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RESEARCH COMMUNICATION

Physicochemical characteristics, viability of starters, total phenolics and antioxidant activities of functional yoghurt supplemented with extracts from *Hylocereus polyrhizus*, *Hibiscus sabdariffa* and *Peristrophe bivalvis*

Nguyen Phuoc Minh

Faculty of Food Science and Technology, Thu Dau Mot University, Binh Duong Province, Vietnam
Email: nguyenphuocminh@tdmu.edu.vn

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ABSTRACT

Hylocereus polyrhizus, *Hibiscus sabdariffa* and *Peristrophe bivalvis* contain a great source of anthocyanin with excellent antioxidant property. Consumers highly appreciate the Yoghurt enriched with phenolics originated from plant. This research evaluated the possibility of yoghurt incorporated with pigment extract from *Hylocereus polyrhizus*, *Hibiscus sabdariffa* and *Peristrophe bivalvis*. Total acidity, syneresis, viability of starter culture, total phenolic content, free radical-scavenging activity of the enriched yoghurt were observed. Results showed that there was significant difference of total acidity, syneresis among yoghurt samples. Meanwhile there were a dramatic increasing of viability of starter culture, total phenolic content, free radical-scavenging activity in the enriched yoghurt compared to the control. *Lactobacillus acidophilus* retained high viability during storage due to functional prebiotic of plant extracts. *Hibiscus sabdariffa* extract would be a promising alternative for the enriched yoghurt with better health benefits.

Introduction

Hylocereus polyrhizus is extensively cultivated in Southeast Asia. *H. polyrhizus* fruit is rich in lycopene, anthocyanin, flavanoid, antioxidants and phytoalbumins (1-5). Its peel occupied 20–25% of the fruit with a great source of phenolics especially betalain but it is usually discarded as waste (6-8). Its peel can be utilized and converted into value-added product by pigment extraction. It can be considered as the natural food colorants not only healthy for human body, but also friendly for society and environment (9-11). *Hibiscus sabdariffa* is one of underutilized ornamental plants widely distributed in the sub-tropical and tropical regions of West, East Africa and South-East Asia. *H. sabdariffa* calyx has beautiful unique anthocyanin pigment with high antioxidant properties (12-14). Its flower contains valuable phytochemical bioactive constituents like phenolics, flavonoids, vitamins greatly contributing to different therapeutic advantages to cure hypertension, hyperlipidemia, cancer and inflammatory diseases; mild the blood viscosity; stimulate milk release and induce intestinal peristalsis (15-18). It's commonly utilized as purple dye for colouring and flavouring agent to enhance the flavour, aroma and overall acceptance of wine, syrup, juice, jam, jelly, pudding, cake, ice cream, tea, confectionery, sauce, marmalade, chocolate (14, 19-27). *Peristrophe bivalvis* is a herbaceous perennial plant widely distributed in cool and humid climate areas (28). It has numerous colors like purple, purple-magenta, red and yellow (29). It contains different photosynthetic pigments, anthocyanins, phenolics, flavonoids. It's useful in food, biological and pharmaceutical industries. It has various therapeutic functions such as anti-hypertension, anti-hyperlipidemia, fungistatic, antibacterial, antioxidant (30-33).

Lactobacillus acidophilus is a gram [+] bacteria with rod morphology, approximately 2-10 µm in dimension. *L. acidophilus* is homofermentative in

