

# THE SHELF-LIFE OF TOTAL POLYPHENOL CONTENT AND THE ANTIOXIDANT CAPACITY OF THE *POLYGONUM MULTIFLORUM* (THUNB.) ROOT EXTRACT AND ITS SPRAY DRIED POWDER ACCORDING TO THE Q<sub>10</sub> METHOD

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**Abstract:** *The main purpose of this research was to investigate the accelerated shelf-life of phenolic compounds in Polygonum multiflorum (Thunb.) root extract and its spray dried products at various temperatures. Using the Q<sub>10</sub> model helped in observing the degradation of the total polyphenol content (TPC) and antioxidant capacity (AC) of the product, while enabling an accurate prediction of the its shelf-life at different storage temperatures. The results showed that the degradation of TPC and AC of the extract was rapid at elevated temperatures. The spray-dried products maintained TPC and AC better than the extract. TPC and AC retention of 85% of the extract were approximately 42.4 and 5.1 days at 30°C, respectively. While TPC and AC retention of 85% of gum arabic were 6.2 and 13.1 days, TPC and AC retention of 85% of maltodextrin (DE 16-19) were 14.68 and 7.16 days, respectively. The obtained model was reliable and easy to use.*

**Key words:** *Extract, Polygonum multiflorum (Thunb.), polyphenol, Q<sub>10</sub>.*

## 1. Introduction

Bioactive compounds, especially phenolic compounds, are relatively sensitive to oxygen, light and temperature. They degrade quickly over a brief period, making storage very difficult. *Polygonum multiflorum* (Thunb.) extract and its products contain a large number of

phenolic compounds such as gallic acid, catechin, resveratrol [10], emodin, physcion [7], rhaponticoside, and torachryson-8-O-β-D-glucoside [15]. These compounds are quite precious, and are used widely in food industry and in the medical fields. They help prevent the development of various health problems such as cardiovascular diseases, cancers,

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neurodegenerative diseases, diabetes or osteoporosis [11]. *Polygonum multiflorum* (Thunb.) is a wild plant found in the mountainous Northern Vietnam, mainly in Cao Bang and Lang Son provinces. It has been used as a medicinal plant for thousands of years [2].

Currently, predicting the degradation of TPC and AC of phenolic compounds in food has been quite difficult because it depends on many factors such as temperature, moisture, types of phenolic compound, and packaging conditions. In addition, consumers also demand food products that have good appearance, taste, flavor, texture, and high retention of nutritional values. Hence, the manufacturers must be able to predict the end of the process preserved by storage conditions. The quality of products is reflected in the food labeling requirements that manufacturers must comply with. The quality of products must be retained within the approved storage time or the shelf-life of product [3]. With increasing requirements for predicting the longevity of food products kept in storage, simple methods for calculating shelf-life become ever more valuable, especially the  $Q_{10}$  method.

The  $Q_{10}$  method is a well-known, indirect predictive technique which relies on the ratio of the times to equivalent damage at two temperatures, usually 10°C apart [6]. The method is generally applicable for saving time because it can predict the shelf-life quickly and accurately. For this reason, it is usually used to predict the shelf-life of a medicine [8].

Until now, no research has explored changes in TPC and AC of the extract or its products from herbal plants by means of the  $Q_{10}$  method. Thus, the main purpose of this research is to develop a method to

determine the changes of TPC and AC of *Polygonum multiflorum* (Thunb.) extract and its products in accelerated temperatures to predict the exact shelf-life of products stored at the appropriate temperature.

## 2. Materials and Methods

### 2.1. Sample Preparation

*Polygonum multiflorum* (Thunb.) roots were harvested from Cao Bang province in Vietnam. The roots were then cleaned with tap water, sliced and dried at 60°C until the moisture level was less than 12%. The slices were then ground into fine powder (diameter less than 0.5 mm) and vacuum-packed.

### 2.2. Chemicals and Reagents

Maltodextrin (MA, DE 16-19) was obtained from GPC company (USA), and gum arabic (GA) was supplied by Tianjin Dengfeng company (China). Folin-Ciocalteu. DPPH (2,2-diphenyl-1-picrylhydrazyl, purity:  $\geq 90\%$ ) was purchased from Merck (Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, purity: 97%) was purchased from Sigma-Aldrich (USA). All the other chemicals and organic solvents used were of analytical grade.

