International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 15-02-2021; Accepted: 08-03-2021: Published: 13-03-2021
www.botanyjournals.com
Volume 6; Issue 2; 2021; Page No. 135-140

Effect of nutraceuticals on physico-chemical properties of mulberry blended juice

Shweta Parida[1*], Kalpana Rayaguru[2], Jogeswar Panigrahi[3], Chandrashree Lenka[4]
[1] Department of Food Science Technology and Nutrition, Sambalpur University, Sambalpur, Odisha, India
[2] Department of Agril Processing and Food Engg, CAET, OUAT, Bhubaneswar, Odisha, India
[3] Department of Biosciences and Bioinformatics, Khallikote University, Berhampur, Odisha, India
[4] Department of Home Science, Sambalpur University, Sambalpur, Odisha, India

Abstract
Mulberry is a ripe, fleshy, delicious fruit that contains low calories and healthy phytonutrients such as polyphenols, minerals and vitamins which are important for optimising the potency of health. The phenolic compounds in mulberry fruit include anthocyanins, flavonols, flavanones, phenolic acids etc. In addition, natural herbs such as basil and mint with ginger, rich source of phytochemicals that provide metabolic functions which for wellness among human-being. Blended juice was prepared with different proportions of herbs and ginger with mulberry. There were 8 different combinations of the juice. Among all the combinations, the sensory score was found best in treatment MBJ T8, followed by the combination MBJ T2. The combination MBJ T8 shown total phenol content, total flavonoid content & total antioxidant capacity as 708.34 ± 11.86 mg GAE, 236.91 ± 5.06 mg QE and 1117.47 ± 5.77 mg TEAC per 100 ml respectively.

Keywords: anthocyanins, flavonoids, phytochemicals

Introduction

Epidemiological evidence is growing day by day, where diets associated with fruits and nutraceuticals have reduced risk of heart disease, cancer, and other chronic illnesses. A diet with increased antioxidant intake, include carotenoids, ascorbate, tocopherols, and phenolics, is now prevalent (Kalt *et al*., 1999; Moyer *et al*., 2002) [11, 21].

Mulberry (*Morus indica*) is a perennial, woody plant that was native to China. Mulberry plants exhibit rapid growth and a short period of proliferation. Mulberry fruit composition depends on their cultivation, diversity, and agro-climatic conditions. These berries were used in medicine for many years. Mulberry contains some phytochemicals that are known as bioflavonoids, which act as antioxidant. The therapeutic properties of mulberry are due to flavonoids and anthocyanin content (Zhishen *et al*., 1999; Naderi *et al*., 2004) [37, 23]. The intensely colored mulberry fruit is rich in phenolic compounds, flavonoids, anthocyanins, and carotenoids (Sofia *et al*., 2014) [32]. This berry's composition and therapeutic properties differ due to the concentration of anthocyanin pigments. Anthocyanin pigments contain colors like red, violet, magenta, and blue. This pigment assists to control pathways against various diseases as dietary modulators. The natural anthocyanin pigments are sensitive to the impact of factors such as pH, temperature, oxygen, and enzymes (Jackman *et al*., 1987; Stintzing *et al*., 2002) [9, 33].

Zingiber officinale, a herb popular as ginger, belongs to the *Zingiberaceae* family. The rhizome (underground stem) is used as a seasoning, medicine, and flavoring agent as well. Ginger has antimicrobial and antioxidant properties, widely used for treating different forms of stomach problems, including bowel movement sickness, stomach upset, pain, diarrhoea, cancer treatment, nausea and vomiting followed by surgery, and loss of appetite. Specific applications include fatigue or muscle soreness pain relief, menstrual pain, infections of the upper respiratory tract, cough, and bronchitis. Ginger is used as a flavoring agent in foods and drinks, in addition to improving nutritional properties. Basil (*Ocimum basilicum*), the gently branched shrub, a holy herb, belongs to the *Lamiaceae* family. Its high antioxidant content reduces cholesterol levels and blood glucose levels, avoiding radiation poisoning and Cataracts. As an anti-aging agent, it is also beneficial for respiratory disorders (Khogare and Lokhande, 2011) [12].

