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The Effect of *Hylocereus polyrhizus* and *Hylocereus undatus* on Physicochemical, Proteolysis, and Antioxidant Activity in Yogurt

Zainoldin, K.H., Baba, A.S.

***Abstract*—**Yogurt is a coagulated milk product obtained from the lactic acid fermentation by the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The additions of fruits into milk may enhance the taste and the therapeutical values of milk products. However fruits also may change the fermentation behaviour. In this present study, the changes in physicochemical, the peptide concentration, total phenolics content and the antioxidant potential of yogurt upon the addition of *Hylocereus polyrhizus* and *Hylocereus undatus* (white and red dragon fruit) were investigated. Fruits enriched yogurt (10%, 20%, 30% w/w) were prepared and the pH, TTA, syneresis measurement, peptide concentration, total phenolics content and DPPH antioxidant inhibition percentage were determined. Milk fermentation rate was enhanced in red dragon fruit yogurt for all doses (-0.3606 - -0.4126 pH/h) while only white dragon fruit yogurt with 20% and 30% (w/w) composition showed increment in fermentation rate (-0.3471 - -0.3609 pH/h) compared to plain yogurt (-0.3369pH/h). All dragon fruit enriched yogurts generally showed lower pH readings (pH 3.95 - 4.03) compared to plain yogurt (pH 4.05). Both fruit yogurts showed a higher lactic acid percentage (1.14-1.23%) compared to plain yogurt (1.08%). Significantly higher syneresis percentage (57.19 - 70.32%) compared to plain yogurt (52.93%) were seen in all fruit enriched yogurts. The antioxidant activity of plain yogurt (19.16%) was enhanced by the presence of white and red dragon fruit (24.97-45.74%). All fruit enriched yogurt showed an increment in total phenolic content (36.44 - 64.43mg/ml) compared to plain yogurt (20.25mg/ml). However, the addition of white and red dragon fruit did not enhance the proteolysis of milk during fermentation. Therefore, it could be concluded that the addition of white and red dragon fruit into yogurt enhanced the milk fermentation rate, lactic acid content, syneresis percentage, antioxidant activity, and total phenolics content in yogurt.

***Keywords*—**Antioxidant activity, Hylocereus polyrhizus, Hylocereus undatus, yogurt

I. INTRODUCTION

MALAYSIA is not a traditional milk producing country and most of its population is not traditional milk consumer. However in the last two decades the consumption of milk and milk products in Malaysia has increased by 3 folds, largely as a direct result of changes in lifestyle and surplus income in the medium-high income population.

The local production of milk, both by cows and goat has steadily increased during this period. This is as a result of encouraging government policy in reducing dependency on the importation of meat and milk. Almost 75% of Malaysian population (Malay and Chinese) are lactose intolerant, thus making the more digestible processed milk (fermented milk) in the form of yogurt would be more suitable for local consumption. In addition, the longer shelf life and value-added nature of these products would be of advantage in the event of temporary overproduction of milk.

Yogurt is a coagulated milk product obtained from the lactic acid fermentation by the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. High levels of live bacterial cultures in yogurt contribute to the nutritional and therapeutic properties. In Malaysia yogurt is called "dadih" and it is consumed occasionally as dessert, in a variety flavoured such as corn, chocolate, strawberry and also plain yogurt.

Proteolysis is the breakdown of large and complex proteins into the smaller and simple peptides. Various functional and bioactive peptides and amino acids will be released due to the proteolytic activity of enzyme proteinase and peptidases especially from lactic acid bacteria. Proteinase activity can be expressed in terms of the amount of peptides produced. Different level of proteolysis will be determined by the enzymatic activities in different temperature and pH condition. The physiological and biotechnological significance of these peptides in dairy products are considered.

Proteolysis in yogurt has been linked to its importance for texture, taste, and flavour development during fermentation and storage period. Changes of the yogurt texture occurred due to breakdown of the protein network.

It also contributed directly to taste and flavour bye the formation of peptides and free amino acids as well as by liberation of such substrates for further catabolic changes and thereby formation of volatile flavour compounds. Peptides can taste bitter or delicious and amino acids can taste sweet, bitter or broth-like.

