การพัฒนาผลิตภัณฑ์พุดดิ้งผักเพื่อผู้สูงอายุและผลของการเก็บรักษาผลิตภัณฑ์ต่อปริมาณโพลีฟีนอลทั้งหมดและฤทธิ์การต้านอนุมูลอิสระ

FORMULATION OF VEGETABLE PUDDING FOR ELDERLY AND THEIR STORAGE EFFECT ON TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITIES

*ชนิกา ฉิมเกิด น้ำผึ้ง รุ่งเรือง ภรัณยา ธิยะใจ ศศิอำไพ พฤฒิพรธานี ยุราพร สหัสกุล ดุลยพร ตราชูธรรม ธัญญ์นลิน วิญญูประสิทธิ์**

***Chanika Chimkerd,** Numphung Rungraung, Parunya Thiyajai, Sasiumphai Purttiponthanee, Yuraporn Sahasakul, Dunyaporn Trachootham, Thunnalin Winuprasith**

สถาบันโภชนาการ มหาวิทยาลัยมหิดล
Institute of Nutrition, Mahidol University.

***Corresponding author, e-mail:** *thunnalin.win@mahidol.ac.th*

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บทคัดย่อ

พุดดิ้งเป็นอาหารที่อุดมไปด้วยสารอาหารต่างๆ ซึ่งมีส่วนประกอบหลักคือ นม น้ำตาล น้ำมัน และสารก่อเจล อีกทั้งพุดดิ้งมีเนื้อสัมผัสที่นุ่มและสามารถกลืนได้ง่าย ดังนั้นผลิตภัณฑ์พุดดิ้งจึงเหมาะสมสำหรับผู้สูงอายุที่มีปัญหาด้านการบดเคี้ยวเนื่องจากมีการสูญเสียฟัน ซึ่งงานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาผลิตภัณฑ์พุดดิ้งที่มีสารอาหารครบถ้วน โดยมีการใช้ผักผง 3 ชนิด ได้แก่ มันเทศหวาน ข้าวโพดหวาน และฟักทอง ที่ระดับความเข้มข้นร้อยละ 8 เติมลงในพุดดิ้งสูตรต้นแบบ เพื่อศึกษาการยอมรับทางประสาทสัมผัส สี ปริมาณโพลีฟีนอลทั้งหมด ฤทธิ์การต้านอนุมูลอิสระ และผลของระยะเวลาในการเก็บรักษาผลิตภัณฑ์ จากการศึกษาพบว่าผลิตภัณฑ์พุดดิ้งผักจะมีสีเข้มกว่าพุดดิ้งสูตรต้นแบบ แต่ผลิตภัณฑ์พุดดิ้งทุกสูตรยังได้รับการยอมรับทางประสาทสัมผัส โดยมีความชอบโดยรวมที่ระดับความชอบปานกลาง สำหรับปริมาณโพลีฟีนอลทั้งหมดและฤทธิ์การต้านอนุมูลอิสระ พบว่าผลิตภัณฑ์พุดดิ้งผักทุกสูตรมีปริมาณโพลีฟีนอลทั้งหมดและฤทธิ์การต้านอนุมูลอิสระมากกว่าพุดดิ้งสูตรต้นแบบ โดยเฉพาะอย่างยิ่งในผลิตภัณฑ์พุดดิ้งข้าวโพดหวานและพุดดิ้งฟักทอง ส่วนผลิตภัณฑ์พุดดิ้งมันเทศหวานมีปริมาณโพลีฟีนอลทั้งหมดใกล้เคียงกับพุดดิ้งสูตรต้นแบบ แต่มีฤทธิ์การต้านอนุมูลอิสระมากกว่าพุดดิ้งสูตรต้นแบบ ผลิตภัณฑ์ทั้งหมดทำการเก็บรักษาที่อุณหภูมิ 35 องศาเซลเซียส เป็นเวลา 12 สัปดาห์ พบว่า ผลิตภัณฑ์มีสีคล้ำขึ้นตามระยะเวลาในการเก็บรักษา ส่วนปริมาณโพลีฟีนอลทั้งหมดของผลิตภัณฑ์ทุกสูตรมีการเพิ่มขึ้นสูงสุดที่สัปดาห์ที่ 6 และหลังจากนั้นมีการลดลงจนถึงสัปดาห์ที่ 12 สำหรับฤทธิ์การต้านอนุมูลอิสระพบว่ามีการเพิ่มขึ้นในระหว่างการเก็บรักษา และมีการลดลงที่สัปดาห์ที่ 12 ดังนั้นในการศึกษานี้จะเห็นได้ว่าการเติมผักผงและระยะเวลาในการเก็บรักษามีผลต่อปริมาณโพลีฟีนอลทั้งหมดและฤทธิ์การต้านอนุมูลอิสระ ผลิตภัณฑ์พุดดิ้งที่พัฒนาขึ้นนี้สามารถเป็นต้นแบบของผลิตภัณฑ์เสริมอาหารสำหรับผู้สูงอายุ ที่มีประโยชน์ต่อสุขภาพ นอกจากนี้ผลิตภัณฑ์นี้สามารถเป็นผลิตภัณฑ์สำหรับเด็กและผู้บริโภคที่ดูแลสุขภาพได้อีกด้วย

