STUDIES ON PROCESSING AND STORAGE OF TENDER COCONUT WATER

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MASTER OF TECHNOLOGY IN AGRICULTURAL ENGINEERING

(PROCESSING AND FOOD ENGINEERING)

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STUDIES ON PROCESSING AND STORAGE OF TENDER COCONUT WATER

BY

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GUNTUR, ANDHRA PRADESH

2016

DECLARATION

I, **K. ANIL KUMAR,** hereby declare that the thesis entitled, "**STUDIES ON PROCESSING AND STORAGE OF TENDER COCONUT WATER"** submitted to **Acharya N.G. Ranga Agricultural University** for the degree of **MASTER OF TECHNOLOGY in AGRICULTURAL ENGINEERING** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: **(K. ANIL KUMAR)**

Date: **I.D. No. BEM14-02**

CERTIFICATE

Mr. K. ANIL KUMAR has satisfactorily prosecuted the course of research and that the thesis entitled "**STUDIES ON PROCESSING AND STORAGE OF TENDER COCONUT WATER"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part there of has been previously submitted by him for a degree of any university.

Date: **Chairperson**

CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON PROCESSING AND STORAGE OF TENDER COCONUT WATER**" submitted in partial fulfillment of the requirements for the degree of **"MASTER OF TECHNOLOGY IN AGRICULTURAL ENGINEERING"** in the major field of **"PROCESSING AND FOOD ENGINEERING"** of Acharya N.G. Ranga Agricultural University, Guntur is a record of the bonafide original research work carried out by **Mr. K. ANIL KUMAR** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. Published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Place:

Date: **(K. Anil Kumar)**

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LIST OF SYMBOLS AND ABBREVIATIONS

MF	:	Microfiltration
UF	:	Ultrafiltration
NF	:	Nanofiltration
RO	:	Reverse osmosis
TCW	:	Tender coconut water
Fig.	:	Figure
POD	:	Peroxidase
PPO	:	Polyphenoloxidase (PPO)
PS	:	Polysulphone
I.U	:	International Unit
ppm	:	Parts per million
MWCO	:	Molecular weight cutoff
dv	:	Cumulative volume
dt	:	Cumulative time
TSS	:	Total Soluble Solids
CFU	:	Colony forming units
TMP	:	Transmembrane pressure

ABSTRACT

Name of the Author	:	**K. ANIL KUMAR**
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Coconut water (*Cocos nucifera* L.) is an ancient tropical beverage whose original properties have drawn the attention of manufacturers as a natural functional drink. The tender coconut water (TCW) technically the liquid endosperm, is the most nutritious wholesome beverage that the nature has provided for the people of the tropics. TCW is rich in essential minerals such as potassium, sodium and natural nutrients like polyphenols. The water inside the nut is sterile but when it is extracted and exposed to air it becomes vulnerable to oxidation besides microbial contamination. Thermal treatments combined with chemical additives are used by the industry but other technologies such as micro and ultrafiltration are yet to be used on an industrial scale. In thermal and chemical processes, taste, aroma and colour are difficult to control and maintain to achieve fresh like taste in the product.

The membrane separation processes such as Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF) and Reverse osmosis (RO) are promising novel alternative non-thermal and non-chemical methods that are relatively less energy intensive and retain heat labile components. MF and UF offer excellent potential in food industry for clarification and pasteurization of liquid foods to replace conventional processing techniques. Therefore a study was conducted to develop process technology for bottling TCW using membrane filtration, pasteurization and chemical additive and suggest a suitable method.

A continuous cross flow flat sheet membrane module was used in the study to process by MF and UF. Initially the permeate fluxes were established using pure water on 0.2µm pore size and 40 kDa and 500 Da molecular weight cut off (MWCO) membranes at various transmembrane pressures (TMPs). The experiments revealed that permeate flux increases with an increase in TMP and membrane pore size or MWCO. The steady state fluxes were relatively higher with MF in comparison to UF and NF at the given TMP. The permeate flux of microfiltered TCW declined from 189.98 L /m^2h and reached a steady flux at 88.51L/m^2h at a TMP of 5.06 kg/cm^2. The flux also

declined from 107.54 to 82.07 L/m^2 h in UF. The flux decline during MF and UF is perhaps due to concentration polarization and consequent fouling.

Five different treatments were investigated to develop process technology and extend shelf life during storage of TCW. In the first treatment, the coconut water was passed through a microfiltration membrane of 0.2 µm pore size at a pressure of 5.06 kg/cm^2 to remove microbes and suspended particles. In the second treatment, coconut water was passed through ultrafiltration membranes of 40 kDa MWCO at pressures about 5.06 kg/cm^2 to remove enzymes such as polyphenoloxidase (PPO) and peroxidase (POD). In the third treatment, the coconut water was bottled and pasteurized at 85 ^oC for 10 min. In the fourth and fifth treatments, the coconut water was filtered through a MF membrane and chemical preservative nisin was added in two concentrations of 5000 I.U. and 2500 I.U. The TCW filtered through muslin cloth was taken as control sample. The control as well as all the treated samples were bottled and stored at 4 °C. The samples were taken at four days interval and their physico-chemical, microbiological and sensory characteristics were determined upto 20 days of storage.

The TSS of TCW generally decreased during storage except for pasteurized samples. Pasteurized TCW did not show any change in TSS compared to all other treatments. The pH generally decreased in all the treatments during storage up to 20 days. The percentage reducing sugars increased for all the samples during storage. However, pasteurized samples recorded lower increase in reducing sugars. The turbidity of the TCW increased during storage as indicated by decrease in the light transmittance values. Turbidity was observed to be relatively low for microfiltered and ultrafiltered TCW suggesting that membranes processes are useful for clarification of TCW. *E.coli*, Fungal and bacterial count were observed to be less in pasteurized samples. Overall based on different quality attributes, pasteurized treatment, MF and UF have been found to give a better quality bottled TCW in that order, the first treatment being the best. It can be concluded that membrane processing of TCW is one of the alternate methods along with thermal processing for producing quality product.

Keywords: Membrane processing, Microfiltration, Ultrafiltration, Permeate flux

Appendix-B

Flow chart for estimation of reducing sugars

Extraction

0.5 to 1.0 g sample / 50 mL Ethanol

(Grind in mortar and pestle ⟶ centrifuged in 3000 rpm for 10 minutes)

Assay

0.1 to 0.2/0.5 mL aliquot

+

0.8-0.9 mL distilled water

↓

Add 1 mL (A) Alkaline copper reagent

↓

Boiling on water bath for 10 min at 100 ℃

↓

A. Cool it and add 1 mL (B) Arseno molybdate reagent

↓

Make volume to 10 mL by adding 7 mL distilled water

↓

Shake well

↓

Read at 540 nm

Calculation

$$\frac{g}{100} g = O.D \times \frac{G.F\ (152)}{\underset{(0.1-0.5\ mL)}{Aliquot}} \times \frac{Total\ volume\ (50mL)}{Sample\ wt. \times 10000}$$

Appendix-A

Permeate flux of samples

a) Permeate flux during microfiltration of tender coconut water at 80 psi

S.No	Cumulative volume (mL), dv	Cumulative time (s), dt	Time (s)	UF Permeate flux (L /m^2h) at 80 psi
1	10	24	12	189.987
2	10	28	38	162.846
3	10	31	67.5	145.804
4	10	34	100	133.395
5	10	35	134.5	129.27
6	10	40	172	112.966
7	10	43	213.5	105.018
8	10	46	258	98.133
9	10	49	305.5	92.125
10	10	51	355.5	88.512
11	10	51	406.5	88.512
12	10	51	457.5	88.512
13	10	51	508.5	88.512
14	10	51	559.5	88.512
15	10	51	610.5	88.512

b) Permeate flux during ultrafiltration of tender coconut water at 80 psi 40 kDa

S.No.	Cumulative volume, dv	Cumulative time, dt	Time (s)	Permeate flux (L /m^2h)
1	10	42	21	107.54
2	10	43	63.5	105.018
3	10	44	105.5	102.593
4	10	46	149	98.133
5	10	49	196.5	92.125
6	10	50	246	90.282
7	10	51	296.5	88.512
8	10	52	348	86.81
9	10	53	400.5	85.172
10	10	54	454	83.594
11	10	55	508.5	82.074
12	10	54	563	82.074
13	10	54	617	82.074
14	10	54	671	82.074
15	10	54	725	82.074

c) Determination of permeate flux

Permeate flux was calculated as $J^* = (1/A)^*(dv/dt)$

Where J^* = permeate flux (L/m^2h)

A = area of the membrane (m^2)

Dv = volume of flow rate (L)

Dt = time of flow rate (h)

$J^* = (1/A)^*(dv/dt)$

$A = 14.5\ cm \times 5.5\ cm = 79.75 cm^2$

$= 7.975 \times 10^{-3}\ m^2$

Dv = 0.01 L

Dt = 24 sec = 0.0066 h

J* = $(1/7.975\times10^{-3}) \times (0.01/0.0066)$

J* = 189.987 L/m^2h

Appendix-C

O.D. values and determination of reducing sugars

a) O.D. values for the determination of reducing sugars of different samples of tender coconut water during storage

Days after storage, (DAS)	O.D Values,					
	T_1	T_2	T_3	T_4	T_5	T_6
0	2.1052	2.0394	2.0394	2.138	2.1052	2.1052
4	2.1644	2.0328	2.1447	2.1618	2.1381	2.1381
8	2.1710	2.0723	2.1644	2.0986	2.0986	2.2105
12	2.2092	2.0986	2.0986	2.125	2.125	2.2368
16	2.2375	2.1065	2.125	2.1710	2.2236	2.2960
20	2.2565	2.1644	2.2065	2.2302	2.2236	2.3552

b) Determination of reducing sugar

$$\frac{G}{100}g = O.D \times \frac{G.F\ (152)}{\text{Aliquot}\ (0.1 - 0.5\ mL)} \times \frac{\text{Total volume (50 mL)}}{\text{Sample wt.} \times 10000}$$

Where O.D = 2.1052

G.F = 152

Aliquot = 0.1

Total volume = 10 mL

Weight of sample = 1mL

Reducing sugars, $(\frac{g}{100}g) =$ 2.1052 × (152/0.1) × (10/(1× 10000)) = 3.2%

Appendix-D

Hedonic scale rating

Name : Date :

Time : Product name:

Procedure:

You have been given 6 coconut water samples. Kindly taste the samples and rate them based on your personal feel according to the numerical value given in the below table for all of its attributes.

S.No.	Feeling/ Attribute	Rating
1	Like extremely	9
2	Like very much	8
3	Like moderately	7
4	Like slightly	6
5	Neither like nor dislike	5
6	Dislike slightly	4
7	Dislike moderately	3
8	Dislike very much	2
9	Dislike extremely	1

Evaluation:

Attribute	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
Appearance						
Flavour						
Overall acceptability						

Chapter-I

Introduction

Chapter-II

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Chapter-III

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Dedicated to
My Parents and Family

Chapter - I

INTRODUCTION

Fruit juices are rich in minerals, fibre, anti-oxidants and have potential for rejuvenating the body and therefore, preferred by consumers as nutritional and refreshing drink. Coconut water (*Cocos nucifera* L.) is an ancient tropical beverage whose original properties have drawn the attention of manufacturers as a natural functional drink (Alexia *et al*., 2011). The tender coconut water (TCW) technically the liquid endosperm, is the most nutritious wholesome beverage that the nature has provided for the people of the tropics to fight the sultry heat. TCW is rich in essential minerals such as potassium, sodium and natural nutrients like polyphenols (Yong *et al*., 2009). In the year 2014-15, coconut plantation occupied an area of 1975.81 ha producing 20439.60 million nuts with productivity of 10345 million nuts per hectare. In the year 2014-15, undivided Andhra Pradesh state stands in the fourth position in the cultivation of coconuts producing 1463.56 million nuts with productivity of 13808 million nuts per hectare (Anonymous, 2015).

Tender coconut water is a refreshing drink with electrolytes (ionic mineral) similar to human plasma. This refreshing drink is filled with many healthy natural nutrients which can enhance the body's metabolism and immunity and is used more as a health supplement. The important significant and useful components in coconut water are cytokinins. The potential anti-cancer properties of specific cytokinins could bring encouraging and novel perspectives in finding cures for the different types of cancers. The recent discovery of other medicinal values of coconut water signifies a good potential in improving human health. Coconut water has recently caught on among athletes, health freaks and urbanites in many developed countries. An increasing international demand for this product could be a highly positive development for thousands of Asian small farmers. The mineral composition and reasonable total sugar content make coconut water a natural isotonic sports drink.

An immature coconut between six to nine months old contains upto 750 mL of water that eventually becomes the flesh. The coconut fruit takes 11 to 12 months to reach maturity. During the fifth month the kernel begins to form a thin layer of jelly around the inside of the endosperm or shell. The shell encloses the tender water, a clear sweet

liquid. At this time, the water is under pressure. During the ripening process, the pressure is released and the water is partially replaced by the kernel. The kernel grows with time and replaces the water by cells storing lipids. Its composition changes as the nut grows. At full maturity (12 months), coconut water represents between 15% and 30% of the weight of the nut. The amount of coconut water that can be harvested from each nut is about 300 mL but depends to a great extent on the stage of maturity and on the variety of coconut (Priya and Ramaswamy 2014). The tender coconut water naturally contains oxidative enzymes in its composition. The water inside the fruit is sterile but when it is extracted and exposed to air it becomes vulnerable to oxidation besides microbial contamination. Thermal treatments combined with chemical additives are used by the industry but other technologies such as micro and ultrafiltration are yet to be used on an industrial scale. Whatever the process, taste, aroma and colour (linked to enzymatic activities) are still difficult to control.

Currently, pasteurization is the main technique used for preservation and microbiological safety. Some studies have been conducted to evaluate the effect of pasteurization on the inactivation of oxidative enzymes, peroxidase (POD) and polyphenoloxidase (PPO) in coconut water. But pasteurization affects some of the heat sensitive aroma and other sensory attributes. Therefore, it is important to establish a preservation process that does not cause a negative effect on the composition and sensory characteristics of coconut water. In this sense, membrane technology is potentially attractive due to its mild processing conditions. In processing using most microfiltration membranes, the permeate fraction can be considered as cold pasteurized as microorganisms are retained by the membrane (Nakano *et al*., 2012).

Preservation by concentration to reduce water activity is one of the important food processing techniques. However, concentration using thermal processing is one of the most energy intensive processes among unit operations in the food industry. So, Membrane separation processes such as Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO) are promising novel alternative non-thermal and non-chemical methods that are relatively less energy intensive and retain heat labile components.

Membrane separation processes are characterized by several advantages as follows: a selective barrier that causes physical separation of the chosen components without addition of any extraneous chemicals, clean and environmental friendly

technology, a lower space requirement, reutilization of by-products or waste products as secondary raw materials, decrease or elimination of processes which are not eco-compatible and optimizing the utilization of energy and water.

Keeping in view of the above aspects, studies were conducted on processing and storage of TCW with the following specific objectives:

Objectives of the Investigation:

1. To process tender coconut water using microfiltration
2. To process tender coconut water using ultrafiltration
3. To process tender coconut water by pasteurization
4. To preserve the membrane processed tender coconut water using chemical additive
5. To conduct the storage studies on the processed tender coconut water
6. To evaluate the quality parameters of the stored product

Chapter - II

REVIEW OF LITERATURE

This chapter deals with review of literature on processing of tender coconut water by membrane processing with different filtration parameters and storage parameters and their effect on physico-chemical and microbial parameters of processed tender coconut water. Various research findings relevant to the present work have been reviewed and presented in this chapter. A very limited research work has been done with tender coconut water using membranes.

2.1 BIO-CHEMICAL COMPOSITION OF TENDER COCONUT WATER

Prakruthi *et al.* (2014) evaluated the physico-chemical characteristics, phytonutrients and stability of coconut water (CW), kernel (CK) at different stages of maturity and commercial coconut products (CCP). The moisture content of CW, CK and CCP were in the range of 95-97 g/100g, 50-85 g/100g and 0.4-3 g/100g respectively. Fat content in CW was low (4-115 mg/100g) whereas in kernel it was high (37-56 g/100g). The CW was acidic in nature (pH 4.5-5.2). Ash content of CK decreased with maturity (1.0%-1.5%) whereas that of CW remained steady (0.3-0.4 g/100g) with maturity. The total sugar content (3.9-4.6 g/100g) and acidity (0.3-0.4 g/100g) of CW did not change with maturity. The phenolics content increased in water (1.4-4.3 mg/100mg) and kernel with maturity (18.5-24.8 mg/100g). The fatty acid composition of the oil extracted from CK had increased saturated fatty acids ($C_{12:0}$) (38-48 g/100mg) and decreased mono saturated fatty acid ($C_{18:1}$) (13-5 g/100g) with maturity. The percentage of medium fatty acids increased with different stages of maturity (47-78 g/100g). The CW and CK contained higher amount of phenolics (1.4-43 mg/100g and 18.5-24-8 g/100mg, respectively) and total tocopherols of CK (0.14-0.59 mg/100g) when compared to CCP. Finally study indicated that CW and CK could serve as valuable raw materials for the preparation of functional food supplement.

Priya and Ramaswamy (2014) reported the typical nutrient, physico-chemical composition and vitamin content present in the tender coconut water (Tables 2.1, 2.2 and 2.3).

