Research Article | Science Technology and Engineering Journal (STEJ) Vol.7, No.2 pages 58-73

Bioactives and Antioxidant Activities of Marigold Flower Juices and the Effect of Different Storage Times

Onanong Phuseerit[1], Colin Wrigley[2] and Sirithon Siriamornpun[3*]

[1] *Department of Science and Technology, Faculty of Liberal Arts and Science, Roi-Et Rajabhat University, Selapoom, Roi-Et 45120, Thailand*

[2] *Centre for Crop Science Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia*

[3] *Research Unit of Process and Product Development of Functional Foods, Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Kuntarawichai, Mahasarakham, 44150, Thailand*

[*] *Corresponding Author: sirithons@hotmail.com, +66 85 7474136*

(Received: 9th September 2021, Revised: 21st November 2021, Accepted: 25th November 2021)

Abstract-The aim of this research was to investigate changes in phenolic compounds, carotenoids and antioxidant activities in marigold flower (*Tagetes erecta* L.) juice with vitamin added and different storage times. The samples were stored for four weeks at 4 °C. The results of this study indicated that changes in the levels of carotenoids, phenolic compounds and the antioxidant activity in marigold juice were affected by storage period. The carotenoids (lycopene, β-carotene and lutein) and total flavonoid contents decreased during the storage period. Total phenolic contents increased during storage for three weeks' storage under the same conditions, but had declined by the end of the storage period (four weeks). The phenolic acids found were ferulic acid and sinapic acid ; the level of each increased during storage while, other phenolic acids and flavonoids decreased during storage. For antioxidant activity (DPPH assay), there was a slight increase in marigold juice with added vitamin E and with a combination of vitamin C and vitamin E. On the other hand, antioxidant activity decreased in marigold juice (no vitamin additives) and in marigold juice with added vitamin C. The present study has provided useful information for industrial production of edible flower juice.

Keywords: Marigold juices, phenolic compounds, flavonoids, carotenoids, effect of storage

1. Introduction

Currently, the awareness in the role of dietary antioxidants in human health has prompted research in the field of food science. Plants are good sources of these bioactives, and there are a number of commercial polyphenol-rich beverages, which base their marketing strategies on antioxidant potency. Marigold (*Tagetes erecta* L.) is a herb of ancient medicinal repute. It grows as a wild and common garden plant throughout Europe, North America and Asia. It has long been used as a food and as an ingredient in animal feed. Marigold is also widely used in traditional and homeopathic medicine as infusions and ointments prepared with its petals. It presents several therapeutic activities, such as anti-mutagenicity, anti-inflammatory, anti-tumorogenic, cicatrizing, antiviral and immunostimulating effects (Gonzalez de Mejıa *et al.*, 1997 ; Hamburger *et al.*, 2003). The pharmacological activity of marigold is related to the content of several classes of secondary metabolites such as essential oils, flavonoids, sterols, carotenoids, tannins, saponins, triterpene alcohols, polysaccharides, a bitter principle, mucilage and resin (Jacobs *et al.*, 1994 ; Piccagliy *et al.*, 1998). According to the fact that marigold contains polyphenols, the assessment of its antioxidant properties is of great interest in understanding the positive effects of these compounds, especially in phytotherapy. Marigold flowers are used as tea, as a food colorant and as an ingredient in cooking. They may be used as the fresh petals or as a dried powder, which can be made into tea, spices and medicines (tinctures, ointments, and creams) (Hamburger *et al.*, 2003). The natural pigmentation of poultry (broilers, in particular) is affected by the composition, especially the carotenoid content, of their diet. An extract of marigold flowers is used commercially as an additive to poultry feed to improve the pigmentation of the bird's fat, skin and egg yolk (Bailey & Chen, 1989). Narahari *et al.* (1981) and Ojeda *et al.* (1983) also reported that marigold petal meal and residue are good sources of xanthophylls (lutein, zeazanthin) for use in layer diets as pigmenting agents for egg yolk coloration. Marigold flower petals are an excellent and most important source of carotenoids, the yellow carotenoids such as carotenes (α and β-carotene) and xanthophylls (lutein, zeaxanthin) (Hojnik *et al.*, 2008 ; Siriamornpuna *et al.*, 2012 ; Abdel-Aal & Rabalski, 2015). Lutein is one of the major constituents of green vegetable, orange fruits and egg yolk, where it exists in its free non-esterified form. It is reported in the literature that, the risk of chronic disease, such as heart disease, cancer, cardiovascular disease, age-related macular degeneration (Slattery *et al.*, 2000, Bhattacharyya *et al.*, 2010) and age-related eye diseases might (Snodderly, 1995) be significantly reduced by diets rich in lutein.

