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Characterization of fatty acid profiles and anti-scanvanging activity of purple sweet potato (*Ipomoea Batatas* L. Lam)-based custard fortified with perilla seed oil (*Perilla frutescens* L. Britton)

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Abstract

A creamy dairy custard was developed in this study. This custard matrixes consists of low-fat milk as main ingredients, purple sweet potato, perilla seed oil, butter, egg yolk, corn starch as well as monk fruit syrup. The aims of this research were to develop a heathy custard product, characterize the fatty acid profiles, anti-scavenging activity and sensory attribute of developed purple sweet potato-based custard fortified with perilla seed oil. The fatty acid profiles of the custard containing 3 % of perilla seed oil provides the highest amount of LA (17.32 %) and ALA (25.88 %). The custard formulation containing 1% of perilla seed oil provides the highest scores in all sensory attribute terms compared to other formulations containing perilla seed oil. The purple sweet potato-based custard containing perilla seed oil provides good antioxidant activities with percent inhibition by DPPH range from 29.8 to 35.1. The result indicates the concentration-dependent of perilla seed oil in the product. The custard developed in this study provides not only an excellent source of polyunsaturated fatty acids but also an excellent source of antioxidant agent.

Keywords: *Custard, Purple Sweet potato, Perilla seed oil, alpha-linolenic acid, linoleic acid*

INTRODUCTION

The food crisis has become a global concern. Many plants have been explored and upscaled in order to serve the global population. Sweet potato (*Ipomoea batatas* L.) is one of the economic crops that originated from Central America. As it can be grown in various types of environmental conditions and produced high yields thus it has been ranked as the seventh most significance food crop in the world. This sweet potato has been cultivated widely in many countries including China, Japan, Korea, United States, Poland as well as Thailand. Tubers and leaves have been used as human diet, livestock feed and raw materials for industry uses (Krochmal-Marczak et al., 2014, Shekhar et al., 2015, Wu et al., 2015) The sweet potato provides 50% higher nutritional values compared to potato. Most of cultivars of sweet potatoes rich in dietary fibre, potassium, copper, manganese, iron, vitamin C, B2, B6 as well as vitamin E. The pharmaceutical properties claimed for sweet potatoes are lower risk of heart attack, lower risk of stroke, low glycemic index which is suitable as antidiabetic, anti-inflamatory, antimicrobial, antioxidant, anticancer (Krochmal-Marczak et al.,

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2014, Mohanraj and Sivasankar, 2014, Shekhar et al., 2015) Thus, it has been utilized in the food industry as a natural food coloring agent, natural antioxidant agent, functional ingredients for producing healthy food products. Due to many health attributes the sweet potato is also introduced in this study as functional food ingredient for making a healthy dairy custard.

Perilla or Nga-Kee-Mon (*Perilla frutescens* L. Britton) is an herbal plant within Lamiaceae family. Both seeds and leaves have been utilized for many therapeutic purposes such as common cold, headache, cough, fish or crab poisoning, some intestinal disorders etc. The perilla seed oil is well known as functional lipids as its riches in polyunsaturated fatty acid (omega-3 and omega-6), sterols as well as tocopherols contents. These functional ingredients tend to provide many beneficial effects to the body includes lowered risk of cancers, cardiovascular diseases, and metabolic disorders (Ahmed, 2018, Dhyani et al., 2019, Yu et al., 2017)

By incorporating this functional oil together with purple sweet potato tend to be a promising candidate for custard making as they are not only providing a good diet with high content of polyunsaturated fatty acids (omega-3: alpha-linolenic acid and omega-6: alpha-linoleic acid) but also provides good natural antioxidant properties which is safe for human consumption. Thus, this research is tended to develop a healthy custard by using purple sweet potato as a base of custard product and then fortified with different concentration of perilla seed oil in order to get a suitable ratio of perilla seed oil in making a tasty custard product.