Table 2.1 Nutrient composition of tender coconut water

Components	Percentage
Water	95.5
Protein	0.1
Fat	< 0.1
Mineral matter	0.4
Carbohydrate	4.0
Calcium	0.02
Phosphorous	< 0.01
Iron in 100 mL	0.5 mg

Table 2.2 Physico-chemical and chemical composition of coconut water at different stages of maturity

Quality parameters	Maturity stage	
	7-8 months	8-9 months
Soluble solids (°Brix)	5.08	0.64
pH	4.83	5.29
Glucose (g/100mL)	2.4	2.9
Fructose (g/100mL)	2.1	2.5
Sucrose (g/100mL)	-	0.4
Total sugar (g/100mL)	5.0	6.3
Potassium (µg/100mL)	198.7	215.8
Calcium (µg/100mL)	14.5	11.5
Magnesium (µg/100mL)	4.6	5.1
Chloride (µg/100mL)	144.6	157.4

Table 2.3 Vitamin Content in tender coconut water

Vitamins	Value
Nicotinic acid	0.64 µg/mL
Pantothenic acid	0.52 µg/mL
Biotin	0.02 µg/mL
Riboflavin	< 0.01 µg/mL
Folic acid	0.003 µg/mL
Thiamin	Traces
Pyridoxine	Traces

Manjunatha and Raju (2013) studied the rheological behaviour of tender coconut (*Cocos nucifera* L.) water as a function of total soluble solid (TSS) content (5.3 to 52.9 ^{o}Brix) and its corresponding water activity (a_w) (0.982 to 0.870) at a wide range of temperature (10 to 85 °C) by controlled stress rheometer using concentric cylinders. The rheological parameter shear stress was measured up to the shear rate of 1000 s^{-1}. The investigation showed that tender coconut water and its concentrate behaved like a Newtonian fluid and the viscosity (η) was in the range 3.80 to 34.88 mPa s depending upon the concentration and temperature used. The temperature dependency on viscosity of tender coconut water was described by Arrhenius equation (r^2>0.96) and the activation energy (E_a) of viscous flow was in the range 5.268 to 20.798 kJ/mol depending upon the total soluble solid content. The effect of total soluble solid content on flow activation energy was described by exponential equation (r^2>0.92, rmse%=11.4, p<0.05) and that of water activity was described by power law equation (r^2>0.98, rmse%=5.54, p<0.01). The effect of total soluble solid content on viscosity of tender coconut water followed second order polynomial exponential equation (r^2>0.99, rmse % < 3.98) at the temperature used. The effect of water activity on viscosity was described by both power law as well as exponential type relationship (r^2>0.99).

Yong *et al.* (2009) reported that the chemical composition and biological properties of coconut water change with maturity (Table 2.4)

Table 2.4 Chemical composition and biological properties of coconut water

Coconut type	**Young**	**Young**	**Mature**
Average weight of coconut(g)	206 (water)	565	393
Age of coconut	-	6 months	12 months
Sugars (g/100g)			
Total	2.61	5.23	3.42
Sucrose	-	0.06	0.51
Glucose	-	2.61	1.48
Fructose	-	2.55	1.43
Inorganic ions (mg/100g)			
Calcium	24	27.35	31.64
Iron	0.29	0.02	0.02
Magnesium	25	6.40	9.44
Phosphorous	20	4.66	12.77
Vitamins	(mg/100g)	(mg/100dm^3)	(mg/100dm^3)
Vitamin C	2.4	7.41	7.08
Thiamin (B1)	0.03	Trace	0.01
Riboflavin (B2)	0.057	0.01	0.01
Niacin (B3)	0.08	ND*	ND*
pH	-	4.7±1	5.2±0.1

ND*: Non detectable

2.2 PROCESSING AND PRSERVATION OF COCONUT WATER

Adelson *et al.* (2015) investigated stability, antibacterial activity and effect of nisin for inactivating microorganisms in fruit juices. However, this preservation method could interfere with the organoleptic characteristics of the product. The approval of bacteriocins as food additives is limited, especially in foods from vegetal origin. The study aimed to verify the stability, its effect on physico-chemical properties, and the antimicrobial activity of nisin in different fruit juices. Nisin concentration remained stable in fruit juices (cashew, soursop, peach, mango, passion fruit, orange, guava, and cupuassu) for at least 30 days at room or refrigerated temperature and did not cause any significant alterations in the physico-chemical characteristics of the juices. Besides, nisin favored the preservation of vitamin C content in juices. The antimicrobial activity of nisin was tested against *Alicyclobacillus acidoterrestris, Bacillus cereus, Staphylococcus aureus and Listeria*

monocytogenes in cashew, soursop, peach, and mango juices. Nisin caused a 4-log reduction in viable cells of *A. acidoterrestris* in soursop, peach, and mango juices after 8 h of incubation, and no viable cells were detected in cashew juices. After 24 h of incubation in the presence of nisin, no viable cells were detected, independently of the juices. At 24 h of incubation in the presence of nisin, viable cells, *S. aureus* were only detected in mango juices, representing a 4-log decrease as compared with the control treatment. The number of viable cells of *B. cereus* at 24 h of incubation in the presence of nisin represented at least a 4-log decrease compared to the control treatment. When the antimicrobial activity of nisin was tested against *L. monocytogenes* in cashew and soursop juices, no reduction in the viable cell number was observed compared to the control treatment after 24 h of incubation. Viable cells were four and six times less than in the control treatment, in peach and mango juices respectively. *A. acidoterrestris* was the most sensitive microorganism to nisin and the least sensitive was *L. monocytogenes*. Still, a reduction of up to 90% of viable cells was observed in peach and mango juices inoculated with *L. monocytogenes*. These results indicate that the use of nisin could be an alternative in fruit juice processing.

Sindhumathi and Amutha (2015) have developed value added products from tender coconut water with addition of spices. Ready to Serve (RTS) beverage prepared from tender coconut water with the blend of ginger and cardamom extracts and packed in glass bottles and stored at room temperature (27±32 °C) and refrigerated temperature (7±1 °C). The prepared RTS is complying with Indian standards for RTS fruit beverages. The blended RTS were analyzed for its different physiochemical as well as sensory quality evaluated by adopting 9 point hedonic scale. The developed RTS was reported to have a shelf life of 120 days for both at room temperature and refrigerated temperature and hence it was safe and fit for consumption.

Manashi *et al.* (2012) have studied green coconut water processing through 2-stage microfiltration system and L-ascorbic acid (25 mg/100mL) was added to it, and then stored at 4 °C for 28 days. Microfiltered coconut water added (AS) or without added (US) ascorbic acid was analyzed for physicochemical, sensorial and microbial changes. With increase in storage time perpetual decrease in nutritional values and sensory qualities of the samples occurred; nevertheless, AS was able to retain these qualities more than US. Added ascorbic acid delayed the decrease of clarity (% transmittance) ($p<0.05$), sensory qualities, and also slowed down the increase of reducing sugar and total fatty acids. A kinetic study of post processing quality loss was also conducted during the storage period. Adequacy of zero and first order kinetic models were dependent on the specific quality

attributes that were studied. Principal Component Analysis (PCA) clearly showed that more drastic color and sensory changes occurred in US compared to AS. Recently, microfiltration of coconut water has been found to be an alternative to thermal sterilization. This study was undertaken to evaluate the effect of L-ascorbic acid addition on the quality attributes of micro-filtered coconut water during low temperature storage (4 °C for 28 days). The study makes a reasonable comparison of the quality attributes of micro-filtered coconut water, with or without added ascorbic acid. Concerted effect of microfiltration and L-ascorbic acid addition proved to be a better method for processing coconut water than microfiltration alone. The shelf-life of the microfiltered coconut water samples was limited upto 4 weeks at 4 °C.

Haseena *et al.* (2010) studied post harvest quality and shelf life of tender coconuts from local tall cultivar, 'West Coast Tall' with husk and intact perianth were stored at room temperature (27±2 °C) and the minimally processed nuts (60% husk removed) were stored both at room temperature as well as refrigerated conditions (13±2 °C) to evaluate the changes in physical and chemical constituents of coconut water during storage. Observations on physiological loss in weight of the stored coconuts, volume and pH of coconut water, total sugars and amino acid, minerals (Na and K) and sensory tests were used to evaluate the quality. The observations were continued till the quality of the nut water deteriorated. It was observed that, to increase the shelf life of the coconuts, the nuts have to be harvested carefully with intact perianth and without any breakage of nuts. The quality of minimally processed nuts deteriorates earlier than non-dehusked nuts during storage.

Chowdhury *et al*. (2009) studied the processing techniques of green coconut water for its preservation. For the study, five treatments (T_1 = Control at 0 °C for 10 min, T_2 = Heating at 70 °C for 10 min, T_3 = Heating at 80 °C for 10 min, T_4 = Heating at 90 °C for 10 min, T_5 = Heating at 100 °C for 10 min) were selected. Heating at 100 °C for 10 min of preserved coconut water was stored in glass bottle at room temperature preformed the best quality up to 6 month of storage according to the taste testing panel. Considering the analysis of findings, it was observed that T_5 (heating at 100 °C for 10 min) preserved in glass bottle was performed the best quality after 6 month of storage at room temperature. This processed coconut water will be suitable for commercial processing.

Barrett *et al.* (2005) reported variation in composition of by-product of copra, desiccated coconut and coconut water depending on the age of the nut. The sugar content

reaches its peak at 2.9% in the 9th month. Bottled coconut water had a pH of 4.2, 14 °Brix, and 0.14% acidity in the form of citric acid. It was reported that in May 2000, the FAO was granted a UK patent for the production of a sterile beverage from coconut water of nine months old nuts. Prefiltration is carried out through a 0.80 µm pore membrane, followed by microfiltration through a 0.20 µm membrane. The sterile product is flushed with nitrogen and aseptically packed retaining its flavour and nutritional characteristics that make it competitive with energy drinks. Adding 0.015% vitamin C ensures color stability of the product that has a minimum shelf life of 8 months.

Chowdhury *et al*. (2005) have studied the processing of coconut water, its storage stability and acceptability. Coconut water collected from sound and green coconut was filtered thorough filtering machine, pasteurized at 85 °C for 10 min and cooled. Effect of citric acid, $K_2S_2O_2$, B-carotene, ascorbic acid and green colour were evaluated. Coconut water was filled into metal cans and glass bottle to access the packaging effect. The change in colour, flavour, TSS, acidity, gas formation and fungal growth was observed. While the sample was stored at room temperature (27- 35 °C) and refrigerated temperature at 4-6 °C observation was made at an interval of 4 months upto 12 months. The colour and flavour process of green coconut water remained unchanged throughout storage period. The addition of acid and preservatives improved the transparency. No gas formation was observed in the canned and bottled water throughout the storage period. The acceptability of pasteurized water without addition of citric acid was highly satisfactory. The canned coconut water was found shelf stable upto 12 months of storage at refrigerator temperature and 10 months of storage at room temperature. The result of sensory evaluation and shelf life of the process green coconut water indicated that the fresh coconut water could be bottled and canned successfully for consumption round the year.

Mahindru (2000) reported that nisin upto 12.5 ppm had been allowed in paneer/channa/cheese-sliced/cut/shredded/processed and in canned rasgulla upto 5 ppm. In prepared coconut water it has been allowed up to 5000 I.U.

Campos *et al.* (1996) observed chemical composition, polyphenoloxidase and peroxidase present in green coconut water. These enzymes showed optimum activity at pH 6.0 and 5.5 and at temperatures of 25 and 35 °C respectively. Among chemical and physical treatments investigated, heating at 90 °C for 550 s and addition of ascorbic acid were, individually, the most efficient for enzyme inactivation. Addition of ascorbic acid did not affect sensory properties, however, heat treatment at 90 °C for longer than 100 s

decreased flavor quality. Combinations of heat treatment with potassium metabisulfite, ascorbic acid or both additives did not affect flavor quality.

Srivatsa and Sankaran (1995) investigated preservation of tender coconut water in plastic pouches of 200 mL capacity and in aluminum beverage cans of 200 mL/ 350 mL capacity. Since tender coconut water is highly susceptible to heating, it was subjected to minimum heating and by the use of additives like nisin to achieve commercial sterility. This has helped in maintaining the natural pH 4.9-5.2 instead of reducing to below 4.5, which reduced the acceptability. Microbial, chemical and organoleptic analyses have been completed upto 3 months storage under ambient conditions. The product was found to be generally acceptable. It remained microbiologically sterile and no significant chemical changes were noticed.

2.3 FACTORS AFFECTING SPOILAGE OF COCONUT WATER

Okolie *et al.* (2011) studied the fungal spoilage of husked and dehusked coconut fruits stored at 10 °C and 30 °C for three months. The husked coconut fruits stored at both 10 °C and 30 °C and the dehusked coconut fruits stored at 10 °C showed no evidence of microbial spoilage at the end of the three months storage period. However, dehusked coconut fruits stored at 30 °C deteriorated. *Aspergillus flavus* and *Aspergillus niger* were the principal fungal agents associated with the spoilage. An investigation of the proximate composition of the dehusked fruits stored at 30 °C indicated a marked significant difference in the percentage composition of moisture, protein, ascorbic acid and carbohydrate content of 3.97±0.28, 3.98±0.07, 0.01±0.002 and 9.27±1.02 respectively as against 46.82±0.43, 3.77±0.05, 2.48±0.15 and 11.89±0.22 obtained for dehusked coconut fruits prior to storage. These results suggest that the deterioration in nutritional composition was due to breakdown of protein and carbohydrate by the spoilage fungi. Further tests confirmed the ability of the isolated spoilage fungi to utilize the different carbohydrate and nitrogen sources as source of carbon and energy. *Aspergillus flavus* showed the ability to grow and utilize more of the various carbohydrate sources than *Aspergillus niger*, although the latter utilized lactose better. Both fungi showed evidence of growth and complete utilization of nearly all the nitrogen sources, except cysteine and L-glutamine, which could not support the growth of *Aspergillus niger*. Likewise, cysteine and L-glutamine, in addition to D-phenylalanine could not support the growth of *Aspergillus flavus.*

Fernanda *et al.* (2009) conducted a study to assess the quality of reconstituted fruit juices and coconut water sold for immediate consumption in bars, restaurants and bakeries, and by street vendors in Belo Horizonte, Minas Gerais, Brazil. Microbial quality was determined by counting coliforms, yeasts, staphylococci and salmonellae. Total titratable acidity, pH and total soluble solids of these beverages were recorded. For coconut water samples, the total reducing sugar content was also determined. The "juices" collected included reconstituted orange, cashew and grape-flavored juice powders and concentrated cashew juice. Sixty samples of these juices and 45 samples of coconut water were collected. More than half (55%) of the juice samples did not comply with current Brazilian legislation, which states that there must be a total absence of coliforms in a 50 mL sample. Sixteen percent of the coconut water samples exceeded the bacterial count limits defined in Brazilian law, with thermotolerant coliform densities above 102 MPN/mL. The high levels of sugar and low pH found in the coconut water were possibly related to the high yeast counts in most samples. Forty seven percent of coconut water samples showed staphylococcal counts above 103 CFU/mL. The numbers of thermotolerant coliforms, yeasts and staphylococci found suggest unsatisfactory hygienic practices during the preparation of these beverages. Salmonellae were not detected in any of the samples. The physical and chemical properties of the drinks varied among the samples. Our results suggest that both the reconstituted juices and coconut waters need better hygienic and sanitary control.

2.4 MEMBRANE PROCESSING OF TENDER COCONUT WATER AND FRUIT JUICES

Junmee and Tongchitpakdee (2015) conducted studies to determine the effects of membrane processing on quality of coconut water. Polysulfone membrane with 0.2 μm pore size was used in microfiltration (MF). After clarification using MF, coconut water was heated using Ultra High Temperature (UHT) process at 140 °C for 4 s. Hunter lab Color (*L*, *a*, *b*), total color difference (Δ*E*), browning index (BI), 5-hydroxymethylfurfural (HMF), haze, pH and total soluble solids (TSS) of clarified coconut water were evaluated during storage at room temperature, 27 °C for 28 days. Average permeate flux for MF was 20 L/m^2 h. Haze formation in coconut water increased with storage time. Initial haze could be significantly removed by MF. Coconut water clarified using MF had lower total color difference (Δ*E*) when compared to initial sample (Day 0). At 28 days of storage, BI and HMF of coconut water clarified using MF were also lowest when compared to control. BI and HMF were positively correlated (r = 0.935). Clarification using MF with 0.2 μm pore

size could help extending shelf life of coconut water. MF treatment did not affect the pH and TSS of coconut water. Clarification using MF with 0.2 μm pore size could extend the shelf life of coconut water by lowering haze, Δ*E*, BI and HMF. Therefore, MF could be an alternative treatment in coconut water processing to obtain high quality of coconut water with extended shelf life.

Nikhil *et al*. (2014) studied non-thermal two-stage microfiltration technique in aseptic conditions to preserve tender coconut water. Concentrations of citric acid (0.02 g/100 mL), ascorbic acid (0.18 g/100 mL) and L-cysteine (0.009 g/100 mL) were standardized according to taste and added to coconut water as natural additives. The coconut water was packed in glass and plastic bottles after flushing the headspace with nitrogen and stored under refrigeration (4 °C). The microfiltered water was studied for microbial, sensory and physicochemical properties for a period of 46 days. The quality of the water packed in glass bottles was better in all respects. The total soluble solids changed from 5.4 to 6 °Brix. The pH changed from 5.7 to 5.8. The soluble sugar concentration increased from 1.9 g/100 mL to 3.1 g/100 mL, free fatty acid content increased from 0.064 mg KOH/g to 2.8 mg KOH/g at the end of 46 days, which was much lower than the changes in control. The protein content decreased in all the samples. Two way ANOVA showed that the storage time had more impact on the sensory properties of the product than the packaging material. The glass bottled product was acceptable on sensory basis till 46 days of storage.

Debien *et al.* (2013) reported that the ultrafiltration (UF) inhibits the enzymatic activity which was responsible for color changes of coconut water without the need for heat treatment. UF performance was evaluated in terms of the permeate flux and enzymatic retention of the coconut water at laboratory unit (LU) and pilot unit (PU). The membranes studied were polyethersulfone 150 kDa (UP150), polyvinylidene fluoride 150 kDa (UV150) and cellulose 30 kDa (UC030). The 150 kDa membrane showed the best permeate flux. The 30 kDa membrane showed the lowest flux, but it resulted in 100% enzymatic retention, while the other membranes showed enzymatic retentions between 71% and 85%. The application of the 30 kDa membrane in the PU resulted in a flux value higher than that obtained in the LU due to the tangential velocity effect. The 30 kDa membrane has proved adequate for industrial applications. From the results it was concluded that the 30 kDa membrane was the best to remove enzymes from coconut water. The permeate obtained from this membrane did not show any color changes since 100% of the enzymes were retained. The application of the 30 kDa membrane resulted in higher permeate flux in cross flow mode.

Chhaya *et al.* (2012) used cross flow ultrafiltration to clarify the pretreated stevia extract. Two practical modes of cross flow ultrafiltration namely steady state under total recycle mode and batch concentration mode were used. It was observed that the significant flux enhancement was achieved with transmembrane pressure drop and cross flow rate. Maximum 200% flux enhancement with cross flow rate and 140% with transmembrane pressure drop were attained in the range of operating conditions studied herein. Effects of cross flow rate on the permeate properties were marginal but that of the transmembrane pressure drop was significant. Recovery of stevioside in the permeate was in the range of 30% to 56% for various transmembrane pressure drop and it was maximum for lower operating pressure, 276 kPa. However, the recovery of stevioside decreased to 38% at 276 kPa pressure after 10 h of operation. Nanofiltration was employed to concentrate the ultrafiltered liquor. During nanofiltration, the ultrafiltered feed was concentrated maximum twice at 1241 kPa and 1500 rpm of stirrer speed within 1 h of operation. Maximum overall purity and recovery of 60% is obtained when ultrafiltration followed by nanofiltration was used for a particular set of operating conditions.