Mint (*Mentha spicata*) acts as a good appetizer since it contains small amounts of limonene, pulegone, caryophyllene, and pinene, which make it more palatable. Mint provides a refreshing flavour to drinks and is good against gastric problems. The purpose of this study was to investigate the chemical and sensory properties of mulberry blended juice with herbs from different plant varieties, to assess their composition, and to obtain some new scientific data on the suitability of the best combination of a mixed juice for the market.

Materials and Methods

Preparation of juice

Mulberry blended juice (MBJ) was prepared from freshly harvested mulberry from Burla, Sambalpur. Fruit was washed with tap water, and the stems were removed manually. A juicer (Bajaj Fx7 699W Food Processor) was used to extract juice for preparation of MBJ. The juice was then clarified by holding the juice for 30min at 4-8 ° C, and filtered through a muslin cloth. Furthermore, ginger rhizomes were cleaned, peeled, and ground to fine paste. Basil, and mint leaves were collected separately and ground. The pastes were sieved by muslin cloth and clear juice was collected. In order to facilitate the consumer, with variance of blended juice, the present investigation was carried out with different combination of mulberry with these three flavour enhancers.

Juice blending proportion

Table

MBJ T1	**= Mulberry (Control)**
MBJ T2	= Mulberry + Ginger (3%)
MBJ T3	= Mulberry + Basil (8%)
MBJ T4	= Mulberry + Mint (16%)
MBJ T5	= Mulberry + Ginger (3%) + Basil (8%)
MBJ T6	= Mulberry + Basil (8%) + Mint (16%)
MBJ T7	= Mulberry + Ginger (3%) + Mint (16%)
MBJ T8	= Mulberry + Ginger (3%) +Basil (8%) + Mint (16%)

Mulberry blended juice samples were heat pasteurized at 70°C for 10 min. According to You *et al.*, 2018[36] this combination of time and temperature was most suitable for the sterilization of mulberry juice to assure at least 5 log reduction in resistant pathogenic microbes.

Estimation of pH, TSS and Acidity

An Abbe Refractometer (Bellingham-Stanley Ltd., England) and a pH meter (Sartorius PB-11, Germany) were used to measure total soluble solid and pH respectively at 20 ° C. Titrable acidity was calculated by IFJU's (1968) [8] process and expressed as 'g citric acid/100 ml juice.'

Estimation of Reducing Sugar Content

The reducing sugar content was estimated by dinitro-salicylic (DNS) acid method. This method is simple, sensitive and adoptable during handling of a large number of samples at a time. 0.5 to 2ml of the sample was pipetted out and the volume was equalized to 3ml with distilled water in all the test tubes. 3ml of DNS was added. Test tubes were heated in the boiling water bath for 5min., 1ml of 40% Rochelle salt solution was added when the test tubes were still warm. The intensity of red colour was read at 510nm. A series of standards using glucose (100mg) and graph was plotted. Tests were expressed as mg of reducing sugar per litre of juice.

Estimation of Vitamin C Content

The ascorbic acid or vitamin C content was determined by titration with 2,6-dichlorophenolindophenol sodium salt solution by the standard procedure given in AOAC,2005 [1] and expressed as mg/100gm. Ascorbic acid in the sample was solubilised or extracted in 2% m-phosphoric acid solution and the method was calibrated against a standard L-Ascorbic Acid (Sigma-Aldrich) solution (1mg/ml) (Ranganna, 2007) [26].

Total anthocyanin concentration

The total concentration of anthocyanin was calculated by the Lee *et al.*, 2005 [18] defined pH differential process. Use UV spectrophotometer (UV-1600) against a blank, the absorbance read at 520 nm and 700 nm, respectively. The TAC was based on C3G molar absorptivity of 26,900 and molecular weight of 449.2, and was expressed as C3G (Cyanidine-3-glucoside) equivalent mg L^{-1}.