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including

Zainoldin, K.H. is with Institute of Biological Sciences, Faculty of Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia (phone: +60379675969; fax: +60379674178; e-mail: hazmi@ um.edu.my).
Baba, A.S.is with Institute of Biological Sciences, Faculty of Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia (phone: +60379675969; fax: +60379674178; e-mail: ahmad@ um.edu.my).

cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables.

Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease.

The dragon fruit (also known as pitaya) is the fruit of several cactus species, especially of the genus *Hylocereus*, but also see *Stenocereus*.. Native to Mexico and Centraland South America, these vine-like epiphytic cacti are also cultivated in Southeast Asian countries such as Vietnam and Malaysia.

Usually, the Pitaya is grown in the tropical lowlands. It is a tropical fruit. It can be grown with organic fertilizer, and without any pesticide and chemical. Therefore, the Pitaya gain its reputation on the market as a healthy fruit.

There are three species of dragon fruit in the genus Hylocereus and one species in the genus Selenicereus. Varieties of *Hylocereus guatemalensis, Hylocereus polyrhizus*, and *Hylocereus undatus* as well as hybrids of these three species are grown commercially worldwide. *Hylocereus undatus* has white flesh with pink skin, *Hylocereus polyrhizus* has red flesh with pink skin while *Selenicereus megalanthus* has white flesh with yellow skin. The fruit can weigh from 150-600 grams and the flesh, which is eaten raw, is mildly sweet and low in calories.

The pitaya fruit is rich in vitamins, it helps the digestive process due to its fiber, prevent colon cancer and diabetes, neutralize toxic substances such as heavy metal, and helps to reduce cholesterol levels and high blood pressure. The red-fleshed varieties contain lycopene, which is a natural antioxidant known to fight cancer, heart disease, and lower blood pressure.

Regularly consuming the pitaya fruit can help against asthma and cough. Dragon fruit is rich in fiber, Vitamin C and minerals. The typical nutritional value per 100g of dragonfruit is as follows: 0.68g ashes, 0.61g fat, 0.9g fiber, 36.1mg phosphorus, 0.012g carotene, 0.229g protein, 83.0g water, 8.8mg calcium, 0.65mg iron, 0.045mg riboflavin, 0.430mg niacin and 9.0mg acid ascorbic.

Dragonfruit is also rich in phytoalbumins which are highly valued for their antioxidant properties. Antioxidants prevent the formation of cancer-causing free radicals. In Taiwan, diabetics use the fruit as a food substitute for rice and as a source of dietary fiber.

The additions of fruits to milk do not only enhance the taste of the yogurt itself, but also the therapeutical values of milk products, and inadvertantly may change the fermentation behaviour. In the present studies, the changes in physicochemical, proteolysis, phenolic content organoleptic properties and antioxidant activity of yogurt upon the addition of *Hylocereus Polyrhizus* and *Hylocereus Undatus* were investigated. The effects of fruit-probiotics interaction on the enumeration of lactic acid bacteria, syneresis, titratable acidity, pH changes, phenolics content, antioxidant activity and peptide concentration were also measured. These aspects are important in evaluating the overall benefits of the addition of fruits into yogurt.

II. Materials and Methods

A. Preparation of starter culture

Pasteurized full cream milk was heated to 41^{o}C. A mixture of probiotic by Chris-Hansen Denmark (which contains *acidophilus, bifidus, casei,* and *thermohilus*) and Biolife Advanced Multi Blend Probiotix (which contains *L. Acidophilus, L. Bulgaricus, L. Casei, L. Rhamnosus, B. Bifidum, B. Infantis, and B. longum*) was mixed thoroughly with the preheated milk followed by incubation for 24 hours at 41^{o}C. The yogurt formed was stored at 4^{o}C and used as starter culture within 14 days.

B. Preparation of fruit-yogurt

Yogurt was made by mixing 10ml of starter culture with 90ml of preheated milk. Dragon fruits-yogurt (red and white) with varying composition (10%, 20%, 30% w/v) was made by adding 10g, 20g, or 30g of gently mashed fruit into 80, 70, 60ml respectively of preheated milk. Total milk solid content for the yogurt was corrected by adding 2g of milk powder for every 10ml mashed fruit used. After 7 hours incubation at 41^{o}C, the yogurt formed was stored at 4^{o}C.