Nakano *et al.* (2012) evaluated the ultrafiltration and pasteurization processes for preservation of coconut water and compared with the fresh product characteristics. Ultrafiltration was conducted in a plate and frame unit composed of 20 kDa membranes. Process was carried out at 5 bars and 15 °C. Permeate flux was measured along the process. Pasteurization was performed in a tubular pasteurizer at 96 °C for 20 s. Samples were collected for determination of physicochemical parameters, microbiological quality and sensory acceptability. Average permeate flux in ultrafiltration was 13 L/m^2h. Both processes were effective for reducing enzyme activity although decreasing total phenolics and soluble solids. Acidity and pH of processed water were similar to fresh one. Microbiological analyses have shown that both processes provided safe products. There was a significant difference in the acceptability of ultrafiltered water in relation to the fresh one and pasteurized water preferred than the ultrafiltered water. The obtained results indicate that both processes may be adequate for processing coconut water, although they need to be optimized regarding their sensory characteristics.

Behnaz *et al*. (2011) studied the clarification of tomato juice through microfiltration process. Influence of transmembrane pressure (1, 2 and 3 bar), cross-flow velocity which corresponds with Reynolds number (300, 1500 and 2500) and temperature (30, 40 and 50 °C) on permeate flux and some properties of clarified juice such as colour, turbidity, density, viscosity, pH and total soluble solid have been studied. The results

revealed that the investigated parameters (i.e. Pressure, Temperature and Reynolds number) had an increasing effect on the permeate flux and colour and the greatest effect on the permeate flux and colour was supplied by cross-flow velocity. The other permeate properties did not significantly change with variations of the operating parameters. The statistical analysis indicated that the interactional effect of cross-flow velocity and TMP on the permeate flux was significant.

Carvalho *et al*. (2010) studied the preservation of pineapple juice which was first hydrolyzed with a commercial pectinase (Ultrazym 100 G) and then clarified by microfiltration. A tubular polyethersulfone membrane with an average pore size of 0.3 μm and a total effective filtration area of 0.05 m^2 was applied. The transmembrane pressures were 1.5 and 3.0 bar respectively and the processes was conducted at room temperature. The results showed that the pineapple juice permeate fluxes were of 57.77 L/m^2h at 1.5 bar and 46.85 L/m^2h at 3.0 bar. Concentration polarization and possibly fouling occurred during the processes. The best clarified juice fluxes were obtained when low transmembrane pressures, 1.5 bar were applied.

Jayanthi *et al.* (2010) reported clarification of tender coconut water in a continuous stirred ultrafiltration cell at transmembrane pressures of 276, 414, 552 and 690 kPa and at stirrer speeds of 800 and 1600 rpm, for each pressure. Permeate flux decline was analyzed using a first-order kinetic model for the development of the polarized layer resistance. Correlations were proposed for the steady-state polarized layer resistance with the operating conditions, e.g. transmembrane pressure difference, RPM and membrane resistance. Decrease in membrane permeability after subsequent experiments was also quantified. Average irreversible fouling resistance was estimated as 7.5×10^{12} m^{-1}. Using the developed design equations of the stirred continuous ultrafiltration system, finally, the performance of such system in terms of productivity as function of operating conditions, membrane area and number of cleaning cycles was also evaluated.

Nagamaniammai *et al.* (2010) studied reverse osmosis as a preservation method to concentrate the tender coconut water and to improve the shelf life with minimum change to its nutritional and other sensory attributes. Trials were made for dummy solution and coconut water to optimize the processing conditions based on their chemical compositions and sensory attributes. The total soluble solid content of concentrated juice was increased from 4.5% to 14.0%. Apart from this, other nutrients also increased 2-2.5 times of its original content. Storage studies were carried out for membrane concentrated tender

coconut water, 25% and 50% upgraded tender coconut water concentrate (i.e. Sucrose 45%, glucose 50%, maltose 4.5% and potassium chloride 0.5% were mixed to increase the concentration) as control, tender coconut water packed in sterile container and with chemical preservative (500 ppm of sodium benzoate). The samples were stored at 30±2 °C and at 12 °C. No changes were observed in the samples kept at 12 °C up to 43 days. At the same time, increase in the acidity, decrease in reducing sugar content and pH were noticed in the samples stored at 30±2 °C within 22 days. Out of all these samples, 14% membrane concentrated tender coconut water and 25% upgraded tender coconut water without preservative in the sterile container at 12 °C with minimum changes in chemical composition was accepted by panelists. The concentration of TCW was increased to its three fold level with less overall acceptability. In this study 25% upgraded TCW sample without preservative at 12 ºC was considered to be the best natural ready-to-serve TCW for more than 48 days.

Pelin *et al.* (2010) evaluated the potential of integrated membrane processes for the clarification and the concentration of apple juice. The fresh apple juice, with a total soluble solids (TSS) content of about 12 °Brix was previously clarified by combined application of fining agents, gelatin and bentonite and UF through 10 kDa or 100 kDa molecular weight cut-off (MWCO) membranes on laboratory scale. The clarified juice was then concentrated by osmotic distillation (OD), membrane distillation (MD), coupled operation of OD and MD or by conventional thermal evaporation up to 65 °Brix. The effect of different clarification and concentration processes on formation of 5-hydroxymethylfurfural (HMF), retention of bioactive compounds (phenolic compounds, organic acids, glucose, fructose and sucrose) and their efficiency in preserving natural color and aroma (trans-2-hexenal, the most relevant compound in apple juice aroma) were evaluated in order to maintain a high quality product. The new membrane based concentration techniques were very efficient since the product characteristics were very similar to that of the initial apple juice especially regarding the retention of bright natural color and pleasant aroma, which are significantly lost during thermal evaporation. Furthermore, among all the concentration treatments applied, only thermally evaporated samples resulted formation of HMF. Phenolic compounds, organic acids and sugars were very stable against all concentration processes, including thermal evaporation. Coupled operation of OD and MD reduced trans-2-hexenal losses drastically tending towards that of the initial juice and hence can be proposed as the most promising alternative to conventional thermal evaporation technique.

Wilson and Maria (2005) evaluated reverse osmosis concentration of orange juice using the spiral wound membrane. Two types of commercial spiral wound polyamide

membranes; OK 4040F and Filmtec XLE 4040 were evaluated. Temperature was kept between 30 and 40 °C. Considering the generally similar temperature ranges, it is possible to observe RO performance characteristics, higher permeate fluxes were obtained for 15 ºBrix experiments, which worked at similar driven pressures used during 18 ºBrix or 20 ºBrix studies. This behaviour was observed for both studied membranes. Membrane XLE 4040 presented lower permeate fluxes and higher volatile aroma losses than OK 4040F. During 15 ºBrix experiments with membrane OK 4040F, permeate fluxes were obtained approximately twice as high as for XLE4040. This ratio decreased to 1.5 times during 20 ºBrix experiments. Summarizing OK 4040F permeate fluxes it is possible to estimate, 5.0 to 6 L/h m^2 (20 ºBrix), 8.0 to 9.0 L/h m^2 (18 ºBrix), and 10 to 14 L/h m^2 (15 ºBrix). This performance could be feasible assuming a multistage system, where the first stages operate at brix lower than the last stage 20 ºBrix to improve productivity.

Cassano *et al.* (2003) studied the clarification and the concentration of citrus (orange and lemon) and carrot juices. The reverse osmosis (RO) process, performed on a laboratory plant unit, was used to preconcentrate the permeate coming from the UF up to 15–20 g TSS/100 g. A final osmotic distillation (OD) step yielded a concentration of the retentate coming from the RO up to 60–63 g TSS/100 g at an average throughput of about 1 kg h m^{-2}. This laboratory unit was mainly used to develop operational parameters for the design of a full-scale plant and, secondly for the production of sample concentrates for their testing and evaluation. On the blood orange juice samples it was demonstrated that the total antioxidant activity (TAA) of juices concentrated by evaporation was lower than that of the fresh juice. During the UF process TAA was maintained, with respect to the fresh juice, both for the permeate and for the retentate. A little decrease of the TAA was observed in the RO treatment, probably on account of the high pressure employed during the process. After this step, the subsequent concentration treatment by OD did not induce any significant changes to TAA independently from the final concentration obtained. Moreover, the juices concentrated by membrane technology retained their colour and large part of their aroma which is on the contrary lost during thermal concentration.

Bottino *et al.* (2002) studied the production of new tomato juices based on the introduction of a MF stage before the RO stage. Ceramic multichannel membranes with an average pore size in the micrometer tenths were used in the MF stage to obtain a clarified serum to be concentrated by RO and a concentrated pulp to be used for remixing with the RO concentrate, in order to formulate new tomato juices. High temperature and pressure resistant spiral-wound RO membranes were used to concentrate the clarified serum up to 14–15 °Brix with permeate fluxes around 20 L/m^2h.

Capannelli *et al*. (1992) studied the UF of Mediterranean orange and lemon juices using different types and configurations of membranes. The UF membranes used were polysuphone (20 kDa to 200 kDa) and polyvinylidene fluoride (15 kDa to 200 kDa). The MF ceramic membranes used had pore sizes of 0.5 to 0.8 µm. Applied pressures were in the range of 50 to 400 kPa, feed temperatures were 20 to 40 °C and feed velocities at membrane surfaces varied from 0.5 to 12 m/s. It was observed that the pulp, pectin and essential oils were completely retained by the membranes. The permeate fluxes for orange juice were 30, 40 and 60 L/m^2h for polysuphone, polyvinylidene fluoride and ceramic membranes respectively. The permeate fluxes for lemon juice were 25, 35 and 50 L/m^2h for polysuphone, polyvinylidene fluoride and ceramic membranes, respectively.

Lira *et al*. (2012) evaluated the physicochemical and microbiological characteristics of coconut water filtered through ceramic MF membranes. The physicochemical characteristics of coconut water remained unaltered after microfiltration. The microbiological analyses indicated that raw and conventionally processed coconut water was unsuitable for human consumption. However, after microfiltration, the product was characterized as commercially sterile and suitable for consumption. Fecal and total coliforms were found to be below 0.3 MPN/mL, mesophilic aerobic microorganisms showed values below 1.0 CFU/mL, and salmonella was absent. These results confirm that treating coconut water by microfiltration through ceramic membranes is a viable procedure to remove microbiological flora without altering the product's original characteristics. It was concluded that the use of ceramic microfiltration membranes of 0.8 µm pore size removes microbiological flora, rendering it fit for human consumption, without changing its nutritional and functional properties of coconut water.

2.5 EFFECT OF OPERATIONAL PARAMETERS DURING MEMBRANE PROCESSING

De Oliveira *et al.* (2012) conducted experiments to study the performance of two membranes viz., tubular ceramic and hollow fiber polyamide under transmembrane pressure of 0.5 and 1 bar for the clarification of passion fruit pulp pretreated by centrifugation and enzymatic treatment at the concentrations of 150 and 300 ppm. Nutritional and sensorial qualities of the clarified juice obtained were evaluated. Thus, it was possible to observe that the most adequate condition for the clarification of passion fruit pulp was with enzymatic treatment at 150 ppm and its posterior microfiltration at the ceramic tubular membrane of 0.3 μm with transmembrane pressure of 0.5 bar. The fouling mechanism was identified by estimation of model parameters according to a nonlinear regression by Bayesian inference. Analysis of the fouling mechanism results revealed that hollow fiber membrane was controlled by a cake filtration mechanism and internal pore blocking fouling mechanism controls ceramic tubular membrane.

Laorko *et al.* (2010) studied the effects of membrane property on the permeate flux, membrane fouling and quality of clarified pineapple juice. Both microfiltration (membrane pore size of 0.1 and 0.2 μm) and ultrafiltration MWCO of 30 and 100 kDa membranes were employed. Membrane filtration did not have significant effects on the pH, reducing sugar and acidity of clarified juice whereas the suspended solids and microorganisms were completely removed. The 0.2 μm membrane gave the highest permeate flux, total vitamin C content, total phenolic content and antioxidant capacity as well as the highest value of irreversible fouling. Based on these results, the membrane with pore size of 0.2 μm was considered to be the most suitable membrane for the clarification of pineapple juice. The optimum operating conditions for the clarification pineapple juice by membrane filtration was a cross-flow velocity of 3.4 ms^{-1} and transmembrane pressure (TMP) of 0.7 bar. An average flux of about 37 Lm^{-2} h^{-1} was obtained during the microfiltration of pineapple juice under the optimum conditions using batch concentration mode.

Chan and Chen (2001) studied the effects of electrolyte concentrations and pH in microfiltration of aqueous solutions of bovine serum albumin. Experiments were performed to investigate the hydrodynamic and solution conditions during incipient fouling stage which lead up to eventual cake formation. Results from flux stepping showed that an increase in wall concentrations coincides with the onset of an apparent

critical flux. This is followed by a time lag before an increase in observed rejection is exhibited. Sub-critical constant flux operation showed that there exists an aggregation and deposition time lag after which the membrane suddenly experiences a rapid increase in hydraulic resistance due to protein aggregates blocking a majority of membrane pores. These incipient fouling conditions were shown to be dependent on pH and ionic strength.

Chilukuri *et al.* (2001) conducted experiments to study fouling mechanisms during cross-flow microfiltration of 0.2% (w/v) bovine lactoferrin solutions under constant flux condition. Fouling resistance curves indicated an initial phase of slow fouling probably by pore plugging or deposition of aggregates. Stable operation with low fouling could be achieved at low flux, e.g. 50 L/m^2h. However, as flux was increased, severe fouling occurred, e.g. at 200 L/m^2 h, probably because lactoferrin formed a concentration induced surface layer. The presence of sodium dodecyl sulphate in the feed caused dramatic decreases in fouling resistance but had no effect on protein transmission. Fouling by protein is highly dependent on the permeate flux and physicochemical properties of the feed. The relation between permeate flux and surface layer formation was highlighted. Aggregates appear to play a major role in fouling of membranes.

Marshall *et al.* (1997) during MF of β-lactoglobulin under constant flux demonstrated that fouling occurred in the immediate vicinity of the pore entrance. It was hypothesized that shear forces on the protein caused denaturation and aggregation leading to deposition at the pore entrance.

Jonsson *et al.* (1996) hypothesised in-situ generation of BSA aggregates during pumping. It was suggested that high denaturation due to pumping led to formation of aggregates that cannot penetrate into pores but form a cake.

Belfort *et al.* (1994) presented different possibilities of fouling mechanisms in MF depending on the protein to pore size ratio. They defined particle diameter d and pore diameters d_p to illustrate possible fouling mechanisms. When $d<<d_p$ the particle could enter most pores and could conceivably close smaller pores thereby reducing the open cross-sectional area for flow.

Kim *et al.* (1992) concluded that aggregate formation during UF is exacerbated by conditions like membrane surface roughness and high convective flows close to the membrane. It was reported formation of aggregates with higher initial flux in UF membranes, but not with lower flux membranes. It was attributed to aggregation due to

conformational changes in BSA molecules associated with high shear rate that exist near the membrane surface. It was hypothesised that these conformational changes exposed hydrophobic regions on BSA molecules which then interacted to form large protein aggregates at the membrane surface.

Blatt *et al.* (1970) reported that pore size distribution of the membrane remains same as for as clean membrane, the shape of the pore size distribution of the gel/cake will most likely change with the time and transmembrane pressure (TMP). Compaction, rearrangement and deposition of smaller particles in the pores of cake/gel could explain this. In this case, slope of the flux versus TMP curve will decrease with increasing pressure.

2.6 NON-THERMAL PROCESSING OF COCONUT WATER

Kathiravan *et al.* (2014) have studied tender coconut water Nannari extract (*Hemidesmus indicus* L.) ready-to serve (RTS) blended beverage. Response Surface Methodology (RSM) was employed to optimize the levels of independent variables (levels of tender coconut water, nannari extract and sugar). The responses of pH, ºBrix, CIE colour (L*, a* and b*) value and overall acceptability (OAA) were studied. The data obtained were analyzed by multiple regression technique to generate suitable mathematical models. The developed blended beverage was processed using pulsed electric field (PEF) with electric field 31.2 kV/cm, 20 pulse widths at 100 Hz frequency to minimise nutritional and sensory attributes losses and compared with conventional thermal pasteurization (96 ºC for 360 s) with p-value of 8.03. Thermal pasteurization showed a significant ($p<0.05$) decrease in colour value, radical scavenging activity and overall acceptability after treatment and also during storage, when compared to PEF treated tender coconut water-nannari blended beverage. PEF treatment also achieved a 3.01±0.69 log inactivation, similar to thermal pasteurization of native micro flora. PEF treated tender coconut water-nannari blended beverage was stable up to 120 days under ambient storage condition (27-30 ℃).

Martina *et al.* (2015) studied high pressure carbon dioxide (HPCD) treatment applied to the pasteurization of coconut water in order to guarantee both its microbial stability and preserve its nutritional and sensory attributes. It was demonstrated that 120 bar, 40 ℃, 30 min were the optimal process conditions to induce a 5-log (CFU/mL) reduction of mesophilic microorganisms, lactic acid bacteria, yeasts and molds and a 7-

Log reduction of the total coliforms. The effect of HPCD on the quality traits of coconut water were investigated by means of physical–chemical and sensory analyses and compared to the Heat Pasteurized (HP, 90 ºC, 1 min) and Fresh Untreated (FU) product. No differences in the basic chemical composition, vitamins and amino acids, were detected between HPCD and FU products. However, differences in the volatile compounds present in the three products were clearly distinguishable; HPCD resulted in a reduction of most of the volatile fractions while HP induced the formation of compounds with a toasted and malty aroma. Nevertheless, few sensory differences were perceived between the FU and the HPCD coconut water, and both were clearly differentiated from the HP product. The ideal process conditions were determined to be 12 MPa, 40 °C, 30 min and induced about 5-Log reductions of mesophilic microorganisms, lactic acid bacteria, yeasts and molds and about 7-Log reductions of total coliforms.