Total phenolics concentration

In determining the MBJ's total phenolic concentration (TPC) the Folin-Ciocalteu approach as defined by Singleton *et al.* 1999 [30] was adopted. TPC was measured as a milligram of Juice equivalent gallic acid per litre.

Total flavonoid concentration

The total concentration of flavonoids (TFC) was determined using the colorimetric method of aluminium chloride, defined by Kwaw *et al.*, 2017[16]. The TFC was measured as a milligram of the corresponding quercetin per litre.

Determination of antioxidant activity

Antioxidant activity for mulberry blends was calculated by plasma ferric reduction (FRAP) assay (Benzie and Strain, 1996) [2]. The mixture was permitted to stand for 10 minutes and UV-visible spectrophotometer was used to test the absorbance at 593 nm. The blank was done using blank and solvent reagents. We used trolox as standard and readings were taken in triplicate.

Sensory analysis

A jury of 30 judges chosen from research students and FSTN department employees, Sambalpur University evaluated the juice for colour, flavor and overall acceptability according to Larmond's (1977) [17] suggested process. Samples were matched by line, and the score was given from 1 to 9, where 1 indicates extreme dislike and 9 indicates extremely liked.

Viscosity and Shear Stress (Flow-behaviour)

The apparent viscosity of the juices was calculated following centrifugation (10 min at 360 xg) using a viscometer from Brookfield (LV DV-E model, Brookfield Technology Laboratories, Stoughton, USA). A 16 mL volume of the juice with corresponding spindle was put in the UL-adapter. Viscosity was measured in the range of 21.5□22.5 ° C at temperatures. Measurements for each sample were performed in triplicate. Shear stress and shear rate were determined based on measured torque and speed of rotation.

Data Analysis

All data is described as triplicate measurements mean ± SD. One-way ANOVA and post hoc Tukey-HSD tests were conducted for each parameter considered to detect discrepancies between the juices. The correlation coefficients of Pearson were determined to determine the effect of the proportions of ginger, basil and mints on the characteristics of the mulberry blended juice. All statistical research was performed using SPSS statistical software (Version 20.0, SPSS Inc., Chicago, Illinois, United States).

Result and Discussion

pH, TSS and titratable acidity

In the analysis pH and ° Brix are slightly changed. The average pH values were 3.68 ± 0.02 and 3.92± 0.01 among all combinations of MBJ T1 to T8. The maximum ° Brix readings ranged from 14.13± 0.09 to14.35 ± 0.2 ° Brix after addition of sugar syrup to maintain the standards of RTS beverages. Differences between all the different combinations in average ° Brix readings represent the sweetness of different herbs. The average acidity value for citric acid ranged from 0.257± 0.007% to 0.264± 0.006%. In MBJ, TSS and acidity among the different combinations was found to be non-significant. Liang *et al.*, 2011[19] found that pH and acidity of raw mulberry fruit was 3.37± 0.06 to 3.82 ± 0.03 and 0.11± 0.00 to 0.72 ± 0.02 respectively. Chottamom *et al.*, 2012, Kim *et al.*, 2010 and Koyuncu *et* *al*, 2014 [3, 13, 15] found the TSS, 5.40 ± 0.34, 5.83± 0.04 and 5.45 to 7.26 °Brix in mulberry fruits.

Reducing Sugar and Vitamin C Content

Table 1 lists the reduction of sugar content in raw mulberry blended juice. Due to variations there were major differences for all of these parameters. Reducing sugar ranged from 5.17 ± 0.12 to 5.59 ± 0.12 g glucose/100 ml from juices. The value of vitamin C ranged from 71.60±0.73 to 81.09±2.52 mg/ 100 ml for different combination of MBJ.