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glycolysis to ferment hexoses and produce D and L-lactic acids (34). It grows well at 30-45 °C and pH 4-5 to produce a great amount of lactic acid, some acetic acid with no hydrogen and no catalase (35). *L. acidophilus* is proved to possess various beneficial effects against health disorders. It has ability to prevent blood cholesterol, mutagenicity, carcinogenicity, constipation, diarrhea, lactose intolerance (36, 37). It's commonly used in the meat, milk, fruits, vegetables and cereal products (38). Yoghurt is a probiotic carrier originated from fermentation of milk. Yoghurt is nutritionally rich in available proteins, minerals, vitamins (39, 40). Yoghurt is extremely superior to milk, specially for people facing lactose intolerance because lactose has been converted to lactic acid by the bacterial starter culture (41, 42). Yoghurt also has useful functional application as probiotic carrier against gastro intestinal disorders (39, 43). Yoghurt can improve gum health, facilitate calcium absorption, limit osteoporosis (44). There has a great demand of symbiotic yoghurt containing prebiotics and probiotics to boost human health and well-being. Different literatures mentioned to yoghurt production enriched by numerous phytochemical sources such as coconut (45), spirulina (46), dietary fiber (47), date (48), milk protein (49), strawberry pulp (50), apple pomace flour (51), artichoke flour (52), olive fruit polyphenol (53), soy bean flour (54), *H. sabdariffa* (55), *H. polyrhizus* (56). Yoghurts are prepared in various styles and varieties (57). However there was any report mentioned to the utilization of *P. bivalvis* to enrich yoghurt. Objective of our study was to examine the possibility of yoghurt incorporated with various pigment extracts from *H. polyrhizus*, *H. sabdariffa* and *P. bivalvis*.

Materials and Methods

Material

Pasteurized milk and skim milk powder were purchased from grocery store. *H. sabdariffa* (Roselle), *H. polyrhizus* (red pitaya), *P. bivalvis* (magenta plant) were collected from January to June of 2018 in local gardens of Ke Sach district, Soc Trang province, Vietnam. They were kept in fresh and quickly moved to laboratory for experiments. *Lactobacillus acidophilus* from Vinmec was kept at 20 °C during transportation and utilized as starter culture for Yoghurt fermentation. Chemical reagents were all analytical grade.

Researching method

H. sabdariffa calyx, *H. polyrhizus* fruit pulp and *P. bivalvis* leaf materials were preliminary dried by convective drying at 50 °C to 18 % moisture content and extracted by ethanol 70 % (at ratio 1:3, material: solvent) by hot extraction and then evaporated under a rotary concentrator to get pigment extracts. 1000 mL of pasteurized milk and 250 gm of skim milk powder were primarily mixed together. 5 gm of *H. sabdariffa*, *H. polyrhizus* and *P. bivalvis* pigment extracts were individually added into the prepared mixture. This mixture was heated at 85 °C for 20 minutes and cooled to 43.5 °C before adding 0.1% *L. acidophilus* (12 log colony forming unit/g or cfu/gm) as starter culture. The incubation was conducted at 43.5 °C for 8 hrs to produce yoghurt. The control group was similarly prepared as the above protocol without pigment extracts. All manufactured yoghurt samples were all examined total acidity, syneresis, viability of starter culture, total phenolic content, free radical-scavenging activity.

Physicochemical, microbial and antioxidant determination

Total acidity (%w/w lactic acid) was estimated by titration with 0.1 N NaOH using phenolphthalein as an indicator. Syneresis (%) was evaluated by draining 50 ml of unstirred yoghurt spread evenly on filter paper at 4 °C for 6 hrs. The obtained whey volume multiplied by 2 was considered as syneresis (58). Viability of *L. acidophilus* (log cfu/gm) was enumerated by 3M-Petrifilm protocol (59). Total phenolic content (mg gallic acid equivalent/ 100 gm or mg GAE/ 100 gm) was examined by the Folin–Ciocalteu protocol (60). Free radical-scavenging activity (%,DPPH) was determined by 2,2 diphenyl-1-picrylhydrazyl supporting by UV/Vis spectrophotometer (60).

