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DEVELOPMENT OF INSTANT PUMPKIN-FINGERROOT DRINK POWDER AND ITS SHELF LIFE MODELING

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Abstract

The objective of the present study was to develop a healthy instant pumpkin-fingerroot drink powder (IPFDP) using freeze-drying technique and establish the kinetic models to predict its shelf life based on two major indices viz., water activity (a_w) and sensory quality. Firstly, freeze-dried instant drink powder in different ratios of pumpkin and fingerroot (50:50, 70:30, 80:20 90:10, 100:0 and 0:100 (w/w)) were prepared and the qualities were investigated. It was found that the a_w of all IPFDP formulas were within the range 0.33-0.35. The instant drink powder prepared from pumpkin and fingerroot in the ratio of 80:20 (formula C) contained significant yields of **β**-carotene (6.25 μg/g) and total phenolic content (206.56 mg GAE/g) and also possessed the strongest 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity (39.15%). This formula also exhibited the highest score of all sensory attributes (7.56-7.98 scores). Based on these results, formula C was selected for consequent storage. The solid samples were packed in the aluminium foil bags under atmospheric pressure and then kept at temperatures of 4 °C, room temperature (32°C) and 45 °C for 70 days. IPFDP underwent increasing a_w values and color changes (*L**, *a**, *b**, total color difference and browning index) causing a lower sensorial consumer acceptance with the increases in storage period and temperature.

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The increase of a_w values and the decrease of overall acceptability scores followed zero-order kinetics and these deteriorations as a function of temperature conformed to the Arrhenius model (R^2>0.9). The activations energies were 19.55 and 13.41 kJ/mol for a_w and overall acceptability, respectively. The results pointed out the overall sensory quality as a limiting criteria for shelf life of IPFDP, thus, the IPFDP had a shelf life of 139 and 81 days at 4 and 32° C, respectively.

Keywords: Shelf life prediction, Kinetics, Pumpkin, Fingerroot, Instant Drink Powder

Introduction

Pumpkin (*Cucurbita* spp.) is an excellent source of nutrients and biologically active components such as phenolic acids, β-carotene, vitamin B and C, and also has pharmacological properties like anti-inflammation, immunomodulatory and antioxidant effects, which help to improve the health in current post COVID 19 period (Hussain et al., 2022). Fingerroot (*Boesenbergia rotunda* L.) contains bioactive compounds, especially panduratin A, an anti-SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) agent and also possesses bioactivities such as antioxidant, anti-inflammatory and anti-carcinogenic properties.

The processing of pumpkin and fingerroot into a functional high value-added drink is an attractive alternative. These materials can be prepared as an instant pumpkin-fingerroot drink powder (IPFDP) by drying and then grinding. Freeze-drying is a well-known technique for the manufacturing of high-quality food powder due to the operation at low temperatures. Many advantages of freeze-drying are preservation of nutrients and thermolabile bioactive compounds, and organoleptic properties, thereby permitting long-term storage (Dermesonluoglu et al., 2015).

During storage, the deterioration of physico-chemical characteristics in dried foods can be color degradation, browning, off-flavor development, moisture absorption and oxidative rancidity (Jena & Das, 2012). Degradation of food quality mainly depends on the storage temperature. A knowledge of the effect of storage temperature helps to predict the suitable storage conditions for maintenance of the quality (Dermesonluoglu et al., 2015). Kinetic modeling is an important approach to predict the changes in physico-chemical and nutritional properties and to estimate the shelf life (Jirasatid et al., 2019).

The kinetics of deterioration of a quality index can be determined from thermodynamic aspects based on the activation energy (E_a) (Kim et al., 2022).

For this, no information on the optimal formation of the IPFDP and the influence of storage temperature on the product's quality have been studied. The value of water activity or a_w (<0.6) was regarded as the threshold limit for shelf life of instant herb drink powder The sensorial consumer acceptance tests have often been used to estimate the shelf life with an index of 6/9 score of hedonic scale (like slightly) for overall acceptability being used as the lower limit (Dermesonluoglu et al., 2015). Therefore, this study proposed to produce and assess the quality and shelf life of IPFDP. Firstly, the effect of the ratios of pumpkin and fingerroot on the physico-chemical, functional and sensory properties of instant drink powders was investigated. Further, the effect of storage temperature on the quality of IPFDP was determined. Kinetic models were developed to evaluate the change quality and consequently predict the product's shelf life based on the excess of one of two thresholds, a_w values and sensory characteristics.

Materials and methods

Materials

Organic plants including ripe pumpkin (*Cucurbita* spp.) and fingerroot (*Boesenbergia rotunda* L.) were obtained from East Nawakaset Group, Chachoengsao, Thailand.

Development of Instant Pumpkin-Fingerroot Drink Powders

Pumpkin fruit was washed in tap water, peeled, cut into pieces 6×3 cm and steamed at 100°C for 45 min. Roots of fingerroot were washed in water to eliminate visible dirt and cut into pieces with 0.5 cm length. Six drink formulations were prepared by mixing of steamed pumpkin and fingerroot in different ratios of 50:50 (A), 70:30 (B), 80:20 (C), 90:10 (D), 100:0 (E) and 0:100 (F) (w/w) in which the formulas E and F were used as the controls. Sterile water was added in the ratio of 1:1 (w/w), homogenized using a blender (Philips HR2221, China) and consequently twice filtered through one layer of cheesecloth. Sweetness was adjusted with 2% (v/v) of stevia syrup (TSS of 25°Brix) on the basis of acceptability sensory testing with 30 untrained panelists using a 9-point hedonic scale.

The juices were drained into a tray with 2 cm thickness and frozen at -18°C for 24 hr in a freezer (MDF-263, Sanyo, Japan). The frozen samples were then freeze-dried

(DW 8-85, Heto Dry winner, Denmark) for 24 hr under vacuum conditions with pressure 132 Pa. Samples were blended using a blender (Philips HR2221, China) and sieved through a 120 mesh to obtain the fine IPFDP.