คำสำคัญ: พุดดิ้งผัก ปริมาณโพลีฟีนอลทั้งหมด ฤทธิ์การต้านอนุมูลอิสระ การเก็บรักษา

Abstract

Pudding is a nutrient-rich dessert commonly served for elderly who is lack of chewing ability due to tooth loss because of its soft texture. Pudding is usually composed of milk, oil, sugar and hydrocolloids. The aim of this study was to formulate ready-to-eat pudding containing essential nutrients with varying types of vegetable powder added, including sweet potato (SP), sweet corn (SC), and pumpkin (PK) powder. The pudding was studied the sensory acceptability, color, total phenolic contents (TPC) and antioxidant activities (AA) measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) assays. The effect of storage time on the color, TPC and AA of each developed formula was also investigated. The vegetable powder was added at 8% (w/w) separately into the control. All developed puddings were accepted by the panelists at like moderately of overall acceptability. Although, the color of vegetable puddings were darker than the control, the panelists still accepted. The TPC and AA of vegetable puddings were greater than that of the control, particularly SC and PK pudding. The TPC content of SP pudding was similar to that of the control, whilst the AA of it was higher than that of the control. The developed products stored at 35 °C for 12 weeks showed that the color was darker. TPC of all formulas were highest at week 6, and then declined until week 12. The AA of the vegetable pudding increase somewhere during storage time, then reduced at the week 12. This study demonstrated that vegetable powder affected TPC and AA. Furthermore, the storage time played an important role on TPC and AA. These products are prototype of supplement for elderly which have health benefits beyond basic nutrition. In addition, these products can be extended to children and health conscious consumers as well.

Keywords: Vegetable Pudding, Total Phenolic Contents, Antioxidant Activities, Storage

Introduction

The situation of Thai older population surveyed by National Statistical Office reported that the proportion of Thais has been changed over the last 20-30 years. The number of children (defined as age under 15) has decreased, while the elderly population (defined as age 60 and older) has rapidly increased. Moreover, this situation seems to continue in the future. The National Statistical Office predicted that the older people would increase from 10 million (14.9% of the population) to 20 million people within ten years. Therefore, the elderly is now more than 10% of population. Thailand is classified as aging society [1]. However, there is an interesting point whether the quality of life including the economic status, well-being, health and nutritional status of this population is good or not.

