Advances in Bioresearch
Adv. Biores., Vol 11 (2) March 2020: 01-10
©2020 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.11.2.110

Advances in Bioresearch

ORIGINAL ARTICLE

Effect on Thermo Sensitive bio available Secondary metabolites of *Rhododendron arboreum* by Cold pressed and centrifuged method of extraction.

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ABSTRACT
This study was conducted to prepare a Juice from (Rhododendron arboreum) petals value added with Ginseng and Aloe barbadensis. By two extractions i.e., cold pressed and traditional centrifugation. The prepared product was evaluated for parameters like physico-chemical functional properties, ascorbic acid, total carotenoids, total anthocyanins, antioxidant activity and organoleptic evaluation. The Thermo sensitive bio available secondary metabolites were documented in Rhododendron arboreum flower petals by cold pressed method were sustained in the juice extract as compared to the regular centrifugation method, Thus, this study strongly recommends cold pressed extraction over centrifugation, to avoid the loss of thermo sensitive bio available secondary metabolites. The storage study reveals the stand of 72 h at room temperature for cold pressed extraction, 48 h for centrifuged extraction.
Keywords: *Rhododendron arboreum, secondary metabolites, cold pressed extraction, Centrifugation extraction, cloud value.*

Received 01.11.2019 Revised 18.01.2020 Accepted 26.02.2020

How to cite this article:
B Jyoti, P Pant, N Choudhary. Effect on Thermo Sensitive bio available Secondary metabolites of *Rhododendron arboreum* by Cold pressed and centrifuged method of extraction. Adv. Biores., Vol 11 (2) March 2020: 01-10

INTRODUCTION

Cold-pressed juices are claimed to contain higher levels of secondary metabolites usually antioxidants and bio available compounds which are thermo sensitive in nature as compared to centrifugation. The conduct and perception of health-enthusiast consumers who are in regular practice of purchasing a service have molded the libation industry to develop and upgrade more improvised functional bracers which are value added with nutritional, exhilarating, ameliorative and invigorative benefits [1]. Moreover, the intensive demand for freshly extracted untreated crude flower/fruit and vegetable juices without much processing clearly specifies the acceptances towards the finished Bracers [2].Therefore, some libation producers have introduced freshly extracted, unprocessed, unpasteurized, cold-pressed juices and have proclaimed that such juices are much improvised and are rich in secondary metabolites as compared to the centrifuged juices. Due to the fact, that in traditional centrifugation, such Juicers are built in such a manner to have a fast-spinning metal blade placed juxtaposed against a mesh filter. These procedures disunite the extract from the pomace by centrifugal torque. When at a very high momentum, the metal blade shear the content heat generates, which adversely impacts the thermo sensitive bio available secondary metabolite of the extract. Whereas in cold-pressed process, fruit/flower are initially crush and then thrust the fruit to extract the juice at a very low momentum. This extraction process does not produce heat and preserves the thermo sensitive compounds. *Rhododendron arboeum, locally* known as "Burans" is an ethno botanical peculiar perennial plant of Himalayan region belongs to the family *Ericaceae.* Along with its idiosyncrasy, The plant exhibits clinical importance its anti-diabetic, anti-inflammatory, anti-diarrheal, hepato-protective, anti-oxidant or adaptogenic activity, cardio protective properties also marks the presence of bio available actinic compounds. It is a high altitude plant latitudes ranging1500-5500m. It is commonly known as state flower of Himachal Pradesh and Nagaland (India).