C. Sample (yogurt water extract) preparations

Yogurt sample (10g) was mixed with 2.5ml distilled water and the yogurt pH was adjusted to 4.0 using 1M HCl. The yogurt was then incubated at 45 ^{o}C for 10 minutes followed by centrifugation (10000rpm, 20 minutes, 4^{o}C). The supernatant was harvested and the pH was adjusted to 7.0 using NaOH. The neutralized supernatant was recentrifuged (10000rpm, 20 minutes, 4^{o}C) and the supernatant was used in analysis.

D. pH measurement

The pH of yogurt was measured by mixing 1 ml of yogurt in 3 ml of distilled water. The pH reading was read using Metler Toledo 320 pH meter. This procedure was performed in triplicate.

E. Total tritatable acidity

Yogurt sample (1 ml) was mixed thoroughly with 9 ml of distilled water Phenolphthalein solution (0.1%, 3 drops) was added and the yogurt suspension was titrated using 0.1 M NaoH. The mixture was stirred continuously and titrated was continued until the indicator changed to a definite pink colour lasting for 30 seconds. The volume of NaOH required to neutralise the yogurt acid was recorded and used to calculate the content of titratable acids (lactic acid percentage equivalent) using the following formula:

$$LA\% = \frac{10 \times V_{NaOH} \times 0.009 \times 0.1}{W} \times 100\%$$

Where
10 = Dilution factor
W = weight of sample for titration
V_{NaOH} = Volume of NaOH used to neutralize the lactic acid
0.1 = Normality of NaOH

F. Syneresis measurement

Yogurt syneresis (the released of whey) was determined by the centrifugation method (Keogh and O' Kennedy, 1998) with some modifications. Yogurt (20g) was centrifuged (640g, 20min, 4°C) and the clear supernatant was harvested and weighed. Syneresis was calculated according to the following equation (Keogh and O' Kennedy, 1998) [2]:

$$Syneresis = \frac{\text{weight of supernatant (g)}}{\text{Weight of yogurt sample (g)}} \times 100\%$$

G. Total phenolic content

The total phenolic content was determined by an assay modified from shetty, et al., (1995) [1]. Homogenized yogurt water extract (1.0ml) was transferred into a test tube and mixed with 1ml of 95% ethanol and 5ml of distilled water. To each sample, 0.5ml of 50% (v/v) Folin-Ciocalteu reagent was added and mixed. After 5 minutes, 1ml of 5% Na_2CO_3 was added and the reaction mixture was allowed to stand for 60 minutes. The absorbance was read at 725 nm and the values were converted to total phenolics, expressed in micrograms equivalents of gallic acid per gram (GAE/g) sample. Gallic acid was used as standard.

H. Antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay

Antioxidant activity of yogurt samples by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition was determined by an assay modified from Shetty, et al., (1995) [1]. Homogenized yogurt water extract (250µl) was added into 3ml of 60 µM DPPH in ethanol. The decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The readings were compared with the controls which contained distilled water (250 µl) instead of yogurt water extract. The inhibition percentage was calculated as follows:

$$\% \text{ inhibition} = \frac{(A_{control} - A_{extract})}{A_{control}} \times 100\%$$

İ. OPA spectrophotometric assay

The OPA reagent was prepared as described by Goodno et al., (1998). The OPA solution was made by combining the following reagents: 50 ml of 100mM sodium tetraborate, 5 ml of 20% (w/w) SDS, and 80 mg of OPA (dissolved in 1 ml of methanol) 200 µl of β-mercaptoethanol. The volume was made up to 100 ml using distilled water. This reagent was prepared fresh prior to assay, was kept in dark bottle, and used within 2 hours of preparation. The solution was mixed briefly by inversion and incubated at room temperature for 2 minutes. Absorbance was determined at 340 nm and the peptide concentration was estimated against tryptone standard curve.

J. Sensory evaluation

Sensory evaluation was assessed by a panel of untrained individuals in the laboratory. All criteria evaluated were given score of 1-10 points. Criteria included sweetness, sourness, overall taste, visual appearance, body texture, and aroma. Descriptions about the criteria evaluated were given prior to evaluation [5].

K. Statistic and analysis

The results were statistically analysed by ANOVA using Minitab 14 software.