Martina *et al.* (2014) investigated and compared the effect of supercritical carbon dioxide (SC-CO_2) alone or in combination with high power ultrasound (HPU) on both the natural microbial flora (mesophilic, lactic acid bacteria and yeast and molds) of coconut water and the pathogenic Gram-negative bacteria Salmonella enterica inoculated in the product. Inactivation kinetics were obtained at 12 MPa, by means of batch apparatus, at different times (from 1 up to 60 min) and temperature conditions (from 25 up to 45 °C). The synergistic effect of SC-CO_2 + HPU was evident and a higher microbial reduction was achieved compared to SC-CO_2 alone: at 12 MPa and 40 °C about 5-log reductions were achieved for natural microbial flora in about 15 min while about 30 min were needed for SC-CO_2 treatment. The storage study highlighted that SC-CO_2 treated coconut water resulted micro-biologically unstable and showed heavy regrowth phenomena during the storage, while, a full shelf life of 4 weeks was assured for SC-CO_2 + HPU treated samples. The storage study indicated that the product remains microbiologically stable after the combined treatment, while heavy regrowth phenomena were observed in the SC-CO_2 treated sample.

Rai *et al.* (2008) studied the shelf life of ultrafiltered juice. Ultrafiltered mosambi juice was collected under aseptic condition in sterilized vials in a laminar flow chamber. The collected filtered juice was stored in amber-colored and transparent vials for 1 month, and its quality parameters (total soluble solid, pH, clarity, color and ascorbic acid) were studied at 5 day intervals. The juice, stored at room temperature, was spoiled in 3 days. The soluble solid and pH of the juice stored at refrigerated temperature in both vials remained unchanged during the storage period. The degradation kinetics of ascorbic acid

and non-enzymatic browning for both the storage vials was established. The clarity of juice decreased with time marginally. Ascorbic acid decreased, and non-enzymatic browning increased in both vials to some extent at the end of storage time (30 days).

Reddy *et al*. (2007) studied the laboratory scale technique for non thermal sterilization of green coconut water. The process consisted of two-stage filtrations under constant pressure using different filter media; namely, low ash filter paper (Whatman 42) in the first stage and cellulose nitrate membrane (0.2 mm pore opening) in the second stage. The water after the second stage of filtration was sterile with no visible growth of microbes on culture plates. The taste of the processed water did not change significantly; however, the flavor and overall acceptability decreased about 9% and 11%, respectively. The water also remained sterile after 1 month in aseptically packed condition, but overall acceptability further decreased by 6%. The filtration reduced different nutrients of the fresh water like fat, ash, total sugar, reducing sugar and protein by 40.0%, 43.9%, 23.4%, 29.2% and 13.3%, respectively; the removal of K, Mg, Ca, Fe and Cu was 10.15%, 16.14%, 19.04%, 20.85% and 22.21%, respectively. Non thermal sterilization of the tender coconut water could be technically feasible by adopting the two-stage filtration process using low ash filter paper and cellulose nitrate membrane, respectively. The normal taste of the water remained unchanged in the filtration. However, some losses of nutrients occurred in both stages, which probably decreased the overall acceptance of the filtered coconut water by 11%. Aseptically packed filtered water stored for 1 month also remained sterile and could maintain the normal taste, but its overall acceptance was 17% lower compared to that of fresh water.

Reddy *et al*. (2005) studied sterile green coconut water produced in a two stage laboratory scale constant pressure filtration system with a prefiltration unit by ordinary filter paper (Whatman No. 4) for removal of suspended particles and a microfiltration unit by cellulose nitrate membrane (0.2 μm pore size) for removal of microorganisms. The filtration performances of the systems were tested at three pressure differentials; 5.33, 10.67 and 16 kPa. Resistances in both the filtration processes and the cake compressibility factors were investigated. The filter medium resistance (R_m) and the cake resistance (α) increased with the increase in applied pressure differentials for both the filtration systems. The values of R_m varied between 3.497×10^{9} and 5.042×10^{9} m^{-1}, and 1.979×10^{10} and 3.076×10^{10} m^{-1} for paper and membrane filter respectively for the entire range of pressure differentials. The corresponding variations were 2.162×10^{12} to 4.280×10^{12} m/kg for paper filter and 2.144×10^{12} to 3.942×10^{12} m/kg for membrane filter. Although the R_m values for

corresponding pressure differentials were high for membrane filter, the values of α were comparable for both the systems. The deposited cakes were compressible with a compressibility factor of 0.4.

Chapter - III

MATERIAL AND METHODS

This chapter deals with raw materials selection, membrane processing of coconut water, operation of membrane module setup, bottling and the storage of the processed coconut water in glass bottles. Description of Equipment, procedures for determining the physico-chemical and microbial properties of the stored coconut water were also presented in this chapter.

3.1 RAW MATERIALS

The tender coconuts were obtained from a local market Bapatla town, Guntur dist, Andhra Pradesh. Nisin (Sigma Aldrich, USA), glass bottles of 250 mL were procured from the market. The coconut variety used locally for tender coconut water sales was chosen as raw material and grown in Eluru region (East coast tall) of Andhra Pradesh. Tender coconuts with good quality and without any pest or disease infestation were selected and pericarp was removed for coconut water extraction (Plate 3.1). The water was extracted fresh just before use for experiment.

Plate 3.1 Tender coconut for extraction of water

3.2 DESCRIPTION OF CROSS FLOW CELL MEMBRANE MODULE SETUP

Experimental setup available at College of Food Science and Technology, Acharya N G Ranga Agricultural University, Bapatla was used in the study. Cross flow cell membrane module setup was developed by Prof. Sirshendu De, Department of Chemical Engineering, IIT Kharagpur, (Fig. 3.1 and Plate 3.2).

Membrane module setup specifications

Name	:	Cross Flow Cell Membrane Setup
Membrane module dimensions (L×B) (Rectangular)	:	14.5 cm × 5.5 cm
Channel gap	:	3 mm
Operating pH range	:	Membrane dependent (Typically 2 to 12)
Cell body	:	316L stainless steel (anti-rust coated)
Maximum transmembrane pressure drop	:	20 bar (300 psi)
Pressure gauges	:	2 nos.
Rotameter	:	0 to 200 Lph
Pump	:	Heavy duty dual piston reciprocating pump

Cross flow cell membrane set up made of stainless steel was used for membrane processing of coconut water. Two neoprene rubber gaskets were placed over the membrane forming the flow channel. The channel height after tightening the two flanges was found to be 3.0×10^{-3} m. The effective dimension of the membrane was 14.5 cm in length and 5.5 cm in width. The cell consisted of two rectangular matching flanges. The inner surface of the top flange was mirror polished. The bottom flange was grooved, forming the channels for the permeate flow. A porous stainless steel plate was placed on the lower flange that provides mechanical support to the membrane. Two flanges were tightened to create a leak proof channel for conducting experiments in cross flow-mode.

The feed was pumped by a high pressure reciprocating pump from the stainless steel feed tank to the cross flow cell (Fig. 3.2). The retentate stream was recycled to the feed tank routed through a rotameter. The pressure and the cross flow rate inside the membrane channel were independently set by operating the valves in the bypass line and that at the outlet of the membrane cell. Permeate samples were collected from the bottom of the cell and were analyzed for physico-chemical and microbial tests.

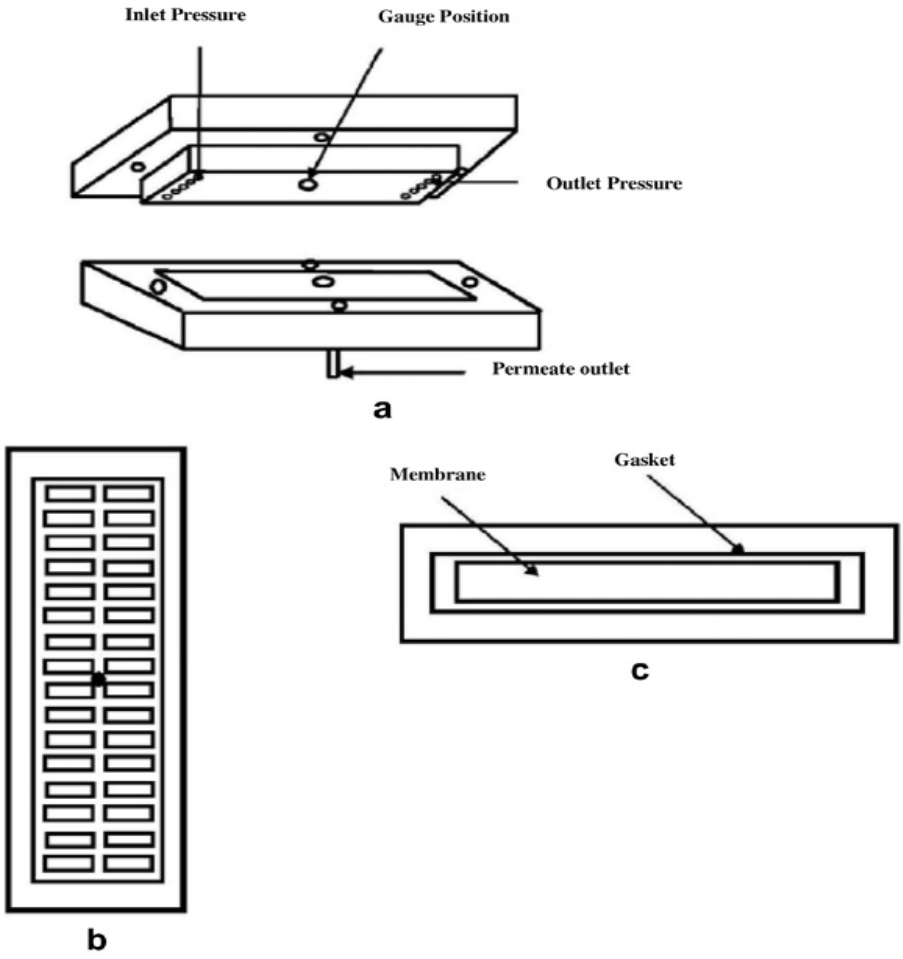

Fig. 3.1Various parts of the membrane cell

(a) Two flanges; (b) the top view of grooved bottom flange; (c) top view of bottom flange after putting the membrane

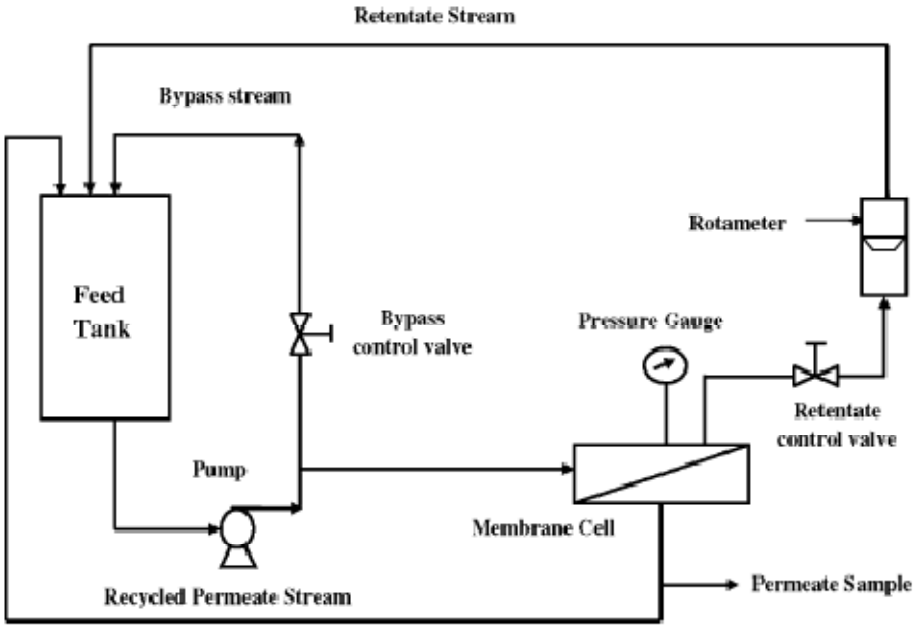

Fig. 3.2 Schematic diagram of the membrane filtration setup

Plate 3.2 Cross flow cell membrane setup

Permeate flux was calculated as

$$J^* = (1/A)*(dv/dt)$$

Where J^*= permeate flux (L /h m^2)

A= area of the membrane (m^2)

dv= volume of flow rate (L)

dt= time of flow rate (h)

3.2.1 Membrane Polymers in Cross Flow Cell

Cross flow cell membranes are extruded in an in-house extrusion machine by the manufacturer. Poly acrylo nitrile (PAN) is used to prepare 0.2 µm microfiltration membranes and Poly Sulfone (PS) is used to prepare ultrafiltration membrane of molecular weight cut off 40 kDa and nanofiltration membrane of 500 Da MWCO. The effective membrane surface area is 79.75 cm^2. The polymeric membranes are asymmetric in nature and have smooth side facing the retentate side (Plate 3.3 & 3.4).

Plate 3.3 Microfiltration membrane of pore size - 0.2 µm

Plate 3.4 Ultrafiltration membrane of MWCO 40 kDa

3.3 DESCRIPTION OF CROWN CORKING MACHINE

Crown corking machine (Plate 3.5) also known as crown capping machine was used in coconut water bottle sealing. Crown corking machine was a compact table top manual operated machine suitable for corking of crowns on the coconut water bottles. Capacity of machine was 600 bottles/h.

Plate 3.5 Hand operated crown corking machine

3.4 EXPERIMENTAL METHODS OF PROCESSING OF TENDER COCONUT WATER

Following different processing methods were investigated to find out best processing technology for preservation of coconut water (Table 3.1)

1. Microfiltration
2. Ultrafiltration
3. Pasteurization at 85 $^{\circ}$C for 10 min
4. Addition of chemical preservative, i.e. nisin to microfiltered coconut water
5. Control, TCW without any treatment were investigated in the study

All the samples of different treatments were refrigerated at 4 °C and quality parameters were studied at an interval of 0, 4, 8, 12, 16, and 20 days of storage.

3.4.1 Microfiltration of Tender Coconut Water

In the first treatment, the coconut water was passed through microfiltration membrane of 0.2 µm pore size at a pressure of 5.06 kg/cm^2 (80 psi) to remove microbes and suspended particles.

3.4.2 Ultrafiltration of Tender Coconut Water

In the second treatment, Fresh coconut was passed through ultrafiltration membranes of MWCO 40 kDa at pressures about 5.06 kg/cm^2 (80 psi) to remove enzymes such as polyphenoloxidase (PPO) and peroxidase (POD). Then coconut water was bottled, refrigerated and quality and storage studies were carried out.

3.4.3 Pasteurization of Tender Coconut Water

In the third treatment, the coconut water was bottled using crown corking machine and pasteurized at 85 ^{o}C for 10 min in a water bath. Heat treatment was suggested to inactivate enzymes and enhances shelf life and ensure microbial safety.

3.4.4 Addition of Chemical Preservative

In the fourth treatment, the coconut water was passed through microfiltration membrane of 0.2 µm pore size at a pressure of 5.06 kg/cm^2 (80 psi) and chemical preservative nisin was added to coconut water. In this treatment two concentrations of Nisin 5000 I.U. and 2500 I.U. were used.

3.4.5 Control tender coconut water without any treatment

Tender coconut water filtered through muslin cloth, bottled and refrigerated as control samples. The control samples were not added any preservative or any thermal treatment given. The controls as well as treated samples were stored at 4 °C (Plates 3.6 to 3.10).

Table 3.1 Different treatments given in tender coconut water processing

Treatment code	Treatment	Method	MEMBRANE	PRESSURE
T_1	Microfiltered coconut water	TCW was prefiltered through muslin cloth and then microfiltered.	0.2 µm pore size made up of Poly acrylo nitrile	5.06 kg/cm^2 (80 psi)
T_2	Ultrafiltered coconut water	TCW was prefiltered through muslin cloth and then ultrafiltered.	UF, 40 kDa MWCO made up of Polysulphone	5.06 kg/cm^2 (80 psi)
T_3	Pasteurization	TCW was prefiltered through muslin cloth and pasteurised at 85 °C for 10 min	-	-
T_4	Microfiltered coconut water addition of nisin	TCW was microfiltered and then chemical preservative nisin 5000 I.U. was added	-	-
T_5	Microfiltered coconut water addition of nisin	TCW was microfiltered and then chemical preservative nisin 2500 I.U. was added	-	-
T_6	Control	No treatment was given	-	-

Plate 3.6 Microfiltered coconut water samples at TMP 80 psi (5.06 kg/cm^2)

Plate 3.7 Ultrafiltered coconut water samples at a TMP 80 psi (5.06 kg/cm^2)

Plate 3.8 Pasteurized coconut water samples

Plate 3.9 Microfiltered coconut water samples preserved using 2500 I.U. concentration of nisin

Plate 3.10 Microfiltered coconut water samples preserved using 5000 I.U. concentration of nisin

3.5 OPERATION OF CROSS FLOW MEMBRANE CELL

3.5.1 Cleaning Protocol after Longterm Inoperation of Plant

1. After an experiment, the membrane was washed following the protocols (as described below) before dissembling.

2. First, clean water (tap water will do, distilled water is better) was circulated for rinsing. For the first few minutes, the bypass and retentate line was recirculated into the feed tank. Once the bypass and retentate line are clear, both were recirculated for 15-20 min (considering 3-4 h of operation). In case of more long duration run, the washing time with clear water was increased proportionately. Approximately 4-5 L of water was sufficient.

3. After washing with water was completed, the system was cleaned with 0.1-0.15 N hydrochloric acid. In this mode, both the bypass and retentate line were circulated. In this case also, washing time was 15-20 min (considering 3-4 h of operation). In case of more long duration run, the washing time was increased proportionately. Approximately 2-3 L of acid was used.

4. After washing with acid is done, the system was washed with 0.2-0.25 N sodium hydroxide solution for 20-25 min (considering 3-4 h of operation). Note, that in case of more long duration run, the washing time was increased proportionately. Approximately 2-3 L of alkali solution was required.

5. Now, depending on the concentration and nature of the product (presence of bio-foulants), the system was rinsed with 1.5%-2% formalin solution for approximately 5-10 min considering 3-4 h of operation). In case of more long duration run, washing time with formalin solution was increased proportionately. Approximately 1-2 L of formalin solution may be required.