Therefore, the aims of this study were to investigate the changes in phenolic compounds, carotenoids and antioxidant activity in marigold flower juice with vitamin added and different storage times were examined.

2. Material and Methods

2.1 Plant Materials

Marigold (*Tagetes erecta* L.) flowers were bought from the markets at Maha Sarakham Province, Thailand. Marigold

flowers were cleaned and the petals were separated. The raw marigold petals were washed and kept at room temperature to drain. The samples were dried by combined far-infrared radiation with hot-air convection. In the drying operations of FIR-HA, the petal samples were placed onto a mesh tray and irradiated with a combination of far-infrared radiation with hot air convection (FIR-HA) at FIR intensities of 5 kW/m2, HA temperature of 40 °C, HA velocities of 1 m/s (Wanyo *et al.*, 2011), and a drying time of 60 min. Irradiation was conducted using a FIR heater (122 · 60 mm, output 250 W, Sang Chai Meter Co., Ltd., Bangkok, Thailand), which emitted radiation in the wavelength range from 15 to 100 μm in a FIR dryer located at the Research Unit of Drying Technology for Agricultural Products, Faculty of Engineering, Mahasarakham University. After drying, these samples were allowed to cool to ambient temperature before used for flower juice.

2.2 Marigold Juice Processing

The dried marigold flowers were extracted using hot water (82 °C) for 2 min (1:10). After boiling, marigold juice was filtered through a 2 mm tea strainer to remove waste. We added sugar (11.0 °Brix), 100 ppm of vitamin C, vitamin E and a combination of vitamins C and E (food grade). The marigold juice was subjected to heat processes at 72 °C for 15 s (mild heat pasteurization). The samples were kept in glass bottle and stored at 4 °C. The samples were analyzed at 0, 7, 14, 21 and 28 days. The phenolic compounds, carotenoids and the antioxidant activity of marigold juice at different storage times were evaluated.

2.3 Determination of Carotenoid Content

The marigold flower juice (20 ml) was placed in a vessel, protected from light, and mixed with 80 ml of extraction solvent (hexane/acetone/ethanol: 50:25:25 v/v/v). The mixture was magnetically stirred during 30 min ; 15 ml of water were added ; the upper layer was placed in a round-bottomed flask, and an aliquot of 10 ml of the extract was evaporated to dryness. The residue was dissolved to a final volume of 4 ml with methanol/ acetonitrile (50:50). The final solution was filtered through 0.45 μm membrane filters and 20 μl were injected for HPLC analysis. Analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A with a diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6×250 mm i.d., 5 μm). The mobile phase consisted of methanol (solvent A)/acetonitrile (solvent B) 90:10 at a flow rate of 0.9 ml/min for 10 min. The column temperature was 30 °C and the absorbance was read at 475 nm.

2.4 Determination of Phenolic Compounds Total Phenolic Contents

Total phenolic content was determined using Folin-Ciocalteu reagent as adapted by Siriamornpun *et al*. (2012) and Chuchird and Pattarathitiwat (2021). Briefly, 0.3 ml of extract was mixed with 2.25 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min ; 2.25 ml of sodium carbonate (60 g/l) solution were added to the mixture. After 90 min at room temperature, absorbance was read at 725 nm using a spectrophotometer. Results

were expressed as µg gallic acid equivalents in 1 ml of sample (µg GAE/ml).