III. Results and Discussions

A. pH and Total titratable acidity

TABLE I
PH, FERMENTATION RATE, AND LACTIC ACID PERCENTAGE

Yogurt	pH	Fermentation Rate	Lactic Acid Percentage
P	4.05± 0.03	-0.3369	1.08± 0.01
W1	4.03± 0.04	-0.3332	1.18± 0.01
W2	4.01± 0.03	-0.3471	1.19± 0.02
W3	3.98± 0.02	-0.3609	1.23± 0.01
R1	4.01± 0.03	-0.3606	1.14± 0.01
R2	3.99± 0.03	-0.3861	1.15± 0.03
R3	3.95± 0.02	-0.4126	1.21± 0.01

For ph measurement during fermentation, it showed that both red and white dragon fruit helps to enhance the milk fermentation rate. After 7.5 hours fermentation, all dragon fruit enriched yogurt showed a lower pH reading compared to plain yogurt and milk fermentation rate in red dragon fruit yogurt for all doses (-0.3606 - -0.4126 pH/h) were increased compared to plain yogurt (-0.3369pH/h) while only white dragon fruit yogurt with 20% and 30% (w/w) composition showed increment in fermentation rate (-0.3471 - -0.3609 pH/h).

In terms of lactic acid percentage (TTA), it showed that all fruit enriched yogurt has significantly different acid lactic percentage compared to plain yogurt. It also suggested that addition of dragon fruit into yogurt may change the acid lactic percentage in yogurt and the percentage of acid lactic between red dragon fruit enriched yogurt and white dragon fruit enriched yogurt is significantly different (P=0.002). After 7.5 hours fermentation, all yogurts with white and red dragon fruit showed a higher lactic acid percentage compared to plain yogurt.

pH measures free H^+ ion whereas the total titratable acidity measure total organic acid that present in yogurt. Both measurements are important because acidification is the key mechanism during yogurt fermentation [3]. The declining of pH during fermentation was due to the protocooperative action of two strain of bacteria *S.thermophilus* and *L.bulgaricus* [3].

The presence milk sugar (carbon source) and milk protein (nitrogen source) in the rich medium of milk and optimum incubation environment (pH 7 and 41^{o}C) encourage the bacterial strain (*S.thermophilus*) to grow rapidly [11]. They transform lactose acid into lactic acid, acetaldehyde, diacetyl, and formic acid. The accumulation of all these fermentation products corresponds to the increasing of acid production during fermentation. The liberation of lactic acids reflects the high metabolic activity of the lactic acid bacteria.

B. *Syneresis measurement*

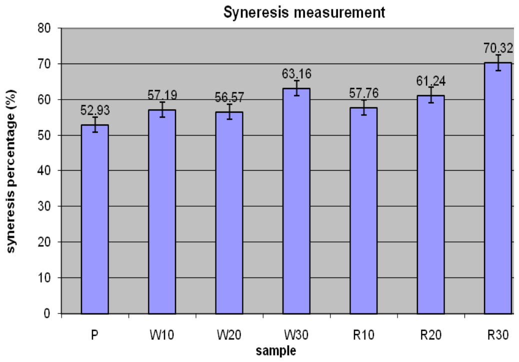

Fig. 1 Syneresis measurement in yogurt

All yogurts with white and red dragon fruit showed a higher syneresis percentage compared to plain yogurt. Yogurt with 30 % (w/w) red dragon fruit showed the highest syneresis (70.32%). This increasing in syneresis is probably due to decreasing in water holding capacity that leds to more releases of whey (Al-Kadamany, Khattar, Haddad, and Toufeili , 2003) [13].

The introduction of red and white dragon fruits did not increase the fiber contents in yogurt, which otherwise would hold the water and thus increase the syneresis. The watery structure of the fruits themselves may lead to more releases of whey in the dragon fruit-yogurt. The higher syneresis shown in dragon fruit-yogurt was most probably caused by higher active water content contributed by the added fruits.

C. *Total phenolic content assay*

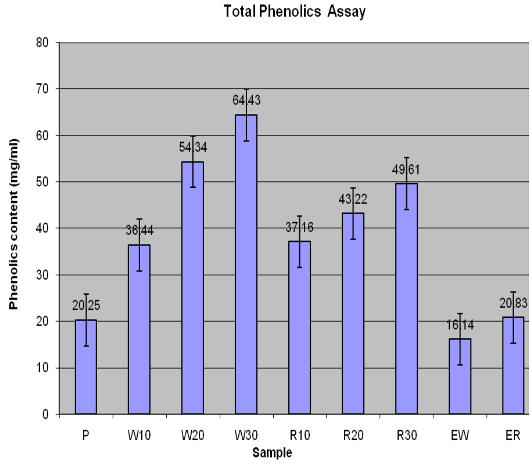

Fig. 2 Total phenolic content in yogurt

All fruit enriched yogurt showed an increment in total phenolic content compared to plain yogurt. White dragon fruit enriched yogurts showed higher increment in total phenolic content than red dragon fruit enriched yogurts. It also showed that there is a significantly different in phenolics content between fruit enriched yogurt and plain yogurt and also suggested that the addition of dragon fruit may change the phenolics content in yogurt.