The aesthetically mesmeric scared flower has a deep impact in religious practices; used for ornamenting purposes within the region. The dried leaves tincture has been practiced in gout treatment and rheumatism. Poultice from the flowers is used in high fever. The flowers extract has a traditional practice to be used as remedy for nose bleeding in the respective region; for blood dysentery the extract is traditionally blended with cow's ghee. Apart from therapeutic medicinal attributes the Flowers of this plant are traditionally customized and utilized by the regional people to prepare local foods and beverages like jelly, sharbat, pickle, juice, jam, syrup, honey, squash, chutney etc., Though *Rhododendron* has multifarious usage, the standardized extract can be incorporated into various foods and beverages through cold pressed technique to secure thermo sensitive compounds. Similarly, *Aloe barbadenis* with the presence of bioactive compound, [3] offers an explanation to show lessen blood glucose level with various useful impacts have been accounted, including immune modulatory, wound and consume mending, hypo glycaemic factor, anticancer, gastro-defensive, antifungal, and calming properties. *Aloe barbadenis* is a colorless gel which does not interfere with the color values of the juice; it also helps to improve nutritive, physiochemical and sensory quality the finished product by protecting off-flavor of *Rhododendron arboreum* juice. Ginseng is another supplement which is specifically incorporated due to its adaptogen: decreases stress by suppressing release of cortisol, Immune modulator, helps in managing menstrual discomfort, improves erectile dysfunction problems, Pre-diabetics and diabetics by glucose regulation, also increases our cognitive ability. Previous studies on the effects of juice extraction methods on the quality of fruit juices. Examined the effects of two different juice extraction methods (centrifugation by a Phillips Electric juice centrifuge vs. squeezing by a Phillips Electric lemon squeezer) on the quality attributes of pomegranate juice. Compellingly, it was found that the two extraction methods did not have any effect on the quality parameters measured, including the composition of sugars and amino acids, color, pH, and anthocyanin content [4] correlated the nutritional value of grape juice prepared by three different household juicers: low-speed masticating juicer, a high-speed centrifugal juicer, and a blender.

It was concluded that the juice extracted by the low-speed masticating juicer had a higher nutritional quality than that of the other two types. Another additional component associated with the quality attributes of juices is the effect of storage conditions, mainly temperature and time-period on the thermo sensitive bio available nutritional components of juices. During storage, the degradation of some bioactive compounds, such as vitamin C, and total carotenoids could occur, which is a critical factor regarding the quality of juices. Generally, bracer producers set a shelf life of shorter time span, during which the physicochemical properties of juices remain conserved. However, during this period, the presence of bio available properties, such as antioxidant capacity and the means by which they are affected by storage conditions remain unclear. However, to consider this issue, few researches have been documented the effect of storage conditions on antioxidant capacity and bioactive compounds of some fruit juices [5],[6],[7], [8].However, to the best of our knowledge, no such information is available on the standardized cold pressed extract of *Rhododendron arboreum* value added with ginseng and *Aleo barbendenis* over the traditional centrifugation method. Furthermore, there is no information published on the thermo sensitive bioavailable compounds extracted by a cold-pressed juicer and normal centrifugal juicer, Moreover, we determined the effect of the storage time-temperature on the quality of cold-pressed juices. The information attained from this study will increase consumer awareness regarding the content of thermo sensitive bio available cold-pressed juices over the traditional centrifugation methods.

MATERIAL AND METHODS

Burans Juice sample Preparation: Two juice extraction procedures have been followed, cold-pressed juice Agaro Imperial Slow Juicer 240 Watts (Agaro, India) Philips viva collection HR 1863/20 centrifugal juicer (Philips, India). Flower of *Rhododendron*, grown in the Kumaon region of Uttarakhand were procured. The flower petals were cleaned and graded according to size and color and after removing the sexual organs, calyx and stalk; the petals were washed under running tap water to obtain the edible portion. This edible portion (petals) was used for further analysis. All the analysis was done in triplicate. The juice extracted by cold pressed method was filtered through a 0.45 µm cellulose ester filter (Merck, Germany) and supernatants used for further analyses. Whereas the juice extracted by centrifugation method was then filled in sterile 50-mLconical centrifuge tubes and centrifuged at 12000 rpm for 15 min at 4 C. The supernatants was filtered as explained above and transferred into a vial for further analyses.

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Fig: 1 Agaro Imperial Slow Juicer 240 Watts (Cold Pressed Juicer) and 2 Philips viva HR 1863/20 (Centrifugation Juicer)

Total Soluble solid content, titrable acidity, reducing and total sugars and pH

Brix was measured at 20°C using an Abbe refractometer (Atago, Tokyo, Japan). Titratable acidity, reducing and total sugar were determined according to the method suggested by Ranganna (1986). The pH of the extract was determined using a pH meter (Ino lab pH 730, Merck Specialties Pvt. Ltd., India).

