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RESEARCH ARTICLE

Isolation of microorganism from the fermented coconut milk and comparison of quality parameters of virgin coconut oil recovered by induced fermentation.

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Abstract

Investigations were carried out at College of Agriculture, Vellayani, Kerala Agricultural University,Kerala, India to produce virgin coconut oil through induced fermentation method during the period 2013-2015.Coconut milk was extracted from grated coconut with coconut water in the ratio 1:1 and was kept for 24 and 36 hours for fermentation and the microorganisms were isolated and screened.*Lactobacillus* isolated at 24 hours showed significantly superior protease and amylase activity compared to other microorganisms. *Lactobacillus* was multiplied and one percent of the culture was used to induce fermentation of coconut milk. The virgin coconut oil recovered from induced fermentation was then compared with the natural fermentation method and wasfound to be significantly superior compared to natural fermentation method.Coconut milk extracted from grated coconut with water in the ratio 1:1, incubated with one percent *Lactobacillus* and kept for 36 hours showed significantly superior oil recovery compared to other treatments. Quality parameters like free fatty acid content, total phenolic content and moisture content did not differ significantly between treatments and was within the limits of APCC Standards. Thus induced fermentation can be taken as a method for the hygienic production of water clear virgin coconut oil.

Introduction:-

Virgin coconut oil is a traditional product of India and enhanced utilization world wide was witnessed in the recent years due to its multifunctional uses.Virgin coconut oil (VCO) is obtained from fresh and mature kernel (12 months old from pollination) of the coconut (*Cocosnucifera L.*) by mechanical or natural means with or without the application of heat, which does not lead to alteration of the nature of the oil(APCC, 2003).Virgin coconut oil can be extracted from the fresh and mature kernel of the coconut meat by several methods (Bawalan and Chapman, 2006). These methods can be divided into wet and dry methods. In wet method, the coconut meat does not undergo drying process while in dry method, the kernel were heated under specific conditions to remove the moisture in it while preventing scorching and microbial invasion. Wet method can be further divided into chilling and thawing, fermentation, enzymatic method or any of these in combination as the main aim is to destabilize the coconut milk emulsion (Raghavendra and Raghavarao, 2010). In dry method, the kernel was dried using controlled heating and subsequently pressed mechanically to obtain the oil. Coconut milk is the natural oil in water emulsion extracted from the endosperm of mature coconut. If properly diluted coconut milk is allowed to stand underfavourable conditions for several hours, the oil naturally separates from the water and protein that binds them together as coconut milk emulsion. The coconut milk when kept for some time result in fermentation. The natural enzymes produced by the microorganismsare responsible for fermentation of coconut milk. The technique if not hygienically practiced can cause contamination. In traditional natural fermentation, settling and subsequent of coconut milk lasted for 36-48 hours(Bawalan and Chapman, 2006).To overcome this, induced fermentation could be done using probiotic microorganisms havingproteolytic, amylolytic and lipolyticcapacities which couldhydrolyse protein,

carbohydrate and lipid components contained in the coconut kernel.Rini*et al* .(2009) observed that the strain of *Lactobacillus bulgaricus* could effectively extract more virgin coconut oil than the other microbial strains like *Saccharomyces cerevisiae*, *Candida rugosa* and *Aspergillusoryzae* when it was introduced into coconut creamunder enzymatic condition at pH 5.0, 45° C and at 5 percent starter concentration.Kumalaningsih and Masdiana(2012) reported the proteolytic and amylolytic enzyme activity of *Lactobacillus* and the potential of using the culture for the production of high quality virgin coconut oil. Satheesh and Prasad (2013) concluded that *Lactobacillus plantarum* NDRI- 184 as the best choice for induced fermentative production of virgin coconut oil.

The quality standards for virgin coconut oil has been put forward by Asian and Pacific Coconut Community Standards (APCC, 2003). The various factors which determine the quality of oil include moisture content, free fatty acids, peroxide content as well as the total plate count. Hydrolytic rancidity in coconut oil had been attributed to the presence of free fatty acids (Fernandez, 1988). A high acid value may indicate a higher tendency to become rancid (Karim, 1997). Moisture content of oil should be kept low as it will increase the shelf life by preventing oxidation and rancidity processes (Mansor*et al*., 2012).

