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Micro filtration of *Moringa Oleifera* juice using hallow fibre membrane technology for clarification

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***Abstract*---**Moringa Oleifera plant is growing many countries including tropical and sub tropical region. It contain numerous nutrition due to vitamins and proteins. The plant leaf extract having phenolics and flavanoids such as gallic acids, chlorogenic acid, ferulic acid, kaempferol, ellagic acid, vanillin and quercetin. The juice is convenient to herbal health drinks combination of different medicinal juices, read to use and serve beverages etc. Moringa Oleifera leave juice has been extracted by grinder then juice were treated with enzyme and centrifuged. The treated juice were clarified by using hallow fibre micro membrane for cold pasteurization. The turbidity, total soluble solid, pH, refractive index, viscosity colour and clarity were analysed on different treatment. It was concluded that best results were obtained in terms of Clarity (47.62%T), Turbidity (5.333 NTU), TSS (5.933°B), pH (5.2725%), Viscosity (1.136667 mPa) for micro filtered juice. The optimum values for the operating condition of micro filtration were 98.0665 kpa pressures with 0.45 µm pore size.

***Keywords*---**Micro filtration, *Moringa Oleifera,* hallow fibre.

Introduction

Moringa Oleifera is a native tree plant from Asia and Africa. It is mostly grown in India, Africa, South America, Mexico and Philippines (Paul & Didia 2012). Now days the Moringa is used as a rich source of food and food products due to its considerable inherent nutritional, antioxidant and phytochemical benefits; as well as its ability to survive in diverse climatic conditions. It has multi-functional utilities in agriculture, medicine, live stock, human and other biological systems (Falowo et al 2018, Ndubuaku et al 2015). The trees many parts such as leaf,

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root, bark, seed, flower and pod have been used for boost nutrition and food securities in developing countries (Popoola et al 2013). The applications of the leaves are rich sources of vitamins, caroteonoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins (Leone et al 2015, Sivasankari et al 2014). The leaves have good source vitamin A (Ferreira et al 2008), Vitamin C (Ramachandran et al 1980), Vitamin E (Borel et al 2013; Efiong et al 2013) and antioxidants (Chambial et al 2013). It is also giving 7 times the Vitamin C of orange, 2 times the protein of yogurt, 4 times the vitamin A of milk, 3 times the potassium of banana (Gopalakrishnan et al 2016). Moringa juice is attracting the new food order due to its wide utility in health beverages and pharmaceutical industry.

Moringa oleifera leaves juice raw juice (R) has a reliving effect in muscular pain (Bhuvaneshwari et al 2013). The R has many pharmacological functions i.e, antimicrobial, antifungal, antihypertensive, antihyperlipademic, antihyperglycemic, antipyretic, wound healing, etc. (Mbikay, 2012; Toma, & Deyno, 2014). The juice has been persevered mostly through thermal preservation techniques such as pasteurization and sterilization. During the thermal preservation the heat sensitive vitamins, polyphenols and others nutrition’s may lost. So the alternate techniques like membrane processing may require for preservation of the nutritional rich juices. Membrane processing is a non thermal green process helpful to retain functional properties present in the juice (Chemat et al 2017). As per the literature survey the microfiltration has been used in clarification and purification of the juice for bitter gourd (Jain et al 2018), banana (Sagu et al 2014), mosambi (Emani et al 2013) etc.

Microfiltration is suitable for separation of micro-organisms, particulates and there is a free flow of protein, reduction of turbidity (Wagner, 2001), (Al-Farsi et al 2003) Microfiltration has some benefits on juice clarification without any changes in the temperature and the pH of the juice and there is no changes in the chemical constituents of the juice during filtration, improvement in juice quality, and solves the waste water treatment problem, decreases production and labour costs (Urošević et al 2017). In this work the fresh *Moringa oleifera* leaves were collected from branches, grinded in mixer and convert into viscous paste and then the viscous paste squeeze in a muslin cloth. The juice obtained is sticky and dark in colour. The juice was clarified by cellulosic enzyme, centrifuged and the micro filtrated. The effect of permeate flux and physic-chemical studies such as Total Soluble Solid (TSS), pH, Viscosity, Turbidity, Colour, Refractive index and Clarity of the different juice samples observations were recorded.

Materials & Methods:
Preparation of Juice

Fresh and tender leaves are purchased from local market. Then the leaves were washed with water and then grinded in a mixer grinder (Bajaj Mixer, Bajaj Rex 500-Watt Mixer Grinder, Hyderabad, Telengana, India). The grinded leaves treated with cellulase enzyme based on the preliminary work with the concentration of 0.1% for 55 min. at 30˚C. The obtained juice (R) was used for further treatment.

Centrifugation

Centrifugation of R juice was performed in a lab scale refrigerated centrifuge (2–16KL; Sigma-Aldrich). The operation was done on batch mode method with 50 mL capacity tubes at 25°C. The independent variables for centrifugation operation were rotational speed (rpm) and time (min). The rotational speed was varied from 6,000 to 8,000 rpm for 20 min and on the basis of preliminary trails. The supernatant was collected for further physicochemical analysis (total soluble sugar [TSS], pH, colour value, turbidity, clarity, refractive index, viscosity) after centrifugation as primary clarified juice (C).