Washing, Cleaning and Sorting of *Rhododendron Arboeum*petals

⬇

Heating with water at 80° C for 5 min)

⬇

Cooling (room temperature)

⬇

Filtration, through 0.45$_u$m cellulose ester filter paper

⬇

Extraction of juice Agaro Imperial Slow Juicer 240 Watts (by cold pressed method)

⬇

Addition of Ginseng and Aloe barbendsis in juice

⬇

Filtration, through 0.45$_u$m cellulose ester filter paper

⬇

Addition of Preservative and stabilizers

⬇

Water bath

⬇ ⬇

Bottling and Capping Further analysis of juice

⬇

Storage (Room and refrigeration temperature)

Flow chart for *Rhododendron arboreum* Juice extraction by Cold pressed Method

Estimation of ascorbic acid, total anthocyanin and total carotenoids;

The ascorbic acid and total carotenoids contents of the samples were determined as per the method suggested by [34]. Results were expressed on mg/ 100 ml. The total monomeric anthocyanin content was determined on a UV-visible spectrophotometer by the pH differential method [35]. The pigment content

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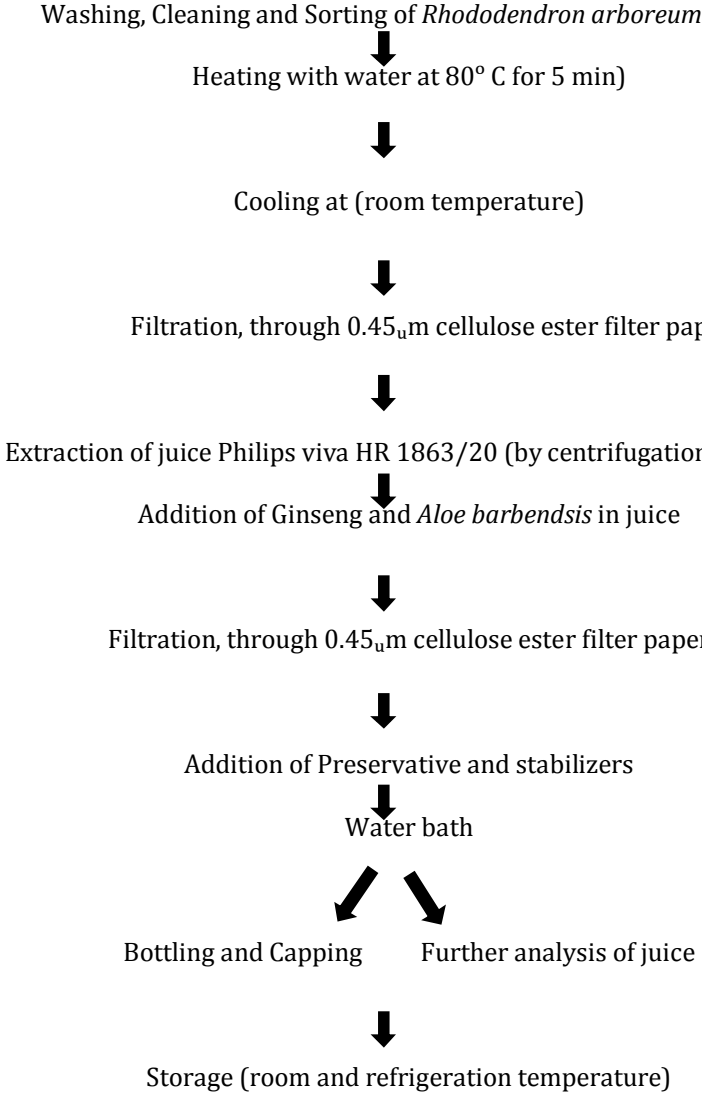
was calculated and expressed as mg cyaniding 3-glucoside (Cyd 3-glu) per L, using an extinction coefficient (€) of 26,900 L/cm/ mol and a molecular weight of 449.2gmol/L.



