however, decrease was significantly lower under refrigerated conditions (decreased from 8.56 to 4.56 and 4.21 mg/100 g in both glass and PET) as compared to ambient conditions (decreased from 8.56 to 6.28 and 6.10 mg/100 g in both glass and PET). Decrease in ascorbic acid content during storage might be due to oxidation or degradation of ascorbic acid into dehydro-ascorbic acid, furfural and hydroxy furfural at above temperatures, therefore its degradation was more in ambient conditions. Our results are in accordance with the findings of Hamid & Thakur (2017) in mulberry appetizer and Chauhan et al. (2019) in wild prickly pear spiced squash. A significant decrease in anthocyanins content of appetizer was recorded during storage (Fig. 2i) which was more in ambient storage conditions (changes from 26.81 to 17.27 and 16.62 mg/100 g in both glass and PET) than refrigerated

conditions (changes from 26.81 to 22.35 and 22.15 mg/100 g in both glass and PET). Decrease of anthocyanins in spiced squash might be due to their high susceptibility to auto oxidative degradation during storage. However, less loss of this attribute in the product might be due to slower rate of its auto oxidation in refrigerated storage conditions as compared to ambient conditions. More retention of anthocyanins of spiced squash packed in glass bottles during storage might be due to the slower rate of reactions in glass bottle than PET as a result of disparity in their thermal conductance properties. Our results are in accordance with the findings of Thakur et al. (2016) in box myrtle appetizer.

A gradual decrease in total phenols content of appetizer was observed during storage (Fig. 2j) which was slower under refrigerated storage conditions (decreased from 36.95 to 33.59 and 33.01 mg/100 g in both glass and PET) than ambient conditions (decreased from 36.95 to 31.69 and 31.03 mg/100 g in both glass and PET). Significant ($p < 0.05$) decrease in total phenols content during storage might be due to their involvement in the formation of polymeric compounds, complexing of phenols with protein and their subsequent precipitations as observed by Abers & Wrolstad (1979) in strawberry preserve. Similar decreasing trend in total phenols have also been reported by Selvamuthukumaran & Khanum (2013) in spiced seabuckthorn mix fruit squash and Thakur et al. (2016) in box myrtle appetizer. A gradual decrease in antioxidant activity of appetizer was observed during storage (Fig. 2k), which was slower under refrigerated storage conditions than ambient. Significant decrease in antioxidant activity during storage might be due to the degradation of color pigment and ascorbic acid during storage period as reported by Chouhan et al. (2019) in wild prickly pear spiced squash. Slower rate of loss of antioxidant activity in refrigerated storage might be due to slower reaction rate in refrigerated conditions as compared to ambient. However, more retention of antioxidant activity of appetizer in glass bottle

might also be because of slower reaction rates in glass bottle, as glass material absorb heat at slower rate as compared to PET.

3.2.2 Sensory characteristics of rhododendron appetizer during storage

The decrease in sensory scores of spiced beverage was observed during storage. However, decrease in these characteristics scores were less in refrigerated storage conditions (Fig. 3) than ambient (Fig. 4). Decrease in colour scores with the advancement of storage might be due to degradation of anthocyanins and browning caused by copolymerization of organic acids of the beverage. While, non-significant decrease in body scores were observed in product during storage. Whereas, a decrease in taste scores of appetizer with advancement of storage period might be due to the loss of sugar-acid-salt blend responsible for taste during storage. Maintenance of higher taste scores in low temperature conditions might be due to the better retention of original sugar-acid-salt-petal extract blend as a result of slow reaction rate contributing change in this blend. The decrease of aroma scores might be due to the potential loss of volatile aromatic compounds during storage. Retention of higher scores of aroma in refrigerated conditions might be due to the lower losses of aromatic compounds at low temperature during storage as compared to ambient conditions. Slight decrease in overall acceptability scores might be due to the loss in appearance, flavour compounds and uniformity of the product during storage. The similar decreasing trend during storage have also been reported earlier by Sharma et al. (2002) in plum appetizer, Selvamuthukumaran and Khanum (2013) in spiced seabuckthorn appetizer, Thakur et al. (2016) in box myrtle appetizer, Thakur et al. (2017) in wild pomegranate appetizer, Hamid & Thakur (2017) in mulberry appetizer and Thakur et al. (2018) in wild aonla appetizer.