Microfiltration

The R juice performed in a laboratory scale filtration attached with hallow fibre microfiltration supplied by CleanSep (Mumbai, India). Clarification was carried out on a tubular microfiltration module using 0.45μm pore size membrane with $0.052m^2$ of permeation area. The membrane was made up of polyether sulfone. The pressure used was 98.0665 kpa at 30°C. The pressure was kept constant and the permeate flux was calculated. When the feed passes through the membrane, filtrate was collected and recirculation of retentate takes place. The filtrate was measured in measuring cylinder. The permeate flux was calculated by

$$\boldsymbol{permeate\ flux = \frac{Vp}{t \times A}}$$

Where VP (L) was permeate collected at a certain time period t (h) for the effective surface area a (m^2) of the membrane. The permeate (ECM-M) and retentate (ECM-R) are evaluated for further physiochemical analysis.

Physiochemical Analysis

Moringa oleifera leaves juice, before and after pre-treatments and microfiltration processes, were analysed for turbidity, colour, total soluble solids (TSS), viscosity, pH, clarity, and refractive index.

Viscosity

The viscosity of the juice was dictated by a U-Tub slim viscometer (zenith glassware, Kolkata, India) at room temperature (28 ± 10°C). The unit of measured viscosity was represent as mPa.s.

Turbidity

Turbidity was measured with a Digital Turbidity-meter (Model 335; Deluxe Company, Delhi, India), and the unit of turbidity was nephelometric turbidity units (NTU).

Colour

Colour was measured as transmittance at 660 nm by a Colorimeter (ColorFlex EZ; Hunter Lab,eston, Virginia, USA).

$\Delta E = \sqrt{(\Delta L*) + (\Delta a*) + (\Delta b*)}$

TSS, Refractive index & pH

Abbe-type digital Refractometer was used for TSS and REFERACTIVE INDEX and expressed in degree Brix (°B).

Clarity

Clarity of the moringaoleifera leaves juice was evaluated by transmittance (%T) at 660 nm utilizing the spectrophotometer (Model: AU 2701; Systronics India, Ltd., Ahmedabad, Gujarat, India).

Result &Discussion

Permeate flux:

Clarification was carried out on a tubular microfiltration module using 0.45µm pore size membrane with 0.052m² of permeation area. The permeate flux was calculated at constant pressure and temperature were 98.0665 kpa and 300C respectively. The variation of permeate flux observed with respect time. The initial filtrate volume was 53 ml in 15 min duration. The filtrate volume was gradually reduced due to blockage of membrane pore or sedimentation of the solid of high molecular weight cut off. The permeate flux ranges between 40.76 L/m2.h to 4.616 L/m2.h in 360 minutes. The study state permeates flux achieved after 150 min. The figure 0i; shows the steady state flux increases with respect to time and after 150 min there are gradual variation on permeate flux. The similar graph patterns were observed by the Jain et al 2018 in the bitter guard juice during membrane filtration.

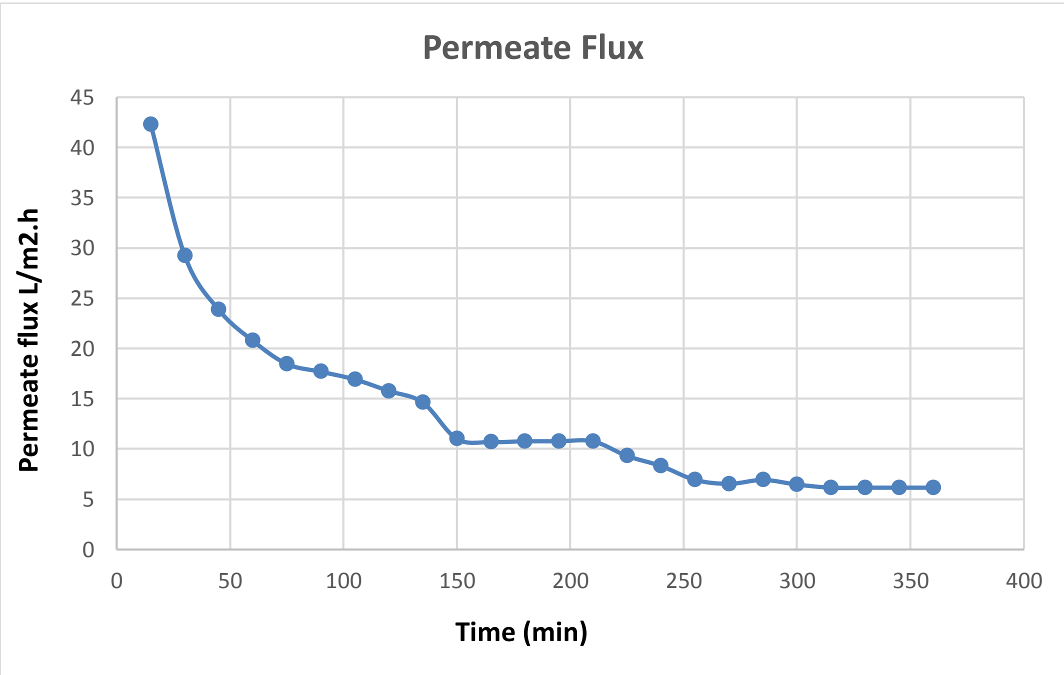


Figure i: Membrane permeate flux variation with respect to time

TSS (Total Soluble Solids):