The physico-chemical (moisture content, water activity and visual color), functional (β-carotene, total phenolic content and DPPH antioxidant activity) and sensory properties of the samples were analyzed. The optimal formulation was chosen based on the functional and sensory properties to perform further storage tests.

Storage Experiments of Instant Pumpkin-Fingerroot Drink Powders

IPFDP (100 g) was packed in a polypropylene zip bag and subsequently packed in an aluminium foil bag. The aluminium foil bag was sealed using a hand sealer under atmospheric pressure. The size of the polypropylene zip bag was 8×11 cm with a thickness of 40 μm. The size of the aluminum laminated polyethylene (ALP) bag was 13×15 cm with a thickness of 80 μm. In order to conduct the accelerated storage of the IPFDP, the sample was stored either at 4°C, room temperature (32±2°C) or 45°C for 70 days (4°C: refrigerator, Toshiba, Japan and 45°C: incubator Model 600, Memmert, Germany). The temperatures inside the refrigerator, incubator and environment were monitored using thermometers (TH-03, Digicon, Japan). During storage, samples were taken out every ten days to examine the moisture content, water activity (a_w), visual color and sensory characteristics.

Analysis of Physico-Chemical Properties

Moisture content of IPFDP was determined using the method described by AOAC. (2000). Water activity (a_w) was measured using a digital water activity meter (EZ-200, Japan). Total soluble solid (TSS) of the reconstituted drink (ratio of instant drink powder:water = 1:1 w/w) was measured using a handheld refractometer (Master-M, Atago, Japan).

Color Measurement

CIE space color parameters of IPFDP were determined with a Hunter colorimeter (CR-400, Konika Minota, Japan). Visual color was expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values. The colorimeter was standardized with black and white plates. Total color difference (ΔE; TCD) between initial and stored samples was calculated using the Hunter-Scottfield equation (1) (Kara & Ercelebi, 2013).

$$\mathrm{TCD} = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

where L_0^*, a_0^* and b_0^* are color values at the initial time and L^*, a^* and b^* are color values at any given time of storage.

Browning index (BI) corresponds to the brown color of the samples, which results from the enzymatic or non-enzymatic browning reactions (Oliveira et al., 2012). BI was calculated by equations (2) and (3):

$$\mathrm{BI} = \frac{100(x - 0.31)}{0.172} \quad (2)$$

where

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)} \quad (3)$$

The dependence between the Hunter color parameters and BI was determined using linear relationships as defined by equation (4) (Kara & Ercelebi, 2013).

$$\mathrm{BI} = \beta_1(\text{Hunter parameter}) + \beta_2 \quad (4)$$

where β_1 and β_2 are constant coefficients.

Analysis of β-Carotene

Beta-carotene of IPFDP was determined according to Sombun et al. (2013) with some modification. Samples (0.1 g) were added to distilled water (1 mL) and left for 12 hr at room temperature in a conical tube. Twenty mL of acetone:hexane solution (2:3 v/v) was added, vortexed for 1 min and left at room temperature for 12 hr. The absorbance of the clear solution was measured at 663, 645, 505 and 453 nm with a spectrophotometer (Shimadzu, UV-1601, Japan) against a blank containing the same liquid chemical solutions used for this analysis without sample. Beta-carotene was calculated using the following formula (5):

$$\text{Betacarotene (mg/100g)} = 0.21A_{663} - 1.22A_{545} - 0.304A_{505} + 0.452A_{453} \quad (5)$$

Analysis of Total Phenolic Content (TPC) and DPPH Radical Scavenging Activity (DPPH RSA)

Firstly, samples (15 g) were extracted by 95% methanol (30 mL), kept in darkness for 24 hours, and then filtered using Whatman No.1 filter paper. TPC were analyzed by the Folin-ciocalteu colorimetric method (Karagozler et al., 2008). The absorbance of the reaction mixtures was measured at 765 nm using a UV-vis spectrophotometer (Shimadzu, UV-1601, Japan). Gallic acid (100-300 mg/mL) was used to prepare the standard curve and the results were expressed in terms of mg gallic acid equivalents (GAE) per g of sample. In addition, antioxidant activities of samples were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Karagozler et al., 2008). The absorbance of the mixtures was measured spectrophotometrically at 517 nm and the percentage of DPPH RSA was calculated using equation (6):

$$\mathrm{DPPH\ RSA\ (\%)} = \left(\frac{\mathrm{A_0} - \mathrm{A_1}}{\mathrm{A_0}}\right) \times 100 \tag{6}$$

where A_0 and A_1 are the absorbance of control and sample, respectively.

Hedonic Sensory Evaluation

Sensory evaluation of aqueous solutions of the IPFDP was performed by 30 untrained panelists between 20 and 35 years of age. The testing panel composed of students and staffs from Rajabhat Rajannagarindra University, Thailand. All panelists were informed about sensory evaluation before the test. The instant drink formulations were reconstituted by adding water in the ratio of 1:1 (w/w) and well stirred for 3 min. Samples (30 mL) were prepared in white plastic cups, coded with a three-digit random number and served at ambient temperature. Evaluations of appearance, color, odor, taste and overall acceptability were performed using a 9-point hedonic scales (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9= like extremely) (Srisuk et al., 2021) In addition, the dependence of sensory score for color on BI or TCD was described by the following equation (4). The sensory evaluation was managed following approval by the Human Ethics Committee of Rangsit University, Thailand (Ethics approval number: RSUERB2021-112).

Kinetics Analysis during Storage

The reaction rate (*dc/dt*) was described using a simple kinetic model following equation (7):

$$\frac{dC}{dt} = \pm kC^n \tag{7}$$

where k is the rate constant, n is the order of the reaction and C is the quantitative value of a_w or overall sensorial acceptability (Jirasatid & Nopharatana, 2021).

A negative sign in equation (7) indicates the degradation of quantitative values, which includes a loss in overall acceptability score with time. A positive sign shows that the quantitative values including a_w increase with time (Jirasatid et al., 2019). Kinetic model was proposed and interpreted to a mathematical model. A differential equation using zero-, first- and second-order kinetics (n = 0, 1 and 2, respectively) was solved by numerical integration and fitted to the experimental data through non-linear regression (Jirasatid & Nopharatana, 2021). The kinetic order of the reaction deduced from the best fit line utilizing the coefficient of determination (R^2) and root mean square percent error (RMSE, %).