Phenolic compounds plays an important role providing protection against pathogens and predators (Bravo, 1998).The beneficial effects derived from phenolic compounds had been attributed to their antioxidant activity (Heim *et al*., 2002).The traditional method of virgin coconut oil in India is by boiling method. The coconut milk extracted is heated to remove the moisture and the solid particles can be separated by sieving.The temperature involved in traditional method is oftenso high that it will decrease the antioxidant activity of the virgin coconut oil (Marina *et al*, 2009).More over the peroxide value of cold extracted virgin coconut oil was lower than the hot extracted virgin coconut oil and was observed as 4.95 and 5.65 meq O_2/kg oil, respectively which increased as a function of storage time in all samples up to 12 months of storage (Srivastava*et al*., 2013).

Hence a study was conducted to isolate microorganisms responsible for inducing fermentation and to compare the physico chemical properties of the virgin coconut oil produced by induced fermentation with the standards specified by APCC.

Materials and methods:-

Isolation of microorganisms:-

Mature nuts of coconut cultivar, West Coast Tall (WCT) of 12 month were harvested from Trivandrum district of Kerala, India stored for two months and were dehusked and shelled. The solid endosperm was grated and ground in the grinder and coconut milk was extracted from the ground mass with water in the ratio 1:1. The ground mass was then transferred to cheese cloth and pressed manually for coconut milk extraction. The coconut milk extracted was kept for 24 and 36 hours for fermentation and microorganisms were isolated by serial dilution and spread plate method.Isolated microorganisms were tested for clear zone formation and quantitatively for amylase (Sadasivam and Manikam,1996) and protease (Enyard, 2008) activity. The microorganism having the highest protease and amylase activity was selected and kept in slants. Mother culture of the selected microorganism was multiplied in broth. To study the multiplication efficiency of the best isolate, it was inoculated in sterilized coconut milk.

Colony count:-

The coconut milk extracted with grated coconut and water in the ratio 1:1 was sterilized at 70°C for 30 minutes. 1ml of mother culture of the best isolate was inoculated into the sterilized coconut milk and colony count was taken.

Induction of fermentation:-

One ml of mother culture of the best isolate was inoculated into sterilised100ml of coconut milk extracted from grated coconut with coconut water in the ratio 1:1, water in the ratio 1:1 and 1:2 and kept for varying hours viz., 18, 24 and 36 hours and the oil recovery of the induced fermentation was compared with the fermentationmethod and the quality parameters were analysed.

Quality parameters:-

The oil recovery was calculated according to the initial weight of coconut milk to the oil extracted after24, 36 and 48 hours of fermentation and dried at 50 ^{o} C (AOAC, 1997).Moisture percentage was determined by hot air-oven method(AOAC, 1997) with slight alteration. Samples were heated at 50^{o}C in a heated and weighed crucible and cooled to a room temperature and re-weighed with the samples inside until constant readings were obtained.Free fatty acid value, peroxide value, iodine value and saponification valuewere determined following the methods of

Sadasivam andManikam (1992).Total phenolic content of the oil samples was determined according to the procedure of Sadasivam and Manikam(1992) with some modifications by (Rammaet al., 2002) using catechin as standard. Refractive index (Ariponnammal,2012),relative density (ISO/FDS 6883,2000), specific gravity (AOAC, 2000) and unsaponifiable matter (AOAC,2000) were also found out.Polenske value of the oil was determined as per ISI (1984). Statistical analysis was carried out using analysis of variance technique (Gomez and Gomez, 1984).