The TSS of MLJ ranges from 3.7 to 6.1 ^{0}Brix. TSS of enzyme treated (E) and raw centrifuge juice (C) is higher as compared to raw juice (R). The minimum amount of soluble solids was present in micro filtrate juice (ECM-F) as compared to raw juice (R) followed by enzyme treated juice (E) and enzyme treated centrifuge (EC). TSS is more value due to enzyme concentration was reported by Schobingeret al 1981 in various enzyme treated juices. R has less TSS than enzyme treated juice. ECM-M has higher TSS as compared to ECM-R. It is observed that juice undergoing the enzymatic treatment demonstrates higher values of reducing sugar and TSS during enzymatic hydrolysis. Enzyme treatment is intensified the degradation of protein and carbohydrates. The same finding is observed by the Sagu et al 2014 in banana juice. During microfiltration, the turbidity of the filtrate reduced & reduction of turbidity is also the reason for decrease in TSS.The reduction of TSS due to microfiltration has been observedby Laorko Aet al 2010 in pineapple juice and Mirsaeedghazi, H. et al 2010 reported in pomegranate juice.
The TSS of MLJ was estimated after extraction as well as after treatment as indicated in materials and methods. TSS of MLJ juices after treatment were found to be significantly lower as compared to raw MLJ. Both the centrifugation and enzymatic treatment were found to decrease the TSS level in raw MLJ (R). Centrifugation was found decrease the TSS more as compared to enzymatic treatment, however, there was no statistical significantdifference in both of them. Interestingly, their combined treatment (EC) was found to cause a further a highly significant decrease in TSS level in MLJ. When microfiltration was applied to EC MLJ, a further significant decrease in TSS level in filtrate was observed. TSS level of retentate (ECM-R) was estimated and found to be significantly higher as compared to R.

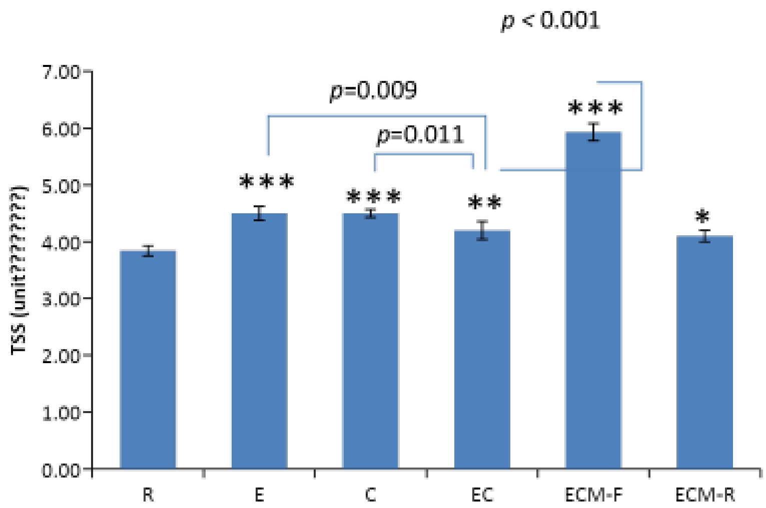


Figure ii: Total Soluble Solid

pH :

The MLJ raw juice (R) is acidic in nature. The overall pH value of MLJ different treated juices ranged between5.25 to 5.57 as described in figure ii. The R has less pH as compared to E followed by R&C, E&EC. The ECM-R has the lowest pH content as compared to ECM-F. The pH value of E was higher than R&C&EC. The growth of microorganisms inhibit due to the low pH. Due to the release of carboxyl groups and galaturonic acid from pectin, there is decrease of pH values notify by the Adubofuor, J. et.al 2010. That is desirable changes in juice for long time preservation. The pH value has a significant role in taste & odour.