The Bureau of Dental Health, Department of Health surveyed the oral health of Thais in 2012. The people with greater than or equal to 20 teeth were 57.8% and 23.5%

in people age 60-74 and 80-89, respectively. In addition, 7.2% and 32.2% of people age 60-74 and 80-89, respectively, have no teeth [2]. Tooth loss affects dietary intake of elderly. Elderly who have this problem cannot consume sticky or solid food such as vegetable, grain, or meat. The National Health and Nutrition Examination Survey or NHANES collected data from 4,622 participants with ages 60 years and over. The results showed that tooth loss was an important factor for less/not enough vegetable consumption [3]. In conformity with the study of Australian adults who had tooth loss or edentulous, they had lower intake of solid food, particularly fruits and vegetables. In contrast, fat intake increased when compared to the one who had 25 teeth or more. This finding showed that selective food avoidance influenced dietary patterns and nutritional intake [4]. In addition, increase in intake of carbohydrate and confectioneries and decrease in consumption of fruits and vegetables in Japanese elderly were observed as well. These resulted in vitamins A, C, B_6, folate, and dietary fiber deficiency, and increased the risk of non-communicable diseases as well [5-6].

Pudding is a sweet dessert composed of nutritionally valuable milk, egg, and sucrose which provides protein, carbohydrates, fat, vitamins, and minerals [7]. Pudding has soft texture and easy to swallow that it can be used as a food or dessert for elderly who have problems with tooth loss, chewing, and swallowing [8]. However, the pudding has no dietary fiber and bioactive compounds. Vegetables are well-known as good sources of dietary fiber, vitamins, minerals, and bioactive compounds [9]. Three types of vegetable powder including sweet potato (SP), sweet corn (SC), and pumpkin (PK) are rich in dietary fiber and high level of bioactive compounds known as carotenoids, were used in this research. Health promotion and prevention of diseases in the elderly who have tooth loss, thus, is necessary. Therefore, this research aimed to develop shelf stable and ready-to-eat vegetable pudding containing essential nutrients as a meal supplement for elderly who have tooth loss. The products can be used as a prototype for developing food for elderly to promote the nutritional status and quality of life.

Objectives

This research aimed to develop shelf stable ready-to-eat vegetable pudding containing essential nutrients as a meal supplement and to evaluate the sensory acceptability, color, total phenolic contents, and antioxidant activities of the vegetable puddings compared with the control. Furthermore, to study the change of color, total phenolic contents and antioxidant activities of vegetable pudding during the storage time.

Methods

Materials

Vegetable powders including sweet potato, sweet corn, and pumpkin powder were purchased from Chiangmai Bioveggie Co., Ltd. Thailand. The nutritional value of the vegetable powders was analyzed by Food and Nutrition Laboratory, Institute of Nutrition, Mahidol University, Thailand as shown in Table 1. Kappa-carrageenan was used as a gelling agent. Milk protein isolate, maltodextrin, modified tapioca starch were certified as food-grade ingredient. Rice bran oil and sugar were purchased from local supermarket In Nakhon Pathom, Thailand.

Table 1. Nutritional value of sweet potatoes (SP), sweet corn (SC), and pumpkin (PK) in the dry basis

Compositions/Nutrients	SP	SC	PK
Energy (Kcal/100g)	381.69	390.01	374.64
Protein (g/100g)	5.43	13.54	8.20
Fat (g/100g)	0.79	5.30	2.40
Carbohydrate (g/100g)	88.22	72.05	80.07
Soluble Dietary Fiber (g/100g)	1.74	2.26	7.38
Insoluble Dietary Fiber (g/100g)	3.94	10.02	12.61
DPPH Value (μmole TE/g)	4.96	13.78	8.18
ORAC Value (μmole TE/g)	0.12	0.56	0.26
TPC (mg GAE/g)	2.15	3.74	3.45
Carotenoid Content (ug/g)	0.15	0.95	2.27

Reagents

Gallic acid and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Fluka (Buchs, Switzerland). Folin–Ciocalteu reagent was obtained from Sigma (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical was purchased from Wako (Wako company, Japan). All solvents used in this research were analytical grade.