Table 1: Effect of treatment mode on some quality attributes of pasteurized MBJ

Attribute	MBJ T1	MBJ T2	MBJ T3	MBJ T4	MBJ T5	MBJ T6	MBJ T7	MBJ T8
pH	3.71±0.02^{cd}	3.79±0.02^{b}	3.82±0.02^{b}	3.92±0.01^{a}	3.68±0.02^{d}	3.77±0.04^{bc}	3.81±0.01^{b}	3.89±0.01^{a}
TSS (°Brix)	14.36±0.1^{a}	14.30±0.11^{a}	14.26±0.09^{a}	14.13±0.09^{a}	14.25±0.07^{a}	14.24±0.27^{a}	14.20±0.16^{a}	14.17±0.15^{a}
Acidity (%)	0.257±0.007^{a}	0.257±0.006^{a}	0.264±0.003^{a}	0.257±0.007^{a}	0.264±0.002^{a}	0.264±0.006^{a}	0.257±0.007^{a}	0.257±0.004^{a}
Reducing Sugar (g/100mL)	5.59±0.12^{a}	5.30±0.14^{abc}	5.19±0.12^{bc}	5.17±0.12^{c}	5.31±0.22^{abc}	5.52±0.11^{ab}	5.50±0.14^{abc}	5.44±0.14^{abc}
Vit. C (mg/100mL)	81.09±0.90^{a}	79.48±1.42^{ab}	79.32±1.70^{ab}	73.96±0.68^{c}	77.52±0.83^{b}	73.19±1.20^{c}	72.21±0.73^{c}	71.04±3.66^{c}
Anthocyanin (mg C3G /100mL)	226.53±2.46^{a}	204.43±3.62^{bc}	208.73±5.62^{b}	197.40±2.62^{cd}	204.30±4.69^{bc}	196.40±2.32^{de}	193.88±3.78^{de}	188.73±1.39^{e}
TPC (mg GAE /100mL)	701.66±6.41^{f}	708.34±11.86^{ef}	717.50±6.90^{de}	737.01±3.92^{bc}	725.12±4.72^{cd}	772.57±4.66^{a}	750.31±4.90^{b}	779.39 ±5.23^{a}
TFC (mg QE /100mL)	232.91±4.13^{d}	236.91±5.06^{cd}	244.57±4.55^{cd}	273.76±7.85^{b}	247.40±4.43^{c}	283.86±6.44^{ab}	277.07±7.43^{ab}	287.55±4.80^{a}
TAC (mg TEAC /100mL)	1099.70±6.56^{f}	1117.47±5.77^{e}	1150.08±4.24^{d}	1128.07±2.67^{e}	1130.81±5.33^{e}	1169.06±6.53^{c}	1198.72±9.44^{b}	1218.34±6.78^{a}

* Along a row different superscripts indicate statistically significant differences ($p < 0.05$)

Pure mulberry juice (control) has the highest vitamin C content. Vitamin C is sensitive vitamin to be preserved during pasteurization as it is very sensitive to heat, which is most important water-soluble antioxidant in cells and an efficient scavenger of reactive oxygen species. According to Kim *et al*., 2004[14] the vitamin C content to be 53.23mg/100gm. Dimitrova *et al*., 2015[5] estimated the reducing sugar to be5.54 ± 0.2 and 5.6± 0.3 among 2 varieties of mulberry fruits. Yazdankhah *et al*, 2019[35] found 4.83% reducing sugar in black mulberry fruits. Our results are greater than Kim *et al*., 2004[14] for Vitamin C, as the varieties differ from one to another.

Anthocyanin, TPC, TFC and TAC Content

Anthocyanin contents of raw mulberry blended juice were found lowest in MBJ T8 and highest in MBJ T1. The values ranged from 188.73±1.39 to 226.53±2.46 mg/L of cyanidin-3-glucoside equivalents. Anthocyanin content got decreased as herbs were included in the formulation. The decrease in anthocyanin depends on the proportion of mulberry get decreased in the formulation. But, in case of TPC, TFC and TAC, the values were increased as the neutraceutical rich herbs had been added. The addition of basil and mint in the MBJ juice increase its phenolic and flavonoid content. Total phenol content ranged from 701.66 ±6.41 to 779.39 ±5.23 mg of gallic acid equivalent (GAE) per litre of juice (mg GAE/ 100ml). The total flavonoid content of MBJ ranged from 232.91±4.13 to 287.55±4.80 mg of quercetin equivalents (QE) per litre of juice (mg QE/100ml). The total anti-oxidant capacity of MBJ ranged from 1099.79±6.56 to 1218.34±6.78 mg of trolox equivalents antioxidant capacity (TEAC) per litre of juice (mg TE/ 100ml). The highest value of TPC, TFC and TAC were found in MBJ T8 where as MBJ T1 scored highest in anthocyanin.