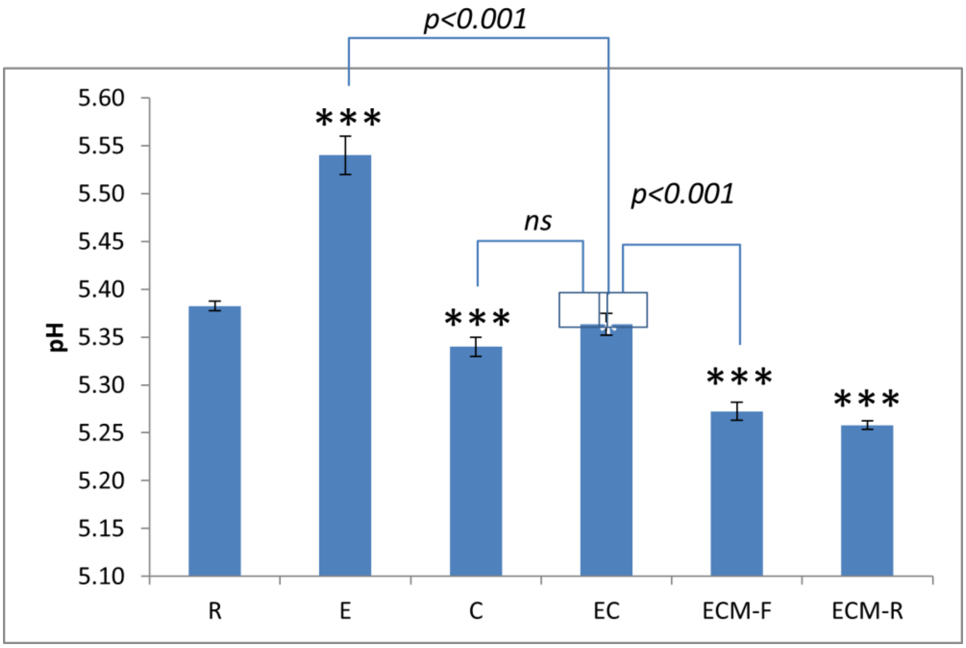


Figure iii: pH values of R, E, C, EC, ECM-F & ECM-R

pH level of MLJ before and after vaious treatments was estimated using standard pH probe method. It was found that enzymatic treatment of MLJ significantly increases the pH level while centrifugation was found to significantly decrease the pH level. MLJ after combined EC treatment was found to restores the pH level deviated by enzymatic or centrifugation treatment and had no significant difference as compared to R. After using microfiltration of EC treated MLJ, filtrate and retentate pH level was observed to further decrease in a statistically significant manner. The pH value has a significant role in taste & odour. Due to the release of carboxyl groups and galaturonic acid from pectin, there is decrease of pH values.

Turbidity:

MLJ turbidity was highest of R as compared to E followed by EC, RC. The EC has less turbidity than R followed by RC&E described in figure 3. The ECM-R has more turbidity as compared to ECM-F because the juice was clarified by the micro filtration the same observation was recorded by Mirsaeedghazi, H. et al, 2010 for pomegranate juice. After micro filtration the clarified juice has low turbidity value. Low turbidity value gives proper clarified juice. The use of enzyme reduces turbidity. Less turbidity shows proper clarification of juice. The less turbidity of juice has more market acceptability.

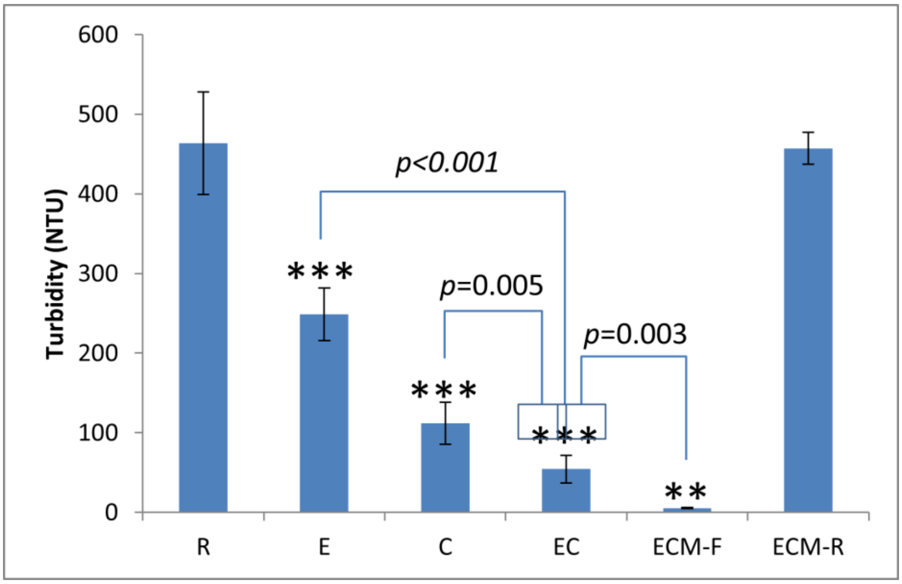


Figure iv: The MLJ's R, E, C, EC, ECM-F & ECM-R treated juice turbidity values in NephelometricTurbidity Units (NTU)

Treatments of MLJ with various methods were found to decrease the turbidity of MLJ. Turbidity of MLJ after centrifugation or enzymatic treatment was found to significantly decrease as compared to raw juice. There was a further significant decrease was observed in the turbidity of MLJ after combined EC treatment. After microfiltration of EC treated MLJ, turbidity was found to be significantly decreased in the filtrate. ECM-R retentate was found to have turbidity comparable with raw MLJ.