Preparation of the Control and Vegetable Pudding

The energy distributions of all formulas were adjusted to be in range of 55-65% carbohydrate, 10-15% protein and 25-30% lipid of total energy. All pudding recipes show in Table 2. In briefly, milk protein was dissolved in water, then left at room temperature for 30 minutes. Single type of vegetable powder was formulated as one flavor of vegetable pudding. Single type of vegetable powder was mixed together with milk protein before dissolving into water. Other dry ingredients, including, modified starch, sugar, maltodextrin, and carrageenan were mixed and dissolved in water, then left at room temperature for 30 minutes. All solution was mixed following to rice bran oil addition. After that, the mixture was homogenized using a rotor-stator (model Ultra Turrax T25, IKA® Works, Inc., Wilmington, NC, USA) at 10,000 rpm for 2 minutes, then heated at 85-90 °C for 10 minutes. The mixture was filled into polypropylene

(PP) plastic cup covered with heat seal lid. These products were sterilized using a water spray retort at 118°C for 33 minutes. The control (C) formula was a pudding without vegetable powder added (Table 2).

Table 2. Ingredient of control and vegetable powder pudding

Ingredient (%)	C	SP conc. 8%	SC conc. 8%	PK conc. 8%
Milk Protein	8.0	3.2	2.3	2.8
Rice Bran Oil	1.0	2.8	2.3	2.8
Maltodextrin	5.0	3.8	3.8	3.8
Sugar	7.0	4.4	4.8	4.8
Carrageenan	0.2	0.2	0.2	0.2
Modified starch	-	0.4	0.4	0.4
Water	78.8	77.2	78.2	77.2

Sensory Acceptability

Thirty completely edentulous elderly panelists, who aged over 60 years old, were representative for sensory acceptability testing. All pudding formulas were served at room temperature in plastic cup with lid. Panelist evaluated all four pudding formulas four times each, once in each session. Presentation order was randomized for each session and between panelists. Appearance, color, hardness, taste, and overall acceptability of all pudding formulas were evaluated using 5-point hedonic scale (1 = dislike very much, 3 = neither like nor dislike, and 5 = like very much).

Storage Test

The control and vegetable pudding were store at 35°C for 12 weeks. The puddings were randomly collected every 2 weeks to determine color. The changes of total phenolic contents and antioxidant activities were analyzed every 3 weeks.

Color Values

The color values were measured using Hunter Lab Digital Colorimeter (ColorFlex EZ, Hunter Associates Laboratory, Inc., Reston, Virginia) in CIE-color system (L*, a*, b*). The color parameters: L* represents lightness from black to white (0 to 100); a*; from green (-) to red (+); and b*; from blue (-) to yellow (+). The total color difference (ΔE) was calculated according to equations as follows:

$$\Delta E = \sqrt{(L - L^0)^2 + (a - a^0)^2 + (b - b^0)^2}$$

Where L and L^0, a and a^0, and b and b^0 corresponds to lightness, redness and greenness, blueness, and yellowness at final and the initial time of storage, respectively. The ΔE can be estimated the changes of color of the products during storage as not noticeable (0-0.5), slightly noticeable (0.5-1.5), noticeable (1.5-3.0), well visible (3.0-6.0), and great (6.0-12.0) [10].

Total Phenolic Contents (TPC) and Antioxidant activities (AA)

1. Sample extraction

Sample was extracted according to the procedures of [11] with slight modification. Six grams of sample was added with 20 mL of 70% (v/v) ethanol, and then shaken for 2 hours at room temperature for extraction. The sample was centrifuged (model Z 400K; HERMLE Labortechnik GmbH, Wehingen, Germany) at 4600 rpm for 10 minutes at 4 °C. Supernatant was collected for determination TPC and AA.