Flow chart representation of *Rhododendron arboreum* Juice extraction Centrifugation Method.

Antioxidant activity (Cupric reducing antioxidant capacity, CUPRAC):
CUPRAC assay was performed according to method developed by [36]. To100µL of sample aliquot, 1mL each of copper (II) chloride solution (10−2M), neocuproine solution (7.5×10−3M) and ammonium acetate buffer solution (pH 7) solution was mixed. The tubes were stoppered and after 1 h and absorbance at 450 nm was recorded against a reagent blank. The antioxidant activity was expressed as mmolTrolox®/ liter, or mM TE.
Organoleptic evaluation for acceptability of the Juice:
Organoleptic evaluation was performed on juice preparations by a ten-member trained panel. For each sensory parameter, such as color& appearance, texture, flavor, taste and overall acceptability, 100 marks were allotted and the products were given to the panelist in coded [9], [10]. The panelists washed their mouths with water intermittently to evaluate samples. Significant differences were determined at the ($P<0.05$) level of significance using the Duncan's multiple range tests.
Microbiological analysis:
Microbiologically the cases were analyzed for total plate count, yeast and mould count. The case for microbial test was drawn aseptically to gain botch free results.
Total microbial count:

Appropriate dilutions of the cold pressed & centrifugation sample (200µl) was plated with (10-15 ml) pre-sterilized melted plate count agar at 45°C, after solidification the petri plates were incubated at 37± 1°C for 24-28 hours. The average count of colonies on petri plates were multiplied by dilution factor and expressed as colony forming units (CFU) per ml. To analysis their data as a 1 d, 3 d, 5 d & 7 d in refrigeration and room temperature.

Yeast & mould count:

Appropriate dilutions of the cold pressed & centrifugation sample (200µl) were transferred aseptically to sterile petri plates in duplicate and mixed well with 15-20 ml of pre-sterilized, melted potato Dextrose agar (pH 3. 1 ±0.1). The petri plates were incubated at 22±1°C for 3-5 days. The average count of colonies present on petri plates were multiplied by dilution factor and expressed as colony forming units (CFU) per ml. To analysis their data as a 1 d, 3 d, 5 d & 7 d in refrigeration and room temperature.

Statistical analysis:

Experiments were laid in complete randomized design with three replications. Duncan's Multiple Range Test was used to determine significant differences. Significance was determined at $P<0.05$. Correlation between total antioxidant capacity and different attributes were computed after cold pressed extraction values and centrifuged extraction values respectively.

RESULTS AND DISCUSSION

In recent years, there is a constant need for improved functional bracers which are not only nutritionally rich in context of freshness, but higher in vitamin content too with minimal processing. Pasteurization is important but has had a negative impact on quality attributes of the juices specifically sensory characteristics of beverages. Though as per the dietary guidelines the conscious consumer is in frequent need of pure flower/fruit juices which has the health-enhancing properties rich in thermo sensitive bio available secondary metabolites plus microbiologically stable. By and large the cold pressed juices have been known as a new generation bracer, time and again proved to be much higher in nutritional aspect as a compared to the traditional centrifuged ones.

Table1.Comparison of Quality attributes during different processing of juice

Quality Attribute	Control	Cold pressed	Centrifuged
Ascorbic acid (mg/100ml)	12.5a	21.9c	17.4b
Total anthocyanins (mg/L)	152.6c	68.76b	28.26a
Total carotenoids (µg/100ml)	2682b	2579a	2680b
Total antioxidant activity (mM Trolox Equivalent(TE)/L)	72.2c	69.6b	61.9a

Values are mean of three independent determinations. Different superscripts in the column with different alphabet are significantly different ($p<0.05$).

Table2. Comparison of chemical analysis during different processing of juice

Chemical analysis	Control	Cold pressed	Centrifuged
TSS	4.78a	7.01b	6.99b
pH	4.0c	3.5b	3.1a
Reducing sugars (%)	3.34a	5.57b	6.01c
Total sugars (%)	7.07a	8.04b	7.98b
Acidity	1.86a	2.12b	2.98c

Values are mean of three independent determinations. Different superscripts in the column with different alphabet are significantly different ($p<0.05$).