### 2.3. Microwave-assisted Extraction (MAE)

Polyphenol from the dried powder of *Polygonum multiflorum* (Thunb.) roots was extracted by microwave system with an acetone concentration of 57.35%, solid/solvent ratio of 1/39.98, extraction time of 289 seconds and microwave power of 127 W. The crude extract was

filtered with Whatman paper. The filtrate was concentrated by evaporation at 45°C until a level of 4% soluble solid was reached. The extract was then stored in a closed container and kept at 4°C [9].

#### 2.4. Spray Drying of *Polygonum multiflorum* (Thunb.) Roots Extract

Gum arabic (GA) and maltodextrin (MD, DE 16-19) were added into the extract to achieve the content of soluble solid of 15% and 25%, respectively. The solution was mixed well before being fed into Lab Plant SD-06 spray dryer (England). The feed flow rate, inlet temperature and air flow speed were approximately set at 500 mL/h, 160°C and 5 m/s, respectively. After the spray drying process completed, the dried powder was collected and vacuum-packed.

#### 2.5. Determination of Total Polyphenol Content (TPC)

The TPC in the extracts was slightly modified and determined by the Folin-Ciocalteu (FC) colorimetric method [12]. Gallic acid was used as the standard chemical. Different concentrations ranging from 1-10 ppm were prepared with deionized water, and the TPC was measured as gallic acid equivalent per gram dry weight basis of fresh sample (mg GAE/g of dry weight). Briefly, 0.1 mL of extract was mixed with 1.5 mL FC reagent (diluted 1/10) and kept for 5 minutes. Then, 4 mL of 20% sodium carbonate solution was added, and topped up to 10 mL with distilled water. After that, this mixture was kept for 30 minutes in the dark, and the absorbance was determined at 738 nm.

#### 2.6. Determination of Antioxidant Capacity (AC)

The AC in the extract was determined by DPPH assay. This method was described by [13] and slightly modified. First, 4 ml of DPPH solution (0.1 mM in ethanol solution) was mixed with 0.1 mL of extract, and the mixture was made up to 5 mL with ethanol. The mixture was then kept for 30 minutes in the dark, and its absorbance was measured at 517 nm. To determine the calibration curve, the absorbance values at 517 nm of some concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were measured. Results were expressed as Trolox equivalent antioxidant capacity (TEAC) ( $\mu\text{mol Trolox/g}$  of dry weight).

#### 2.7. Determination of Shelf-life of Products

The extract was obtained from a 1 g initial sample after extraction process, then it was evaporated and made up to 50 mL by adding distilled water. The extract was stored in dark bottles while the spray-dried product was packed in vacuum (30 g/packet). All samples were preserved at 60°C and 70°C, at a humidity of 85% for 6 days in the humidity cabinet. Changes in TPC and TEAC were measured every day. According to [6] and [14], the  $Q_{10}$  values were determined by the equation below:

$$Q_{10} = \frac{\text{Shelf life at } T^0}{\text{Shelf life at } (T^0 + 10^{\circ}\text{C})}$$

and

$$f_2 = f_1 \cdot Q_{10}^{(T_1 - T_2)/10}$$

where:

- $T_1$  and  $T_2$  are the given temperature and the required temperature for storage, respectively;
- $f_1$  is the given shelf-life at the given temperature ( $T_1$ );
- $f_2$  is the estimated shelf-life.

## 2.8. Data Analysis

The experimental data were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ( $p < 0.05$ ) were determined by Fisher's least significant difference (LSD) procedure using the Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean  $\pm$  standard deviation (SD).

## 3. Results and Discussion

### 3.1. Determining the shelf-life of TPC and TEAC of the extract

The obtained results showed that TPC and TEAC of *Polygonum multiflorum* (Thunb.) roots extract exhibited significant differences at various storage temperature ( $p < 0.05$ ). Both TPC and TEAC degraded quite significantly. At 60°C, the TPC and TEAC retention rates were higher than those at 70°C. However, the degradation of TPC and TEAC at the same storage temperature were not similar. The TEAC retention rate decreased more rapidly than that of TPC. For instance, the TEAC retention rate was 16.33% at 60°C after 6 days of storage, while the TPC retention rate was 67.94% in the same condition. Similar results were obtained at 70°C: The TPC and TEAC retention rates were 52.31% and 10.86%, respectively

(Figure 1 and 2). These degradations increased quickly when the extract was stored for a long time, especially at a high temperature, because phenolic compounds were released from the initial material and were quite sensitive to light, temperature and oxygen in ambient air.