Clarity:

Clarity is the one of the prominent characteristics of the juice. The R has the least clarity than E followed by C, EC, ECM-F & ECM-R. Clarity was highest in micro filtered juice because after micro filtration the juice becomesclearer. Clarity is the significant indicator of clarified juice. The clarified juice shows minimum absorbance as reported by Sin, H. N. et al, 2006 forclarification of sapodilla juice.

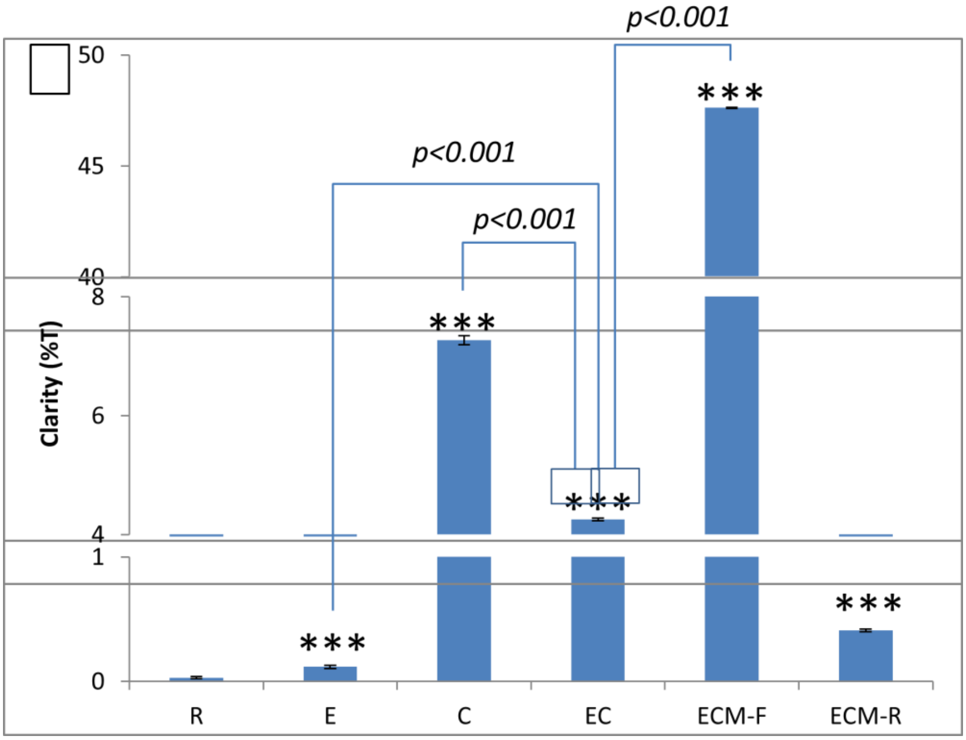


Figure v: Clarity in transmittance (%T) of R, E, C, EC, ECM-F & ECM-R

Claritiy of MLJ were estimated and presented in %T. Clarity of raw MLJ was found to be very low which was found to be significantly augmented after enzymatic treatment. Centrifugation was found to have a high degree of clarifyingeffect on MLJ. Combined EC treatmentwas also found to favour the clarity of MLJ as compared to R or E treatment alone. However, clarity of EC-treated MLJ was found significantly lower as compared to centrifugation only. Microfiltration of EC treated MLJ was observed to have the highest clarity among all the treatment regimens. Interestingly, the retentate after ECM also had a significantly higher level of clarity as compared to raw MLJ.

Viscosity:

The R juice has higher viscosity as compared to E followed by EC & C. The E&C have lowest viscosity as compared to R due to high enzyme concentration decreases the viscosity values are shows in figure V. It is observed that juice undergoing the enzymatic treatment demonstrates higher value of values of reducing sugar and TSS during enzymatic hydrolysis. Enzyme treatment is intensified the degradation of protein and carbohydrates. The findings are similar to guava juice enzymatic treatment done by Ninga et al 2018.The viscosity value is highest in ECM-R as compared to ECM-F. Lowest viscosity of juice is more suitable in enzymatic clarification of juice due to the high viscosity of juice leads to problem during filtration. It has also observed by the Sin et al 2006 in sapodilla juice.