Results and discussion:-

Isolation and screening of microorganisms:-

Themicroorganisms were isolated from the fermented coconut milk extracted from coconut gratings and water in the ratio 1:1 and kept at 24 and 36 hours. There was a significant difference in the population of isolated microorganisms from coconut milk extracted with coconut gratings and water in the ratio 1: 1 and kept for fermentation at 24 and 36 hours after fermentation. The maximum bacterial population of 44.60×10^6per mlwas observed at 24 hours after fermentation compared to 36 hours. However there was no significant difference in colony forming units of *Lactobacillus* and yeast isolated.Soeka*et al*. (2008) reported that the fermentation of coconut cream occurred when the enzymatic starter had been employed for processing. Crude coconut oil was formed due to a phenomenon of protein digestion that played a role to stabilize emulsion of the coconut cream into a soluble material. The enzymatic starter with high capacity of amylolytic and proteolytic activity could hydrolyse carbohydrate and protein which contained in the coconut cream as its substrate into soluble sugar and amino acid and peptide.

Protease and amylase activity:-

Protease and amylase activity of microorganisms isolatedwas determined. *Lactobacillus* isolated at 24 hours showed significantly superior protease (184.200µg tyrosine released in 2 hour) and amylase (26715.000µg of maltose produced during 5 minutes) activity compared to other treatments. The lower protease and amylase activity was observed for yeast isolated at 36 hours.According to Kumalaningsih and Masdiana (2012) lactic acid bacteria have highest proteolytic and amylolytic enzyme activity which indicated that lactic acid bacteria has the potential to be used as pure culture for the production of virgin coconut oil of high quality oil.*Lactobacillus* isolated at 24 hours was used as the mother culture. According to Satheesh and Prasad (2012) the inoculum concentration of 1, 2 and 5 percent of *Lactobacillus plantarum*onyield of virgin coconut oil revealed 5 percent as more efficient with an efficiency of 82.91 percent compared to one percent inoculum concentration which showed an efficiency of only 65.67 percent.

Count of Lactobacillus:-

The *Lactobacillus*count increased with time and maximum population of 94×10^4cfu per ml was noticed after 36 hours. A higher population at 36 hours showed that the activity was maximum at that period compared to 18 and 24 hours and hence the maximum recovery of oil at that period can be attributed to its higher population at that period.

Quality parameters:-

The effect of induced fermentation and fermentation on oil recovery was analysed. Oil recovery by induced fermentation and fermentation method showed significant difference. The virgin coconut oil recovered from induced fermentation method was significantly superior (17.733 percent) compared to fermentation method (17.3410 percent). Among treatments, the treatmentcoconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours (21.780 percent) showed significantly superior oil recovery compared to other treatments.

Free fatty acid content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and inoculated with 1 per cent of *Lactobacillus*culture and kept for fermentation for 18, 24 and 36 hours and dried at 50°C did not show any significant difference between treatments.High free fatty acid in the oil is an indication of change caused by oxidation resulting in the rancidity of the oil. The fatty acids were in the range of 0.200 to 0.233mg KOH/ g of oil in the treatments. Thus the results were of indication that extraction of virgin coconut oil by any of the methods followed would not alter the free fatty acid content of the virgin coconut oil. Free fatty acid valueof virgin coconut oil recovered by adding microbial inoculum (*L. bulgaricus*) as enzymatic starter was recorded as 0.22 percent (Rini*et al*., 2009).

The total phenolic content of virgin coconut oil recovered from coconut milk extracted by different methods and inoculated with one per cent *Lactobacillus* culture did not differ significantly.. This might be because of low temperature (50º C)which was used for drying.

The moisture content of virgin coconut oil did not differ significantly among treatments. Moisture contentof virgin coconut oil recovered by adding microbial inoculum (*L. bulgaricus*) as enzymatic starter was recorded as 0.30 percent (Rini*et al*., 2009).

Thequality parameters like moisture content, specific gravity, relative density, acid value, peroxide value, iodine value, polenske value,unsaponifiable matter, colour and odourof virgin coconut oil produced by induced fermentation method wasfound out to be within the within the range of APCC standards(2003).