2.4.1 Total flavonoid content

Total flavonoid content was determined using the colorimetric method described by Siriamornpun *et al.* (2012). Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by the addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3 \cdot 6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well by vortex. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as µg rutin equivalents in 1 ml of sample (µg RE/ml).

2.4.2 Identification and quantification of phenolic compounds

RP-HPLC system for analysis of phenolic compounds HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A with a diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6×250 mm i.d., 5 µm). The composition of solvents and used gradient elution conditions were described previously by Uzelac *et al.* (2005) and Butsat *et al*. (2009) with some modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 ml/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B ; from 5 to 15 min, 9% solvent B ; from 15 to 22 min, linear gradient from 9 to 11% solvent B ; from 22 to 38 min, linear gradient from 11 to 18% solvent B ; from 38 to 43 min, from 18 to 23% solvent B ; from 43 to 44 min, from 23 to 90% solvent B ; from 44 to 45 min, linear gradient from 90 to 80% solvent B ; from 45 to 55 min, isocratic at 80% solvent B ; from 55 to 60 min, linear gradient from 80 to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38 ˚C, injection volume, 20 µl, UV-diode array detection at 280 nm (for hydroxybenzoic acids), 320 nm (for hydroxycinnamic acids) and 370 nm (for flavonols). Phenolic acids in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method.

2.5 Determination of Antioxidant Activity

The antioxidant activities were DPPH and FRAP assay

2.5.1 DPPH radical-scavenging activity

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH (Gulluce *et al.*, 2007). The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, which is a method adapted from Wanyo *et al.* (2015) and Srisaikham & Rupitak (2021), Braca *et al*. (2001). Aqueous extract (0.1 ml) was added to 2.9 ml of a 0.004% DPPH in methanol. Absorbance at 517 nm was determined after 30 min, and the percent

inhibition of activity was calculated as [(Ao-Ae)/Ao]×100 (Ao=absorbance without extract ; Ae=absorbance with extract).

2.5.2 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe3+ to Fe2+. The formation of the blue-coloured Fe2+-TPTZ complex (Fe2+ tripyridyltriazine) increases the absorbance at 593 nm. The method of Butsat and Siriamornpun (2010) was used with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl3·6H2O solution. The fresh working solution was warmed to 37 ºC before use. Flower extracts (100 µl) were allowed to react with 1.9 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction (Butsat & Siriamornpun, 2010). The results were calculated by standard curves prepared with known concentrations of FeSO4, and were expressed as mmol FeSO4/ml DW.

2.6 Statistical Analysis

The results were analyzed using analysis of variance (ANOVA) and reported as mean ± SD. Duncan's new multiple range test was used to determine significant differences. Statistical significance was declared at $p<0.05$.

3. Results and Discussion

3.1 Changes in Carotenoids During Storage

Carotenoids (lycopene, β-carotene and lutein) contribute to preventing degenerative diseases, such as cardiovascular diseases, diabetes, and several types of cancer (Baker & Gunther, 2004). The identification of the carotenoid components (as chromatographic peaks) was carried out by comparing retention times with those obtained with a standard mixture of lycopene, β-carotene and lutein. The quantification was performed using calibration curves made by injecting different amounts of lycopene, β-carotene and lutein in proportions similar to those in the samples. Concentrations of the major carotenoids extracted from marigold juice after different storage times are presented in Figure 1 (a), (b) and (c). The sample of marigold juice with vitamin E added at the initial stage had the highest amount lycopene (16.09 mg/ 100 ml) followed by marigold juice with added vitamins C and E with a concentration of 14.02 mg/100 ml (Figure 1 (a)).