The dependence of the rate constant on temperature can be analyzed using the Arrhenius equation (8):

$$k = k_0 exp\left[-\frac{E_a}{RT}\right] \tag{8}$$

where k is the rate constant, k_0 is the Arrhenius constant, T is the absolute temperature (K), R is the ideal gas constant (8.3145 J/mol•K), and E_a is the activation energy (J/mol), which was calculated by multiplying R by the slope in the plot of ln k versus $1/T$ (Jirasatid & Nopharatana, 2021; Babosa-Canovas et al., 2007).

The half-life time ($t_{1/2}$) is the time required for a 50% of decrease or increase of the initial value of a given quantitative parameter. The half-life values for zero-order reactions were calculated by equation (9):

$$t_{1/2} = \frac{C_0}{2k} \tag{9}$$

where C_0 is an initial content and k is the rate constant

Statistical Analysis and Model Evaluation

The goodness of model fitting to the experimental data was tested by considering the R^2 (equation (10)) and RMSE values (equation (11)), obtained from the kinetic plots with n = 0, 1 and 2, respectively. The highest of R^2 and lowest of RMSE were accepted as the best model fitting to the experimental data. R^2 values close to 1 indicated that the curve followed closer to the data.

$$\mathrm{R}^2 = 1 - \frac{\sum_{i=1}^{\mathrm{n}}(\mathrm{P_{obs}}-\mathrm{P_{pred}})^2}{\sum_{i=1}^{\mathrm{n}}(\mathrm{P_{obs}}-\mathrm{P_{mean}})^2} \tag{10}$$

$$\mathrm{RMSE\%} = \sqrt{\frac{1}{\mathrm{n}}\sum_{i=1}^{\mathrm{n}}\left(\frac{\mathrm{P_{obs}}-\mathrm{P_{pred}}}{\mathrm{P_{obs}}}\right)^2} \times 100 \tag{11}$$

where P_{obs} is the observed value, P_{pred} is the predicted value, P_{mean} is the average value from all observed values and n is the number of observation data.

The experiments were performed with a completely randomized design (CRD). Sensory data were carried out with a Randomized Complete Block Design (RCBD). Statistical analysis was determined by analysis of variance (ANOVA) using Minitab statistical software version 18 (Minitap Pty Ltd, Australia). Significant difference of the mean values was established according to Tukey's multiple range test at $p \leq 0.05$.

Results and Discussion

Development of Instant Pumpkin-Fingerroot Drink Powders

In this study, it was found that the ratios of pumpkin and fingerroot did not significantly affect a_w and moisture content of instant drink powders ($p>0.05$), but influenced the visual color and antioxidant properties of the products ($p \leq 0.05$) (Table 1 and 2). The a_w and moisture content of all instant drink formulas varied within the range 0.33-0.35 and 5.52-5.75%, respectively. The a_w and moisture content of dried food are important factors for the quality and shelf life. With a_w about 0.3, instant drink powders were quite stable with respect to lipid oxidation, browning reaction, enzyme activity and various microbial growths. Values of a_w <0.6 and/or moisture content <10% are ideal for dried foods such as instant drink powder (Akther et al., 2020).

The IPFDP were light yellow in color. The L^* values (brightness) of all IPFDP were almost similar (62.9-69.2) as well as the $+a^*$ (redness) values (7.2-8.9) (Table 1). The high values L^* of freeze-dried instant drink powders indicated the preservation of color characteristics resulting from the protection of possible browning reactions during freeze-drying technique. The visual $+b^*$ values of IPFDP ranged from 12.6 to 52.1 (Table 1). It was obvious that sample F had the lowest b^* (12.6) due to the low content of β-carotene (5.36 μg/g), while the other formulas containing pumpkin showed higher values of b^* and β-carotene. This was because pumpkin contained higher amounts of β-carotene, which resulted in the color appearing more yellow to red, compared to fingerroot. Murkovic et al. (2002) reported that the carotenoids in pumpkin varieties ranged from 0.6 to 74 μg/g for β-carotene, 0 to 75μg/g for α-carotene and 0 to 170 μg/g.

Table 1 Water activity (a_w), moisture content and visual color of instant pumpkin (P)-fingerroot (F) drink powders with different formulations.

Sample (P:F)	a_w ^{ns}	Moisture content (%) ^{ns}	Color		
			L^*	a^*	b^*
A (50:50)	0.34±0.01	5.61±0.10	69.2±0.9^{a}	8.5±1.3^{b}	52.1±0.1^{c}
B (70:30)	0.35±0.01	5.66±0.08	68.7±0.8^{c}	7.9±0.9^{c}	54.8±1.0^{b}
C (80:20)	0.34±0.00	5.59±0.12	67.7±0.4^{d}	7.5±0.5^{d}	55.1±0.9^{a}
D (90:10)	0.35±0.01	5.52±0.04	69.0±0.3^{b}	7.5±0.2^{d}	49.3±1.3^{d}
E (100:0)	0.35±0.01	5.67±0.03	62.9±0.5^{e}	7.2±0.1^{e}	47.4±1.2^{e}
F (0:100)	0.33±0.00	5.75±0.05	67.5±0.6^{d}	8.9±0.7^{a}	12.6±1.3^{f}

Remark Values are means ± SD. Values with different small letters in a column are significantly different at $p \leq 0.05$. ^{ns} not significant in a column ($p > 0.05$).

Sample C contained the highest amount of TPC (206.56 mg GAE/g), which were in concordance with the strongest antioxidant activity (39.15%). In contract, sample F had the lowest TPC (194.69 mg GAE/g) and also corresponded to the weakest antioxidant activity (36.68 %) (Table 2). However, all samples exhibited high TPC resulting from both pumpkin and fingerroot being rich in TPC (Table 2). Zdunic et al. (2016) reported that pumpkin fruit contained TPC and total carotenoids of 0.906 mg GAE/g and 86.3 µg/g, respectively. Additionally, Jirakiattikul et al. (2021) found that the shoot base and rhizome of fingerroot had high accumulation of TPC with approximately 168.98-172.21 mg GAE/g.