LITERATURE REVIEW

2.1 Sweet potato (Ipomoea Batatas L.)

Sweet potato is one of dicotyledonous plants botanically classified under *Convolvulaceae* family. This plant originated in Central America. This crop is tolerant to various environmental conditions thus it has been cultivated in many parts of the world especially in a warm, hot climate zone or some temperate zone. This plant has been utilized as food, animal feed as well as raw materials for industry uses. This plant produces a tuber which gives a sweet taste and good aroma. The shape of tuber can be spherical, oval, spherical-oval, or fusiform. The tuber flesh could be varying from white, cream, yellow, orange, red, claret to purple depending on a cultivar (Krochmal-Marczak et al., 2014, Shekhar et al., 2015, Wu et al., 2015). The purple sweet potato indicated to reducing risk of death of ischemic heart attack, stroke, anticancer, antidiabetic, antiinflamatory, antimicrobial, antioxidant (Sulistiani et al., 2018)

2.2 Perilla (Perilla frutescens L. Britton)

Perilla frutescens L. Britton is one of the aromatic plants within *Lamiaceae* family. It is originated from China and has been propagated in South Korea, Japan, and Thailand (Dhyani et al., 2019). It has its unique mint like odor that derived from the characteristic of its family. It has a four-sided stem that is covered with short hairs. The leaves vary from green to purple, ovate and glossy with downy-haired. The perilla contains a small flower with bell- shaped that varying from white to purple. It bears a tiny gray-brown fruit. The perilla can be divided into two varieties which are *P. frutescens var. frutescens* (seed oil crop), and *P. frutescens var. crispa* (leafy vegetable crop). This two perilla varieties can be differ by their morphology which are green and purple (Pandey and Bhatt, 2007). Many phytochemicals were found in perilla, and the most abundant phytochemicals are rosmarinic acid, luteolin, chrysoeriol, quercetin, catechin, caffeic acid and ferulic acid. In additions, the perilla seed also contains many other compounds like Phytosterols, Tocopherols, Squalene, and polyunsaturated fatty acid (Ahmed, 2018, Dhyani et al., 2019). As a result, the perilla has been utilized for food and therapeutic purposes.

RESEARCH METHOD

3.1. Materials and chemical reagents

Sweet potato (*Ipomoea Batatas* L. Lam) was collected from Suwincha Farm, Nakhon Rachasima Province, Thailand. Cold pressed perilla seed oil was purchased from local market, Bangkok, Thailand. All organic solvents were purchased from Merck (Darnstadt, Germany). Standard phenolic compounds including DPPH, Butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, USA).

3.2. Preparation of creamy dairy custard

The purple sweet potatoes were cleaned and boiled. Then the cooked flesh sweet potatoes were added and homogenized by blender. A total of 100 g of creamy dairy custard was prepared. 4 g of butter and egg yolk were mixed manually with a whisk for 1 min and then 0.07 monk fruit syrup was mixed for 2 min. After that, 57.97 g of low-fat milk was heated at 80 °C and gradually added and mixed for 5 min. Then, the mixtures were filtered prior to heat at 80 °C for 15 min and the mixtures were cooled down at room temperature and then different concentration of cold pressed perilla seed oil was added for each formulation. The custard samples were then kept at 4 °C until further analysis.

3.3. Characterization of fatty acid profiles in custard by GC-FID

The custard formulations containing different concentrations of perilla seed oil, and cold pressed perilla seed oil were further characterized for fatty acid profiles according to Santiworakun et al. (2022). Two grams of samples were added into the test tube and extracted with dichloromethane: methanol (2:1, v/v). The solutions were mixed every 15 min for 1 h. The mixtures were then filtered with What paper (No.1) and transferred into new test tubes. Then, the KCl (0.1 mol/L) was added into test tubes containing filtrate and was centrifuged at 2000×g for 10 min (Himac CF7D2, Hitachi, Tokyo, Japan). After centrifugation the mixtures were separated into two layers. The upper layer was discarded while 200 μL of lower layer was then transferred into new test tube and converted into fatty acids methyl esters (FAMEs) using 2 mL of 0.5 M NaOH-methanol.