According to [4], food deteriorates during storage on account of different mechanism and modes. The mode determining its shelf-life depends on the product properties (raw materials, formulation and ingredients), packing methods, processes and storage conditions. In general, the loss of food quality or shelf-life is evaluated by measuring some characteristic quality indexes which are described by kinetic equation as the exponential, hyperbolic, linear, quadratic and complex model. However, Figures 1 and 2 show that TPC and TEAC retention rate were suitable for the quadratic model at 60°C and 70°C because they achieve the highest  $R^2$  values ( $> 0.95$ ).

The TPC and TEAC retention rate of 85% was chosen to calculate the shelf-life model. Based on the regression equation (Figures 1 and 2), the shelf-life of TPC of 85% was 2.28 days and that of TEAC retention of 85% was 2.03 days at 60°C. Meanwhile, these shelf-lives were 0.86 days for TPC and 1.49 days for TEAC at 70°C.

The  $Q_{10}$  values were calculated by means of the formula below [14]:

$$Q_{10-TPC} = \frac{\text{Shelf life at } T^0}{\text{Shelf life at } (T^0 + 10^{\circ}C)} = \frac{2.28}{0.86} = 2.65$$

$$Q_{10-TEAC} = \frac{\text{Shelf life at } T^0}{\text{Shelf life at } (T^0 + 10^{\circ}C)} = \frac{2.03}{1.49} = 1.36$$

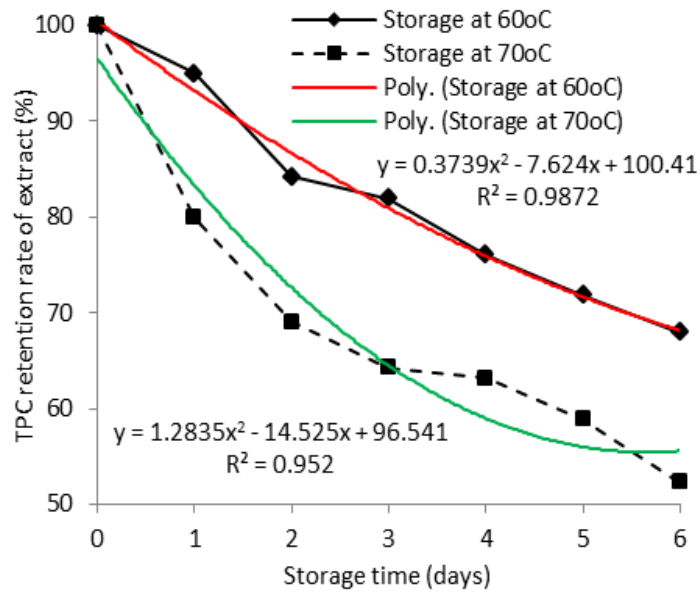


Fig. 1. Changes of TPC in the extract at different storage temperatures

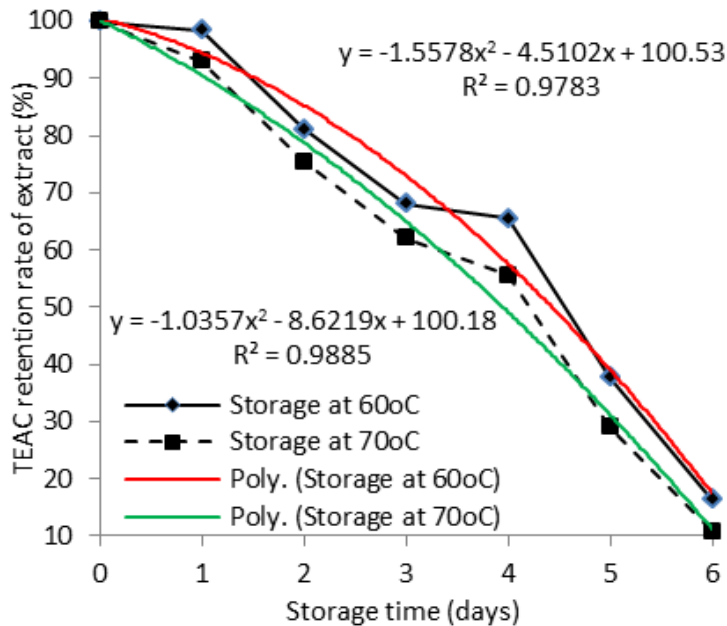


Fig. 2. Changes of TEAC in the extract at different storage temperatures

Food quality may have one or many characteristic quality indexes such as sensory quality, nutrient loss or perishable time. This leads to one or many  $Q_{10}$  values. The  $Q_{10}$  value increases thus increasing the shelf-life and decreasing the level of