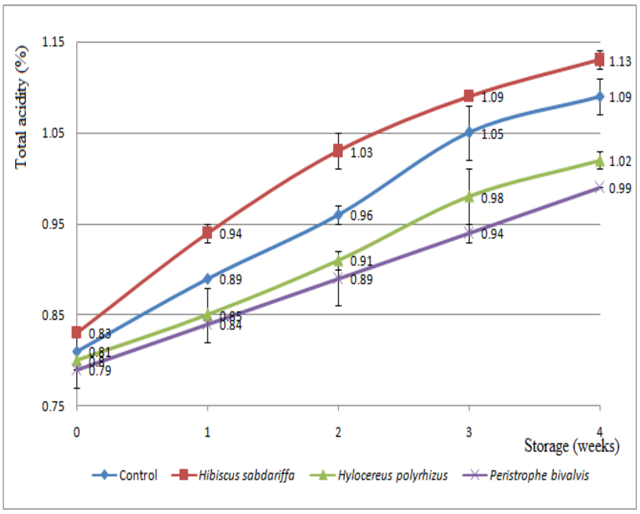

Fig. 1. Total acidity (% w/w) of yoghurt enriched by *Hibiscus sabdariffa, Hylocereus polyrhizus, Peristrophe bivalvis* extracts.

Results and Discussion

Fig. 1 shows the effect of *H. sabdariffa, H. polyrhizus, P. bivalvis* extracts on the total acidity of the enriched yoghurt. Total acidity had an increasing trend during storage. During the fermentation of milk into yogurt, the high metabolic activity of yogurt bacteria decreased with cooling whereas enzymatic activity continued. Hence, an accumulation of total acidity was noticed during storage (61). Yogurt supplemented with *H. sabdariffa* had the highest total acidity (0.83÷1.13%). Meanwhile the yoghurt incorporated with *P. bivalvis* had the lowest total acidity (0.79÷0.99%). This may be attributed to the higher acidity of *H. sabdariffa* extract compared to other ones. According to one study, total acidity of *H. sabdariffa* extract was 4.20±0.01% (in fresh calyx), 12.73±0.09% (in concentrated fresh roselle extract) and 11.96±0.34% (in concentrated dried roselle extract) (62). Supplementation of *H. sabdariffa* calyx extract into the reconstituted low fat milk yoghurt resulted in significant reduction of pH 4.4÷4.16 vs. 4.54÷4.31 as control (63). *H. sabdariffa* L. flowers marmalade significantly increased titratable acidity of formulated yoghurt (64). Addition of *H. sabdariffa* calyx extract into the probiotic yoghurt resulted to total acidity 0.81÷1.14% (65). Yogurts enriched by *H. polyrhizus* resulted a higher lactic acid percentage (1.14÷1.23%) compared to plain yogurt (1.08%) (56).

Fig. 2 presented the influence of *H. sabdariffa, H. polyrhizus* and *P. bivalvis* extract on the syneresis of the enriched yoghurt. Syneresis had an increasing trend during storage. Yogurt supplemented with *H. sabdariffa* had the highest syneresis (30.4-9.3%). Meanwhile the control yoghurt had the lowest syneresis (29.3-5.9%). Addition of *H. sabdariffa* calyx extract into the reconstituted low fat milk yoghurt resulted in significant reduction of syneresis 0.8-1.9 vs. 1.3-2.6 as control (63). Similar results were observed by adding cranberry paste and pumpkin fiber into yoghurt (66). Supplementation of *H. sabdarifffa* Calyx extract into the probiotic yoghurt resulted to syneresis 18.85 to 24.90 ml/50 gm of sample (65). Yogurts enriched by *H. polyrhizus* resulted a higher syneresis percentage (57.19-70.32 %) compared to plain yogurt (52.93 %) (56).

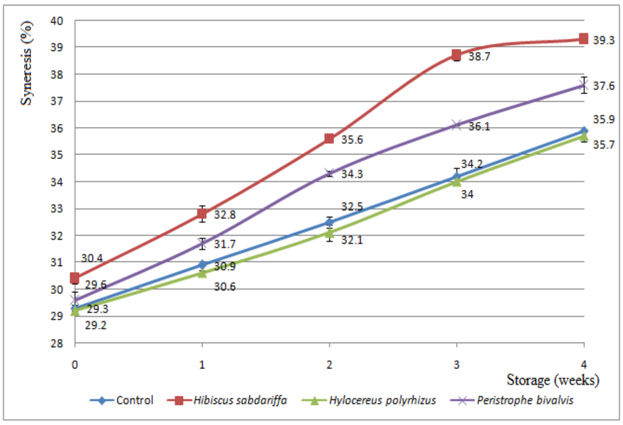

Fig. 2. Syneresis (% w/w) of yoghurt enriched by *Hibiscus sabdariffa, Hylocereus polyrhizus, Peristrophe bivalvis* extracts.