The mixtures were later heated at 100 ◦C for 15 min and cooled down at room temperature. 2 mL of 14% BF 3-methanol was then added into the test tube containing mixtures, heated at 100 ◦C for 1 min and cooled down at room temperature. After that, 500 µL of hexane and 5 mL of saturated NaCl were added into test tube and centrifuged at 1000×g for 5 min. The 20 µL of FAMEs were then collected from the upper phase of the solution and injected into Gas chromatography (GC-FID 2010; Shimadzu) equipped with a DB23 column (30 m × 0.25 mm i.d.x 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA). Helium was used as a carrier gas in GC-FID system with total flow rate of 62.9 mL/min, split 1:50. The set-up conditions for GC-FID were as follows: the initial and final column temperatures were set as 80 ◦C and 220 ◦C, respectively. The injector and detector temperatures were 250 ◦C and 300 ◦C, respectively. The FAMEs standards were used and injected in the system in order to identify the fatty profiles of the samples. The results are expressed as the relative percentage of each individual fatty acid present in the sample.

3.4 Sensory Evaluation

The four formulation of creamy dairy custard was evaluated by trained panelists using 9-Point Hedonic scale. An evaluation form was developed and used to evaluate the attributes (such as overall acceptability, flavor, color, taste, texture, viscosity, compatible with bread, and smoothness).

3.5 Determination of anti-scanvanging activity using DPPH assay

Antioxidant activity of cold press perilla seed oil and custard fortified with purple sweet potato and different concentrations of perilla seed oil were measured on the basis of its scavenging activities towards the stable of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Briefly, various concentrations of samples (0.1 mL) and 0.208 mM DPPH dissolved in methanol solution (0.1 mL) were added. After 30 min of incubation in the dark at room temperature, the absorbance was measured against a blank (methanol) at 515 nm (Cheng et al., 2006) using a UV/Visible spectrophotometer. Inhibition of DPPH radical was calculated as a percentage (%) using the formula:

% Inhibition = $[\ (A_0 - A_1)\ A_0] x 100$

Where A_1 = absorbance of the test sample and A_0 = absorbance of control. Each assay was carried out in triplicates.

3.6. Statistical analysis

All experiments were done in triplicate and all results are presented as mean±SD. The statistical analyses of collected data were performed using SPSS version 22.0 (IBM). One-way analysis of variance (ANOVA) and multiple comparisons by Tukey's were performed to analyze the difference among data. Statistical differences were significant at $P<0.05$.

FINDINGS AND DISCUSSION

4.1 Characterization of fatty acid profiles in custard by GC-FID

Fats and lipids play an important role in human diet. Each type of them may give different benefits to health depending on their functions. Functional lipids have become public attention as they have a potential to boost an immune system, prevent and treatment for varieties of diseases including obesity, blood pressure, diabetes, bone-related diseases, cardiovascular-related diseases as well as depression (Bhat, 2021). Cold pressed perilla seed oil is one of well-known herbal oil which has been considered as functional oil as it richs in many phytochemical ingredients and functional lipids. Especially polyunsaturated fatty acids (PUFA) in which they occupied more than 50 % of total lipids found in perilla seed oil. The previous studied revealed that Thai perilla seed oil contains 61.51% of alpha linolenic acid (ALA), 16.55% of linoleic acid (LA)and 11.62 % of oleic acid (Kangwan et al., 2021). Similarly, in this study the ALA (53.18%) was report to be the most aboundunt fatty acids found in perilla seed oil, linoleic acid (22.17%), and oleic acid (14.90%). For the purple sweet potato-based custard fortified with different concentration of perilla seed oil, the oleic acid (24.07-34.41%) showed to be the most abundant fatty acids found in all formulations. The highest amount of LA (17.32%) and ALA (25.88%) was found in the custard fortified with 3% of perilla seed oil. The LA and ALA indicated the concentration-dependent, as the amount of fortified perilla seed oil increase, the amount of LA and ALA also increase. Thus, the fortified custards were rich in functional lipids from perilla seed oil.