degradation. Frozen poultry products have  $Q_{10}$  values of approximately 20, while those of the fresh fruits and vegetables range between 2 and 3. Those of fried snack foods range between 1.5 and 2.5. Some  $Q_{10}$  values of nutrient loss index are low, such as lipid oxidation ( $Q_{10}=1.5$  to 2) [6] and total carotenoid loss (30%) in gac powder ( $Q_{10}=2.44$ ) [1]. These results are in agreement with the findings of this study because the phenolic compounds degrade easily at high temperatures during long storage period. The resulted  $Q_{10}$  values of TPC and TEAC were 2.65 and 1.36, respectively.

The extract was stored at 30°C, the shelf-life of TPC and TEAC retention rate of 85% was estimated as:

$$f_{2-TPC} = f_{1-TPC} \cdot Q_{10}^{\Delta/10} = 2.28 * 2.65^{\frac{60-30}{10}} = 42.4 \text{ days}$$

$$f_{2-TEAC} = f_{1-TEAC} \cdot Q_{10}^{\Delta/10} = 2.03 * 1.36^{\frac{60-30}{10}} = 5.1 \text{ days}$$

The results show that TEAC retention rate decreased rapidly, the estimated shelf-life of TEAC retention rate of 85% was only 5.1 days while the estimated shelf-life of TPC retention rate of 85% was 42.4 days. The reliability of the achieved model was evaluated from day 0 to the testing days at 30°C.

The differences between estimated retention values (85%) and actual retention values (Table 1) were not significant, and they were 10.31% for TPC and 4.68% for TEAC. This obtained model is significant and may be applied to many different cases. The prolonged storage process was not an advantage and it might lead to phenolics oxidation due to light, oxygen exposure at high temperature and especially the structure of phenolic compounds.

Testing for the reliability of shelf-life extract model

Table 1

Testing days	TPC [mg GAE/g DW]	TPC <sub>retention</sub> [%]	TEAC [ $\mu$ mol TE/g DW]	TEAC <sub>retention</sub> [%]
0	48.04 $\pm$ 0.32 <sup>a</sup>	-	341.22 $\pm$ 5.67 <sup>a</sup>	-
5.1	-	-	274.06 $\pm$ 6.23 <sup>b</sup>	80.32
42.4	35.88 $\pm$ 0.27 <sup>b</sup>	74.69	-	-

Different superscript letters in the same column denote significant differences ( $p < 0.05$ ).

"-": not tested.

### 3.2. Determining the Shelf-life of TPC and TEAC of Spray Dried Products

The TPC and TEAC of the spray dried products also decreased with the increase of storage time at different temperatures. At 70°C, the degradation levels of TPC and TEAC were higher than those of TPC and TEAC at 60°C but lower than that of the extract. The degradation of TPC and TEAC at the same storage temperature were

different from another. For instance, TPC and TEAC retention of GA were of 67.79% and 75.34% (Figures 3 and 4), TPC and TEAC retention of MD were of 81.57% and 66.87% (Figures 5 and 6); and TPC and TEAC retention of the extract were of 52.31% and 10.86% at 70°C after 6 days of storage, respectively.

The degradations of TPC and TEAC of spray dried product were often modeled by a quadratic function, and degradations

of TEAC of MD at 70°C had exponential models. Those models were chosen based on the  $R^2$  value. The  $R^2$  values for these response variables were remarkably close to 1, denoting that the regression models provide excellent explanations of the relationship between the storage time and the retention rate of TPC or TEAC.