The highest content of *β*-carotene was found in marigold juice with added vitamins C and E (22.3 µg/ 100 ml), while the lowest level of β-carotene was identified in B (19.1 µg/ 100 ml) (Figure 1 (b)).

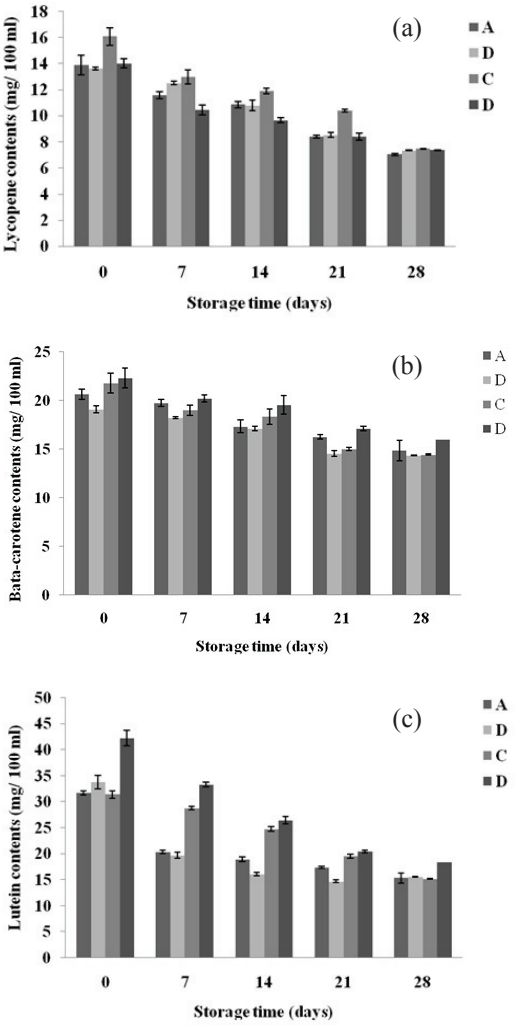

Figure 1. Lycopene (a), β-carotene (b) and Lutein (c) contents of marigold juice at different storage times (A: control ; B: marigold juice with added vitamin C ; C: marigold juice with added vitamins E and D: marigold juice with added vitamins C and E).

With different marigold processes, lutein levels were 31.7, 33.8, 31.4 and 42.3 µg/100 ml in the marigold juice (alone), in the marigold juice with added vitamin C, in the marigold juice with added vitamin E and in the marigold juice with added vitamins C and E, respectively (Figure 1 (c)).

Although the marigold juice with added vitamins C and E sample had higher contents of lutein, there were significant differences among all samples ($p< 0.05$). Our results for stored marigold juice indicate that storage time significantly affects ($p<0.05$) lycopene, *β*-carotene and lutein contents. The contents of carotenoids were found to decrease following increases in storage time. Lycopene losses were 25% after only 7 days in the case of marigold juice with added vitamins C and E, while in the case of marigold juice with added vitamin C loss was only 8% after 7 days. In the case of *β*-carotene, the losses were less than 33% at the end of the storage period, in all cases. On the other hand, lutein content was more than 37% reduced in only 21 days, reaching over 50 % loss at the end of the experiment, for all samples. Decreases in carotenoids by storage time have been reported in many studies, mostly in juice (Lin & Chen, 2005 ; Chen *et al.*, 1996). Lin and Chen (2005) found that the contents of lutein, *β*-carotene and its cis isomer in tomato juice decreased following increases in storage temperature and storage time. This result further demonstrated that both isomerisation and degradation of β-carotene may proceed simultaneously, which may lead to inconsistent changes of concentration. This phenomenon was also observed by Pesek *et al.* (1990) and Chen *et al.* (1996), who reported that the dominant reaction, isomerisation or degradation, may be dependent on many factors, such as temperature, illumination intensity and storage environment. Sharma and Le Magure (1996) demonstrated that the reaction rate of lycopene during storage of tomato puree at 25 °C was 2.7 times greater than at 5 °C. In a study dealing with storage of dried tomatoes (10% H_2O) at 37 °C,