3.5.2 Operation of Membrane Plant for Conducting Experiments

A fresh membrane was compacted at a pressure higher than the maximum operating pressure for 3 h using distilled water and then its permeability was measured. The raw material was placed in a feed tank of 5 L capacity. A high pressure reciprocating pump was used to feed the TCW into the cross-flow membrane cell. Volumes of permeate were collected at 10 mL incremental values during the experiment and corresponding time was noted. A bypass line was provided from the pump delivery to the feed tank. Retentate and bypass control valves were used to vary the pressure and flow rate accordingly. The permeate stream after collecting required amount of sample was recycled to the feed tank to maintain a constant concentration in the feed tank under total recycle mode. The recirculation flowrate of TCW was kept constant at 100 Lph. The corresponding cross flow velocity of the retentate is 1.68 m/s. Once an experimental run was over, the membrane was thoroughly washed, in situ, with distilled water for 30 min applying a maximum pressure of 30 psi. The cell was dismantled and the membrane was rinsed with distilled water and was dipped in 2% sodium dodecyl sulphate solution overnight. Next day, the membrane was washed carefully with distilled water to remove traces of surfactant. The cell was reassembled and the membrane permeability was again measured using distilled water. After that, the set up was ready for the next experiment product. All the experiments were conducted at a room temperature of 33±2 °C. Average values of time for the collection of two successive cumulative permeate volume was considered (Appendix-A) for plotting flux versus time graph. All the experiments were triplicated and averages were presented. The holdup volume in the plant is 1.5 L. Minimum volume of feed required to conduct experiments is 4 L.

Following are the important points to operate cross flow membrane plant:

1. Maximum safe operating pressure is upto 150 psi with the system.
2. Maximum cross flow rate is upto 150 Lph.
3. Bypass line and the retentate line are to be recirculated in the feed tank.
4. Both the bypass and retentate valves are to be simultaneously adjusted to attain the desired pressure and flow rate. Note that closing the bypass valve will increase the flow rate as well as pressure whereas, closing the retentate valve (located at the exit of the membrane module) the pressure will rise but flow rate will decrease.
5. It is to be remembered that the bypass valve is more sensitive to pressure while the retentate valve is more sensitive to flow.
6. Control of both the valves is not linear, which means that the system will start to respond to the closing of the valve and it may change drastically after a particular point.
7. There are two modes of filtration – total recycle mode and batch concentration mode.
8. In the total recycle mode the permeate is recycled back to the feed tank. In this case, the feed concentration remains constant and therefore during ultrafiltration, the system achieves steady state after some time.
9. In case of batch concentration mode, the permeate is not recycled back, therefore the feed volume decreases and subsequently the concentration increases.
10. In case the solution (to be filtered) has high debris/fibers or large particulates, it is be filtered first with a fine mesh cloth before feeding into the membrane.
11. All the membranes are to be stored in 1% to 2% formalin solution when not in use for more than 2 days. In case the membranes are not used for long time, the storing liquid must be changed after every 15 days.

3.6 DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES OF RAW AND PROCESSED TENDER COCONUT WATER

The physico-chemical properties of the processed TCW such as °Brix, pH, reducing sugars, turbidity were determined by the following methods.

3.6.1 Determination of °Brix of Processed Tender Coconut Water

Total soluble solids (TSS) of TCW were measured by placing a drop of the TCW sample on the prism of the Pocket Refractrometer (ATAGO make, range 0%-93%) and expressed in terms of °Brix (Plate 3.11). The TSS of TCW was measured at an interval of four days until the end of 20 days storage period. The changes were recorded for all treatments during the storage period (Ranganna, 1986).

Plate 3.11 Pocket Refractrometer

3.6.2 Determination of pH

The pH was measured using a digital pH meter (Systronics µ pH system, Model 362) using a glass electrode (Plate 3.12). Electrode was dipped in the thoroughly mixed samples of TCW and the value was registered once it had stabilised (Ranganna, 1986). This pH was measured at an interval of four days until the end of 20 days storage period. The changes were recorded for all treatments during the storage period.

Plate 3.12 Digital pH meter

3.6.3 Determination of Turbidity

Turbidity was measured using a Systronics Spectrophotometer 166 (Plate 3.13) at transmittance of 610 nm (Prakruthi *et al.*, 2014) using a transparent cuvette. The coconut water sample was filled in cuvette, then cuvette was placed in cubicle in the path of light and the value was registered once it had stabilised. This turbidity was measured at an interval of four days until the end of 20 days storage period.

Plate 3.13 Digital Spectrophotometer

3.6.4 Determination of Reducing Sugar

Principle involved is that when reducing sugars heated with alkaline copper tartrate, the copper converts from cupric to cuprous state and thus cuprous oxide was formed, when cuprous oxide is treated with arsenomolybdic acid, the reduction of molybdic acid to molybdic blue takes place. The blue colour absorbs maximally at 540 nm (Sadashivam and Manikam, 1992).

Reagents

A. Alkaline copper reagent

1. 4 g copper sulphate
2. 24 g Na_2CO_3 anhydrous
3. 12 g sodium potassium tarterate
4. 16 g sodium bicarbonate
5. 180 g anhydrous sodium sulphate (mol. wt. 142.04)

} Dissolved in 1 L distilled water

B. Arseno molybdate reagent

1. 50 g ammonium molybdate (mol. Wt. 1235.90)
2. 2.1mL H_2SO_4 AR
3. 0.3 g sodium arsenate (mol. wt. 312.02)

} Dissolved in 1 L distilled water and kept for 2 days and filtered through whatman 2 filter paper

Method

A sample of 1 mL TCW sample was taken and the estimation of reducing sugars was carried out according to the flow chart given in Appendix-B and the calculations for reducing sugars are shown in Appendix-C.

Calculations

Reducing sugars % = (G.F. (152) × Total volume (50 mL)) / (wt sample × 10000)

3.7 ASSESSMENT OF MICROBIOLOGICAL QUALITY OF PROCESSED TENDER COCONUT WATER

Presence of microorganisms viz bacteria, fungi and *E.coli* were determined by performing standard dilution technique using suitable media.

Composition of Nutrient agar for Bacterial count:

Peptone	–	5 g/L
Beef extract	–	3.0 g/L
Glucose	–	5.0 g/L
Nacl	–	3.0 g/L
Agar	–	16.0 g/L
Distilled water	–	1000 mL

Composition of EMB agar for *E.coli* count:

Peptic digest of animal tissue	–	10 g/L
Di potassium phosphate	–	2 g/L
Lactose	–	5 g/L
Sucrose	–	5 g/L
Eosin Y	–	0.4 g/L
Methylene blue	–	0.065 g/L
Agar	–	13.5 g/L
Final pH	–	7.2 ± 0.2
Distilled water	–	1000 mL

Composition of potato dextrose agar for fungal count:

Potatoes infusion form	-	200 g/L
Dextrose	-	20 g/L
Agar	-	15 g/L
Distilled water	-	1000 mL

Appropriate quantities of respective agars were prepared, autoclaved at 121 °C at 15 psi for 15 min, and then poured into sterile petri plates. TCW samples were serially diluted using sterile distilled water by standard dilution technique. 0.1 mL of serially diluted TCW sample (10^{-6} dilution) was spread into petri plates and left in incubator for 24 h at 37 °C for bacteria,

48-72 h at 27 ℃ for fungi and 24-36 h at 37 ℃ for yeast. Microbial count was done using in a digital colony counter (Plate 3.14) and specific CFU were determined using the following formula.

$$\text{Colony forming units (CFU) per mL} = \frac{\text{Number of colonies}}{\text{Dilution} \times \text{volume plated}}$$

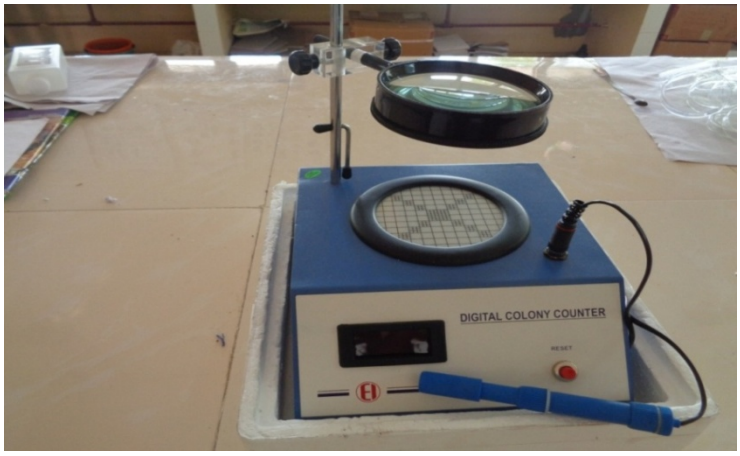

Plate 3.14 Digital colony counter

3.8 SENSORY EVALUATION

Sensory evaluation of samples was carried out for consumer acceptance and preference using 10 untrained panelists selected at random. Appearance, flavour, overall acceptability of the samples were rated using a 9-point Hedonic scale where 9 and 1 represent like extremely and dislike extremely respectively as per the standard proforma (Appendix D). Sensory evaluation was carried out at ambient conditions in a comfortable and quiet area without disturbance under fluorescent lighting. Water was supplied to cleanse palate between samples. Three sensory attributes stated below were considered for the study.

I. Appearance

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4

Dislike moderately	3
Dislike very much	2
Dislike extremely	1

II. Flavour

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

III. Overall acceptability

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Chapter - IV

RESULTS AND DISCUSSION

In this chapter the details of the results of the effect of TMP on flux for different membranes and various treatments given to TCW to enhance shelf life have been presented. Physico-chemical and microbiological properties of stored TCW have been evaluated during the storage period and significant observations are discussed.

4.1ESTABLISHMENT OF PURE WATER FLUX WITH DIFFERENT MEMBRANES AT VARIOUS TRANSMEMBRANE PRESSURES

Permeate flux was established using for pure water during microfiltration with pore size 0.2 µm membrane at 20, 40, 60 and 80 psi (Table 4.1 and Fig. 4.1). Initially the flux was observed to be 372.39 L/m^{2}h at 20 psi and then increased gradually to 932.12 L/m^{2}h as transmembrane pressure was increased upto 80 psi.

Table 4.1 Permeate flux of pure water during microfiltration with different TMPs

S.No.	Transmembrane Pressure, psi	Permeate flux, L/m^{2}h
1	0	0
2	20	372.39
3	40	642.82
4	60	796.91
5	80	932.12

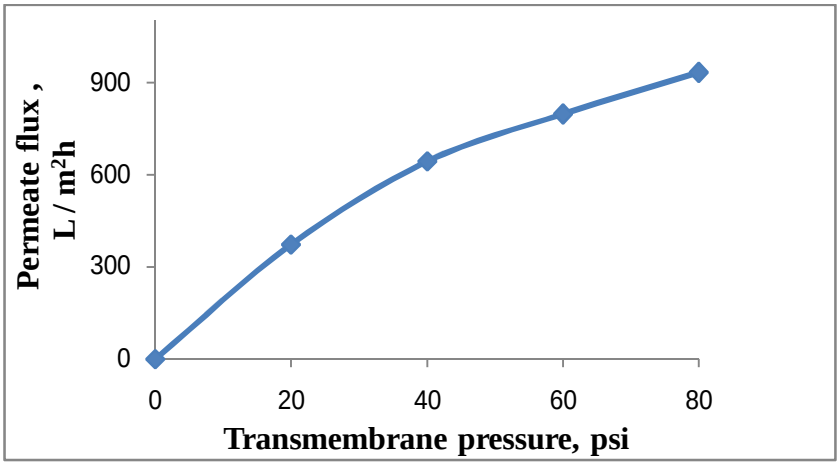

Fig. 4.1 Variation of pure water flux during microfiltration at different TMPs

Permeate flux was established using for pure water during ultrafiltration with MWCO 40 kDa at 20, 40, 60 and 80 psi (Table 4.2 and Fig. 4.2). Initially the flux was observed to be 153.34 L/m^2 h at 20 psi and then increased gradually to 462.28 L/m^2 h as transmembrane pressure was increased upto 80 psi.

Table 4.2 Permeate flux of pure water during ultrafiltration with different TMPs

S.No.	Transmembrane Pressure, psi	Permeate flux, L/m^{2}h
1	0	0
2	20	153.34
3	40	258.33
4	60	370.13
5	80	462.28

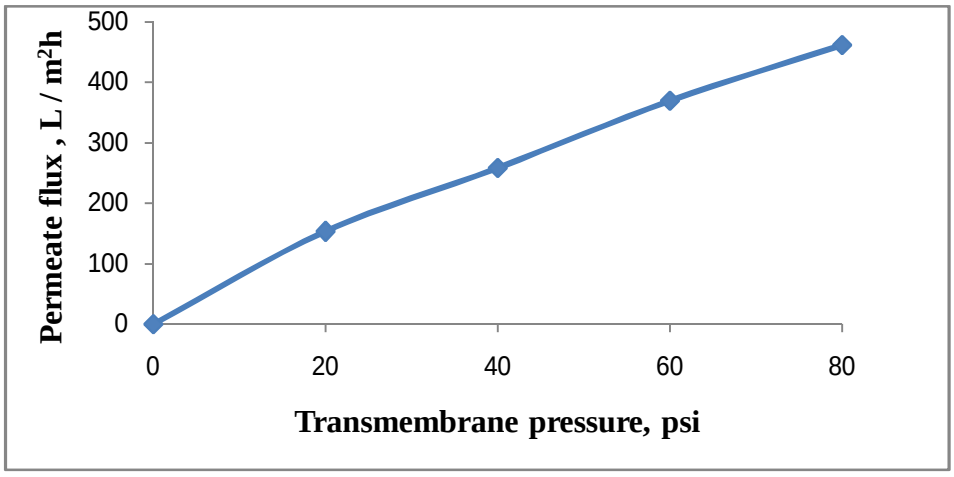

Fig. 4.2 Variation of pure water flux during ultrafiltration at different TMPs

Permeate flux was established using for pure water during nanofiltration with MWCO 500 Da at 20, 40, 60 and 80 psi (Table 4.3 and Fig. 4.3). Initially the flux was observed to be 64.20 L/m^{2}h at 20 psi and then increased gradually to 163.91 L/m^{2}h as transmembrane pressure was increased upto 80 psi.

Table 4.3 Permeate flux of pure water during nanofiltration with different TMPs

S.No.	Transmembrane Pressure, psi	Permeate flux, L/m^{2}h
1	0	0
2	20	64.20
3	40	97.44
4	60	131.43
5	80	163.91

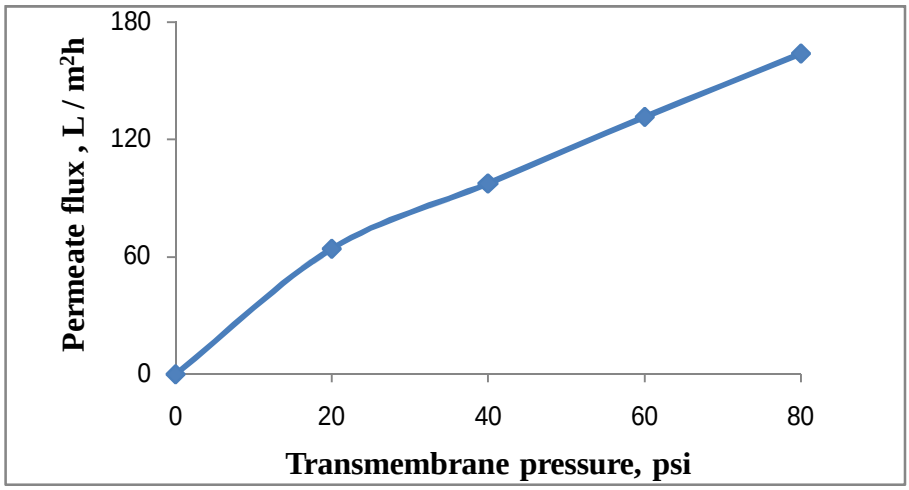

Fig. 4.3 Variation of pure water flux during nanofiltration at different TMPs

A comparison of fluxes on three different membranes at four TMPs is given to establish the effect of pore size and TMPs on fluxes (Table 4.4 and Fig. 4.4)

Table 4.4 Permeate Fluxes of MF, UF and NF at different TMPs

S.No.	TMP, psi	MF, Permeate flux, L/m^2h	UF, Permeate flux, L/m^2h	NF, Permeate flux, L/m^2h
1	0	0	0	0
2	20	372.39	153.34	64.20
3	40	642.82	258.337	97.44
4	60	796.91	370.132	131.43
5	80	932.12	462.2875	163.91

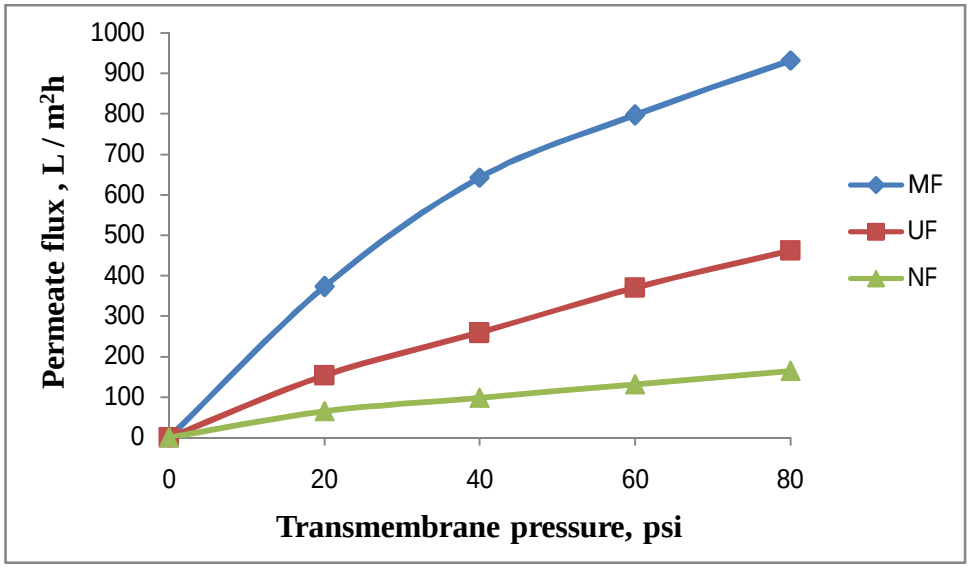

Fig. 4.4 Comparison of Permeate Flux using different membranes with different TMPs

It is clear that pure water flux increased as TMP increased on any membrane. This is as per the Darcy's law which relates driving force with permeate flux. Also permeate flux increased with an increase in pore size or MWCO at any TMP. This is due to decrease in membrane resistance as pore size or MWCO increases. It is also reported that higher TMPs and higher pore sized membranes give higher fluxes (Belfort *et al.*, 1994 and Marshall *et al.*, 1997).

4.2 VARIATION OF PERMEATE FLUX OF PURE WATER DURING MEMBRANE FILTRATION

Permeate flux during microfiltration was recorded for pure water (Table 4.5 and Fig. 4.5). There was no significant change in the permeate flux observed during the experiment perhaps due to no solids to accumulation or plug pore on the membrane surface. The permeate flux was observed to be in the range of 964.55 to 1139.92 L /m^2h initially and reached a steady flux 964.55 L/m^2h.