4 Conclusion

-Rhododendron appetizer developed by mixing 35 % extract, 40 °B TSS, 1.20 % acidity and with a spice extract -(10 %)- of cardamom (1 g), cumin (2.5 g), black pepper (2.5 g), common salt (5 g), mint juice (1 %) and ginger juice (1.5 %) have got highest sensory characteristics scores, which could be stored safely for a period of 6 months under both storage temperatures and also in both packaging materials like PET and glass bottles. The best quality of this beverage could be maintained in glass bottle stored under refrigerated storage conditions as compared to PET bottle. As rhododendron flowers have short shelf life after harvest is one of the major factors that give the necessity of developing a cheap and efficient preservation process for value-addition of these flowers. Hence, rhododendron flowers can be successfully utilized for the production of good quality and nutritionally enriched products.

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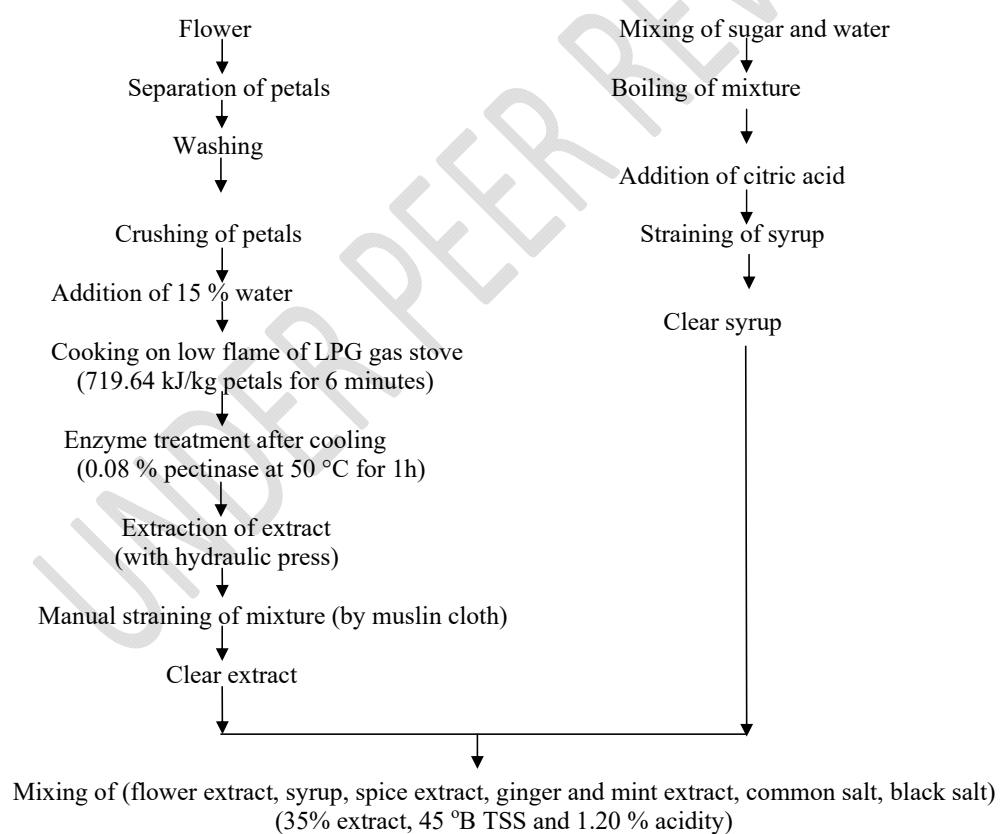
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Table 1. Treatment detail of appetizer

Treatment	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈
Flower extract (%)	25	30	35	40	25	30	35	40
TSS (° B)	40	40	40	40	45	45	45	45



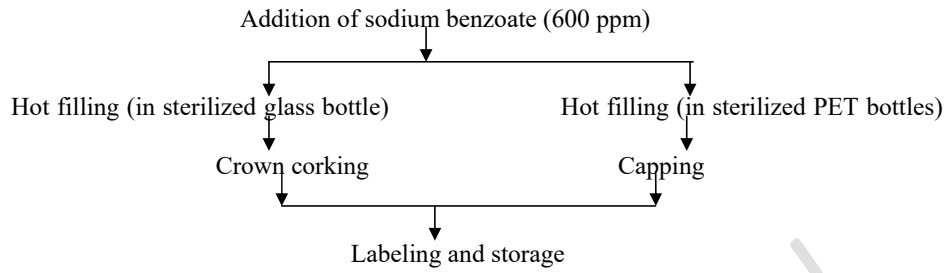


Figure 1. Process flow chart for the preparation of rhododendron appetizer

UNDER PEER REVIEW

Table 2: Physico-chemical characteristics of different recipes of rhododendron appetizer