2. Total phenolic contents (TPC)

Total phenolic contents (TPC) were measured using Folin-Ciocaltue method [12]. The method was performed in 96-well flat-bottom plate. Twenty-five microliter of the ethanolic extract was mixed with 50 µL of Folin–Ciocalteu reagent (10% v/v). After 5 minutes of stand in dark at room temperature, sodium carbonate (7.5% w/v, 200 µL) was added, and mixed well. The mixture was then incubated in dark at room temperature for 2 hours. The absorbance was measured at 765 nm using the microplate reader (BioTek Instruments, Inc., Winooski, VT). Gallic acid was used as a standard. The TPC was showed as gallic acid equivalents (GAE) per gram.

3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH assay was performed according to the method of Fukumoto and Mazza [13] with some modification. Twenty-two microliter of the ethanolic extract was reacted with 200 µL of DPPH solution (150 µM in 70% (v/v) ethanol), in a 96-well flat-bottom plate before being incubated in dark at room temperature for 30 minutes. The absorbance was monitored at 520 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT). The DPPH value was showed as µmole trolox equivalent (TE) per grams of sample.

4. Oxygen radical absorbance capacity (ORAC) assay

ORAC assay was performed according to the method of Ou; et al. [14] with some modification. The reaction mixture consisted of ethanolic extract (25 µL), fluorescein solution (30 mM, 150 µL) and AAPH solution (153 mM, 25 µL) in 75 mM potassium phosphate buffer (pH 7.2). The assay was performed in 96-well flat-bottom black plate. The mixture of sample extract and fluorescein was incubated at 37 °C for 30 minutes prior to AAPH solution addition. The fluorescence intensity was monitored for 90 minutes using a microplate reader (Bio–Tek Instruments, Inc., Winooski, VT) with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The ORAC value was showed as µmole trolox equivalent (TE) per grams of sample.

Statistical Analysis

All parameters were analyzed in 3 replicates and reported as mean values ± standard deviation. One-way variance analysis (ANOVA) with Duncan's new multiple range test using SPSS software for Windows version 22.0 (SPSS Inc., Illinois, U.S.A.) was used to compared means of each parameter at a 5% level of probability.

Results

Vegetable puddings were developed as a prototype of ready-to-eat meal supplement for the elderly people. They did not only contain essential nutrients (protein, carbohydrates, fat, vitamins, and minerals), but also contain vegetable powder. Therefore, the elderly could increase their vegetable consumption which could be an alternative source of bioactive components. The puddings were sterilized using water spray retort to obtain the shelf stable product which can be stored at room temperature. In addition, they were filled in the polypropylene (PP) plastic cup covered with heat seal lid which could protect them from light. Figure 1. shows the appearance of the control and vegetable pudding.

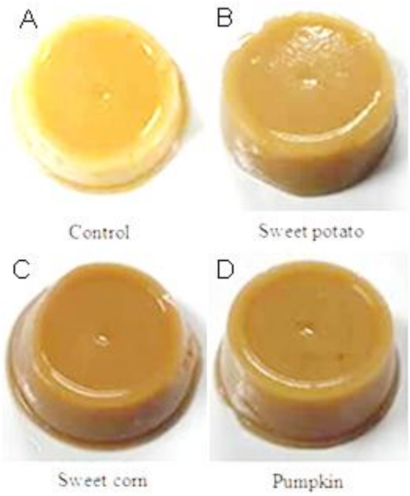

Figure 1. Photographs of control (A), sweet potato (B), sweet corn (C), and pumpkin (D) pudding.

Sensory Acceptability

The scores of sensory acceptability including appearance, color, hardness, taste, and overall acceptability are shown in Table 3. There was no significant difference among the mean values of appearance, taste, and overall acceptability in vegetable pudding samples compared with the control. From figure 1, the color of the developed products was darker. Therefore, the mean scores of color slightly decreased in PK and SC pudding, while this value significantly reduced in SP pudding compared to the control. Addition of vegetable powder improved the texture of the pudding, thus, the mean values of hardness of the developed pudding was significantly higher than that of the control. All vegetable puddings were accepted by elderly panelists with the mean score of overall acceptability at 4.1-4.5 (like moderately).