In general, although the TPC and TEAC values of spray-dried product may be lower than the initial extract due to the sensitivity to high temperature of the phenolic compounds, the bioactive compounds, especially the remaining polyphenol, were quite stable under high temperature treatment. Therefore, these spray-dried products can slow down the decrease of TPC and TEAC during storage, in comparison to the initial extract. Besides, the degradation of polyphenol compounds depends on the type of carrier agent and on the composition of materials. As a result, there was a difference between the degradation of the extract, GA and MD during the storage time. In addition, microcapsules can protect the bioactive compounds from light, heat and oxidation during storage time. Alternatively, the spraying method reduces water activity, storage and transport costs, and it equally ensures a microbiological stability of products [5]. Using GA and MD as carrier agent in this study was more effective than using ethyl cellulose which was used for spray drying polyphenol from bayberry extract in the same storage conditions at 60°C and 70°C [16].

Choosing TPC and the TEAC retention of 85% of all spray dried product the shelf-life models were calculated. Based on the regression equation (Figures 3, 4, 5 and 6), the shelf-life of TPC and the TEAC retention of 85% were determined:

TPC<sub>GA-60</sub>: 4.5 days, TPC<sub>GA-70</sub>: 4.04 days

TEAC<sub>GA-60</sub>: 5.96 days, TEAC<sub>GA-70</sub>: 4.58 days

TPC<sub>MD-60</sub>: 5.66 days, TPC<sub>MA-70</sub>: 4.12 days

TEAC<sub>MA-60</sub>: 2.15 days, TEAC<sub>MA-70</sub>: 1.44 days.

According to [6], the  $Q_{10}$  values were calculated as below:

$$Q_{10-TPC-GA} = \frac{4.5}{4.04} = 1.113$$

$$Q_{10-TPC-MD} = \frac{5.66}{4.11} = 1.374$$

$$Q_{10-TEAC-GA} = \frac{5.96}{4.58} = 1.301$$

$$Q_{10-TEAC-MD} = \frac{2.15}{1.44} = 1.493$$

The spray dried products were stored at 30°C, the shelf-life of TPC and the TEAC retention rate of 85% was estimated as follows:

$$f_{2-TPC-GA} = f_{1-TPC-GA} \cdot Q_{10-TPC-GA}^{\frac{60-30}{10}} = 4.5 * 1.113^{\frac{60-30}{10}} = 6.2 \text{ days}$$

$$f_{2-TEAC-GA} = f_{1-TEAC-GA} \cdot Q_{10-TEAC-GA}^{\frac{60-30}{10}} = 5.96 * 1.301^{\frac{60-30}{10}} = 13.12 \text{ days}$$

$$f_{2-TPC-MD} = f_{1-TPC-MD} \cdot Q_{10-TPC-MD}^{\frac{60-30}{10}} = 5.66 * 1.374^{\frac{60-30}{10}} = 14.68 \text{ days}$$

$$f_{2-TEAC-MD} = f_{1-TEAC-MD} \cdot Q_{10-TEAC-MD}^{\frac{60-30}{10}} = 2.15 * 1.493^{\frac{60-30}{10}} = 7.16 \text{ days}$$