Table 4.5 Permeate flux during microfiltration of pure water at 80 psi

Time (s)	Permeate flux (L /m^2h)
5	964.55
10	964.55
14	1139.92
19	964.55
23	964.55
28	964.55
32	964.55
36	1139.92
40	1139.92
45	964.55
50	964.55
55	964.55

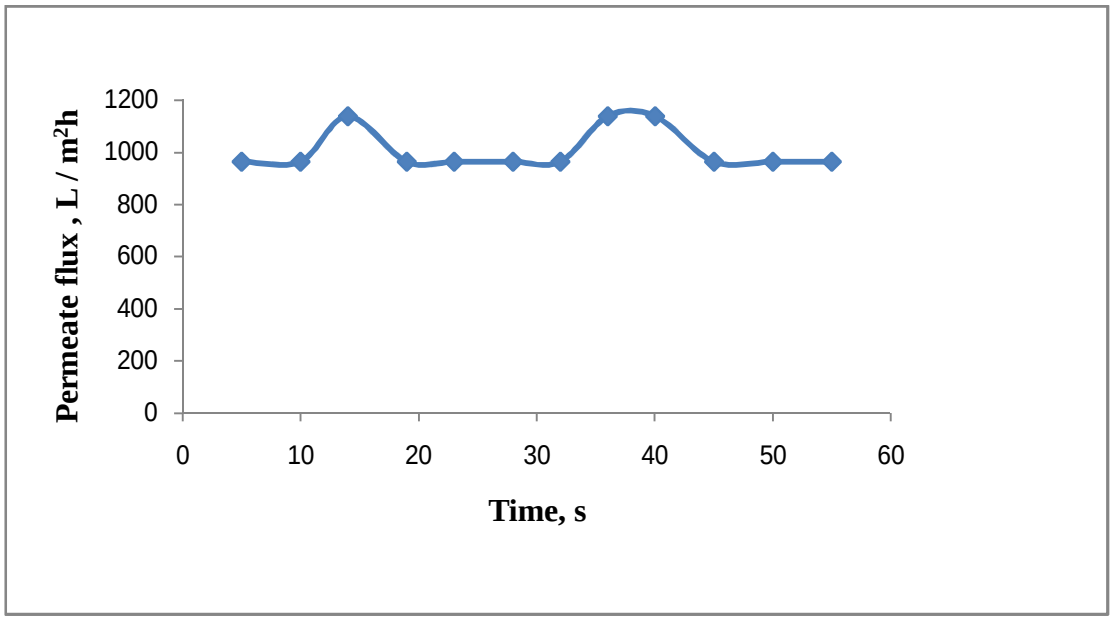

Fig. 4.5 Variation of pure water flux during microfiltration at 80 psi (5.06 kg/cm^2)

Similarly the permeate flux during ultrafiltration of pure water was observed (Table 4.6 and Fig. 4.6). Permeate flux was observed to be 417.97 L/m^{2}h. It is observed that under same TMP of 80 psi; MF membrane gave a steady flux of 964.55 L/m^{2}h which is higher than 417.97 L/m^{2}h that obtained on UF membrane. This is obvious as membrane pore size increases, flux will also increase. Similar observations were made by many researchers (Kim *et al.*, 1992; Davis *et al.*, 1987 and Jonsson *et al.*, 1996).

Table 4.6 Permeate flux during ultrafiltration of pure water at 80 psi (5.06 kg/cm^2)

Time (s)	Permeate flux (L /m^{2}h)
11	417.97
23	379.97
34	417.97
46	379.97
57	417.97
68	417.97
79	417.97
91	379.97
103	379.97
114	417.97
125	417.97
136	417.97
145	417.97

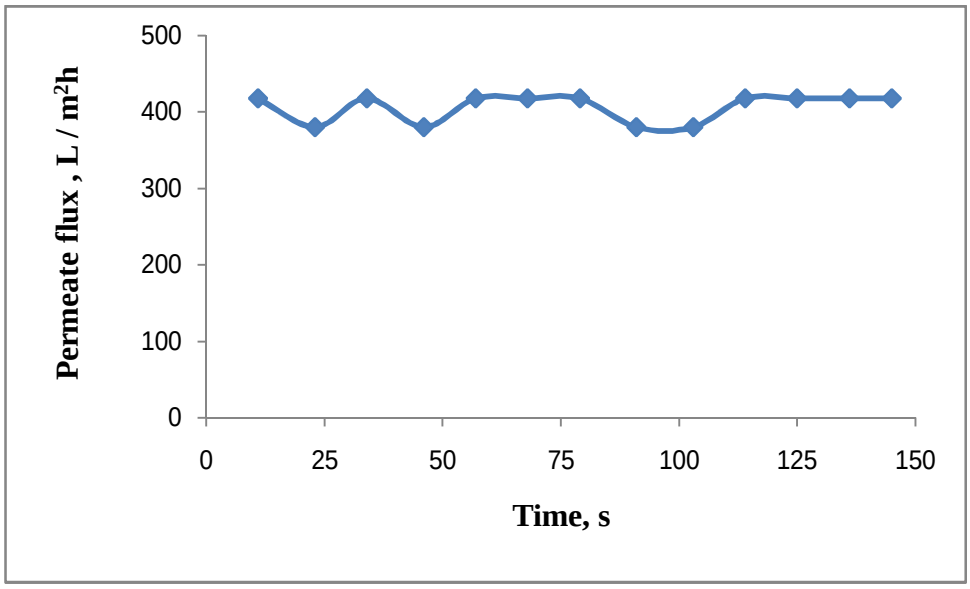

Fig. 4.6 Variation of pure water flux during ultrafiltration at 80 psi (5.06 kg/cm^2)

4.3 VARIATION OF PERMEATE FLUX DURING MEMBRANE FILTRATION OF TENDER COCONUT WATER

Five different treatments (Table 3.1) were investigated to enhance shelf life of bottled TCW. TCW samples were stored at refrigerated conditions (4 °C) and physico-chemical properties during storage period were determined. At an interval of four days, the stored samples of TCW were taken for their quality evaluation i.e. TSS, pH, reducing sugars and turbidity. Microbiological tests i.e. *E.coli* count, fungal count and bacterial count were also conducted to find out the spoilage in TCW samples. Based on these characteristics, the shelf life of tender coconut was assessed.

In the first treatment, the coconut water was passed through microfiltration membrane of 0.2 µm pore size at a pressure of 5.06 kg/cm^2 (80 psi) to remove microbes and suspended particles.

Permeate flux during microfiltration of TCW gradually decreased from 189.98 to 88.51 L /m^{2}h and reached a steady state flux at 88.51 L /m^{2}h within 6 minutes (Table 4.7 and Fig. 4.7). The decline in permeate flux during MF of fruit juices was also reported earlier (Pelin *et al.*, 2010; Cassano *et al.*, 2003; Bottino *et al.*, 2002 and Capannelli *et al.*, 1992).

Table 4.7 Permeate flux during microfiltration of tender coconut water at 80 psi (5.06 kg/cm^2)

Time (s)	UF Permeate flux (L /m^{2}h) at 80 psi
0	189.98
12	162.84
38	145.80
67.5	133.39
100	129.27
134.5	112.96
172	105.01
213.5	98.13
258	92.12
305.5	88.51
355.5	88.51
406.5	88.51
457.5	88.51
508.5	88.51
559.5	88.51

The decline in flux is attributed to concentration polarization and consequent fouling (Blatt *et al.,* 1970). Most of the constituents after muslin cloth filtration of TCW are probably less than few nm as they are vitamins, sugars and salts. It is surprising to observe that 0.2 µm (200 nm) membrane is getting flux decline. This may due to pore fouling (Marshall *et al*., 1997).

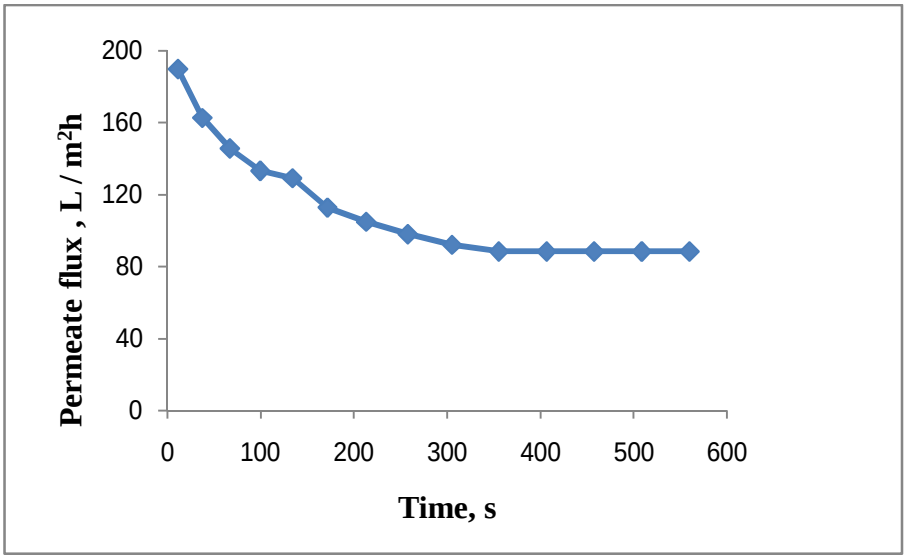

Fig. 4.7 Permeate flux during microfiltration of tender coconut water at 80 psi (5.06 kg/cm^2)

In the second treatment, Fresh coconut was passed through ultrafiltration membrane of MWCO 40 kDa at pressures of 5.06 kg/cm^2 (80 psi) to remove enzymes such as polyphenoloxidase (PPO) and peroxidase (POD). Then coconut water was bottled, refrigerated and quality and storage studies were carried out.

There is a general decrease in flux with time even in the case of ultrafiltration of TCW (Table 4.8 and Fig. 4.8) at transmembrane pressure of 5.06 kg/cm^2 (80 psi). The flux declined from 107.54 to 82.07 L/m^{2}h. The decline in flux during membrane filtration is due to fouling via concentration polarization of solute particles. Fouling may have occurred due to pore narrowing by smaller particles that may have accumulated on the pore walls or by pore plugging (Chilukuri *et al.*, 2001).

Table 4.8 Permeate flux during ultrafiltration of tender coconut water at 80 psi 40 kDa (5.06 kg/cm^2)

Time (s)	Permeate flux (L /m^{2}h)
0	107.54
21	105.01
63.5	102.59
105.5	98.13
149	92.12
196.5	90.28
246	88.51
296.5	86.81
348	85.17
400.5	83.59
454	82.07
508.5	82.07
563	82.07
617	82.07
671	82.07

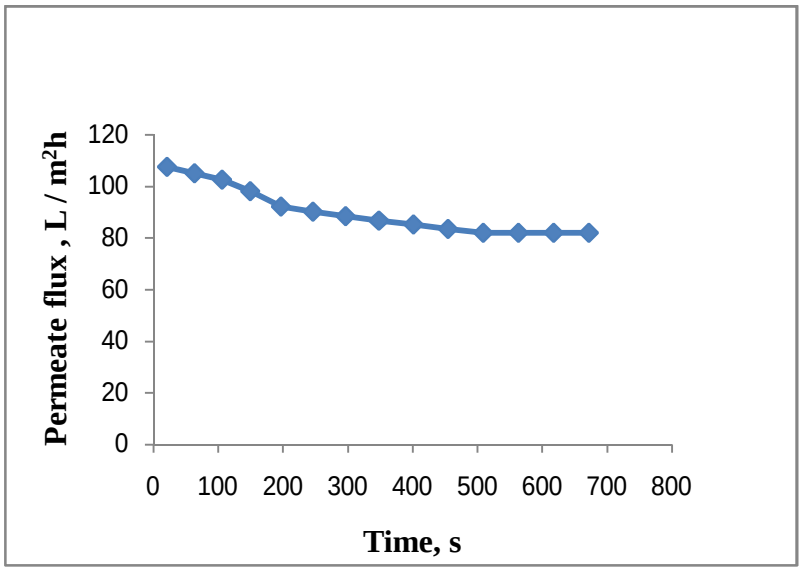

Fig. 4.8 Permeate flux during ultrafiltration of tender coconut water at 80 psi

4.4 EFFECT OF DIFFERENT TREATMENTS ON PHYSICO-CHEMICAL PROPERTIES OF STORED TENDER COCONUT WATER

4.4.1 Changes in Total Soluble Solids

Total Soluble Solids (TSS) were found to be in the range of 4.95 °Brix to 5.29 °Brix initially, then the values gradually decreased with time in all treatments. The TSS of control, T_6 was very high initially and then decreased from 5.29 to 4.456 °Brix. The TSS of treatment T_1 was also initially 4.95 °Brix and then after 20th days of storage it was observed to be 4.736 °Brix. The TSS decreased from 4.96 to 4.726 °Brix for treatment T_2. There was no significant change observed in TSS of treatment T_3. Similar observation was made by Chowdhury *et al.* (2009) for heat treated coconut water samples at 85 °C for 10 minutes. TSS decreased from 5.2 to 4.539 °Brix, from 5.15 to 4.8 °Brix for treatments T_4 and T_5, respectively. Both microfiltered and ultrafiltered samples were observed to have slightly lower TSS perhaps due to removal of some suspended particles from the tender coconut water. It is observed that TSS generally decreased on storage for all the treatments in the study (Table 4.9 and Fig. 4.9).

Table 4.9 Changes in TSS of different treatments of tender coconut water during storage

Days after storage, (DAS)	Total Soluble Solids, °Brix					
	T_1	T_2	T_3	T_4	T_5	T_6
0	4.95	4.96	5.1	5.2	5.15	5.29
4	4.9	4.9	5.06	5.25	5.012	5.2
8	4.85	4.81	5.1	4.95	4.9	5.15
12	4.7	4.79	5.133	4.756	4.95	5.05
16	4.8	4.75	5.1	4.7	4.85	4.83
20	4.736	4.726	5.1	4.539	4.8	4.456

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)); T_2 – ultrafiltration (40 kDa, 5.06 kg/cm^2 (80 psi)); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80psi)) + Preservation with Nisin 2500 I.U.; T_6- control

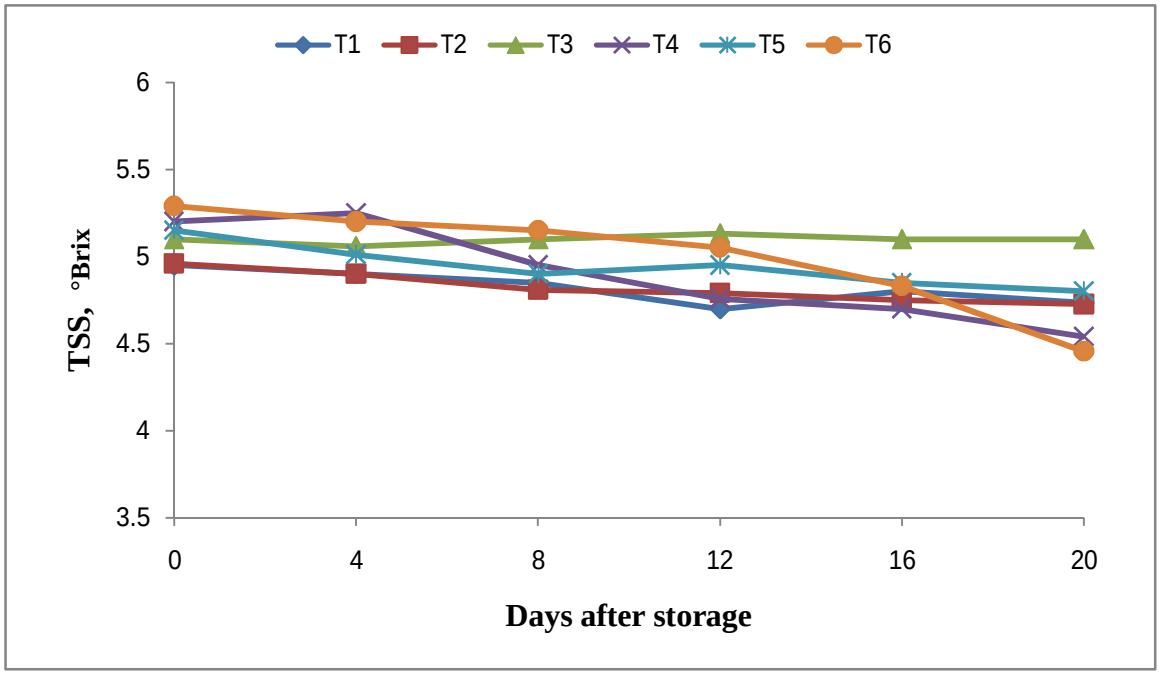

Fig. 4.9 Variation of TSS of different treatments of tender coconut water during storage

The tender coconut water comprises of sugars, minerals and other nutrients. The initial TSS of the treatments T_1 & T_2 were found to be slightly low because some of the complex sugars and cloud forming solids may have been retained in filtration because of their higher molecular size. TSS in all treatments decreased during storage probably due to fermentation process. Similar observation was made by Rosa *et al*. (2012). Treatment T_3 exhibited less change in TSS because of resistance to fermentation process due to thermal treatment (Chowdhury *et al*., 2009). The TSS of treatments particularly

untreated TCW reduced probably more due to the conversion of carbohydrates into sugars, organic acids and other soluble materials by metabolic processes during storage (Manashi *et al*., 2012).

4.4.2 Changes in pH

The pH of tender coconut water generally decreased upon storage in the study (Table 4.10 and Fig. 4.10). The pH in treatments T_1 and T_2 decreased from 4.864 to 4.562 and 4.951 to 4.64 respectively. It was observed high initially after four days of storage in these treatments because of removal of most of the colloidal particles which might cause acidity in TCW and then the pH decreased during storage probably due to fermentation process as it was completely non-thermal process. The pH of treatment T_6 decreased from 4.921 to 4.25. The probable reason for the decrease in pH in control treatment may be due to microbial growth. It was very low on 20th day compared to all other samples because it was not given any treatment (control) due to which fast fermentation reactions may have occurred. The pH of treatment T_3 decreased relatively less from 4.81 to 4.563 perhaps due to thermal treatment by which fermentation process might have been delayed. The pH in general decreased upon storage which could be due to the production of free acids by microbial growth or by polysaccharides (Manashi *et al*., 2012).