The *Rhododendron arboretum* Juice recipe has been standardized with the value addition of Ginseng and ***Aloe barbendsis*** and juice has been extracted by both the methods i.e., cold pressed and centrifugation. Various research studies have been done to analyze the total anthocyanins, total caroteniods, antioxidant capacity and ascorbic acid. When we measured and compared the values of cold pressed juice with centrifuged ones the content of thermo sensitive bio available secondary metabolites have restored remarkably in case of cold pressed extraction. This result provide convincing evidences that the above mentioned claim is not misleading, such studies have been done earlier too, which explains similar observation [11] who considered grape juice extract and found the cold pressed juice extract is more nutritious as compared to other high speed centrifugation extract. Storage conditions, (i.e, time-

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temperature) combination, could affect the thermo sensitive bio available secondary metabolites and antioxidant capacity related to the different juicing methods. Previous studies have considered this phenomenon and have found that the storage plays a crucial role in the quality of the juices. Under the refrigerated conditions (i.e, 40C) the nutritional quality and antioxidant capacity of juices remains unchanged as compared to be stored at room temperature [12], [13], [14]. Coherently in this study it has been found that storage of juice at room temperature adversely effects the quality of cold pressed juice within 72h, and 48h to that of centrifuged ones [15], [16], [17]. But due to the presence of ***Aloe*** *barbendsis* and its antifungal properties there is a delayed degradation in the structure of bio available compounds.

However the high curative and remedial value of *Rhododendron arboreum* value added with ginseng marks to the presence of several antioxidants, carotenoids, anthocyanins etc.[18] As a result our research aims to provide a bracer which is antioxidant rich and can be used as a "mood-enhancer" drink due to the presence of anti-depressant ginseng, Future perspective study with minimum processing effect, by using new age cold pressed extraction procedure.

The analysis of our designed experiment clearly indicates the high content of antioxidants (Table1) in cold pressed extraction as compared to centrifugation. It is suggested to preserve fruit juices in a controlled time-temperature atmosphere i.e., refrigeration and consuming it in acceptable time limit is suggested. Few researches have noticed that such storage could affect the TPC and antioxidant activity of the extracted juice.[19], however, a slight decrease in ascorbic acid and total caroteniod values of some fruit beverages which are kept in refrigerator are having a stand of 8 days [20]. Thus, as a natural phenomenon the quality of freshly prepared unpasteurized, untreated extracts have detrimental effect on its quality attributes. Thus, there is no certain scale to measure the exact shelf life or stand of freshly prepared unpasteurized, untreated juices extracted by cold pressed and centrifugation methods respectively, kept under home-refrigeration. However most of the bracer manufactures claim to a shelf life of 3-5 days. Thus, in the present study the experiment has been designed to examine the quality of cold pressed juice and centrifuged juice under home-refrigeration process. The samples were taken from (1 d), (3 d), (5 d), (7 d, usual suggested shelf life of bracers). No changes were noted for the (total bacterial and yeast) i.e., total microbial count under the home refrigeration storage (Table 3) on the 7 d at 40C, signifying the stability of extract during storage. Furthermore the physico-chemical properties i.e., (TSS, pH) of the juice extracted by the cold pressed and centrifugation was slightly differing from each other. Titratable acidity increase in both case due to the presence of ginseng.

Clarity is another important terminology associated with the juices, used to determine the cloud value in the juice extractions, such as proteins, lipids, cellulose and hemicelluloses and other minor particles [21]. The acceptance of any bracer majorly depends upon the cloud value, it is a visual parameter interconnected with the flavor and color, thus plays major role in OAA of the juice [22]. In the present study the cloud value of *Rhododendron arboreum* juice value added with ginseng and *Aloe barbendsis* did not reveal the presence of any ascribed particles; no change during the storage has been noticed in cold pressed juice which clearly indicates the stability of the juice, during the storage at 40C. Whereas there is a significant increase in the cloud value of *Rhododendron arboreum* juice extracted by the centrifugation process on 7 d, the increase of cloud value marks the splitting of large molecules resulting in higher number of suspended particles.