Table 3. Sensory acceptability score of control and vegetable pudding.[1, 2]

Formula	Appearance	Color	Taste	Texture	Overall acceptability
C	4.28 ± 0.68[a]	4.24 ± 0.66[b]	4.58 ± 0.58[a]	2.73 ± 0.67[a]	4.54 ± 0.25[a]
SP	3.97 ± 0.81[a]	3.79 ± 0.73[a]	4.17 ± 0.95[a]	4.33 ± 0.80[bc]	4.23 ± 0.86[a]
SC	4.27 ± 0.69[a]	3.93 ± 0.83[ab]	4.43 ± 0.73[a]	4.53 ± 0.68[c]	4.43 ± 0.73[a]
PK	4.10 ± 0.96[a]	4.10 ± 0.76[ab]	4.23 ± 0.90[a]	4.03 ± 0.96[b]	4.17 ± 0.91[a]

[1]Values are expressed as means ± S.D. (n = 30).

[2]Different letters in the same column indicate significant difference at $p \leq 0.05$.

Color Values

The color values of control and vegetable puddings are given in Table 4. The control had the highest value of lightness (L*), while redness (a*) and yellowness (b*) was the lowest. This indicated that the control puddings were lighter than all vegetable formulas as shown in figure 1. Among vegetable pudding, SP pudding was brighter than others due to greater lightness value and lower redness and yellowness than others. Effect of storage on the color values are illustrated in figure 2. The lightness of all pudding tended to decrease, while redness and yellowness slightly increased along the storage time. This implied that the products were darker when they were kept longer. The total color difference (ΔE) at week 12 compared to initial time is shown in figure 2. The results demonstrated that well visible differences were obtained in SC (ΔE = 5.74) and PK (ΔE = 4.24) pudding. In case of SP pudding, the great difference was observed (ΔE = 7.66).

Table 4. Color, DPPH, ORAC and TPC value of control and vegetable pudding.[1, 2]

Formula	Lightness L*	Redness a*	Yellowness b*	DPPH value (µmole TE/g)	ORAC value (µmole TE/g)	TPC (mg GAE/g)
C	72.60 ± 0.11[a]	8.64 ± 0.02[d]	26.15 ± 0.04[d]	0.274 ± 0.00[d]	3.803 ± 0.02[b]	0.287 ± 0.00[b]
SP	58.55 ± 0.03[b]	11.17 ± 0.00[c]	34.18 ± 0.01[c]	0.349 ± 0.01[c]	6.672 ± 0.54[a]	0.300 ± 0.01[b]
SC	45.81 ± 0.08[d]	14.27 ± 0.04[a]	35.63 ± 0.03[b]	0.757 ± 0.02[a]	6.947 ± 0.27[a]	0.344 ± 0.01[a]
PK	46.87 ± 0.07[c]	13.41 ± 0.01[b]	38.02 ± 0.07[a]	0.511 ± 0.02[b]	6.540 ± 0.09[a]	0.336 ± 0.01[a]

[1]Values are expressed as means ± S.D. (n = 3).

[2]Different letters in the same column indicate significant difference at $p \leq 0.05$.