a high loss of 50% was found after a 30-day storage and 70% after 90 days (Zanoni *et al.*, 1999). Light and oxygen are two other important factors during food processing and storage, and should be avoided during long time storage (Xianquan *et al.*, 2005). On the other hand, other studies showed higher losses of carotenoids during processing and/or storage (Chen *et al.*, 1996 ; Lin & Chen, 2005). In fact, the stability of carotenoids in foods is variable. This happens not only because of extrinsic factors, such as the severity of heat treatment, presence or absence of light, temperature of storage, packaging, amongst others, but also because of the characteristics of the food matrices, such as their chemical composition, the oxygen dissolved in the samples, size of the particles, and the physical state of the carotenoid in the food (Marx *et al.*, 2003 ; Rodriguez-Amaya, 1999 ; Vasquez-Caicedo *et al.*, 2007).

3.2 Total Phenolic Compounds

Phenolic compounds are widely distributed in fruits and vegetables (Li *et al.*, 2006). They have received considerable attention, due to their potential antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications for human health (Imeh & Khokhar, 2002). TPC was determined in comparison with gallic acid as standard and the results were expressed in terms of gallic acid equivalents (µg GAE/ ml). The total phenolic content of the marigold juice ranged from 26.42 to 34.58 µg GAE/ml. Total phenolic contents increased during storage for three weeks storage at 4 °C (Figure 2 (a)) while, they had declined by the end of the storage period. The polyphenol content of plants is associated with antioxidant capacity and is normally measured with the Folin-Ciocalteu reagent, which can react with all types of antioxidants through electron transfer-based antioxidants including non-phenolic compounds such as vitamin C, dehydroascorbic acid, amino acids and carotenoids. The current results correspond with the experiments of Moussaid (2000), who reported that the total polyphenol content in coated mature oranges increased during nine weeks of storage at 20°C.

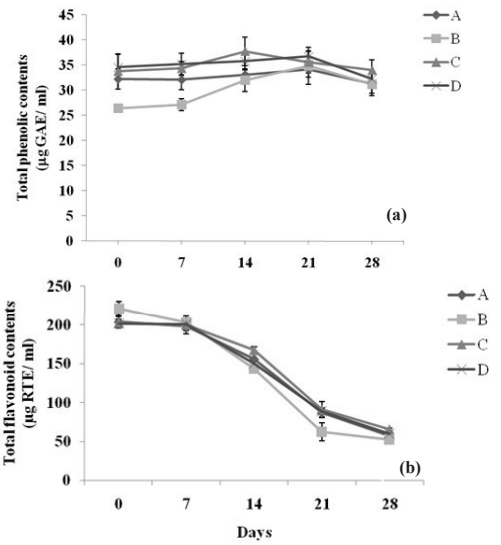

Figure 2. Total phenolic (a) and total flavonoid (b) contents of marigold juice at different storage times. (A: control ; B: marigold juice + vitamin C ; C: marigold juice + vitamins E and D: marigold juice + vitamins C and E).

3.3 Total Flavonoid Content

Flavonoids, as one of the most diverse and widespread groups of natural compounds, are probably the most important natural phenolics (Prasad *et al.*, 2009). A multitude of biological effects *in vitro* and *in vivo* results from the consumption of flavonoid-containing foods. Epidemiologic studies show that increased

consumption of flavonoids reduces the risk of cardiovascular disease and certain types of cancer (Arts & Hollman, 2005 ; Koga & Meydani, 2001). However, these potential benefits may be reduced according to the results described here, based on the significant losses detected in the amounts of flavonoids remaining after the storage of juice samples (Figure 2 (b)). The total flavonoid contents in all samples decreased moderately during increases in storage time at 4°C. The total flavonoid contents were less than 50% after 3 weeks of the storage period in all samples. Van Buren *et al.* (1976) found that flavonol glycoside content in juice decreased upon 90 days of storage at 30 °C.