The ascorbic acid content of *Rhododendron arboreum* juice was 21.9mg/100 ml in cold pressed extraction and 17.4mg/100 ml in centrifuged extraction respectively (Table 1). *Rhododendron arboreum* juice had vitamin C contents even more than some other fruits like mangosteen, 1.0 µg/g; red jambu, 1.6 µg/g; European plum, 1.8 µg/g and banana, 2.2µg/g fresh weight of fruits [23] ascorbic acid is thermo sensitive in nature, heat treatment adversely affects the ascorbic acid content and make it unstable. The lower value of ascorbic acid signifies its instability during the heat treatment [24].

Anothocyanins are thermo sensitive pigments and are relatively unstable during processing and storage. pH, temperature, ascorbic acid and oxygen are considered to be significant element which directly impacts the degradation or stability of the compounds [25]. Total anthocyanin in cold pressed juicing i.e., 68.76mg/L and 28.26 mg/L, was marked during centrifugation extraction, the lower values of total anthocyanins in centrifugation process exhibits the destruction of molecules due to heat generated by high speed spinning of the blades. In earlier studies too, [26] explained the heating effect of anthocyanins, their decomposition and transformation, which leads to the formation of open structure of anythocyanins with the formation of chalcones (degraded brown products). This may, however noted to have less anthocyanin content in the centrifuged *Rhododendron arboreum* juice.

The anothcyanins degradation is being accelerated due to the presence of ascorbic acid and higher pHvalues. Moreover the interaction of anthocyanins with ascorbic acid leads to the condensation reaction [27] which adversely affects the compounds of both. Furthermore the stability of thermo sensitive

anthocyanins is strongly influenced by the pH [28]. In previous studies also higher pHand low acidity has been noticed and which considerably affects the anthocyanin stability adversely [29].

Total caroteniod of *Rhododendron arboeum* flower extract during cold pressed centrifugation is found to be 2579 µg/100ml, due to prolong heating [30], during the processing when thermal treatment was given a sudden raise in the values of carotenoid content is being noticed [31] facilitating the degradation of caroteinod- associated protein related structures, whereas the value of centrifuged extraction was noted to be 2680 µg/100ml, higher than to that of cold pressed extraction.

Total antioxidant activity:"CUPRAC" method is being used to measure the antioxidant activity, this method is being found to be convenient to identify the antioxidant activity of a particular compound of *Rhododendron arboreum* extract, the total antioxidant activity of prepared juice was found to be more in cold pressed juice as compared to the centrifuged ones. The reason behind the difference in values is due to degradation of thermo sensitive bio available secondary metabolites[32]. The declining rate of antioxidant activity is co related with the decrease in ascorbic acid content, total carotenoids. Thus, in the present study the antioxidant activity was higher in cold pressed juice as compared to centrifuged ones.

Organoleptic evaluation: Feedback from trained sensory panel was complied. The result reveals the acceptance value for the parameters of color, appearance, flavor and texture upon the sensory evaluation of the extracted juices by cold pressed and centrifugation method respectively. There is ($P<0.05$) differences in appearance, color, flavor and texture upon sensory evaluation cold pressed juice to that of centrifuged juice. At (0 d) storage on home- refrigeration the flavor enhancement has also been noticed, due to the addition of ginseng but on the (7 d) Color stability is another considerable quality feature associated with the storage of juice, by different means of extractions. However in this present study almost negligible difference in color was observed in cold pressed juice and centrifuged juice respectively, also both the value additives i.e., ginseng and *Aloe barbendsis* hardly brings about any change in the color values of the *Rhododendron arboreum* juice.