Treatments	Colour (TCU)		Apparent viscosity (Min.)	pH	Sugars (%)		Ascorbic acid (mg/100 ml)	Anthocyanins (mg/100 ml)	Total phenols (mg/ 100 ml)	Antioxidant activity (%)
	R	Y			Total sugars	Reducing sugars				
T ₁	16.71	2.40	21.27	3.51	37.81	28.71	5.01	19.01	26.71	18.96
T ₂	17.43	1.97	24.15	3.56	37.89	28.86	6.53	22.86	31.81	22.86
T ₃	18.93	1.53	27.16	3.58	37.91	28.91	8.56	26.81	36.95	26.99
T ₄	18.36	1.20	30.05	3.61	38.16	29.06	9.13	30.89	42.15	31.21
T ₅	16.98	2.35	23.15	3.52	42.65	33.81	5.06	19.05	26.75	18.98
T ₆	17.60	1.95	26.21	3.56	42.71	33.89	6.55	22.89	31.83	22.87
T ₇	18.36	1.48	28.36	3.59	42.81	34.01	7.99	26.86	36.97	27.02
T ₈	19.12	1.11	30.31	3.62	43.26	34.21	9.15	30.93	42.19	31.25
CD _{0.05}	0.12	0.09	0.38	0.01	0.10	0.12	0.11	0.17	0.19	0.21

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Table 3: -Sensory characteristics (scores) of different recipes of rhododendron appetizer

Treatment	Colour	Body	Taste	Aroma	Overall acceptability
T₁	7.90	8.00	8.00	7.17	7.73
T₂	8.03	8.07	8.07	7.32	7.83
T₃	8.40	8.30	8.50	7.52	8.30
T₄	8.30	8.20	8.30	7.40	7.97
T₅	8.00	8.01	8.01	7.22	7.84
T₆	8.13	8.11	8.11	7.33	7.93
T₇	8.15	8.20	8.28	7.50	8.05
T₈	8.30	8.20	8.31	7.40	8.07
CD_{0.05}	0.09	0.08	0.16	0.09	0.13

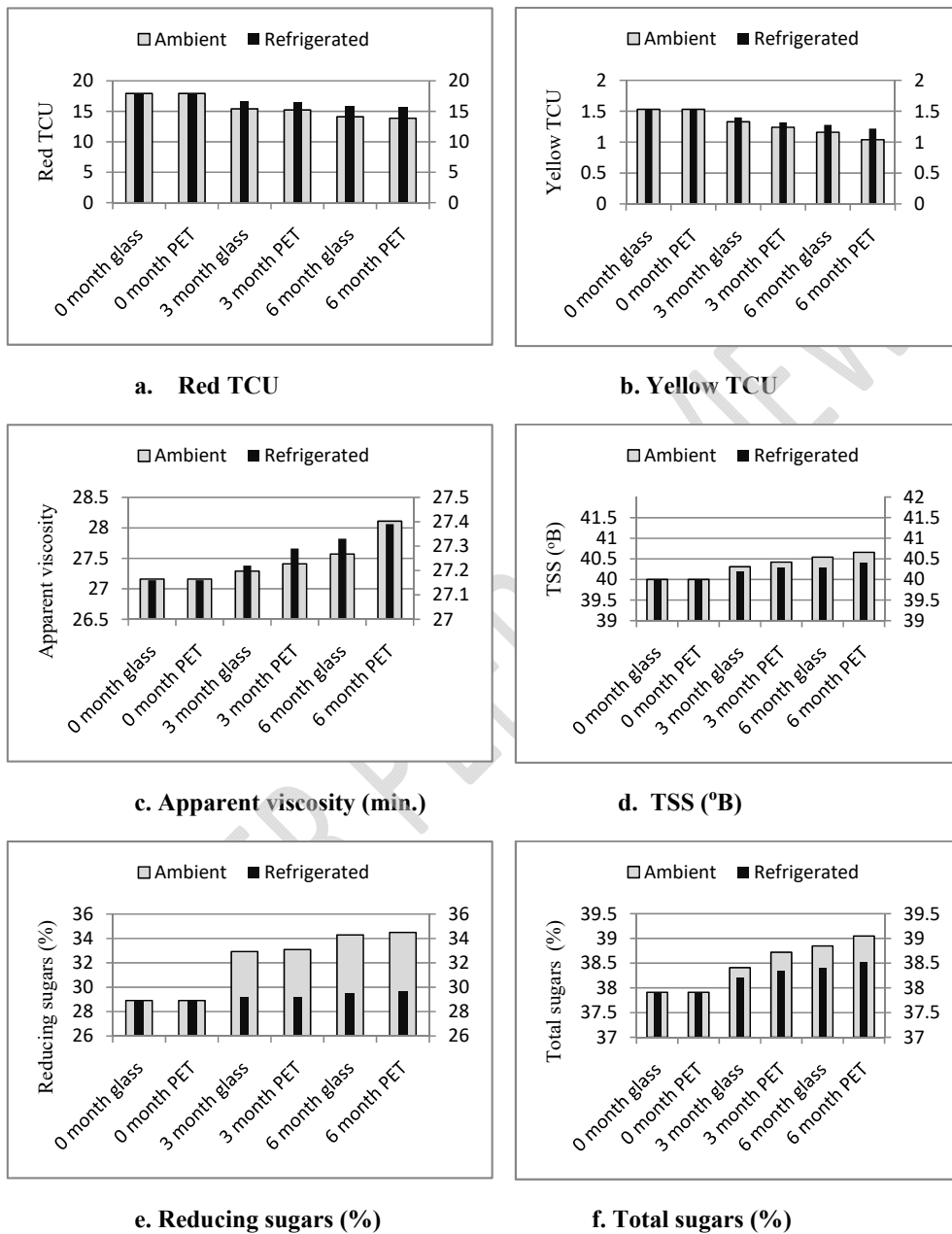
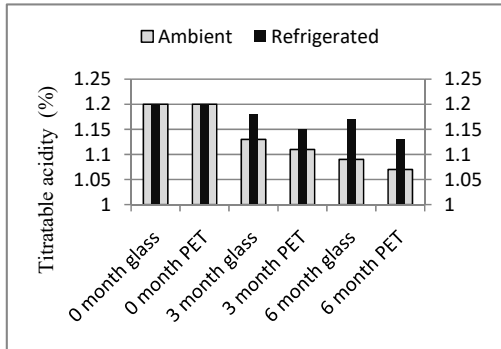
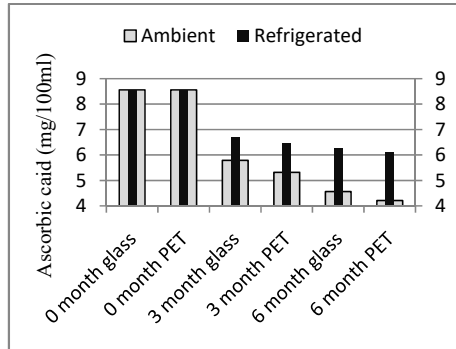