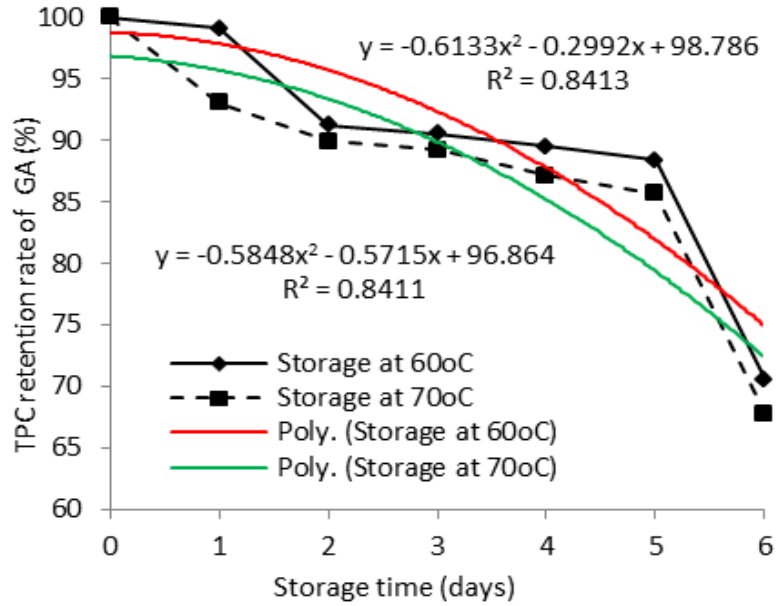


Fig. 3. Changes of TPC of gum arabic at different storage temperatures

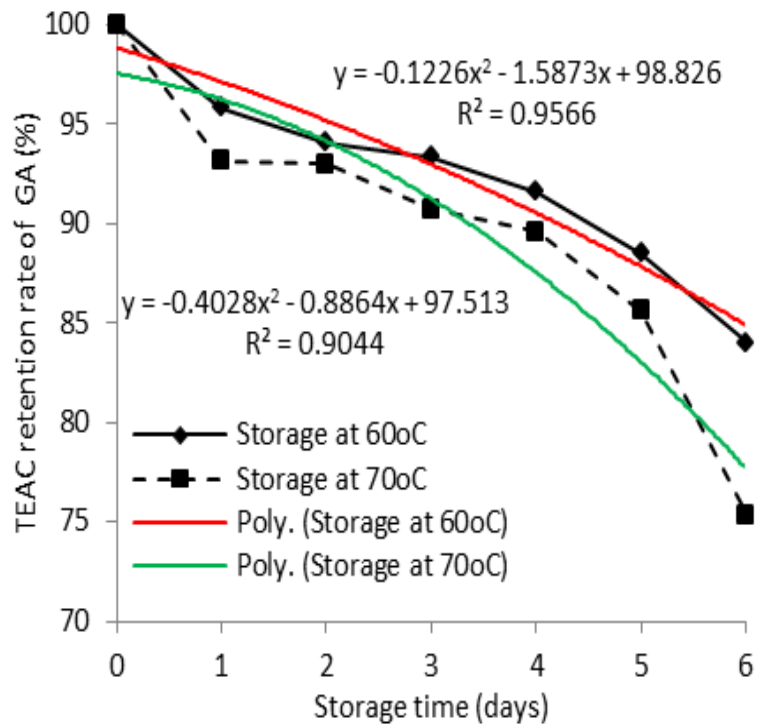


Fig. 4. Changes of TEAC of gum arabic at different storage temperatures



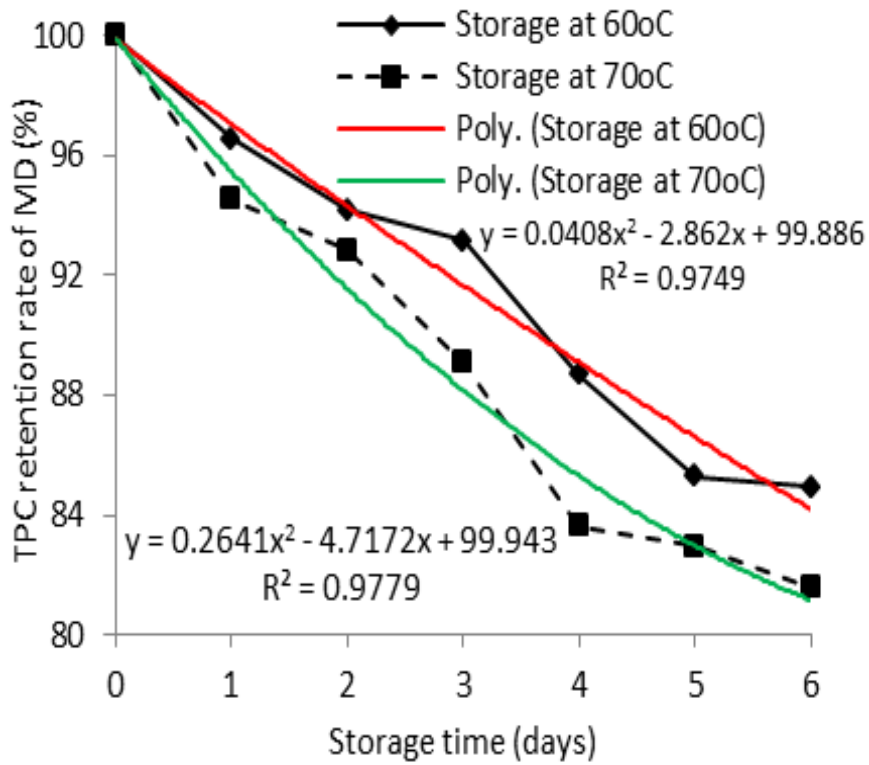


Fig. 5. Changes of TPC of maltodextrin at different storage temperatures

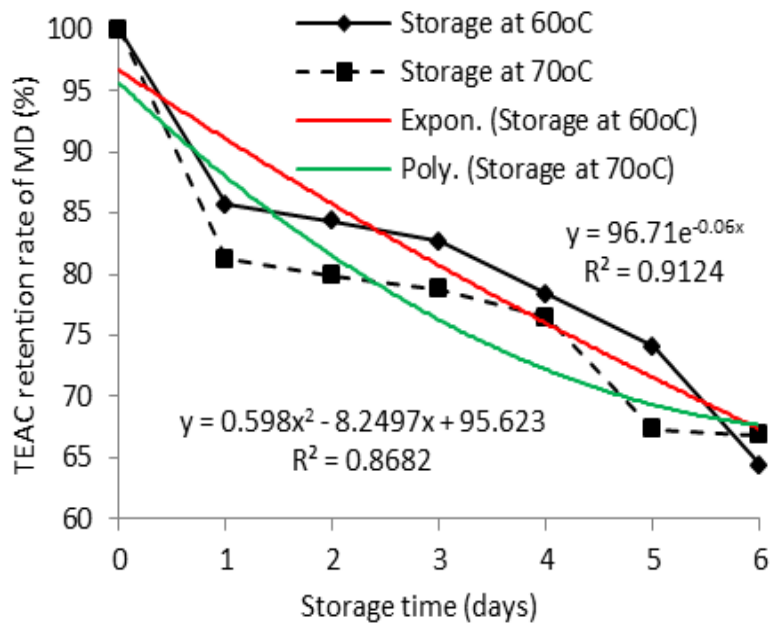


Fig. 6. Changes of TEAC of maltodextrin at different storage temperatures

The results showed that the shelf-life of TPC and TEAC of GA and MD were opposite. The TPC degradation of GA was more rapid than that of MD and the TEAC degradation of GA was slower than that of MD. The reliability of the achieved model was evaluated from day 0 to the testing days at 30°C.

Table 2 shows that there was a difference between the estimated retention values (85%) and the actual retention values. However, these

differences were not significant either. For GA, the difference in TPC was 4.64% and that in TEAC was 6.87%; while for MD, the difference in TPC was 3.95% and that in TEAC it was 4.05%. Hence, this is a reliable model that can be useful in the pharmaceutical and food industry to predict the degradation of bioactive compounds during the storage time.

Testing for the reliability of shelf-life spray dried products model Table 2

Testing days	Gum arabic			