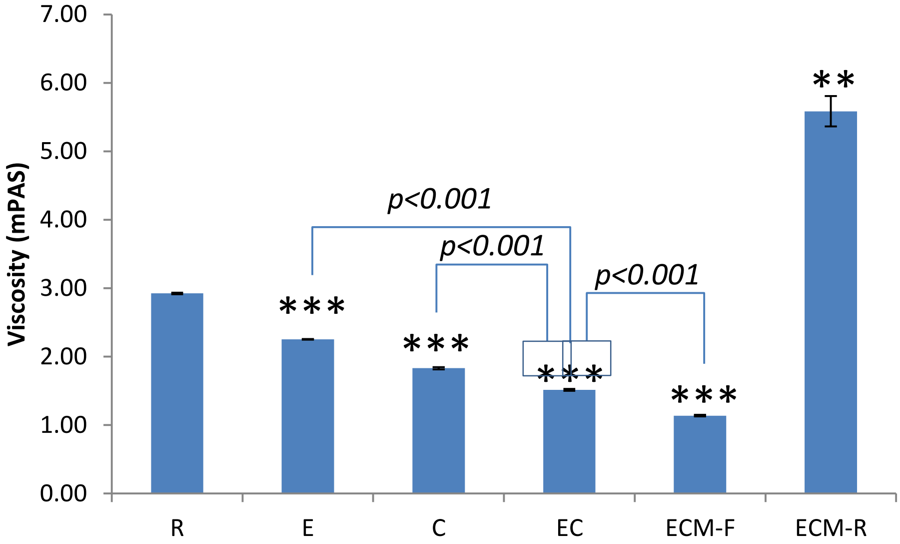
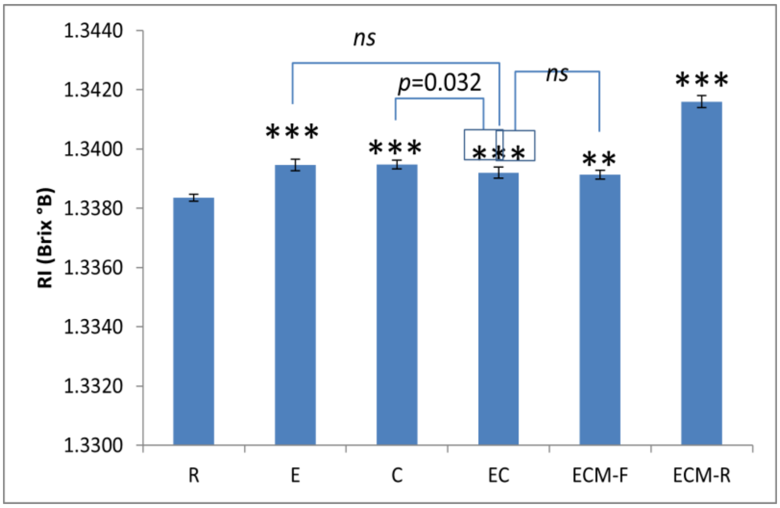


Figure vi: Viscosityin mPaS of R, E, C, EC, ECM-F & ECM-R

Treatments were found to decrease the viscosity of MLJ. Both the enzymatic and centrifugation treatment was found to significantly decrease the viscosity of MLJ as compared to R. Among both of these, viscosity level was lower in MLJ after centrifugation. When combined treatment (EC) was applied, viscosity level of treated MLJ was found even significantly lower as compared to single enzymatic or centrifugation treatment. Additional application of microfiltration of EC-treated MLJ was found to cause statistically significant decrease in viscosity. Viscosity of ECM-F was found to be least among any of the treatment either alone or in combination.

Refractive index:

The ECM-R has maximum refractive index 1.343^{0}Brixand the R has minimum 1.33^{0}Brix values compared to all types of sample. The sugar content of ECM-R increases due to microfiltration. The similar finding was observed by Contreras et al 1992 in the sugar content in various juices. The RI of E,C, EC & ECM-F were slightly increases in compare to R due to enzymatic treatment on juices. The figure vi shows the variation of RI on various samples were recorded. There is no significant variation found on E, C, EC& ECM-F on RI values.



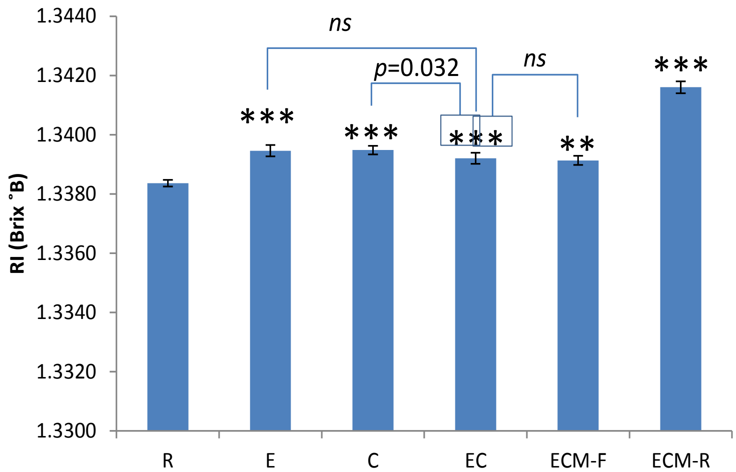


Figure vii: Refractive Index in^{0}Brix of R, E, C, EC, ECM-F & ECM-R

RI of raw MLJ and after treatments were estimated and presented in Fig.6. As indicated, enzymatic and centrifugation treatment were found to augment the RI of MLJ as compared to raw juice. MLJ obtained after combined EC treatment also had a significantly higher RI as compared to those of raw juice. Interestingly, MLJ after combined treatment had a lower RI as compared to single treatment either enzymatic or centrifugation. However, the difference between RI of MLJ after combined treatment and singlecentrifugation treatment was found to statistically significant. The combined EC treatment and enzymatic treatment alone were not found to significantly differ in their RI level of MLJ. Moreover, additional microfiltration of EC-treated MLJ was not found to significantly alter the RI level. Nevertheless, retentate afterECM treatment was found to have significantly higher level of RI as compared to untreated (raw) or treated MLJ.