Plate 1.Plate showing growth of yeast and *Lactobacillus*

a) Yeast **b) *Lactobacillus***

Plate 2. Microscopic view of stained *Lactobacillus*and yeast separated from fermented coconut milk

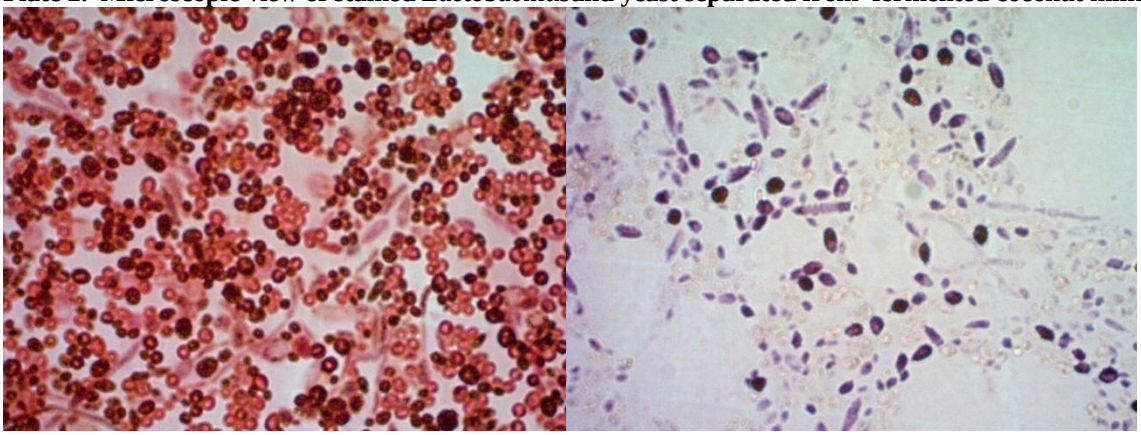

Fig.1. Protease activity of microorganisms isolated

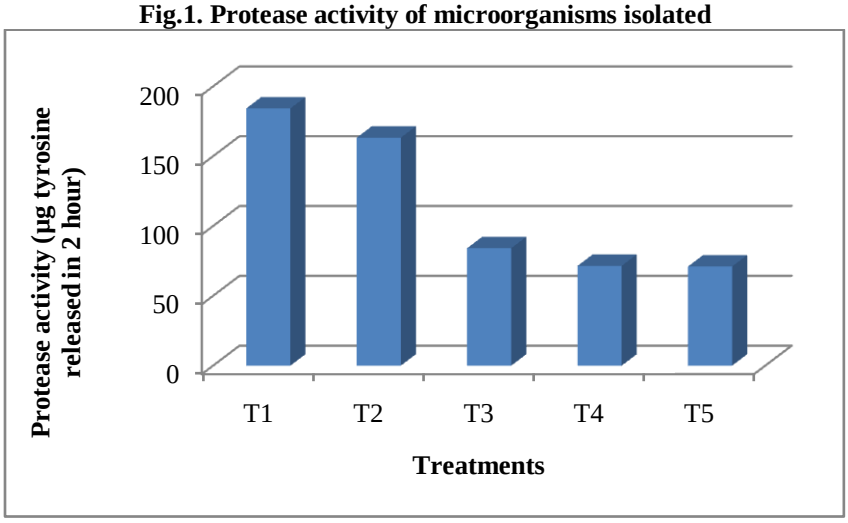

Fig.2. Amylase activity of microorganisms isolated

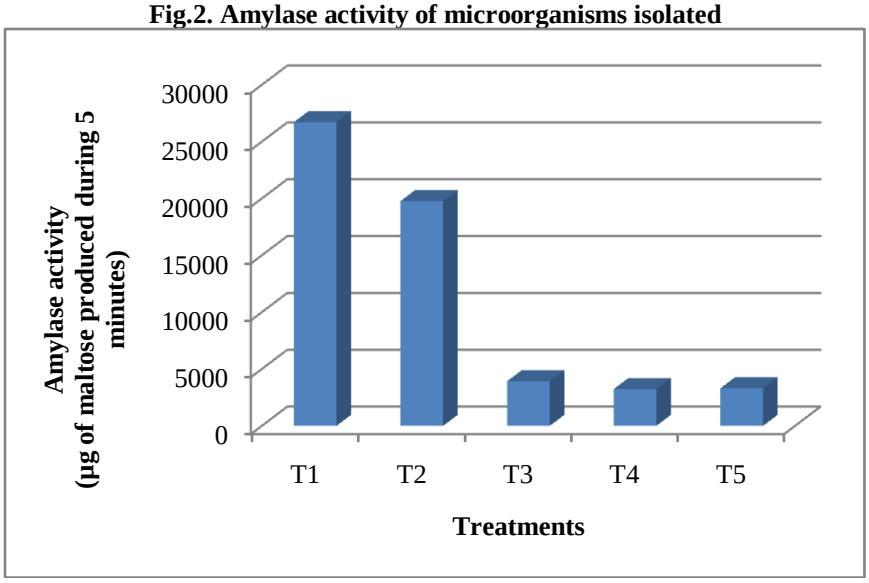