Fig. 3 reveals the impact of *H. sabdariffa, H. polyrhizus* and *P. bivalvis* extracts on the *Lactobacillus acidophilus* viability (log cfu/gm) in the enriched yoghurt. *L. acidophilus* viability had an increasing trend in the first two weeks and decrease afterwards. Yogurt supplemented with *H. sabdariffa* had the highest *L. acidophilus* viability (11.4-3.7 log cfu/gm). Meanwhile the control yoghurt had the lowest *L. acidophilus* viability (11.0-11.6 log cfu/gm). Bamidele (63) found higher lactic acid bateria 1.2-1.5 x 10^7 cfu/gm on the day 7^{th} in yoghurt incorporated with *H. sabdariffa.* There were no statistically differences between control and *H. sabdariffa*-supplemented groups in terms of lactic acid bacteria (64). *H. sabdariffa* calyx was demonstrated to be beneficial on the quality of *L. casei* incorporated probiotic yoghurt. Yeast and mold proliferation were absent from the *Sabdariffa* yoghurt (65).

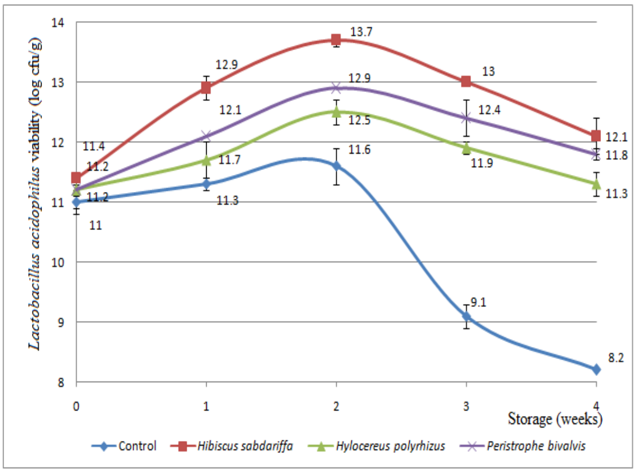

Fig. 3. *Lactobacillus acidophilus* viability (log cfu/gm) of yoghurt enriched by *Hibiscus sabdariffa, Hylocereus polyrhizus, Peristrophe bivalvis* extracts.

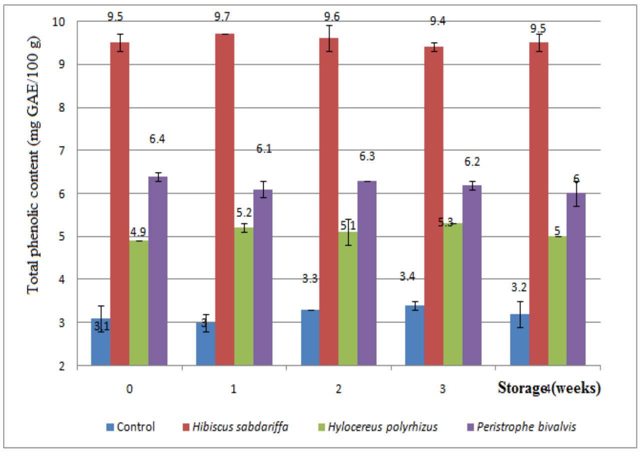

Fig. 4. Total phenolic content (mg GAE/100 gm) of yoghurt enriched by *Hibiscus sabdariffa, Hylocereus polyrhizus, Peristrophe bivalvis* extracts.