3.4 Identification and Quantification of Phenolic Compounds

RP-HPLC analysis was used to identify the phenolic compounds of marigold juice samples after different storage times by comparison with standard compounds. In the marigold juice analysed, it was possible to identify ten phenolic acids, namely, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, as well as five flavonoids, namely rutin, myricetin, quercetin, apigenin and kaempferol. The distribution of phenolic acids in the samples is presented in Table 1. The major phenolic acid found in juice of marigold was identified as ferulic acid, followed by sinapic acid, chlorogenic acid and gallic acid, while vanillic acid was not detected. The phenolic acids increased slightly during storage at 4°C, but declined again slightly at the end of the storage period. With other juices, such as tomato, the soluble phenolic acid content increased slightly during 10 days of storage at 7, 15 and 25°C (Toor & Savage, 2006). Caffeic acid increased in the first two weeks and declined slightly until the end of storage. The contents of protocatechuic acid, p-hydroxy benzoic acid, syringic acid and p-coumaric acid decreased steadily during two weeks' storage at 4 °C ; however, after three weeks, the compounds were not detected. The level of sinapic acid and ferulic acid increased during storage at 4 °C ; the content of these compounds was higher at the end of storage (Table 1). Generally, phenolic acids were reported to be synthesised in plants as a defensive response mechanism to stress (Naczk & Shahidi, 2006). At low temperature stress levels, L-phenyalanine is converted to tran-cinnamic acid catalysed by phenyalanine ammonia-lyase (PAL), followed by hydroxylation and/or methylation of p-coumarate, eventually yielding caffeic, ferulic and sinapic acids.

The major flavonoid in the samples was quercetin, ranging from 196.2 µg/ ml to 206.7 µg/ml (Table 2). While kaempferol was found in smaller amounts, ranged from 3.2 µg/ ml to 4.0 µg/ ml. The flavonoids in all samples decreased slightly during storage at 4°C. Apigenin and kaempferol were not detected after storage during two weeks. Our findings are similar to the data from a study by Rapisarda *et al.* (2008), who reported that flavanones of orange (Valencia variety) decreased after storage during 20 days. A decrease of flavonol glycosides in juice concentrates upon 90 days of storage at 30 °C has been reported by Van Buren *et al*. (1976). During the storage of the juice, various changes may occur, for example, as a result of browning and degradation reactions. Spanos *et al.*

(1990) compared apple juices prepared from concentrates that were not stored, with concentrates that were stored at 25 °C for 9 months ; they showed that quercetin glycoside and phloridzin concentrations decreased at 54 and 32%, respectively, as a result of this period of storage (Spanos *et al.*, 1990). In the apple juice from the not-stored concentrate, 2.9 mg/ l of catechin and 6.1 mg/ l of epicatechin was present, but in the juice from the stored concentrate these compounds were not detected at all (Spanos *et al.*, 1990).

3.5 Antioxidant Activity

3.5.1 DPPH radical scavenging activity

The DPPH assay is a preliminary test to investigate the antioxidant potential of extracts. This assay has been widely used to test the free radical scavenging ability of various samples (Shimoji *et al.*, 2002 ; Sakanaka *et al.*, 2005). DPPH, a free radical compound, is a stable organic radical with a characteristic absorption at 517 nm ; it was used to study the radical scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, thus neutralising its free radical character (Naik *et al.*, 2003). The colour changes from purple to yellow and its absorbance at wavelength 517 nm decreases. The DPPH radical-scavenging activity (percentage inhibition) of marigold juice after different storage times is given in Figure 3 (a). In the test with DPPH radical, there was a slight increase in antioxidant activity during 4 weeks of storage at 4 °C in marigold juice with added vitamin E and with a combination of vitamin C and vitamin E.