Fig. 3.Count of *Lactobacillus* in coconut milk at different hours of fermentation under sterile conditions

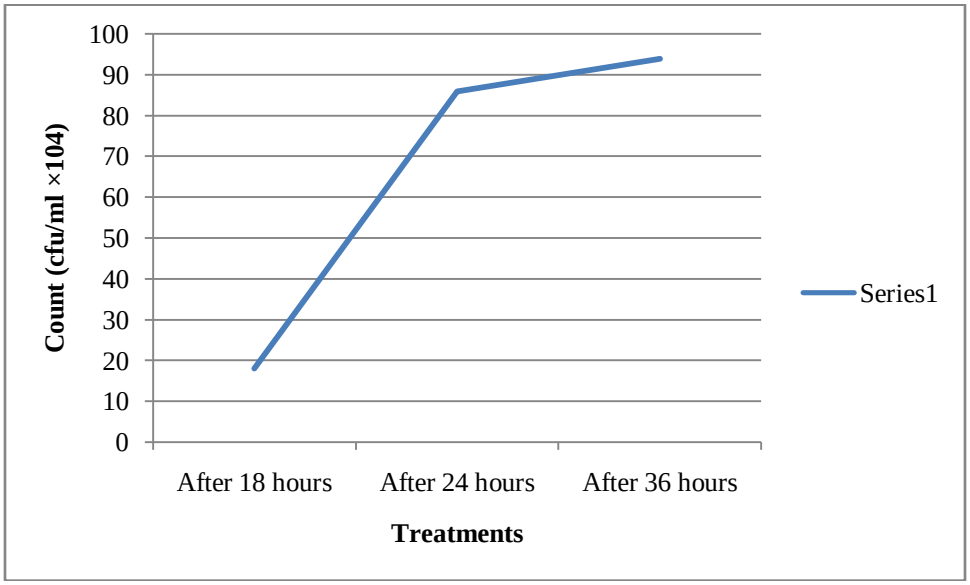

Table: 1. Microorganisms isolated from coconut milk fermented at 24 and 36 hours

Time	Bacteria $\times 10^6$cfuml^{-1}	*Lactobacillus* $\times 10^3$cfuml^{-1}	Yeast $\times 10^3$cfuml^{-1}
24h	44.60	33.12	5.41
36h	38.10	37.00	5.96
SE	2.25	2.24	0.48
CD(0.05)	4.74	NS	NS

Table: 2. Effect of induced fermentation and fermentation on oil recovery (%)