	TPC [mg GAE/g DW]	TPC <sub>retention</sub> [%]	TEAC [ $\mu$ mol TE/g DW]	TEAC <sub>retention</sub> [%]
0	39.1 $\pm$ 0.37 <sup>a</sup>	-	146.21 $\pm$ 2.53 <sup>a</sup>	-
6.2	35.05 $\pm$ 0.71 <sup>b</sup>	89.64	-	-
13.12	-	-	114.24 $\pm$ 3.11 <sup>b</sup>	78.13
Testing days	Maltodextrin			
	TPC [mg GAE/g DW]	TPC <sub>retention</sub> [%]	TEAC [ $\mu$ mol TE/g DW]	TEAC <sub>retention</sub> [%]
0	20.21 $\pm$ 0.11 <sup>a</sup>	-	126.26 $\pm$ 2.27 <sup>a</sup>	-
7.16	-	-	112.44 $\pm$ 3.01 <sup>b</sup>	89.05
14.68	16.38 $\pm$ 0.21 <sup>b</sup>	81.05	-	-

Different superscript letters in the same column denote significant differences ( $p < 0.05$ ).

"-": not tested.

#### 4. Conclusions

The  $Q_{10}$  method is useful in predicting the shelf-life of a food product and has many advantages such as safety, accuracy, reduced cost and time efficiency. It could be used to construct the shelf-life model for extract or spray-dried products. *Polygonum multiflorum* (Thunb.) extract and so that its polyphenol microcapsules were successfully modeled by the  $Q_{10}$  method using GA and MD as a carrier agent. After being microcapsulated, phenolic compounds were more stable than their un-encapsulated counterpart.

The results of this study would be beneficial to the industrial application of plant polyphenol.

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