Table 1. Fatty acid compositions in perilla seed oil and custard containing different concentrations of perilla seed oil.

Fatty acid	Relative percentage composition of fatty acid (%)				
	Perilla oil (PSO)	Custard F1 (PSO 0 %)	Custard F2 (PSO 1 %)	Custard F3 (PSO 2%)	Custard F4 (PSO 3%)
Lauric Acid (C12:0)	-	13.51 ± 0.01^{b}	10.41 ± 0.48^{b}	6.67 ± 0.04^{a}	3.43 ± 0.08^{a}
Myristic Acid (C14:0)	-	7.44 ± 0.01^{a}	5.97 ± 0.27^{a}	4.21 ± 0.04^{a}	2.72 ± 0.08^{a}
Palmitic Acid (C16:0)	6.64 ± 0.31^{b}	28.58 ± 0.07^{a}	26.53 ± 1.07^{a}	22.45 ± 0.19^{a}	20.46 ± 0.27^{a}
Stearic Acid (C18:0)	3.11 ± 0.11^{a}	8.85 ± 0.08^{a}	8.00 ± 0.04^{a}	7.63 ± 0.09^{a}	6.12 ± 0.04^{a}
Oleic Acid (C18:1cis)	14.90 ± 0.45^{b}	34.41 ± 0.23^{a}	29.02 ± 2.84^{a}	28.42 ± 0.18^{a}	24.07 ± 0.67^{a}
Linoleic Acid (ω6) (C18:2cis)	22.17 ± 0.28^{a}	7.22 ± 0.08^{a}	11.38 ± 0.50^{a}	13.84 ± 0.14^{a}	17.32 ± 0.17^{a}
AlphaLinolenic Acid (ω3) (C18:3n3)	53.18 ± 0.17^{e}	0.00 ± 0.00^{a}	8.68 ± 0.48^{b}	16.78 ± 0.24^{c}	25.88 ± 0.22^{d}

Mean ± standard deviation. Values followed by the same letter did not differ statistically according to the Tukey test at 5% ($P \leq 0.05$).

4.2 Sensory Evaluation

The consumers' acceptability is one of the important factors that need to concern when formulating new food product. Four formulations of developed custard were evaluated for sensory attributes with ten panelists. According to the results, the developed custard without perilla seed got the highest scores in all terms compared to other custard formulations (Table 2). The results also indicated that the perilla seed oil and the amount of perilla added were significantly affect the panelist preference on the custard product. This might due to its nutty aroma and taste which were detrimental for liking. Among the three formulations containing perilla seed oil, the custard containing 1% of perilla seed oil provides the highest score compared to other custard containing perilla seed oil. Thus, the custard with 1% of perilla seed oil shown to be a good candidate for developing custard product.

Table 2. Acceptibility scores of custards containing different concentrations of perilla seed oil

Attributes	Average score±SD (n=10)			
	PSO 0.0%	PSO 1.0%	PSO 2.0%	PSO 3.0%
Color	5.60±1.56	5.70±1.25	5.60±1.43	5.50±1.43
Flavor	6.00±1.94	5.50±1.84	4.70±1.64	4.40±1.78
Taste	6.40±1.78	5.50±1.43	4.70±1.06	4.50±1.84
Texture	6.40±1.84	6.40±0.97	5.60±1.26	5.30±1.83
Smoothness	7.30±1.06	4.70±1.83	5.70±1.25	5.60±1.65
Viscosity	6.60±1.65	6.50±1.08	5.70±1.34	5.40±1.51
Compatible with bread	6.40±1.71	5.60±0.97	4.70±1.06	4.80±1.48
Overall acceptability	6.40±1.90	5.80±1.32	4.80±0.92	4.60±1.51