Treatments	Oil recovery (percent)		
	Induced fermentation (Group1)	Fermentation (Group 2, Control)	Mean
T_1 (CG +CW 1:1+ 18h)	16.000	15.567	15.780
T_2 (CG +CW 1:1+ 24h)	18.833	18.467	18.650
T_3 (CG+ CW 1:1+ 36h)	21.300	21.067	21.180
T_4 (CG +W 1:1 + 18h)	16.567	15.733	16.150
T_5 (CG + W 1:1 + 24h)	19.167	18.933	19.050
T_6 (CG+W 1:1 + 36h)	21.967	21.600	21.780
T_7 (CG+W 1:2 + 18h)	13.633	12.933	13.280
T_8 (CG +W 1:2 + 24h)	15.200	15.400	15.300
T_9 (CG +W 1:2 + 36h)	16.933	16.367	16.650
Mean	17.733	17.341	-
CD (Groups)	0.322		
CD (Treatments)			0.152
CD (Induced fermentation Vs fermentation)	0.456		

Table: 3. Effect of induced fermentation on quality parameters of virgin coconut oil

Treatments	Free fatty acid (mg KOH/g of oil)	Total phenolic content (mg catechin equivalent /kg of oil)	Moisture content (%)
T_1 (CG +CW1:1 + 18h)	0.220	57.666	0.066
T_2 (CG +CW1:1 + 24h)	0.226	66.000	0.080
T_3 (CG+ CW1:1 + 36h)	0.220	60.000	0.080
T_4 (CG +W 1:1 + 18h)	0.200	65.000	0.073
T_5 (CG + W 1:1 + 24h)	0.226	48.333	0.053
T_6 (CG+W 1:1 + 36h)	0.200	62.333	0.073
T_7 (CG+W 1:2 + 18h)	0.220	63.666	0.073
T_8 (CG +W 1:2 + 24h)	0.233	71.000	0.073
T_9 (CG +W 1:2 + 36h)	0.226	67.333	0.073
SE	0.045	7.816	0.025
CD(0.05)	NS	NS	NS

Table 4.Comparison of essential composition of virgin coconut oil produced by induced fermentation with APCC standards

Parameter	Desirable Amount (APCC Standard, 2003)	VCO by induced fermentation method
Moisture (percent)	Max 0.1	0.073
Free Fatty Acid (percent)	Max 0.2	0.020
Peroxide Value meq/kg	Max 3	0.220
Relative density	0.915 – 0.920	0.917
Refractive index at 40^0 C	1.4480 – 1.4492	1.468
Saponification Value	250 – 260 milligram	262.428
Iodine Value	4.1 -11	5.380
Unsaponifiable matter (percent) by mass, max	0.2 - 0.5	0.398
Specific gravity at 30 deg./30 deg. C	0.915 – 0.920	0.917
Polenske Value, min	13	13
Total Plate Count	< 0.5	0
Colour	Water clean	Water clear
Odour and Taste	Natural fresh coconut scent,free of sediment, free from rancid odour and taste	Natural fresh coconut scent, free of sediment, free from rancid odour and taste
Food Additives	None permitted	Not added

Conclusion:-

In the present study the isolation and screening of microorganisms involved in production of virgin coconut oil from fermented coconut milk was carried out. The *Lactobacillus* culture showed maximum proteolytic and amylolytic activity and hence one percent of the *Lactobacillu*s culture was used for inducing fermentation. The oil recovery was significantly superior in the induced fermentation method compared to natural fermentation method and the moisture content, free fatty acid content, peroxide value, iodine value, polenske value, specific gravity,unsaponifiable matter , colour and odour were within the range specified by the APCC standards and hence can be induced fermentation can be recommended as a suitable method for the virgin coconut oil production.