Table 4.10 Changes in pH of different treatments of tender coconut water during storage

Days after storage, (DAS)	pH of tender coconut water samples					
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
0	4.864	4.951	4.81	4.851	4.81	4.921
4	4.91	5.015	4.61	4.89	4.883	4.85
8	4.85	5.05	4.681	4.78	4.6	4.67
12	4.8	4.894	4.749	4.56	4.406	4.48
16	4.739	4.78	4.521	4.44	4.45	4.43
20	4.562	4.64	4.563	4.32	4.36	4.25

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)); T_2 – ultrafiltration (40 kDa, 5.06 kg/cm^2 (80 psi)); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)) + Preservation with Nisin 2500 I.U.; T_6- control

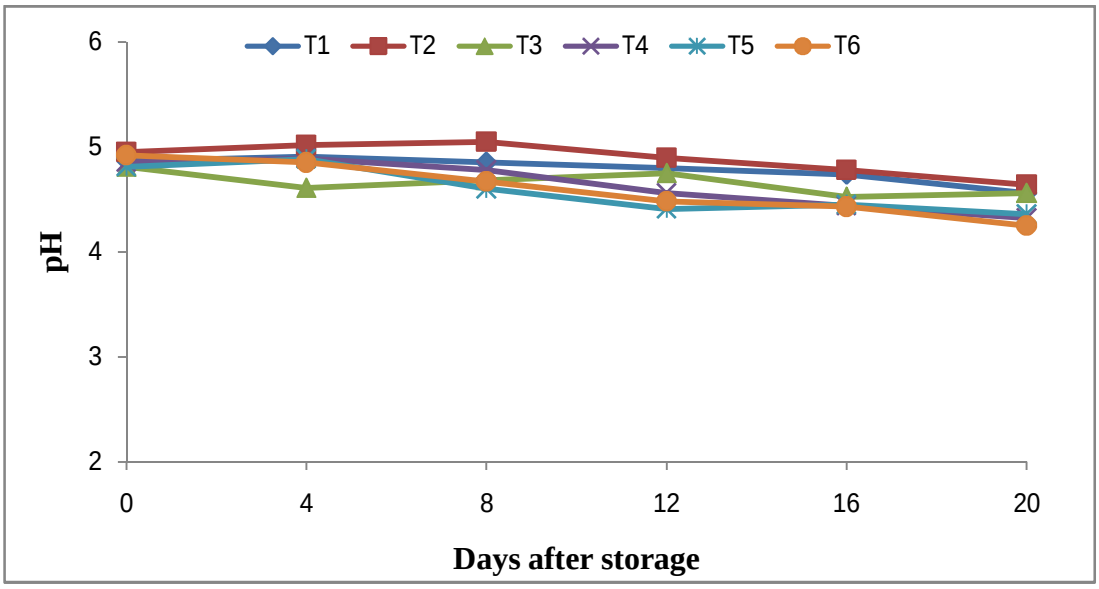

Fig. 4.10 Variation of pH of different treatments of tender coconut water during storage

4.4.3 Changes in Reducing Sugars

There is an increase in percentage reducing sugars in all the treatments upon storage (Table 4.11 and Fig. 4.11). The reducing sugars for treatment T_6 were observed to increase from 3.2% to 3.58% upon storage. The reducing sugars were low for treatment T_3 i.e., 3.1 and increased to 3.254% upon storage. The values of reducing sugars for all other treatments T_1, T_2, T_4 and T_5, increased from 3.2% to 3.43%, 3.1% to 3.29%, 3.25% to 3.39% and 3.2% to 3.38%, respectively.

Table 4.11 Changes in reducing sugars of different treatments of tender coconut water during storage

Days after storage, (DAS)	reducing sugars%					
	T_1	T_2	T_3	T_4	T_5	T_6
0	3.2	3.1	3.1	3.25	3.2	3.2
4	3.29	3.09	3.26	3.286	3.25	3.25
8	3.3	3.15	3.29	3.19	3.19	3.36
12	3.358	3.19	3.19	3.23	3.23	3.4
16	3.401	3.202	3.23	3.3	3.38	3.49
20	3.43	3.29	3.254	3.39	3.38	3.58

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2(80 psi); T_2 – ultrafiltration (40 kDa,5.06 kg/cm^2 (80 psi); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2(80 psi) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2(80psi) + Preservation with Nisin 2500 I.U.; T_6- control

The presence of more reducing sugars is an indication of conversion of sugars into simple sugars. The more % reducing sugars indicates degradation of quality.

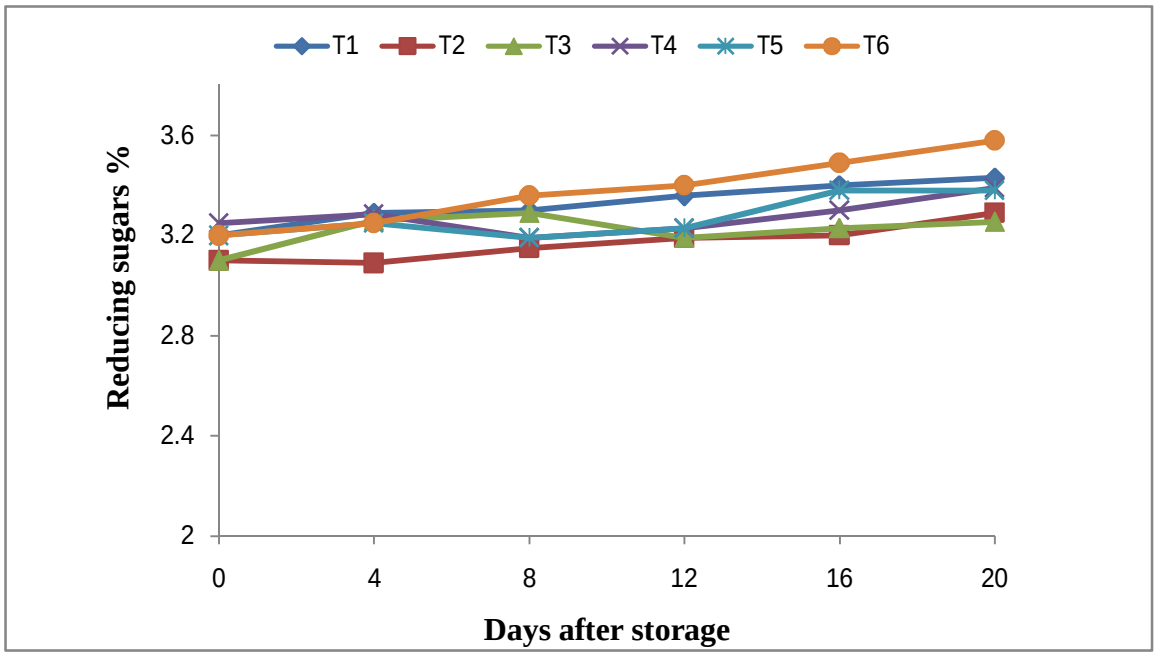

Fig. 4.11 Variation of reducing sugars of different treatments of tender coconut water during storage

The reducing sugars found to be increasing in all the treatment including control because of the breakdown of total sugars into reducing sugars. The reducing sugars might have also increased because of the hydrolysis of non-reducing sugars to reducing sugars. Similar observations were made by Manashi *et al.*, 2012 and Nikhil *et al.*, 2014. The increase of reducing sugars for treatment T_3 was relatively low perhaps due to thermal treatment that delayed the fermentation process of conversion of total sugars to reducing sugars.

4.4.4 Changes in Turbidity

The Turbidity of the tender coconut was measured in terms of transmission of light at a wavelength of 610 nm (Prakruthi *et al.*, 2014) using a spectrophotometer (Table 4.12 and Fig. 4.12). Turbidity is inversely related to light transmission values. It was evident that treatment T_6 exhibited a greater decrease in transmittance values from 88.37% to 65.589% during the period of storage. In contrast, less decrease in transmittance values from 89.023% to 83.23% were noted for treatment T_2 during storage. The transmittance values for other samples were also observed. Transmittance values were observed to decrease from 92.1% to 84.52% after 20 days of storage for treatment T_1. Transmittance values decreased from 91.023% to 81.3% for treatment T_3.

The values also decreased for treatments T_4 and T_5 from 88.9% to 75.3% and 89.3% to 74.365% respectively on 20 days of storage. The increase in turbidity is an indication of changes in biochemical constituents of TCW. Similar observations were made by Manashi *et al*. (2012).

Table 4.12 Changes in light transmission (%) of different treatments of tender coconut water during storage

Days after storage, (DAS)	Transmittance values, %					
	T_1	T_2	T_3	T_4	T_5	T_6
0	92.1	89	91.023	88.9	89.3	88.37
4	90.56	87.63	88.666	87.23	87.033	83.5
8	87.6	86.53	87.56	83.73	86.48	78.63
12	85.6	83.36	86.2	79.3	82.65	72.6
16	83.56	82.69	83.3	77.36	79.3	68.36
20	84.52	83.23	81.3	75.3	74.365	65.589

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80psi)); T_2 – ultrafiltration (40 kDa, 5.06 kg/cm^2 (80 psi)); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80psi)) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)) + Preservation with Nisin 2500 I.U.; T_6- control

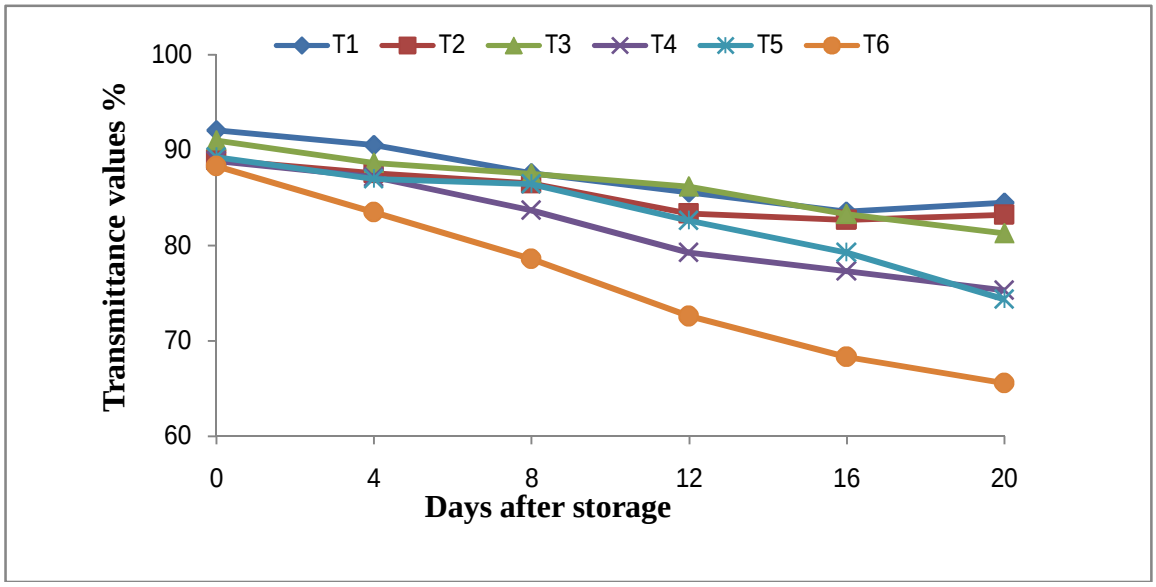

Fig. 4.12 Variation of transmittance (%) of different treatments of tender coconut water during storage

The turbidity of tender coconut water samples was found to increase during storage. The turbidity was found to be more for sample T_6. Faster fermentation process would have taken place and breakdown of sugars would have led to high turbidity in control sample. Clarity of coconut water is one of the important attributes. Consumers rate clear beverages as the most thirst quenching and opaque beverages as least. The

transmittance value generally decreased with storage time. The turbidity of stored samples can be attributed to the increasing microbial load and other cell debris (Manashi *et al.*, 2012). The treatment T_1 had very high transmittance value because of removal of colloidal particles by microfiltration. Treatment T_2 also recorded high transmittance value when compared to treatment T_6 because of ultrafiltration by which most of solids were removed and the samples were less turbid. The experiments using MF and UF suggest that turbidity could be reduced and a clear TCW is possible using membrane filtration process.

4.5 CHANGES IN MICROBIOLOGICAL QUALITY OF STORED TENDER COCONUT WATER

There was no *E.coli* count found initially in all the treatments. In treatment T_1, *E.coli* count increased to 1.02 x 10^6 CFU / 10 mL in storage. There was no *E.coli* in treatments T_3 even on 20^{th} day of storage period. However in treatment T_6 higher colonies were observed (Table 4.13 and Fig. 4.13). Fernanda *et al*. (2009) reported similar microbial changes during storage in reconstituted stored coconut water.

Table 4.13 *E.coli* count of different treatments of tender coconut water during storage

Days after storage, (DAS)	***E.coli* count (x 10^6 CFU / 10 mL of sample)**					
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
0	ND	ND	ND	ND	ND	ND
4	0.14	ND	ND	ND	0.14	1.23
8	0.21	0.02	ND	0.03	0.48	2.67
12	0.35	0.05	ND	0.45	0.69	2.80
16	0.43	0.13	ND	1.21	1.28	3.12
20	1.02	0.19	ND	2.03	2.64	3.24

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80psi)); T_2 – ultrafiltration (40 kDa, 5.06 kg/cm^2 (80psi)); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80psi)) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2(80 psi)) + Preservation with Nisin 2500 I.U.; T_6- control

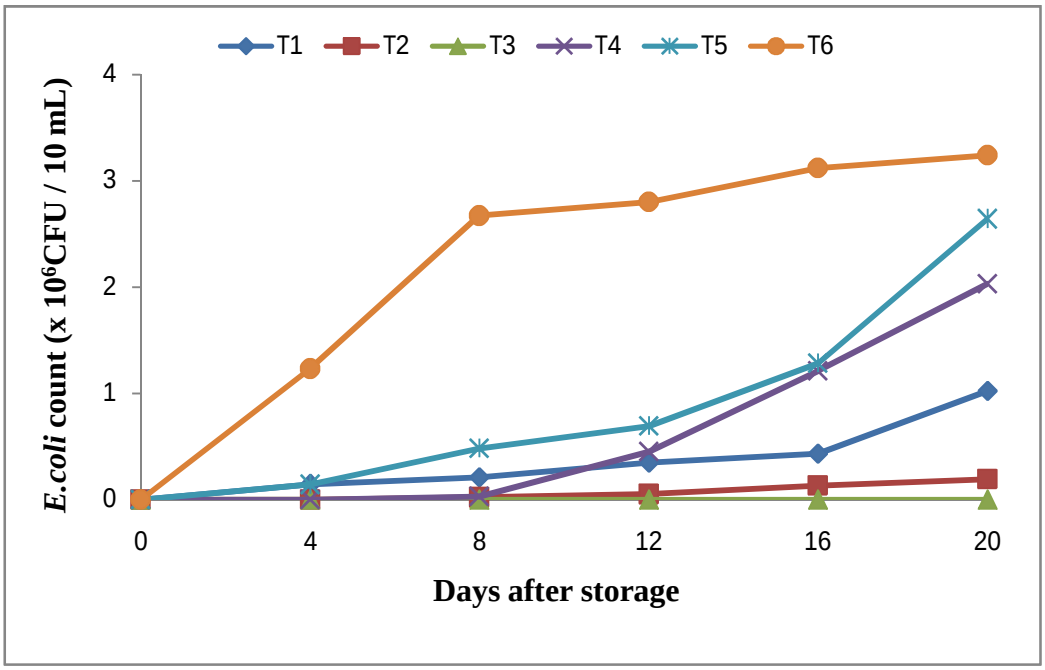

Fig. 4.13 *E.coli* count of different treatments of tender coconut water during storage

Fungal count indicated no growth in all treated samples except in sample T_6 in which it increased to 2.73 x 10^6 CFU/10 mL on 20^{th} day (Table 4.14 and Fig. 4.14). Similar observations were made by Fernanda *et al*. (2009).

Table 4.14 Fungal count of different treatments of tender coconut water during storage

Days after storage, (DAS)	Fungal count (x 10^6 CFU/10 mL of sample)					
	T_1	T_2	T_3	T_4	T_5	T_6
0	ND	ND	ND	ND	ND	0
4	ND	ND	ND	ND	ND	0.49
8	ND	ND	ND	ND	ND	1.67
12	ND	ND	ND	ND	ND	2.47
16	ND	ND	ND	ND	ND	2.68
20	ND	ND	ND	ND	ND	2.73

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)); T_2 – ultrafiltration (40 kDa, 5.06 kg/cm^2 (80 psi)); T_3 – pasteurization at 85 °C, 10min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi)) + Preservation with Nisin 2500 I.U.; T_6- control

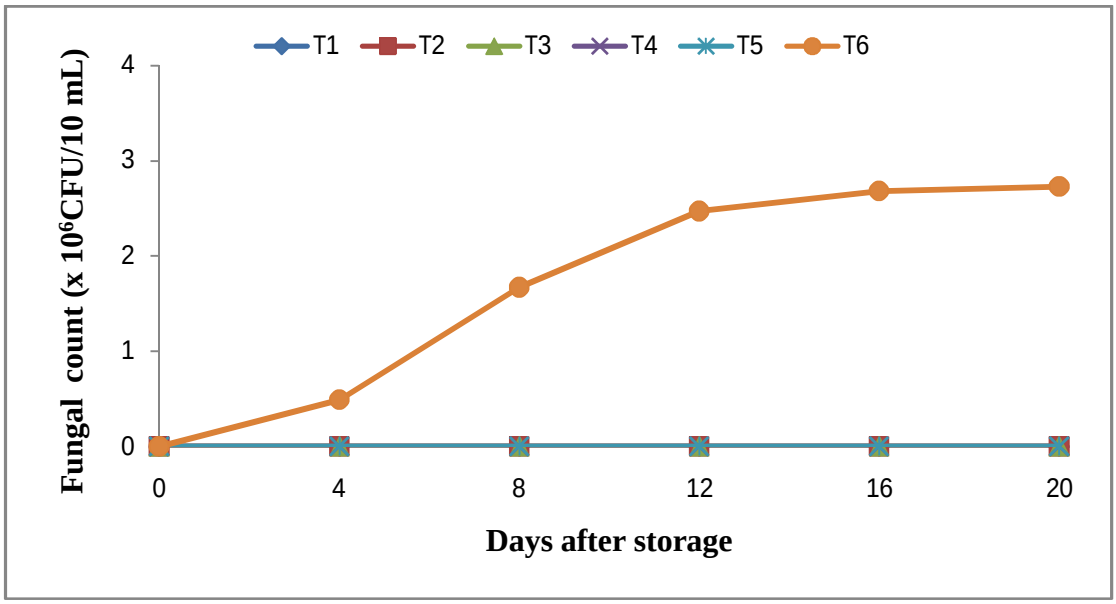

Fig. 4.14 Fungal count of different treatments of tender coconut water during storage

Bacterial count other than coliform was observed to be more in treatment T_6 upon storage (Table 4.15 and Fig. 4.15). Bacterial count was observed to be less for samples T_2 and T_3. Initially there was no growth in treatment T_2, then increased from 0.01 x 10^6 CFU/10 mL on 8th day to 0.19 x 10^6 CFU/10 mL on 20th day. The treatment T_3 had bacterial count of 0.02 x 10^6 CFU/10 mL on 20th day. The treatment T_4 had bacterial count of 0.06 x 10^6 CFU/10 mL on 4th day to 2.74 x 10^6 CFU/10 mL on 20th day. The treatment T_5 had bacterial count of 0.18 x 10^6 CFU/10 mL on 4th day to 2.89 x 10^6 CFU/10 mL on 20th day. Fernanda *et al*. (2009) reported similar microbial changes during storage in reconstituted stored coconut water.