Besides, there are also significant different in phenolics content between white dragon fruit yogurt and red dragon fruit yogurt (P=0.014) which showed that white dragon fruit yogurt give higher increment in phenolics content compared to red dragon fruit yogurt.

The addition of dragon fruit increased ($P<0.05$) the total phenolics content in yogurts compared to plain yogurt with white dragon fruit yogurt showed higher total phenolic content ($P<0.005$) than that in red dragon fruit yogurt. This might be due to the addition of both white and red dragon fruits that content high phenolic compounds such as phenolic acids and polyphenols which are commonly known to be found in plants.

Betacyanins, the pigments found in Hylocereus cacti, also contributed to the total phenolics, due to a phenol structure in the molecule [17]. Betacyanins are a class of water-soluble pigments that provide the colours in a wide variety of flowers and fruits. They cannot be found in plants containing anthocyanin pigments, and structurally they are unrelated [17].

D. Antioxidant activity by 1, 1-diphenyl-2- picrylhydrazyl radical (DPPH) inhibition assay

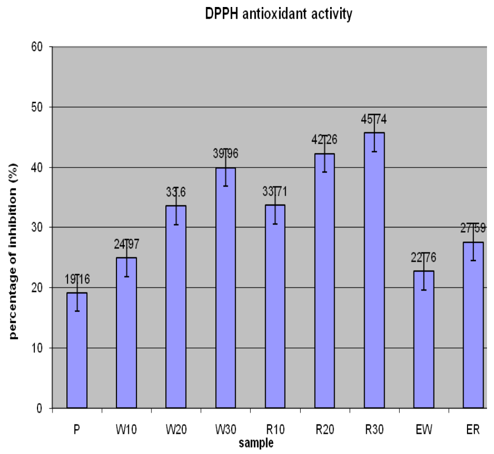

Fig. 3 Percentage of antioxidant inhibition in yogurt

Using the DPPH radical scavenging method, it was shown that all fruit enriched yogurts showed an increment in percentage of inhibition compared to plain yogurt. It also shown that all fruit enriched yogurt showed a significant different in the percentage of inhibition compared to plain yogurt and it is suggested that addition of dragon fruit into yogurt may change or enhance the percentage of inhibition. However, the difference in the percentage of inhibition between red dragon fruit yogurt and white dragon fruit yogurt is not significant (P=0.062).

The higher antioxidant activity of both dragon fruit yogurt is a desirable characteristic that may enhance the therapeutic values of yogurt. This might be due to the addition of both white and red dragon fruits that contained vitamins, phytoalbumins, and lycopene which are highly valued for their antioxidant properties [10].

Furthermore, the increment in antioxidant activity in dragon fruit enriched yogurts might be due to the increment in total phenolics content that shown in Fig 4 as we know that Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants [10].

E. Peptide concentration in yogurt by OPA assay

The entire fruit enriched yogurt showed no significant different in peptide concentration compared to plain yogurt (P=0.061). It also suggested that addition of dragon fruit into yogurt may not change the peptide concentration in yogurt. This result might be due to low protein content in both white and red dragon fruits which are commonly known to have only about 0.229g protein in every 100g of them.