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Literature cited:-

1. AOAC (Association of Official Agricultural Chemists). 1997. Official Method of Analysis.(16thEd.). AOAC International Washington DC.
2. AOAC [Association of Official Agricultural Chemists]. 2000. Official Method of Analysis. (17thEd.). AOAC International, Washington DC, 2200p.
3. APCC [Asian and Pacific Coconut Community]. 2003. *APCC Standards for virgin coconut oil* [on-line]. Available: http://www.apccsec.org/standards.htm.
4. Ariponnammal, S.A. 2012. Novel method of using refractive index as a tool for finding the adultration of oils.*Res. J. Recent Sci.* 1(7): 77-79.
5. Bawalan, D.D. and Chapman, K.R. 2006.*Virgin Coconut Oil- Production Manual for Micro- and Village-Scale Processing*. Food and Agricultural Organisation, United Nations, 80p.
6. Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Res.* 56: 317-333.
7. Enyard, C. 2008. Sigmas non- specific protease activity assay- casein as a substrate.*J. Visualised Exp*. 19: p.899.
8. Fernandez, W.L. 1988. Microbial examination of mature coconut fruit.*Philipp. Agric.* 71(1):13-20.
9. Gomez, K.A. and Gomez, K.A. 1984. *Statistical Proceedings for Agricultural Research*(2nd Ed.). John Willey and Sons Inc., Singapore, 262p.
10. Heim, K.E., Tagliaferro, A.R. and Bobilya, D.J. 2002. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. *J. Nutr. Biochem.*13: 572-584.
11. ISO/FDIS 6883. 2000. Animal and vegetable fats and oils - determination of conventional mass per volume (litre weight in air). In: International Standard: Agricultural food products, animal and vegetable fats and oils. Geneva: International Organization of Standard.
12. Karim, M.R. 1997. Purification and characterization of proteins from mulberry silkworm *Bombyxmori* L. and biochemical analysis of two varieties of pupae bivoltein and multivoltein, M.Sc. thesis, Dept. of Biochemistry University of Rajshahi, 98p.
13. Kumalaningsih and Masdiana, P. 2012. The utilization of microorganisms isolated from fermented coconut milk for the production of virgin coconut oil. *J. Basic. Appl. Sci. Res.* 2(3): 2286-2290.
14. Mansor, T.S.T., CheMan, Y.B., Shuhaimi, M., Afiq,M.J. and Ku Nurul, F.K.M. 2012.Physicochemical properties of virgin coconut oil extracted from different processing methods. *Int. Food Res. J.19 (3): 837-845.*
15. Marina, A.M., CheMan, Y.B., Nazimah, S.A.H.and Amin, I. 2009. Chemical properties of virgin coconut oil.*J. Am. Oil Chem. Soc*. 86: 301-307.
16. Raghavendra, S.N.andRaghavarao, K.S.M.S. 2010.Effect of different treatments for the destabilization of coconut milk emulsion.*J. Food Eng.* 97: 341-347.
17. Ramma, L.A., Bahorun, T., Soobrattee, M.A. and Aruoma, O.I. 2002.Antioxidant activities of phenolic, proanthocyanidin and flavonoid components in extracts of *Cassis fistula.J. Agric. Food Chem*. 50(18): 5042-5047.
18. Rini, H., Sulistyo,J.andRahayu, D.R. 2009. Extraction of coconut oil (*Cocosnucifera* L).*Biodiversitas.*10(3): 151-157.
19. Sadasivam, S. and Manickam, A. 1992.*Biochemical Methods for Agricultural Science.*Wiley Eastern Ltd., New Delhi, 246p.
20. Sadasivam, S. and Manickam, A. 1996.*Vitamins, In: Biochemical methods.* (2nd Ed.). New Age International (P) Ltd., New Delhi, pp. 185-186.
21. Satheesh, N.and Prasad, N.B.L. 2012.Induced fermentative production of virgin coconut oil.*Asian. J. Food AgroInd.* 5(5): 355-363.
22. Satheesh, N.andPrasad, N.B.L.2013.Optimization of parameters for fermentative production of virgin coconut oil by*Lactobacillus* sp. *Ann. Food Sci. and Technol.* 14 (2): 312-317.
23. Soeka, Y.S., Sulistyo, J. E. and Naiola. 2008. Analisisbiokimiaminyakkelapahasilekstraksisecarafermentasi. *Biodiversitas.*9(2): 91-95.
24. Srivastava, Y., Semwal, A.D. and Sharma, G.K. 2013.Studies on storage stability of hot extracted (HEVCO) and cold extracted virgin coconut oil (CEVCO) in different flexible and rigid packaging system. *Int. Food Res. J*. 20(4): 1971-1976.