Table 4.15 Bacterial count other than coliform group of different treatments of tender coconut water during storage

Days after storage, (DAS)	Bacterial count other than coliform (x 10^6 CFU/10 mL of sample)					
	T_1	T_2	T_3	T_4	T_5	T_6
0	ND	ND	ND	ND	ND	ND
4	0.26	0	ND	0.06	0.18	1.23
8	0.31	0.01	ND	0.31	0.42	2.67
12	0.48	0.07	ND	0.49	0.79	2.80
16	0.63	0.16	ND	1.02	1.26	3.12
20	0.78	0.19	0.02	2.74	2.89	3.24

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi); T_2 – ultrafiltration (40 kDa,5.06 kg/cm^2 (80 psi); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin 2500 I.U.; T_6- control

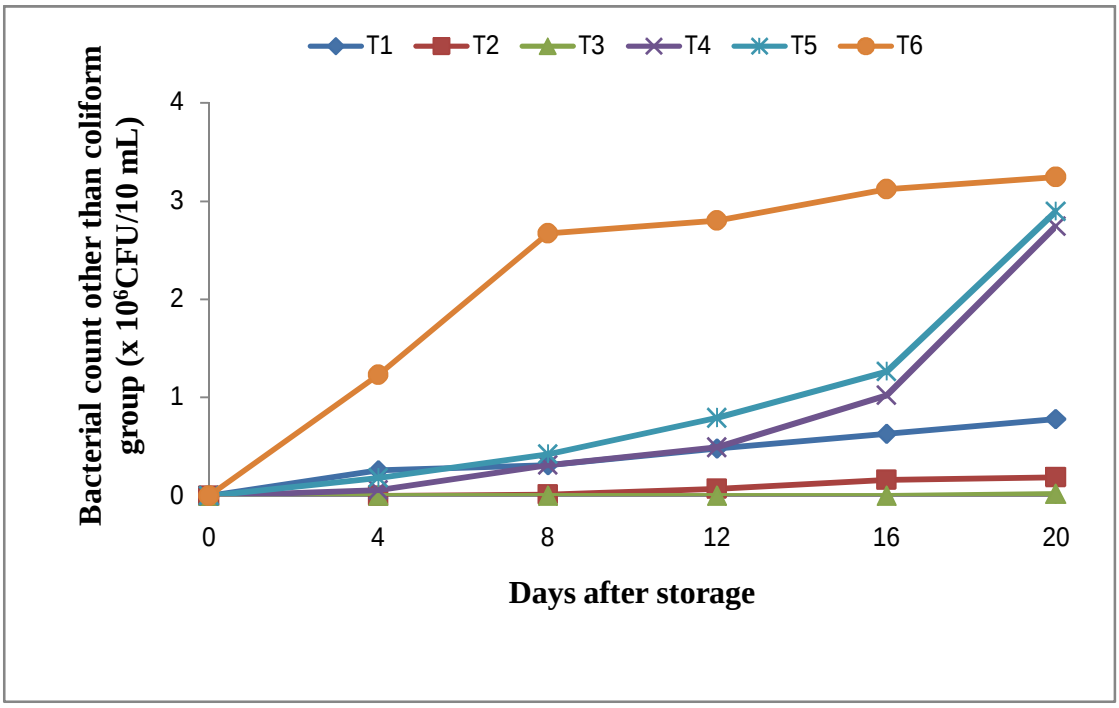

Fig. 4.15 Bacterial count other than coliform group of different treatments of tender coconut water during storage

4.6 SENSORY EVALUATION OF TENDER COCONUT WATER SAMPLES

Sensory evaluation of treatments was carried out for consumer acceptance and preference using 10 untrained panelists selected at random. Appearance, flavour, overall acceptability of the samples were rated using a 9 point Hedonic scale where 9 and 1 represent like extremely and dislike extremely respectively as shown in Appendix-D. Sensory evaluation was carried out at ambient conditions in a comfortable and quiet area without disturbance under fluorescent lighting. Water was supplied to cleanse palate between samples.

Membrane filtered, Pasteurized and untreated (control) tender coconut water samples were evaluated by panelists. The evaluated data assessed by panelists by filling a form is shown in Appendix-D. Appearance rating has been compiled (Table 4.16 and Fig. 4.16). The treatments T_1, microfiltered, T_3, pasteurized and T_2, ultrafiltered tender coconut water scored higher rating on hedonic scale than other treatments.

Table 4.16 Appearance rating of sensory evaluation of different treatments of tender coconut water during storage

Treatments	Appearance						
	Days after storage, (DAS)						
	0	4	8	12	16	20	Avg.
T_1	8.4	8.4	8.0	8.0	7.0	7.0	7.8
T_2	8.4	8.4	8	7.5	7.0	7.0	7.716
T_3	8.4	8.4	8.4	8.0	7.5	7.5	8.03
T_4	8.4	8.0	7.5	7.5	7.0	7.0	7.56
T_5	8.4	8.0	7.5	7.0	7.0	7.0	7.483
T_6	8.4	7.5	7.5	7.0	7.0	7.0	7.4

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi); T_2 – ultrafiltration (40 kDa,5.06 kg/cm^2 (80 psi); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin 2500 I.U.; T_6- control

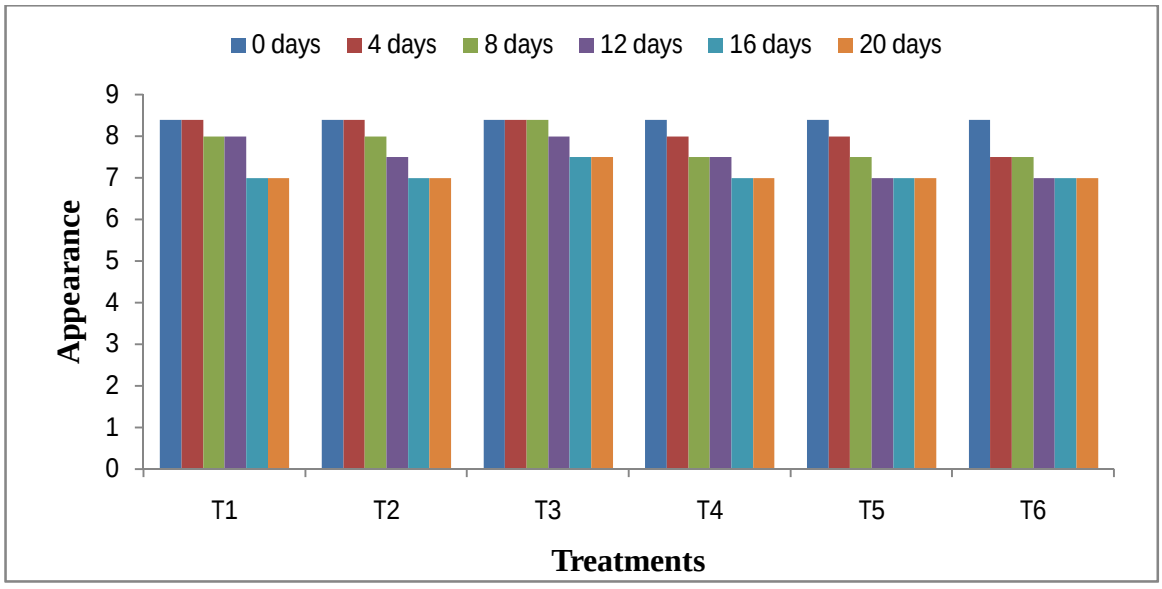

Fig. 4.16 Appearance values of different treatments of tender coconut water during storage

Sensory quality assessed by panel of experts for establishing flavour rating was also compiled (Table 4.17 and Fig. 4.17). The treatment T_3 pasteurized TCW scored highest rating on hedonic scale than other samples.

Table 4.17 Flavour rating of sensory evaluation of different treatments of tender coconut water during storage

Treatments	Flavour						
	Days after storage, (DAS)						
	0	4	8	12	16	20	Avg.
T_1	8.4	8.0	8.0	7.5	7.5	7.0	7.733
T_2	8.4	8.0	8.0	7.5	7.0	7.0	7.65
T_3	8.4	8.4	8.0	8.0	7.5	7.5	7.966
T_4	8.4	8.0	7.5	7.5	7.0	7.0	7.566
T_5	8.4	8.0	7.5	7.0	7.0	7.0	7.48
T_6	8.4	7.0	6.5	6.0	5.0	4.0	6.15

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80psi); T_2 – ultrafiltration (40 kDa,5.06 kg/cm^2 (80 psi); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin 2500 I.U.; T_6- control

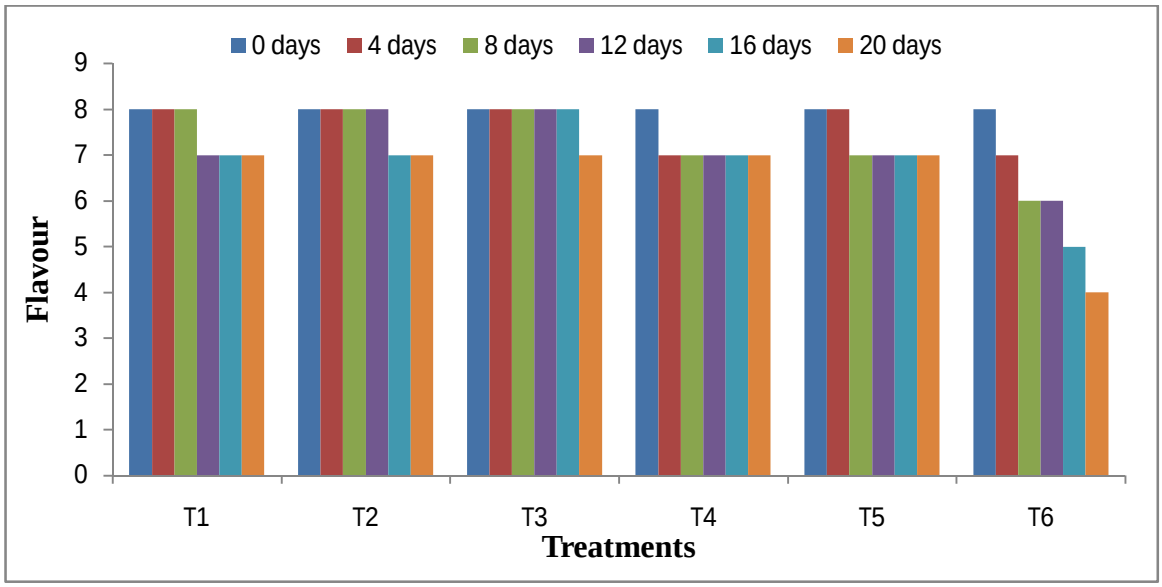

Fig. 4.17 Flavour values of different treatments of tender coconut water during storage

Similarly the sensory quality assessed by panelists for establishing overall acceptability rating has been compiled (Table 4.18 and Fig. 4.18). The treatments T_1, T_2 and T_3 scored higher rating on hedonic scale than other treatments.

Table 4.18 Overall acceptability rating of sensory evaluation of different treatments of tender coconut water during storage

Treatments	Overall acceptability						
	Days after storage, (DAS)						
	0	4	8	12	16	20	Avg.
T_1	8.4	8.0	7.0	7.0	7.0	7.0	7.4
T_2	8.4	8.0	7.5	7.0	7.0	7.0	7.48
T_3	8.4	8.0	8.0	7.5	7.0	7.0	7.65
T_4	8.4	7.5	7.0	7.0	7.0	7.0	7.31
T_5	8.4	8.0	7.5	7.0	7.0	7.0	7.48
T_6	8.4	7.5	7.0	6.0	5.0	4.0	6.31

T_1 – microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi); T_2 – ultrafiltration (40 kDa, 5.06 kg/cm^2 (80 psi); T_3 – pasteurization at 85 °C, 10 min; T_4 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin (5000 I.U.); T_5 – Microfiltration (0.2 µm, 5.06 kg/cm^2 (80 psi) + Preservation with Nisin 2500 I.U.; T_6- control

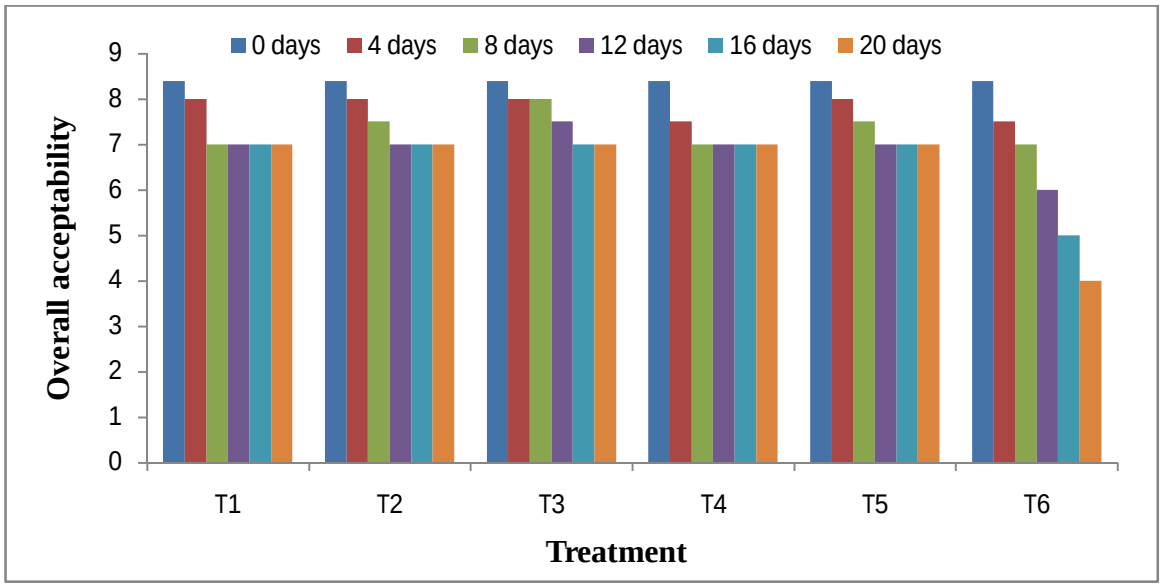

Fig. 4.18 Overall acceptability values of different treatments of tender coconut water during storage

Although there are some benefits of maintaining TSS and percentage reducing sugars in Nisin treated samples, there was no observed significant benefit of improving microbial and sensory attributes investigated in the study. Overall based on the experimental data, it can be concluded that among all the treatments T_3, T_1 and T_2 exhibited good physico-chemical, microbiological and sensory properties for the processed tender coconut water during storage upto 20 days.

Chapter-V

SUMMARY AND CONCLUSIONS

A study on preservation of tender coconut water (TCW) using microfiltration (MF), ultrafiltration (UF), pasteurization at 85 °C for 10 minutes and preservative nisin at two concentrations of 2500 and 5000 I.U. was undertaken with an aim to develop process technology and improve the shelf life of bottled tender coconut water. The samples were bottled using crown corking machine and kept in storage at 4 °C for 20 days to assess the changes in physico-chemical, microbiological and sensory qualities.

The following conclusions were drawn from the study.

1. Studies using pure water to establish permeate flux on different membrane pore sizes and molecular weight cut off (MWCO) at various transmembrane pressures (TMP's) revealed that permeate flux increased with an increase TMP and membrane pore size or MWCO. The steady state fluxes are relatively higher with MF in comparison to UF and NF at the given TMP.

2. There is a general decrease in flux with the time in both the MF and UF experiments using TCW. The permeate flux during MF of TCW decreased gradually from 189.98 L /m^2h and reached a steady flux at 88.51 L /m^2h. The permeate flux during UF of TCW also decreased gradually from 107.54 to 82.07 L /m^2h and reached a steady flux at 82.07 L /m^2h.

3. TSS content in all treatments generally decreased during storage. The TSS content in TCW was initially high for pasteurized samples than the membrane processed TCW perhaps due to removal of some cloud forming solids in membrane filtration.

4. The pH value of all the samples decreased during storage. Higher pH was recorded for ultrafiltered sample, microfiltered and pasteurized samples on 20^{th} day of storage indicating that these treatments resulted in relatively better pH stable product.

5. Percentage of reducing sugars increased during storage. Pasteurized, UF and MF samples exhibited lower increase in percentage reducing sugars in that order whereas untreated samples exhibited higher conversion into reducing sugars suggesting spoilage of untreated samples on storage.

6. Membrane filtered samples recorded less turbidity (high light transmission) than other treatments. However untreated samples were more turbid suggesting that membrane processing is useful to produce clarified product.

7. Microbial analysis indicated by *E.coli* count, fungal count and bacterial count revealed that pasteurized, UF and MF samples are relatively better. Untreated samples exhibited values higher than permissible limits.

8. Sensory evaluation indicated that the pasteurized samples scored highest rating followed by UF and MF samples on hedonic scale by panelists in terms of overall acceptability.

9. It could be concluded that pasteurized, microfiltered and ultrafiltered treatments are better in that order, the first being the best among all the treatments upto a storage period of 20 days under refrigerated conditions based on the physico-chemical, microbiological and sensory data.

Suggestions for Future Work

Based on experiments conducted in the present study, it is suggested that storage studies could be conducted on membrane filtered samples and addition of other suitable range of preservatives or in combination beyond 20 days. Further studies may be conducted to investigate flux decline during membrane filtration using TCW and elucidate fouling mechanisms. Operating conditions such as cross flow velocity, TMP and membrane parameters such as pore size/ MWCO, type of membrane material may be further studied to optimize the membrane flux and selectivity.

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Note: The literature is cited as per the "Thesis Guideline" prescribed by Acharya N.G. Ranga Agricultural University, LAM, Guntur.