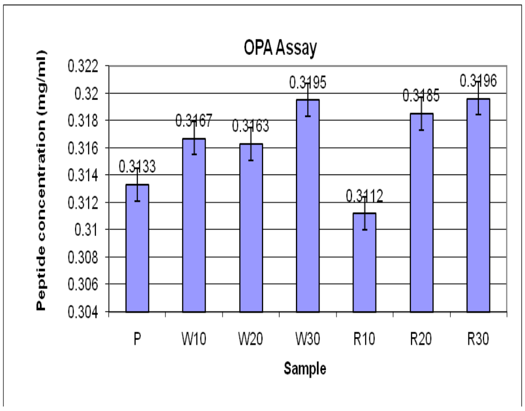

Fig. 4 Peptide concentration in yogurt

F. Sensory evaluation

Red dragon fruit yogurt showed a significant better score for virtual appearance (P=0.018), overall taste (P=0.05) compared to plain yogurt while both white and red dragon fruit yogurt showed a significant better score (P=0.03 and 0.049) for sweetness compared to plain yogurt. This might be due to the addition of both dragon fruits that enhanced the yogurt taste (contains more sugar) and the pigmentation (caused by the content of flavonoids) of red dragon fruits that gave better visual appearance to the yogurt.

IV. Conclusion

The addition of white and red dragon fruit into yogurt enhanced the milk fermentation rate, lactic acid content, syneresis percentage, antioxidant activity, total phenolics content and organoleptic properties in yogurt.

References

[1] Apostolidis, E., Kwon, Y.,-I., Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innovative Food Science and Emerging Technologies*, 8, 46-54.

[2] Aryana, K., J., McGrew, P. (2007). Quality attributes of yogurt with *Lactobacillus casei* and various probiotics. *LWT-Food Science Technology*.

[3] Brabandere, A., G., Baerdemaeker, J., G. (1999). Effects of process conditions on the pH development during yogurt fermentation. *Journal of Food Engineering*, 41, 221-227.

[4] Coisson, J. D., Travaglia, F., Piana, G., Capasso, M., Arlorio, M. (2005). *Eurtepe Oleracea* juice as a functional pigment for yogurt. *Food Research International*, 38, 893-897.

[5] Cueva, O., Aryana, K., J. (2007). Quality attributes of a heart healthy yogurt. *LWT-Food Science Technology*.

[6] Davis, J., G. (1973). Yogurt manufacture. *Fd Mf.* June,p.23.

[7] Farnsworth, J.P., Li, J., Hendricks, G.M., Guo, M.R. (2006). Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. *Small Ruminant Research*, 65, 113–121.

[8] Guggisberg, D., Eberhard, P., Albrecht, B. (2007). Rheological characterization of set yoghurt produced with additives of native whey proteins. *International Dairy Journal*, 17, 1353-1359

[9] Joel Isanga., Guonong Zhang. (2009). Production and evaluation of some physicochemical parameters of peanut milk yoghurt. *LWT-Food and Science Technology*, 42, 1132-1138.

[10] Li-chen Wu , Hsiu-Wen Hsu, Yun-Chen Chen, Chih-Chung Chiu, Yu-In Lin, Ja-an Annie Ho. (2006). Antioxidant and antiproliferative activities of red pitaya. *Food Chemistry*, 95, 319-327.

[11] Lourens-Hattingh, A., Viljoen, B. C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal*, 11, 1-17.

[12] McCue, P., P., Shetty, K. (2005). Phenolic antioxidant mobilization during yogurt production from soymilk using Kefir cultures. *Process Biochemistry*, 40, 1791-1797.

[13] Penna., A. L. B., Sivieri, K., Oliviera, M. N. (2001). Relation between quality and rheological properties of lactic baverages. *Journal of Food Engineering*, 49, 7-13.

[14] Saint-Eve, A., Levy, C., Le Moigne, M., Ducruet, V., Souchon, I. (2008). Quality changes in yogurt during storage in different packaging materials. *Food Chemistry*, 110, 285-293.

[15] Shihata, A., Shah, N. P. (2000). Proteolytic profiles of yogurt and probiotic bacteria. *International Dairy Journal*, 10, 401-408.

[16] Vargas, M., Chafer, M., Albors, A., Chiralt, A., Gonzalez-Martinez, C. (2008). Physicochemical and sensory characteristic of yoghurt produced form mixture of cows' and goat's milk. *International Dairy Journal*, 18, 1146-1152.

[17] Vasco, C., Ruales, J., Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits in Ecuador. *Food Chemistry* 11, 816-823.

[18] Vijayendra, S. V. N., Palanivel, G., Mahadevamma, S., Tharanathan, R. N. (2008). Physico-chemical characterization of an exopolysaccharide produced by a non-ropy strain of Leuconostoc sp. CFR 2181 isolated from dahi, an Indian traditional lactic fermented milk product. *Carbohydrate Polymers*, 72, 300-307.