Colour

ΔE (colour difference) was increased for E as compared to R followed by C, &EC the observations were shown in figure 7 (a). ΔE (color difference) was increased for ECM-F as compared to ECM-R. As the observations shown in Fig.7 (b), Chroma value for E& ECM-F was maximum. As shown in Fig.7(c), Hue value was maximum negative for R as compared to E followed by EC&C. ECM-R has positive hue value & negative for ECM-F. ΔE has a qualitative value in a three dimensional space, Chroma signifies the quantitative attribute characteristics; Hue is the qualitative representation of the chromatic nature of the colour. The similar colorvariations were recorded by Ghosh et al 2018 in jamun juice.

Colour differences of MLJ at raw level and after treatment measured through various method described in materials and methods. As indicated in fig.7(a) and 7(b), colour (L*) and Colour (b) had a similar trend after treatments. Enzymatic treatment was found to increase both of these colour parameters while centrifugation had a highly significant but reverse effect. A combined EC treatment also found to have a decreasing effect on both of these colour parameters. However, colour (L*) and colour (b) levels of combined treated MLJ was significantly higher as compared to centrifugation only treatment. Microfiltration after EC treatment (ECM) was found to further significantly decrease the colour (L*) and colour (b) level in filtrate. Interestingly, retentate (ECM-R) had a highercolour (L*) and colour (b) level compared to filtrate (ECM-F), however, it was found be significantly lower than those of raw MLJ.

Interesting results were obtained for the Hue of the MLJ after treatments (fig.7c). Raw and enzymatic treated MLJ had negative values. The enzymatic treatment was found to have significantly less negative value for hue as compared to raw MLJ. Centrifugation was found to increase the hue of MLJ which was recorded with positive values. After combined EC treatment of MLJ, hue value was found to significantly higher as compared to any of the single treatments. However, additional application of microfiltration was observed to significantly decrease the hue of MLJ as compared to those of EC treated. Retentate after ECM was found to have the highest level of hue among any of the treated or raw MLJ.

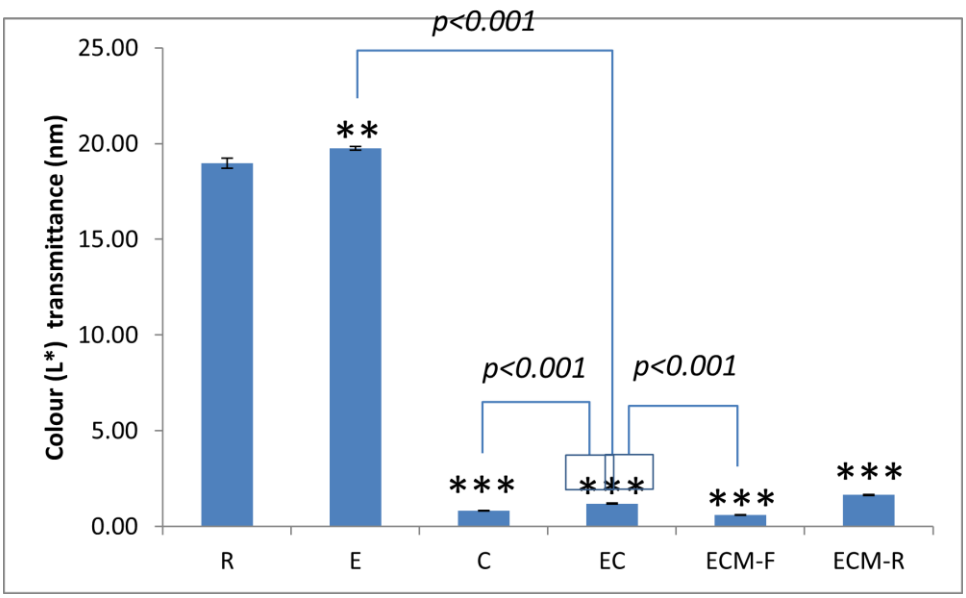


Figure viii (a): Variations in colour (L*) transmittance in nm of R, E, C, EC, ECM-F & ECM-R