In contrast, there was a decrease in antioxidant activity in marigold juice and marigold juice with added vitamin C. The results presented are in line with the data obtained by Piga *et al.* (2002), Del Caro *et al.* (2004). The described a slight decrease in the TEAC value obtained by DPPH method for orange juice stored in 4 °C. In contrast to Arena *et al.* (1999) showed increases in antioxidant activity after 2 months of storage in orange juices reconstituted from concentrate. If the decrease in the antioxidant activity may be linked to a lower content of phenolic compounds and vitamin C in stored juice as compared to fresh, the increase in the antioxidant activity is usually ascribed to Maillard's reaction products.

Table 1. Phenolic acids of marigold juice *at different storage times*

Sample	Storage time (days)	Phenolic acids (µg/ ml)										Total
		GA	PCCA	p-OH	VA	ChA	CFA	SyA	p-CA	FA	SNA	
A	0	16.4±1.1	6.7±0.5	4.1±0.2	ND	20.3±0.6	12.3±0.6	8.2±0.3	6.2±0.3	39.1±2.7	38.2±2.5	151.5
	7	15.0±1.2	7.8±0.2	3.5±0.1	ND	18.2±1.5	13.1±0.3	7.5±0.3	7.6±0.2	40.5±2.0	40.1±2.6	150.3
	14	13.2±1.1	5.2±0.3	2.3±0.2	ND	10.3±1.6	16.2±0.7	4.7±0.3	6.3±0.3	43.2±2.5	40.3±2.1	132.7
	21	13.3±1.3	ND	ND	ND	11.1±0.7	14.3±0.3	ND	ND	55.1±3.6	42.3±2.5	128.1
	28	10.0±0.8	ND	ND	ND	7.7±0.5	13.3±0.7	ND	ND	60.2±3.4	45.4±2.8	136.6
B	0	8.3±0.3	8.1±0.2	3.5±0.1	ND	21.1±0.3	13.1±0.9	7.1±0.1	5.9±0.1	42.6±2.4	40.1±2.8	149.8
	7	9.1±0.3	7.1±0.5	2.8±0.1	ND	20.1±0.9	14.3±0.5	6.3±0.5	2.3±0.1	41.1±1.2	42.3±3.2	140.4
	14	9.2±0.5	6.3±0.3	2.1±0.1	ND	19.2±1.3	16.8±0.3	6.0±0.2	2.0±0.0	45.1±2.1	42.6±2.4	139.3
	21	8.1±0.6	ND	ND	ND	16.9±1.5	15.9±0.1	ND	ND	48.7±2.8	45.9±3.1	125.5
	28	8.7±0.7	ND	ND	ND	10.2±1.1	12.5±0.9	ND	ND	45.2±3.0	48.3±2.9	122.9
C	0	13.2±1.0	7.2±0.2	3.8±0.2	ND	20.5±1.2	12.8±0.6	7.8±0.2	6.5±0.2	35.3±1.3	37.8±3.1	144.9
	7	14.6±1.2	8.1±0.3	3.9±0.2	ND	17.9±1.3	14.1±0.7	7.2±0.7	5.9±0.2	38.4±1.7	36.2±2.7	146.3
	14	12.7±0.7	7.4±0.2	4.0±0.2	ND	10.8±0.8	15.6±1.0	6.1±0.5	4.3±0.3	40.0±1.8	41.8±2.5	137.7
	21	9.0±0.4	ND	ND	ND	8.8±0.5	15.1±0.2	ND	ND	45.8±1.9	44.2±2.7	116.9
	28	11.9±0.5	ND	ND	ND	11.1±0.6	18.4±1.3	ND	ND	52.4±2.0	46.7±3.0	140.5
D	0	15.0±0.8	6.8±0.3	4.1±0.3	ND	19.6±1.0	12.3±0.6	8.0±0.1	6.3±0.2	40.0±2.1	35.2±2.7	147.3
	7	13.4±0.8	7.2±0.3	3.4±0.3	ND	20.5±2.0	13.2±1.0	6.7±0.3	6.0±0.3	42.0±1.5	37.8±2.1	150.2
	14	12.9±1.1	6.5±0.1	2.9±0.2	ND	21.0±2.3	14.7±0.7	6.0±0.2	5.5±0.2	44.2±1.5	39.4±3.2	150.1
	21	12.9±1.1	ND	ND	ND	15.3±1.2	13.5±0.3	ND	ND	51.0±3.7	40.8±2.3	129.5
	28	10.7±0.9	ND	ND	ND	13.8±1.0	12.2±1.2	ND	ND	53.6±2.1	43.1±2.4	137.4