Table 2 Functional properties of instant pumpkin (P)-fingerroot (F) drink powders with different formulations.

Sample (P:F)	β-carotene (µg/g)	TPC (mg GAE/g)	DPPH (%)
A (50:50)	6.02±0.01^{d}	199.76±0.00^{b}	37.16±0.01^{c}
B (70:30)	5.28±0.02^{f}	198.92±0.02^{bc}	36.98±0.02^{c}
C (80:20)	6.25±0.00^{c}	206.56±0.00^{a}	39.15±0.01^{a}
D (90:10)	6.42±0.01^{b}	198.56±0.01^{bc}	37.72±0.02^{c}
E (100:0)	6.83±0.00^{a}	196.45±0.02^{c}	38.45±0.00^{b}
F (0:100)	5.36±0.01^{e}	194.69±0.04^{d}	36.68±0.01^{d}

Remark Values are means ± SD. Values with different small letters in a column are significantly different at $p \leq 0.05$. TPC: total phenolic content; DPPH: DPPH radical scavenging activity.

Simple correlation pointed out the interrelationship between bioactive compounds (TPC and β-carotene) and DPPH antioxidant activity of IPFDP. The changes in TPC of IPFDP was positively correlated with the changes in DPPH antioxidant activity (r = 0.687) as well as β-carotene and DPPH antioxidant activity (r = 0.742).

The hedonic term for the overall acceptability of all IPFDP ranged between “like slightly” to “like very much” (6.09-7.98 scores), indicating the acceptance of the samples by the panelists (Table 3). In appearance, color, odor and taste, there were significant differences among the assessed samples, impacting the significant difference in overall acceptability ($p \leq 0.05$). This demonstrated that the ratio of pumpkin and fingerroot in instant drinks greatly influenced sensory attributes. The sweet taste of all products was improved by stevia, a zero calorie natural sweetener. TSS of reconstituted instant drinks ranged from 20.0 to 20.3°Brix ($p > 0.05$) (data not shown). The gratifying taste of the products caused consumer acceptance. Sample C had the highest score of overall acceptability (7.98 scores), which was presumably a result of the panelists’ liking for its odor, color and taste.

The above results were in agreement with the study of Rittisak et al. (2022) who reported that the instant beverage prepared from broken riceberry had an a_w of 0.354, moisture content of 4.28% and DPPH antioxidant activity of 45%. Based on our results, sample C was chosen to perform further storage tests. The consumption of 200 g formula C beverage (100 g of instant drink powder and 100 g of water) obtained high bioactive compounds like β-carotene (625 μg) and TPC (20.6 g). Thus, the product was confirmed as a functional drink promoting health benefits.

Table 3 Sensory attributes of reconstituted instant pumpkin (P)-fingerroot (F) drink powders with different formulations.

Sample (P:F)	**Sensory attributes (scores)**				
	Appearance	Color	Odor	Taste	Overall acceptability
A (50:50)	6.17 ± 0.83^{e}	6.46 ± 1.19^{c}	6.37 ± 1.31^{c}	6.04 ± 0.76^{d}	6.49 ± 1.31^{c}
B (70:30)	6.13 ± 1.31^{e}	6.37 ± 1.19^{c}	6.64 ± 0.59^{d}	6.06 ± 1.00^{d}	6.09 ± 1.09^{c}
C (80:20)	7.56 ± 0.93^{a}	7.89 ± 1.16^{a}	7.98 ± 0.53^{a}	7.79 ± 1.48^{a}	7.98 ± 1.30^{a}
D (90:10)	6.36 ± 0.82^{d}	6.44 ± 1.07^{d}	6.40 ± 0.97^{c}	6.21 ± 1.00^{c}	7.40 ± 0.97^{b}
E (100:0)	7.15 ± 0.35^{b}	7.03 ± 0.95^{b}	6.96 ± 0.42^{b}	6.28 ± 0.48^{c}	7.08 ± 1.23^{b}
F (0:100)	6.76 ± 0.76^{c}	6.93 ± 0.38^{bc}	6.23 ± 0.71^{c}	6.63 ± 0.57^{b}	6.28 ± 1.34^{c}

Remark Values are means ± SD. Values with different small letters in a column are significantly different at $p \leq 0.05$.

Storage Experiments of Instant Pumpkin-Fingerroot Drink Powders (Formula C)

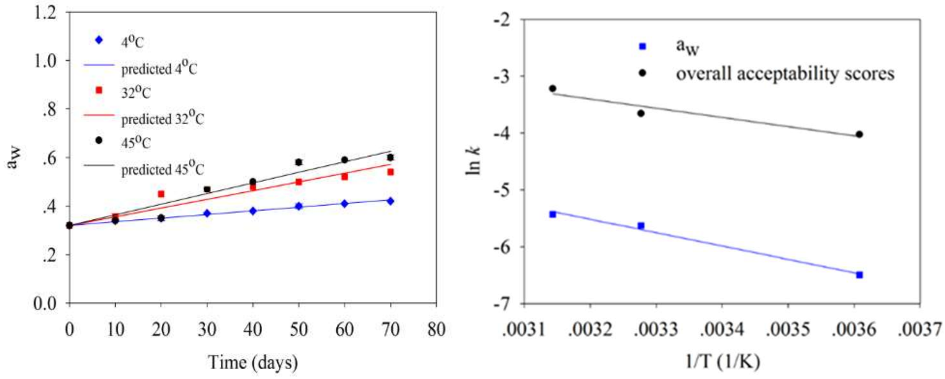
Moisture Content

The results revealed that the storage temperature greatly influenced the moisture content. The initial moisture content of IPFDP was 5.64±0.02%. At the end of storage for 70 days, the moisture contents of the assessed samples at 4, 32 and 45°C increased to 5.90, 9.25 and 15.23%, respectively. The moisture content of the stored samples at 4°C increased slightly ($p \leq 0.05$), while there were dramatic increases in the moisture content for samples stored at 32 and 45°C ($p \leq 0.05$). This indicated that transmission of water vapor from environment into the packaging resulted in an increase of the moisture content of the instant drink powders. Samples absorbed higher moisture under higher storage temperatures. This result was in agreement with Razak et al. (2018), the storage of encapsulated *Orthosiphon stamineus* spray-dried powder, where the storage condition under low temperature was an efficient method to retain the quality of dried food products due to the reduction of transmission of water vapor.