Sensory evaluation is widely used in food science and technology to estimate the quality of a product with respect to mouth feel, odor, taste, color. Considering, analyzing the sensory characteristics of freshly extracted unpasteurized cold pressed juices during home-refrigeration storage is of most benefit for obtaining ideal storage conditions that would satisfy the health enthusiast zeal of consumer acceptance. Hence, the parameters measured in the present study has adequate substantial knowledge regarding the effect of storage conditions on the quality of cold-pressed juice over centrifuged juice.

However, to expand our knowledge, flavanols, total flavonoids, and total phenol in concern of extract collected from cold pressed and centrifugation juicing procedures could be a subject for future investigation. In summary, we compared the content of thermo sensitive bio available secondary metabolites and antioxidant capacity of cold-pressed juices to those of the traditional centrifugation ones. Our results thus provide evidence that claim higher nutritional quality, retention of thermo sensitive bio available secondary metabolites, of cold-pressed juices over traditional centrifugation. Moreover, the physicochemical functional properties, antioxidant capacity and content of thermo sensitive bio available compounds of cold-pressed juices remained unchanged until day 5 of storage under home refrigerated conditions, whereas centrifuged ones stands for 2 days only, However, at day 7, most of the measured values started to decline and reached their lowest levels at day 7 of storage. This observation indicates that storage of juices in refrigerators could negatively affect the quality of cold-pressed juices and centrifuged juices, thus questioning the claim regarding the longer shelf life of juices kept in home refrigerators by any way of extraction.

Fructose is a reducing sugar, which indirectly enhance the anthocyanin content of the extract, this happens majorly due to the degradation during heating, also 5-(hydroxymethyl)furfural formation takes place which uniformly accelerates the rate of anthocyanin degradation [33].

Microbiological

Total plate count: The data presented in Table 3indicates that the addition of *Aloe barbadenis* the microbial count samples in comparison to control samples. The *Rhododendron arboreum* juices with cold pressed and centrifuged stored at room temperature and had higher count as compared to the samples stored at refrigeration temperature. During storage, total plate count slightly increased.Significantly interaction among the preservatives, storage temperature and storage period showed that the total plate was higher.

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Table 3 Total plate count (cfu/ml) for Cold press and centrifuged samples at different treatments and storage conditions

Treatment	Storage Temperature	Cold Press				Centrifuged			
		Storage Period (Days)				Storage Period (Days)			
		1	3	5	7	1	3	5	7
Control	Room Temperature	-	98	159	208	-	120	187	269
	Refrigeration temperature	-	45	106	170	-	68	148	198
Sodium benzoate	Room Temperature	-	55	96	102	-	59	98	124
	Refrigeration temperature	-	-	60	80	-	-	76	93

Table 4 Yeast and Mould Count (cfu/ml) for Cold press and centrifuged samples at different treatments and storage conditions

Treatment	Storage Temperature	Cold Press				Centrifuged			
		Storage Period (Days)				Storage Period (Days)			
		1	3	5	7	1	3	5	7
Control	Room Temperature	-	10	23	46	-	24	34	65
	Refrigeration temperature	-	-	14	34	-	-	23	45
Sodium benzoate	Room Temperature	-	-	8	12	-	-	10	14
	Refrigeration temperature	-	-	3	7	-	-	5	11

Yeast and Mould count: The yeast and mold count of *Rhododendron arboreum* are presented in table 4. The data showed that all the samples were devoid of any yeast and mould count during first day of storage. The yeast and mould count appeared after two days of storage and increase thereafter.
Thus, the addition of *Aloe barbadenis* lowered yeast and mould count in comparison to the control. The *Rhododendron arboreum* juices with cold pressed and centrifuged stored at room temperature higher count as compared to the sample stored at refrigeration temperature. Significantly interaction among the preservatives, storage temperature and time showed that yeast and mould count was low when the sample treated with *Aloe barbadenis* and stored at refrigeration temperature.

CONCLUSION

The present study suggested the extraction of juices via cold pressed extraction due to the sustaining of thermo sensitive bio available secondary metabolites in comparison to the centrifugation process.

ACKNOWLEGEMENT

We thank Dr. Prity Pant Professor, Department of Agriculture, Swami Vivekananda University Sagar, Madhya Pradesh, India for providing laboratory facilities.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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