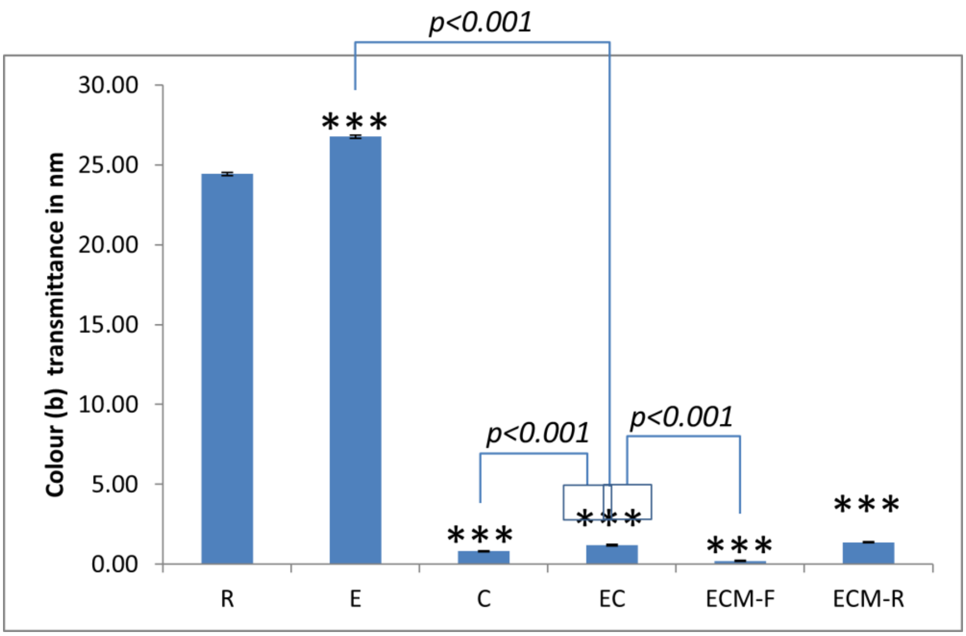


Figure viii (b): Variations in colour (b) transmittance in nm of R, E, C, EC, ECM-F & ECM-R

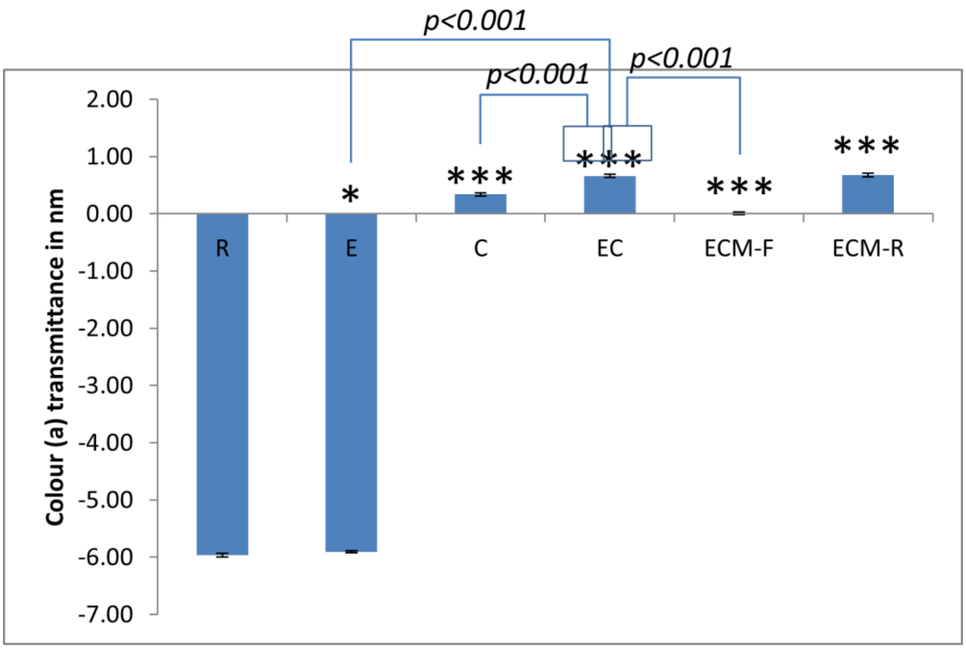


Figure viii (c): Variations in colour (a) transmittance in nm of R, E, C, EC, ECM-F & ECM-R

Conclusion

Moringa is thought to have the potential of providing vital nutrition as well as health and well being to consumers. In this work, fresh Moringa leaves juice extract was envisaged as a good vehicle of spreadin g its nutraceutical benefits. Clarification of MLF juice was carried out using both centrifugation and MF. Comparison of physicochemical properties. It was concluded that best results were obtained in terms of Clarity (47.62%T), Turbidity (5.333 NTU), Tss(5.933°B), pH(5.2725%), Viscosity (1.136667mPa)for micro filtered juice. The optimum values for the operating condition of micro filtration were 98.0665 kpa pressure with 0.45 μm pore size. Good restoration of the membrane was achieved by cleaning the membrane with acid and alkali. Fouling was mainly due to the presence of fibres and macromolecules. Considering quality and quantity, MF is found to be the most appropriate method for the clarification of MLE juice.

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