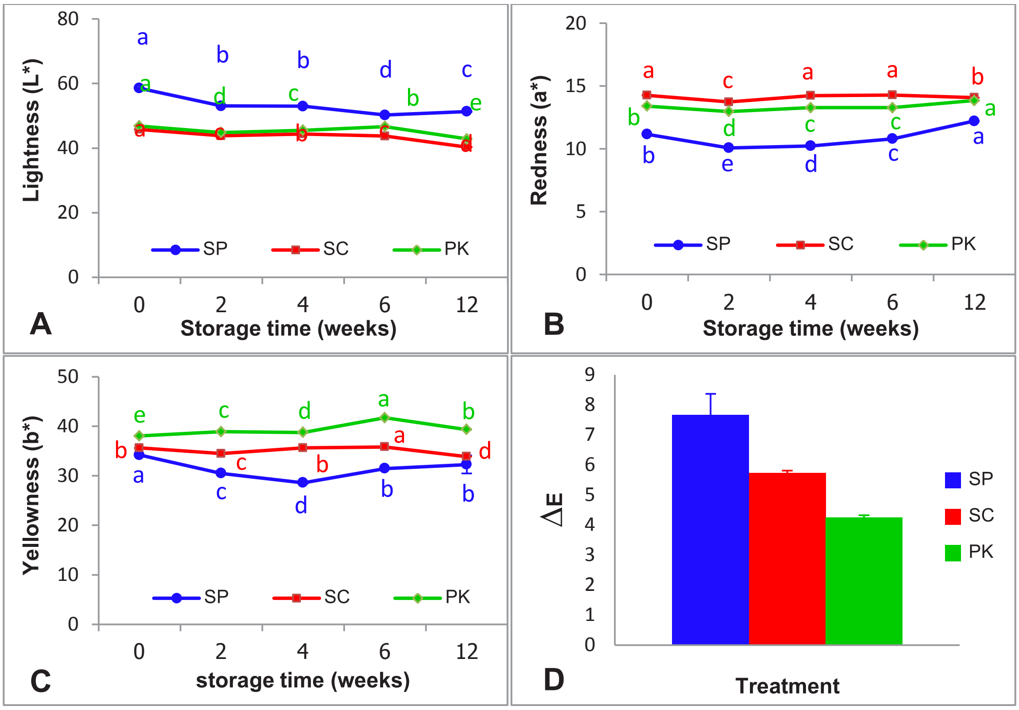

Figure 2. Lightness (A), Redness (B), Yellowness (C), and ΔE (D) of vegetable pudding during storage at 35 °C for 12 weeks. Different letters indicate significant differences amongst the storage time.

Total Phenolic Contents (TPC) and Antioxidant Activities (AA)

TPC and AA measured by DPPH and ORAC assay of control and vegetable puddings are presented in Table 4. As expected, addition of vegetable powder into the pudding formula increased TPC and AA. Pudding added SC and PK powder contained greater amount of TPC compared to the control and SP pudding. These results were in accordance with AA. The TPC content of SP pudding was similar to that of the control pudding, whilst the AA of it was higher than that of the control pudding.

The changes of TPC and AA of vegetable puddings during storage at 35 °C for 12 weeks are presented in figure 3. There was a trend that TPC increased to the maximum levels at week 6, then declined to the lowest amount at week 12 in vegetable pudding, except SC pudding.

The SP and SC pudding had the maximum DPPH value at week 6, and declined until week 12. On the other hand, the lowest DPPH value of pudding added PK powder was found at week 3, then gradually increased during storage time. In case of ORAC value, the different trend was observed. ORAC values of pudding added SP and SC increased from week 0 until week 3 and 6, respectively, after that the activities decreased. In contrast, PK pudding slowly declined along the storage time.