Water Activity

Water activity is the amount of available water for microbial growth, chemical and biochemical changes. It is one of the critical parameters used to estimate the shelf life of food products because specific changes in color, texture and sensory characteristics of many products are related to narrow a_w ranges Figure 1a illustrates the changes of a_w of IPFDP during storage at various temperatures. The a_w of all samples increased progressively with storage time ($p \leq 0.05$) and also increased with temperature. Initial a_w for IPFDP was 0.32 and rose to 0.42-0.6 at the end of storage at temperature of 4-45°C. A mechanistic explanation was that the diffusion of water vapor from the environment into the system (packaging) throughout the observation period resulted in an increase of vapor pressure in foods. The increase of a_w or water content could tend to dissolve and consequently mobilize reactant species This suggests that as a_w increased from 0.3 to 0.6, the probability of deterioration increased by lipid oxidation, browning reactions and enzyme activity, but it was free from microbial growth such as mold, yeast and bacteria (Dilrukshi & Senarath, 2021). The changes of a_w values were in concordance with the changes of moisture content. The highest increase in a_w presented after 70 days of storage at 45°C and was approximately 1.9 times of an initial value, whereas the smallest increase was approximately 1.3 times of stored sample at 4°C.

Non-linear regression analysis found that the increasing of a_w of IPFDP at the storage temperatures of 4-45°C followed zero-order reaction kinetics based on the relatively high R^2 and low RMSE (R^2=0.893-0.992, RMSE=0.925-7.135%), conversely, lower R^2 and higher RMSE were achieved using first- (R^2=0.845-0.985, RMSE=1.341-7.946%) and second-order kinetic plots (R^2=0.790-0.975, RMSE=1.838-9.933%) as seen in Figure 2 and Table 4. The reaction rate constants (k) increased at higher temperature, confirming that the increase of a_w value was dependent on temperature (Table 4). The linear correlation of $\ln k_{aw}$ vs $1/T$ showed that the rate constants followed the Arrhenius model as shown by the high coefficient of determination (R^2=0.929) (Figure 1b). With respect to the studied temperatures (4-45°C), the activation energy (E_a) for the increasing of a_w values was calculated to be 19.55 kJ/mol. The time for a 50% increase ($t_{1/2}$) of a_w for the IPFDP within the studied temperatures varied from 36.6 to 106.0 days (Table 4). An increase of temperature caused the half-life time of the IPFDP to diminish. The kinetics of changes of a_w of food products has been studied by many researchers. Weiss et al. (2018) found that the changes of a_w in spreadable processed Gouda cheese during storage at 8-30°C fitted a first-order reaction model and the activation energy was 79.31 kJ/mol. Zardetto et al. (2021) reported that a_w of fresh egg pasta during storage at 0-10°C decreased by a pseudo-zero order reaction with an activation energy of 28 kJ/mol. Similar to the results obtained here, the rate constant increased with higher temperature. Moreover, the lower activation energy for changes of the a_w value implied that the increase of a_w in IPFDP was less susceptible to change in temperature as compared with the changes of a_w in Gouda cheese or fresh egg pasta.



(A) (B)

Figure 1 Zero-order kinetic plot of increases of a_w values during storage of instant pumpkin-fingerroot drink powders (A). and Effect of temperature on deterioration of a_w and overall acceptability scores following the Arrhenius model for instant pumpkin-fingerroot drink powders during storage (B).

Table 4 Kinetic parameters for deterioration quality of a_w and overall acceptability scores of instant pumpkin-fingerroot drink powders during storage.

Reaction	Temperature (˚C)	k	R^2	RMSE (%)	$t_{1/2}$ (days)	Arrhenius model		
						E_a (kJ/mol)	k_0	R^2
a_w	4	0.0015	0.992	0.925	106.0	19.55	7.44	0.989
(zero order)	32	0.0036	0.893	6.147	44.6			
	45	0.0044	0.937	7.135	36.6			
overall	4	0.018	0.795	3.345	234.6	13.41	5.80	0.917
acceptability	32	0.026	0.946	2.024	162.7			
(zero order)	45	0.040	0.907	4.976	104.9			

Remark k: reaction rate constant, day^{-1} for change of a_w, scores/day for loss in overall acceptability; R^2: coefficient of determination; RMSE: root mean square error; $t_{1/2}$: half-life time; E_a: activation energy; k_0: Arrhenius constant, day^{-1} for change of a_w, scores/day for loss in overall acceptability.