Lee *et al*., 2005[18] reported the concentrations of total anthocyanins, total phenolics, total flavonoids in the extracts of mulberry to be 487.5±0.58mg C3G/100gm, 2027.4±18.5 mg GAE/100gm and 336.3±5.8 mg QE/ 100gm. Shivashankara *et al*., 2010[28] found that anthocyanin content was between 4,103.93 to 4,255.65 mg/100 g, total phenol content varied from 538.32 to 5,054.04 mg of gallic acid equivalents per 100 g, total flavonoid content was between a range of 8.72 to 157.71 mg QE/ 100gm and highest ferric reducing antioxidant potential 4,224.24 to 4,515.75 mg ascorbic acid equivalent antioxidant capacity. Negro *et al*., 2019 [25] found that Mulberries are a good source of anthocyanin about 300 mg/100 g FW, phenols 424 to 485 mg Gallic Acid Equivalent (GAE)/100 g FW, and a good antioxidant capacity by FRAP with 21μmols Trolox equvalent/g FW. Jiang *et al*., 2013[10] reported that anthocyanins, total phenolic acids and total flavonoid contents of ten varieties mulberry were determined where the highest content was 272.00 ± 1.20 mg cyanidin-3-glucoside/g, 690.83 ± 7.38 mg GAE/g and 965.63 ± 4.90 mg RE/g FW, respectively. Liang *et al*., 2012[20] studied 8 mulberry cultivars and found anthocyanins content (114.67-193.00 mg/100mg), total polyphenols content (189.67-246.00 mg GAE/100mg) and total flavonoid content (40.94 □ 150.32 mg/100mg). There was a good linear correlation between antioxidant activity and total polyphenols content.

Blended juices with the highest antioxidant activity and polyphenols have the strongest nutritional properties. However, polyphenolic contents are well known to be linked to the astringency and bitterness of juice, so that a high level of polyphenols can cause organoleptic properties to be defective. For this purpose, a sensory study of the juices was carried out so that the general acceptability among consumers could be calculated. The hedonic scale test, which offers a responsive assessment of any sensory changes (Meilgaard, Civille & Carr, 1999) [22], examined possible sensory variations in juices. By taking one way ANOVA, all different physico-chemical attributes like pH, TSS, acidity, protein, reducing sugar and other therapeutic components including vitamin C, anthocyanin, TPC, TFC and total anti-oxidant capacity (TAC) had been compared for 8 different compositions of the juice. From the ANOVA, it was found that there was a significant difference among the 8 different composition of mulberry blended juice on the basis of all the attributes except acidity and TSS.

Sensory Analysis

Sensory assessment is a valuable tool for product designing, lowering prices, quality assurance, and assessing storage stability. The food selection and purchasing of goods is affected by sensory evaluation. One goal of the sensory assessment is to reliably predict customer preferences. The sensory evaluation is the experience of taste, smell, touch and hearing perception that results from a complex of assessment and observations of food components by one or more individuals. Qualified panels and experts may be used to assess quality management targets and to facilitate the production of appropriate formulations and advancement of commodities. Untrained panels are generally judged for consumer reactions and/or product acceptance while evaluating the organoleptic attributes for a food. Sensory approaches to product optimisation have been used (Sidel and Stone, 1983 and Fishken, 1983) [29, 6]; quality assurance (Reece 1979; Nakayama and Wessman, 1979; and Wolfe, 1979) [27, 24, 34] and certainty of product□s shelf life (Dethmers, 1979) [4]. Flavor is an important parameter in drinks. Flavour is described as a sensation by the USA Flavor Chemist Society as being a product of the characteristics of mouth that stimulate senses of taste, smell, tactile sensations, and temperature felt in the mouth (Heath, 1978) [7]. The perception of taste, smell and mouthfeel are the chemical stimulations of taste buds, olfactory organs and mouth palate, throat, and nose when the food is consumed. The sensation experienced in the sniffing of an object as a sensation of the nose whereas sensing factors such as cooling, burning, and pungency is a combined effect of nose, mouth and throat. As the sensory analysis graph shows, a comparison of similarity values, which leads to draw a conclusion that taste is "highly significant□ attribute for MBJ juice, and therefore it is considered the most essential parameter among four variables. Both the mouthfeel and the flavour were equally significant (Sinija and Mishra, 2011) [31]. Hence, the data collected in the sensory study helps to approve the formulation. The overall acceptability of all these attributes has been summarised in the Fig.4.1. The star diagram of all above attributes represents highest score in combination of MBJ T8 followed by MBJ T2. In most of the judges there was a conflict related to strong mint flavour in MBJ T8, and preferred MBJ T2 because of its soothing astringency of ginger, followed by MBJ T4. Owing to the leafy aroma in the sample the score was least in cases of MBJ T6.