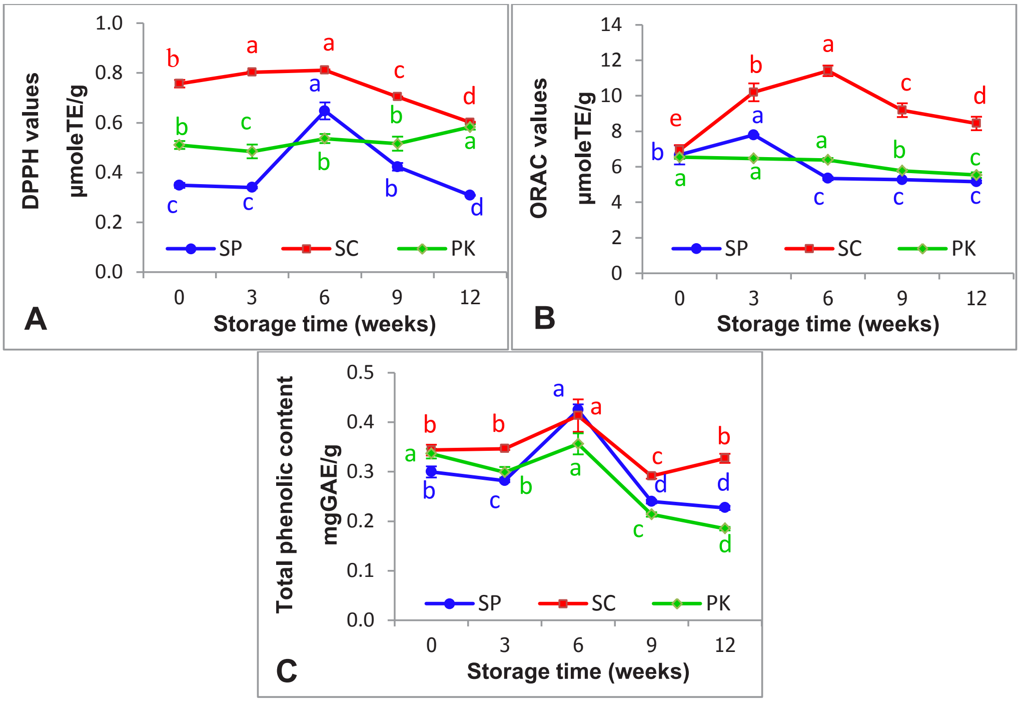

Figure 3. DPPH values (A), ORAC values (B) and TPC (C) of vegetable pudding during storage at 35 °C for 12 weeks. Different letters indicate significant differences amongst the storage time.

Conclusions and Discussion

Vegetable puddings were developed as a prototype ready-to-eat meal supplement containing essential nutrients for the elderly. All vegetable puddings were accepted by elderly panelists with overall acceptability score at like moderately. The color change of products during storage might be contributed by several factors, such as heat, light, and oxygen, which were prone to oxidation and chemical degradation [15]. The products were darker when they were kept longer that might be due to the Maillard products. The Maillard reaction occurs from sugar and protein interaction. These important phenomena happen during the processing, cooking or storage of much kind of foods [16].

The possibility of increase in TPC and AA during storage is the formation of some compounds such as Maillard products. Maillard products, for example reductones and melanoidins can be generated during thermal processing and storage. These compounds can be detected by Folin-Ciocaltue method and have the antioxidant, antimicrobial and anti-hypertensive activities [16-18]. Alternatively, reduction of TPC and AA may due to the gradation of bioactive components which act as antioxidants during the storage [19]. In addition, the storage temperature, light exposure, and oxygen permeability may cause the decline in TPC and AA during storage [20]. The different trend of AA observed from 2 methods may be because of the difference of mechanism. ORAC assay evaluated base on hydrogen atom transfer mechanism, while DPPH assay measured under mixed mechanism (hydrogen atom transfer and single electron transfer mechanism) [21].

Nutritional status of the elderly who is lack of chewing ability due to tooth loss is one of the biggest problems in aging society. Vegetable pudding is an alternative choice which is not only serves the basic nutrients, but also contains other health benefits such as AA. Bioactive compounds, for example phenolic compounds are responsible for this activity. The development of the pudding added vegetable powder including SP, SC and PK powder showed an enhancement of the phenolic compounds and AA in the pudding. The products were well-accepted form the elderly. The slightly reduction of TPC and AA was observed after kept the products at 35°C for 12 weeks. The vegetable pudding is a prototype product which can improve the nutritional status and quality of life of the elderly or one who have chewing problems.

Furthermore, carotenoid contents will be further conducted and the information of nutritional status of the elderly before and after consuming the vegetable pudding will be collected in order to confirm that these products can help to improve the nutritional status and quality of life of the elderly.

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