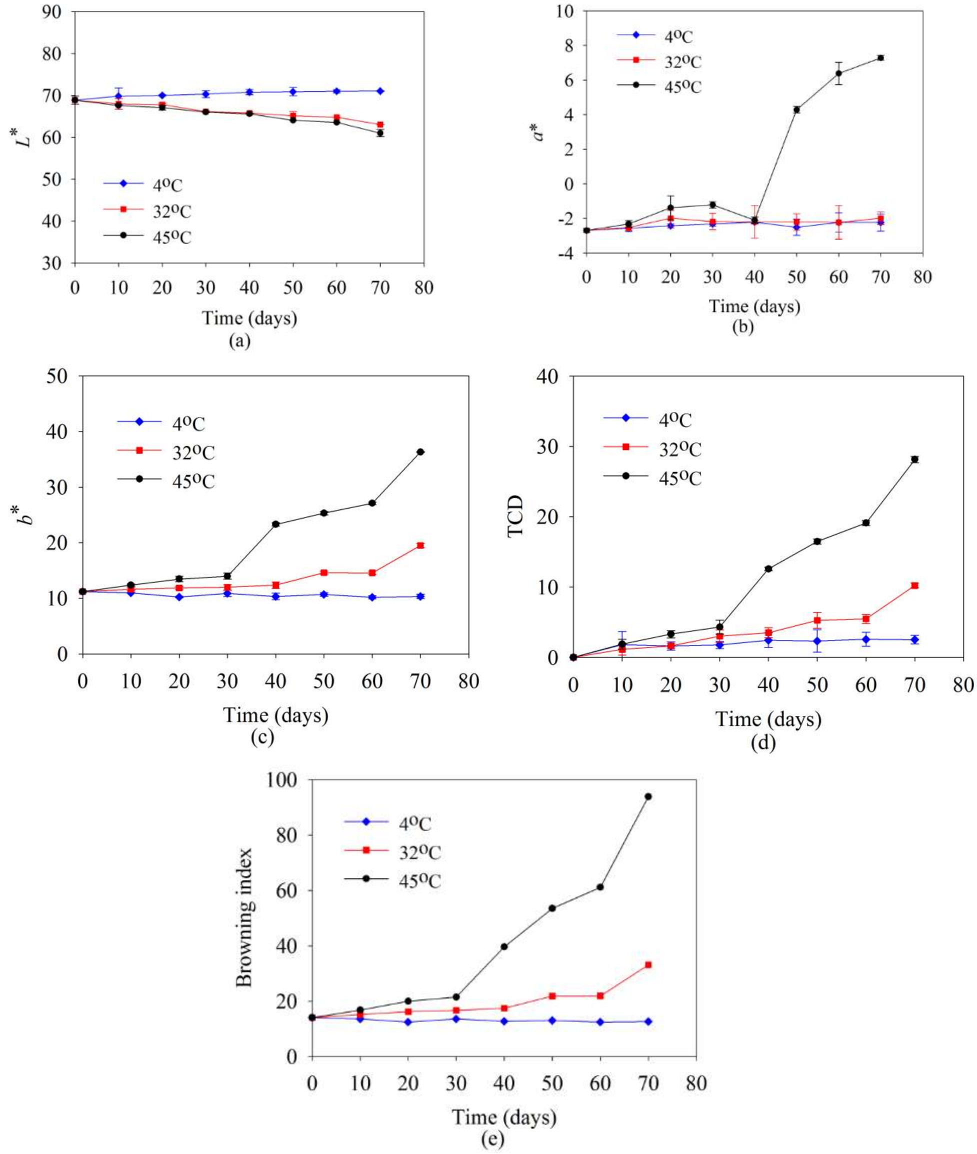


Figure 2 Changes in (a) *L**, (b) *a**, (c) *b**, (d) total color difference (TCD) and (e) browning index of instant pumpkin-fingerroot drink powders during storage at 4, 32 and 45˚C.

Color Parameters

Color parameters including *L**, *a**, *b**, TCD and BI of IPFDP were analyzed once every 10 days for a total of 70 days at various temperatures and the changes are presented in Figure 2(a) to (e). Hunter *L** values (lightness) decreased significantly as a function of storage time ($p \leq 0.05$) and temperature, except for the sample stored at 4˚C

($p>0.05$) (Figure 2(a)). The decrease of the *L** value was reflected in a browning of the sample. The sample darkened when the storage temperature and time increased. At the end of storage, *L** values were about 100, 91 and 85% of the initial value in samples kept at 4, 32 and 45 ˚C, respectively.

Color parameter *a** rose only slightly from -2.69 to -2.23 at 4 ˚C and -1.99 at 32˚C after 70 days, indicating greenness of the IPFDP ($p>0.05$). On the contrary, a significant increase of *a** value was observed under storage at 45 ˚C ($p\leq0.05$). It increased from -2.69 to +7.29, indicating a shift from green to red hues of the IPFDP (Figure 2(b)).

The relative visual *b** values increased directly with storage time at temperatures of 32 and 45˚C ($p\leq0.05$), showing a general change to a yellower hue. Hunter *b** values of the samples varied within the ranges 11.21-10.35, 11.21-19.49 and 11.21-36.30 at temperatures of 4, 32 and 45 ˚C, respectively (Figure 2(c)).

Total color difference (TCD) is the color difference between the stored sample and the initial sample. TCD < 1, the color differences could not be seen by the human eyes, 1 < TCD < 3 , the human eyes can see minimal color differences subject to the angle, while TCD> 3, the color differences are visible by the human eyes Urbina et al. (2021) The results showed that TCD increased with time and temperature, especially at 32 and 45˚C ($p\leq0.05$) resulting from the changes of *L**, *a** and *b** values (Figure 2(d)). Color changes of 2.55, 10.21 and 28.17 units were obtained after storage at 4, 32 and 45˚C, respectively. This represented a significant difference in the visible color by the human eyes for the products stored at 32 and 45 ˚C.

Similar to TCD, BI increased greatly with time and temperature, and varied from 14.03 to 33.14 at 32˚C and to 93.85 at 45˚C. However, there was not found to be a browning in the sample stored at 4˚C (Figure 2(e)). This suggests that the main portion of the changes in color values during storage at 32 and 45˚C were obviously because of the formation of browning pigment. Browning could be due to non-enzymatic browning reaction like Maillard. It can be concluded that the color parameters were influenced significantly by temperature. However, the color parameters were stable at 4˚C obvious from results above.

Correlation between the Hunter color parameters (L^*, a^*, b^* and TCD) and BI of IPFDP was described using a linear relationship as shown in Table 5. BI indicated a high negative correlation with L^* (r = -0.931, $p<0.01$), but BI indicated a high positive correlation with a^*(r = 0.922, $p<0.01$), b^* (r = 0.966, $p<0.01$) and TCD (r = 0.973, $p<0.01$). High values of correlation coefficients in the above relationship indicated that BI had a stronger relationship with TCD changes than that of the changes of b^*, L^* and a^* values, respectively. This result clearly confirmed that the decrease of L^* and the increases of a^* and b^* values were related to the formation of browning pigments, which resulted in the increase of intensity of darkness, redness and yellowness in IPFDP. A similar relationship was also reported by Lee & Nagy (1988).