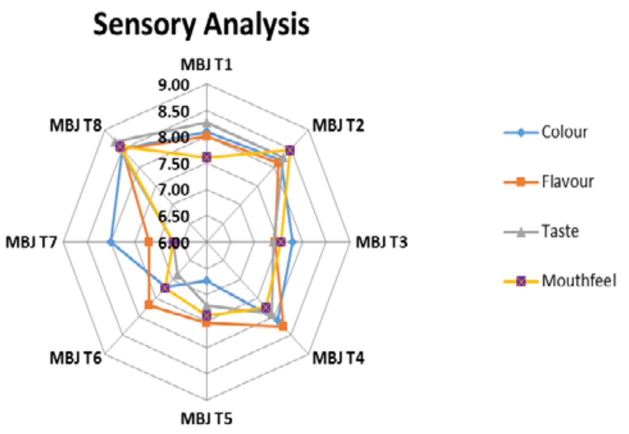

Fig 1: Sensory attributes score for different combination of mulberry blended juice

By taking one way ANOVA, 5 different sensory attributes like colour, flavour, taste, texture and overall acceptability (OA) had been compared for 8 different compositions of the juice. From the ANOVA table, it was found that there is a significant different among the 8 different compositions of mulberry blended juice on the basis of colour, flavour, taste, mouthfeel and OA. By comparing the overall acceptability of the MBJ juices, it was found that highest score was obtained in the formulation MBJ T8 with OA score 8.5 among all the combinations. The people who don□t like the strong mint flavour, the blend of mulberry with ginger in combination MBJ T2 with OA score 8.2 was recommended.

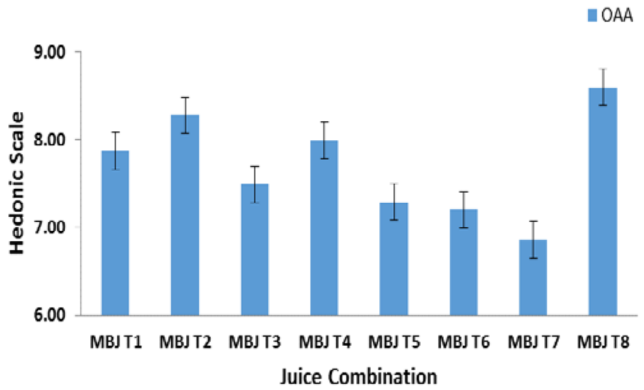

Fig 2: Overall Acceptability sensory score for different combination of MBJ juice

Viscosity and Shear Stress (Flow-behaviour)