Fig. 4 shows the effect of *H. sabdariffa* and *H. polyrhizus, P. bivalvis* extracts on the total phenolic content in the enriched yoghurt. The total phenolic contents were nearly stable during 4 weeks of storage. Yogurt supplemented with *H. sabdariffa* had the highest total phenolic content (9.4-9.7 mg GAE/100 gm). Meanwhile, the control yoghurt had the lowest total phenolic content (3.0-3.4 mg GAE/100 gm). *H. sabdariffa* L. flowers marmalade significantly increased total phenolic content (5.57–14.69 mg GAE/ 100 gm) of formulated yoghurt (64). *H. sabdariffa* was reported as an excellent source of antioxidants and total phenolics (67-69). The total phenolic content in the yogurt enriched by *H. sabdariffa* was noticed at 15.21 mg GAE/100 gm (70). Yogurts enriched by *H. polyrhizus* resulted a higher total phenolic content (36.44-64.43mg/ml) compared to plain yogurt (20.25 mg/ml) (56). Our results were similar to the findings in another study (71). Yoghurt supplemented with aronia, blueberry and grape juice would receive great advantage of probiotic with excellent phenolic intake.

Fig. 5 shows the effect of *H. sabdariffa, H. polyrhizus* and *P. bivalvis* extracts on the antioxidant

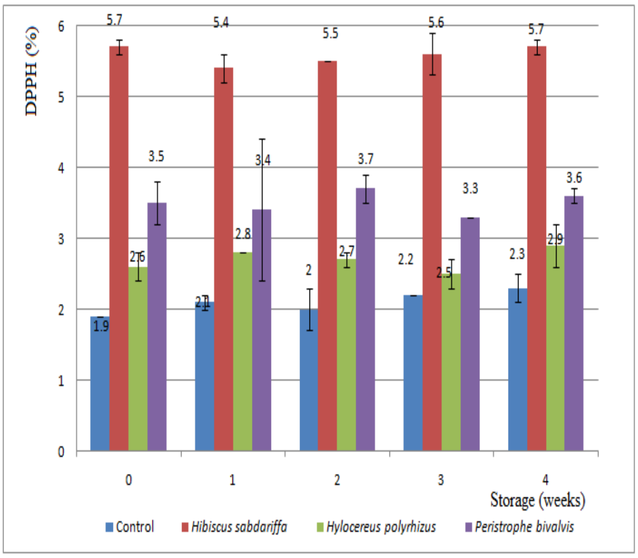

Fig. 5. Antioxidant activity (%, DPPH) of yoghurt enriched by *Hibiscus sabdariffa, Hylocereus polyrhizus, Peristrophe bivalvis* extracts.

activity (%, DPPH) in the enriched yoghurt. The free radical-scavenging activity in the enriched yoghurt were nearly stable during 4 weeks of storage. Yogurt supplementd with *H. sabdariffa* had the highest DPPH (5.4-5.7 %). Meanwhile, the control yoghurt had the lowest DPPH (1.9-2.3%). *H. sabdariffa* L. flowers marmalade significantly increased antioxidant capacity (5.92–26.73 mg TE/100 gm) of formulated yoghurt (64). Supplementation of *H. Sabdariffa* extract in yoghurt enhanced the total antioxidant property with DPPH in range from 12.32 to 59.43 % (65). Yogurts enriched by *H. polyrhizus* resulted a higher antioxidant activity (24.97-45.74 %) compared to plain yogurt (19.16 %) (56).

Conclusion

Yoghurt is one of the most common fermented dairy products with a great consumer acceptability due to its functional advantages. There is a trend of using yoghurt incorporated with plant pigments as bioactive constituents that are safe and potential health benefits. In this research, extracts from *H. polyrhizus*, *H. sabdariffa* and *P. bivalvis* were added into yoghurt as functional ingredients. *H. sabdariffa* revealed as a promising candidate to improve total acidity, syneresis, *L. acidophilus* viability, total phenolic content and free radical-scavenging activity in the enriched yoghurt. Consumer will have more chance to consume one kind of the healthy yoghurt enriched with phytochemical source.

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Authors' contributions

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

Conflict of interests

The authors confirmed that this study was conducted without any conflict of interest.

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