Figure 2 (a-f). Effect of storage on physico-chemical characteristics of rhododendron appetizer

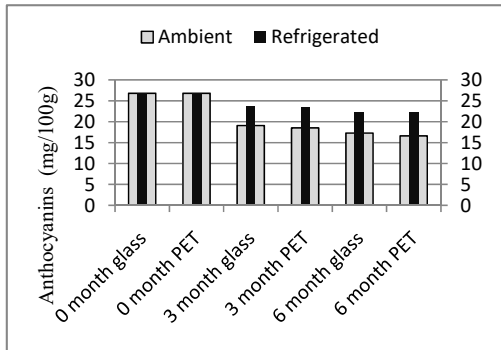
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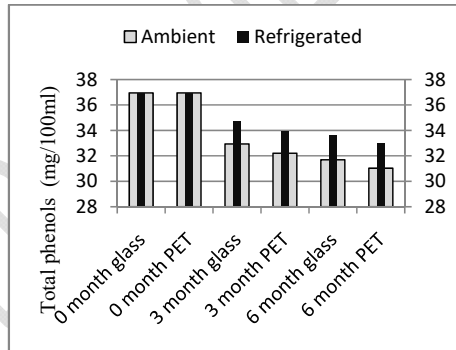
g. Titratable acidity (%)



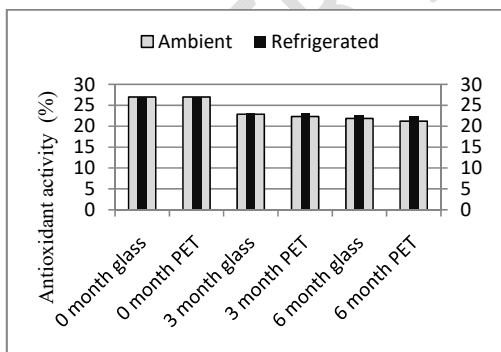
h. Ascorbic acid (mg/100ml)



i. Anthocyanins (mg/100 ml)



j. Total phenols (mg/100ml)



k. Antioxidant activity (%)

Figure 2 (g-k). Effect of storage on physico-chemical characteristics of rhododendron appetizer