A key parameter of flow behaviour is the viscosity of fluid foods. It essentially influences the overall mouth sensation and affects the taste strength and perception of flavour. Furthermore, researchers and industrialists have been interested in the viscosity of liquid food for many decades. For industrial quality control the connection between sensory and instrumental values may be used in order to ensure a good customer acceptance of sensory characteristics by viscosity within a range. It is important not only to obtain an excellent quality, but also to establish a data basis that is crucial for the optimisation of the installation design and transformation process itself, to understand the physical properties values depending on the temperature and the solids content during manufacturing phase (Zuritz *et al*., 2005) [38]. Owing to different combination treatment (Fig.4), viscosity changed just slightly among the combinations. The mean viscosity for MBJ T1 juices was 4.5 MPa.s, 20 °C in control sample and minimum in MBJ T8. In general, ginger, basil and mint juices mixture treatments did not change the viscosity and texture of the juice, which retains the characteristics of blended juice. A typical shear stress-shear rate curve was obtained in the Fig.3 for MBJ at 20 °C. There has been obviously non-newtonian behaviour of a pseudoplastic (shear-thinning) fluid. For all variations, similar curves have been obtained. Viscosity of MBJ T2, T5 and T7 were found to follow a similar pattern, where as MBJ T3 and T4 follow another parallel pattern.

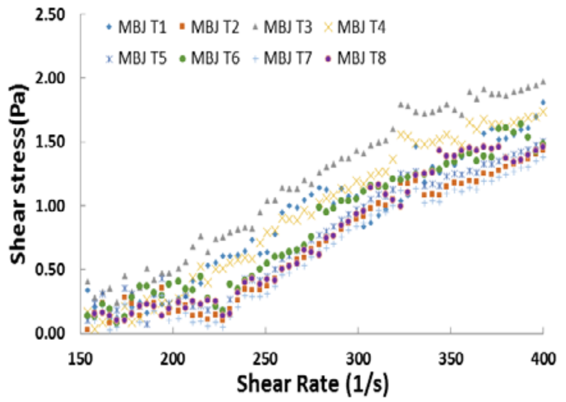

Fig 3: Flow curves of mulberry-blended juice at different treatment

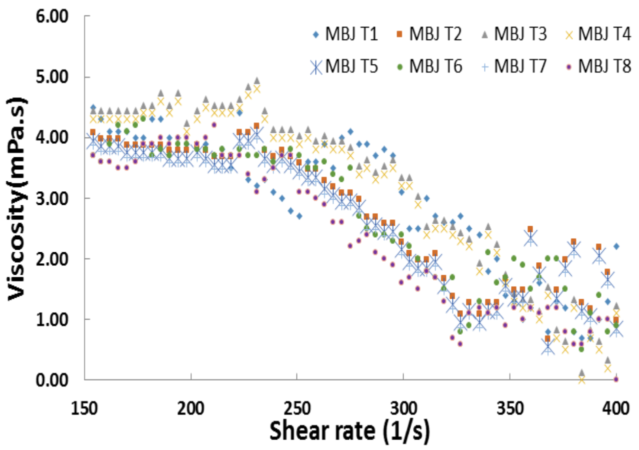

Fig 4: Viscosity curves of mulberry-blended juice at different treatment

Conclusion

As a conclusion, it is possible to improve the quality of single juice by blending with various herbs like ginger, basil and mint. The study revealed that the quality characteristic (except sensory) of mulberry blended juice could be improved by blending with various nutraceutical herbs like ginger, basil, and mint either individual or in combination at different proportion. Analysing the biochemical, therapeutic and sensory properties of mulberry blended juice with 3% ginger, 8% basil and 16% mint was confirmed to be best among all the treatment under study. However, followed by the best treatment in MBJ T8 (OA Score 8.50) the combination MBJ T2 with 3% ginger juice only can also be chosen as an alternative with sensory score of 8.2 as well as with a compromise value of total phenol content (TPC), total flavonoid content (TFC) & total antioxidant capacity (TAC) as 708.34 ± 11.86 mg GAE, 236.91 ± 5.06 mg QE and 1117.47 ± 5.77 mg TEAC per 100 ml respectively. The flow behaviour curve of shear stress verses shear rate for all the variants of treatments shown a non - newtonian behaviour indicating increase in shear stress at a reduced rate with increase in shear rate. But all the curves started from origin indicating pseudo-plastic behaviour of juice. The viscosity of all the juice of all the treatments reduced with increase in shear rate. The present research could provide useful data on formulation of blended juice for commercial mulberry juice production.

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