Table 5 Correlation coefficients (r) for the relationship between the color parameters (L^*, a^*, b^* and TCD) with BI, and TCD and BI with the sensorial color score for instant pumpkin-fingerroot drink powders during storage.

Index	BI	Sensory score for color
L^*	-0.931*	
a^*	0.922*	
b^*	0.966*	
TCD	0.973*	-0.880*
BI		-0.847*

Remark *$p<0.01$

Hedonic Sensory Evaluation

Changes in sensory scores of reconstituted drinks during storage at 4 °C, 32 °C and 45°C for 70 days of IPFDP are shown in Figures 3 and 4. Initially, the sensorial scores for appearance (7.34 score), color (7.35 score), odor (7.95 score), taste (7.99 score) and overall acceptability (8.39 score) were described within the level of 'like moderately' to 'like extremely'. Nonetheless, the scores of all sensory characteristics declined continuously with the progression of storage time ($p \leq 0.05$). This was ascribed to the displeasing color and rancid flavor in the reconstituted drinks caused by the formation of browning pigments and lipid oxidation, particularly under storage at 45 °C. In Table 5, the relationship between the scores for color and TCD was found to be negatively correlated (r = -0.880, $p<0.01$) as well as the scores for color and BI (r = -0.847, $p<0.01$).

This indicates the increase of TCD or BI significantly related to the decrease of color scores. The results for the sensory and color characteristics of the products clearly revealed that IPFDP stored at low temperature (4°C) was preserved excellently resulting in the prolongation of shelf life.

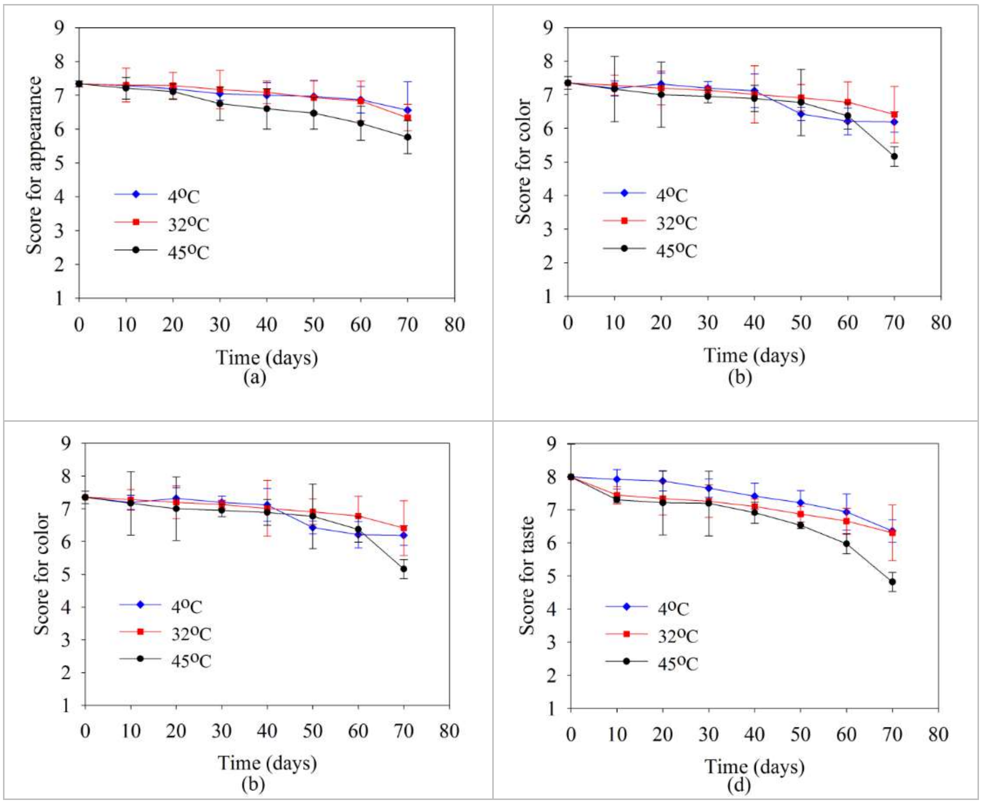


Figure 3 Sensory scores for (a) appearance, (b) color, (c) odor and (d) taste for instant pumpkin-fingerroot drink powders during storage at 4, 32 and 45°C.

Consumer acceptance based on the sensory properties is an important direct quality index for estimation the shelf life of food products. In general, overall sensorial acceptability is used to substitute for all sensory characteristics (Shukla et al., 2020). The kinetics of deterioration of overall acceptability scores was thus determined. As seen in Figure 4, non-regression analysis showed that the overall acceptability scores fitted the best with zero-order reaction kinetics on the basis of high R^2 and low RMSE (R^2=0.795-0.946, RMSE=2.024-4.976%) as compared with first-(R^2=0.781-0.956, RMSE=1.757-5.640%) and second-order models (R^2=0.768-0.963, RMSE=1.548-6.258%) (Table 4). The deterioration rate (*k*) rose systematically with temperature (Table 4). These values were higher than the *k* values of changes of a_w (0.0015 to 0.0044), indicating a higher deterioration rate of sensorial overall acceptability of samples. The activation energy was estimated from an Arrhenius plot and was 13.41 kJ/mol (Figure 1b). The model showed a good fit to the temperature dependence of *k* values (R^2=0.917). Like these results, many studies found that the decline of overall acceptability scores in food products followed zero-order kinetic reaction (Dermesonluoglu et al., 2015).