4.3 Determination of anti-scanvanging activity using DPPH assay

An antioxidant compound in food plays a crucial role in health protection and prevention from many diseases. Both synthetic and natural occurring antioxidants have been introduced to many foods. So, natural ingredients which are rich in antioxidants were also implemented in the custard developed in this study. A colorimetric method for the determination of antioxidant activity in purple sweet potato-based custard was applied in this study. The method involved the extraction of the antioxidants and then the extracts were dissolved in methanol solution prior to reacting with 2,2′-diphenyl-1-picrylhydrazyl (DPPH). The colorimetric rapid reaction development was based on the presence of antioxidants in the product. The reaction was measured by UV-vis spectrophotometer at absorbance of 515 nm. Theoretically, high total of phenolic compounds will provide high values of antioxidant activities (Zheng and Wang, 2001). The antioxidant activities of both purple sweet potato and perilla seed oil were studied by many researchers (Shekhar et al., 2015, Dwiyanti et al., 2018). Previous studies reveal that the purple sweet potato tuber provides high antioxidant activities. This antioxidant property was attributed to the number of anthocyanins, phytophenolics, and carotenoids presented in the tuber. Meanwhile, the perilla seed oil also possesses high antioxidant activity which could be derived from phenolic acids, polyphenols, as well as flavonoids. The antioxidants contained in custard resulted in the color change of DPPH solutions. This was due to the DPPH radical reacting with the hydrogen atoms donated by antioxidants in the custard and leading to reduced DPPH. The percentage of inhibition was measured from each custard formulations which were 29.8, 31.5, 35.1 and 34.47 for custard containing 0%, 1%, 2% and 3% of perilla seed oil respectively (Figure 1). The antioxidant activity of control custard formulation could possibly come from the antioxidant compound presented in purple sweet potato. However, the antioxidant activity was increased after the addition of perilla seed oil. The antioxidant efficacy of custard containing 2% and 3% were statistically different compared to control and 1% fortified custard formulations (p<0.05). Consequently, the result indicated that the developed sweet potato-based custard was enriched with antioxidant agent which is benefit as health promoting agent.

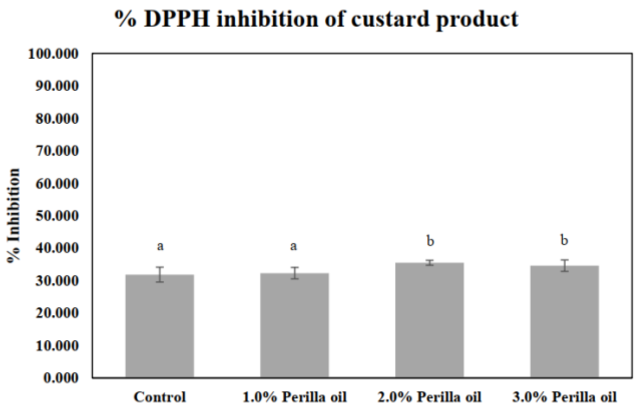

Figure 1. The % inhibition of custards containing different concentrations of perilla seed oil.

CONCLUSION AND FURTHER RESEARCH

A creamy dairy custard fortified with perilla seed oil was developed in this study. The outcome revealed that the purple sweet potato-based custard developed was excellent source of functional lipids such as alpha-linolenic acid which is an important precursor of polyunsaturated fatty acid synthesized in the human body. In addition, the developed custard products were also excellent sources of antioxidant agents. Further research needs to be taken especially the study on the suitable proportions of perilla seed oil added in sweet potato-based custard in order to get not only a heathy custard but also provide high sensory scores from the subjects. Moreover, the purple sweet potato with high content of anthocyanins should screening and selected in order to get the best functional ingredient to make a sweet potato-based custard.

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