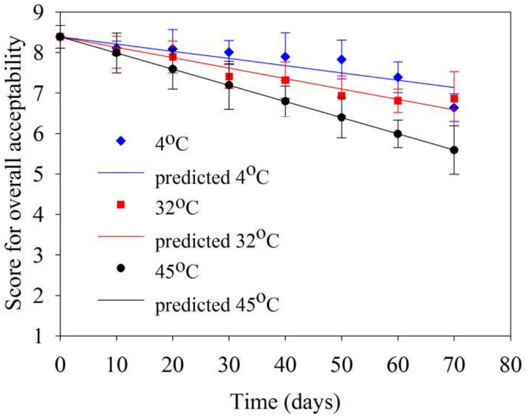


Figure 4 Zero-order kinetic plot of declining sensory scores for overall acceptability for instant pumpkin-fingerroot drink powders during storage. Experimental data and simulation data are the symbols and solid lines, respectively.

Shelf Life Predictions

The mathematical models for the prediction of the shelf life of IPFDP were developed based on two major critical indices viz., a_w value and overall acceptability scores. According to Thai Community Product Standard (TCPS) a_w value of 0.6 was regarded as the threshold limit for instant drink powder. A sensory acceptability score of 6 corresponding to ‘like slightly’ would be the minimum acceptable score for assessing of shelf life (Bunkar et al., 2014). Simple equations for shelf life calculation can be expressed based on a zero-order kinetic reaction (equations (12) and (13)).

$$t_{SL} = \frac{a_{w1} - a_{w0}}{k_0 \exp\left[\frac{-E_a}{RT}\right]}$$ equation (12) based on a_w value

$$t_{SL} = \frac{S_0 - S_1}{k_0 \exp\left[\frac{-E_a}{RT}\right]}$$ equation (13) based on sensory score

where t_{SL} is shelf life of instant drink powder (days), a_{w1} is the limiting a_w value (0.6), a_{w0} is the initial a_w value (0.32), S_1 and S_0 are the limiting (6 score) and initial sensory scores (8.39 score) for overall acceptability, respectively, k_0 and E_a are the Arrhenius constant and activation energy, respectively, of the change in each quality index (Table 4) and R is the gas constant (Jirasatid et al., 2019).

Table 6 Shelf life (t_{SL}, *days*) calculation for instant pumpkin-fingerroot drink powders stored at 4 and 32 °C based on the quality indices (a_w = 0.6 and overall acceptability score = 6).

Temperature (°C)	a_w, t_{SL} (day)	Sensory, t_{SL} (day)
4	182	139
32	84	81

Table 6 shows the predicted shelf life value of the stored IPFDP at refrigerator (4°C) and room (32°C) temperatures. The shelf life at 32° Calculated values based on a_w and sensory evaluation was similar, but significantly different as compared to the sample stored at 4°C. The shelf life values estimated based on sensory evaluation were shorter than those estimated by the kinetic modeling of a_w. Thus, the shelf life should be determined from the more conservative sensory evaluation criteria. Therefore, it was concluded that the shelf life of IPFDP under storage at 4 and 32°C were 139 and 81 days, respectively. This finding showed the importance of storage of IPFDP at low temperature in order to restrict changes to qualities such as a_w, color parameters and browning, and thus extend the shelf life of the product.

Validation of Kinetics Modeling

The suitability of the kinetic modeling to estimate the experimental results was analyzed. The Arrhenius model was used to predict the k values from changes of a_w values and overall acceptability scores. The a_w values and overall acceptability scores at any time of storage at various temperatures were then calculated using a zero-order kinetic reaction equation. The resulting predictions and the actual deterioration obtained experimentally for a_w and overall acceptability scores are displayed in Figure 5(a) and (b), respectively. The Arrhenius model fitted reasonably well with the experimental data for a_w with high R^2 of 0.892-0.992 and low RMSE of 0.968-7.543% and overall acceptability scores with R^2 and RMSE intervals of 0.796-0.946 and 3.017-6.024%, respectively.

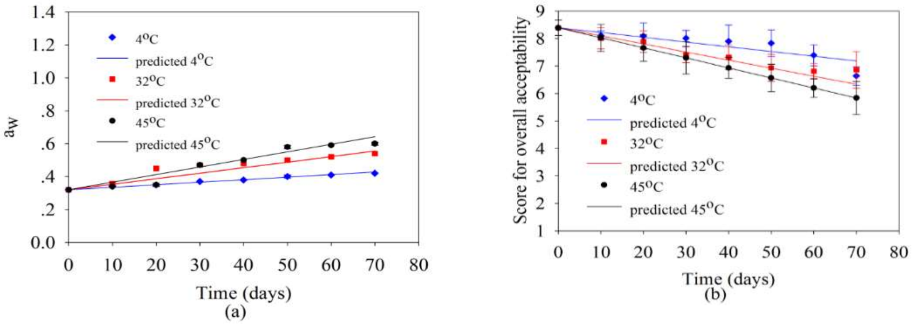


Figure 5 Kinetic model validation using zero-order reaction for changes of (a) a_w and (b) overall acceptability scores for instant pumpkin-fingerroot drink powders during storage. Experimental data and simulation data are the symbols and solid lines, respectively.

Conclusions

This study outlined the development of IPFDP using the freeze-drying technique with good taste and quality. The instant drink powder prepared from pumpkin and fingerroot in the ratio 80:20 w/w (formula C) contained significant amounts of β-carotene (6.25 μg/g) and TPC (206.56 mg GAE/g) and also exhibited potential for DPPH antioxidant activity (39.15%). The changes of quality of formula C IPFDP during storage revealed a significant deterioration of the a_w values, color parameters and sensory characteristics with the increasing of time and temperature. Kinetic modeling based on the Arrhenius relationship of the deterioration changes of the index parameters (a_w and overall acceptability scores) in IPFDP during storage for 70 days were developed for predicting the shelf life under an accelerated condition at temperature of 4-45 ˚C. The a_w values and overall acceptability scores followed zero-order kinetic models. The shelf life prediction of the IPFDP under storage 4˚C and ambient temperature (32˚C) was 139 and 81 days, respectively based on the sensory evaluation criteria, which is the most index parameters influencing consumer acceptance for IPFDP. Overall, IPFDP can be introduced as a functional drink, which is a good source of bioactive compounds and also was sensorial accepted throughout its shelf life.

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