Values are expressed as mean ± SD of triplicate measurements. ND: not detected ; GA: gallic acid ; PCCA: protocatechuic ; p-HO: p-hydroxy benzoic acid ; ChA: chlorogenic acid ; VA: vanilic acid ; *CFA: caffeic acid ;* SyA: syringic acid ; p-CA: p-coumaric acid ; FA: ferulic acid ; SNA: sinapic acid. A: control ; B: *marigold juice + vitamin C ; C: marigold juice + vitamins E* and D: *marigold juice + vitamins C and E.*

Table 2. Flavonoids of marigold juice *at different storage times*

Sample	Storage time (days)	*Flavonoids (µg/ ml)*					*Total*
		Rutin	**Myricetin**	**Quercetin**	**Apigenin**	**Kaempferol**	
A	0	*156.6±3.9*	5.4±0.3	*199.8±2.0*	10.2±0.8	3.4±0.1	375.4
	7	*145.5±3.0*	6.3±0.1	*187.2±3.4*	3.4±0.1	*ND*	342.4
	14	*132.4±4.2*	3.8±0.3	*200.1±1.4*	*ND*	*ND*	336.3
	21	*121.2±7.3*	*ND*	*166.5±5.1*	*ND*	*ND*	287.7
	28	*110.1±8.7*	*ND*	*141.4±8.0*	*ND*	*ND*	251.5
B	0	*143.2±2.3*	4.9±0.2	*206.5±2.1*	10.3±0.5	4.0±0.2	368.8
	7	*121.9±1.9*	5.8±0.1	*208.0±5.7*	4.0±0.2	*ND*	339.7
	14	*119.7±5.0*	4.2±0.2	*253.2±1.5*	*ND*	*ND*	377.1
	21	*108.5±9.0*	*ND*	*173.8±3.2*	*ND*	*ND*	282.3
	28	*95.7±9.5*	*ND*	*157.2±3.7*	*ND*	*ND*	252.9
C	0	*145.1±1.8*	5.1±0.5	*196.2±4.3*	10.3±0.3	3.8±0.3	360.5
	7	*122.4±2.0*	5.4±0.3	*190.1±4.2*	3.8±0.3	*ND*	321.7
	14	*120.2±4.7*	4.3±0.1	*210.5±4.4*	*ND*	*ND*	335.0
	21	*111.0±8.4*	*ND*	*134.2±5.7*	*ND*	*ND*	245.2
	28	*90.9±8.7*	*ND*	*121.1±1.4*	*ND*	*ND*	212.0
D	0	*151.3±4.7*	4.8±0.3	*206.7±7.8*	10.2±0.1	3.2±0.1	376.2
	7	*134.1±4.1*	4.6±0.2	*201.0±9.0*	3.2±0.1	*ND*	342.9
	14	*112.5±3.8*	3.5±0.5	*205.7±4.0*	*ND*	*ND*	321. 7
	21	*104.7±11.2*	*ND*	*157.8±7.9*	*ND*	*ND*	262.5
	28	*100.2±10.1*	*ND*	*129.0±3.0*	*ND*	*ND*	229.2

Values are expressed as mean ± SD of triplicate measurement. ND: not detected. A: control ; B: *marigold juice + vitamin C ; C: marigold juice + vitamins E* and D: *marigold juice + vitamins C and E.*

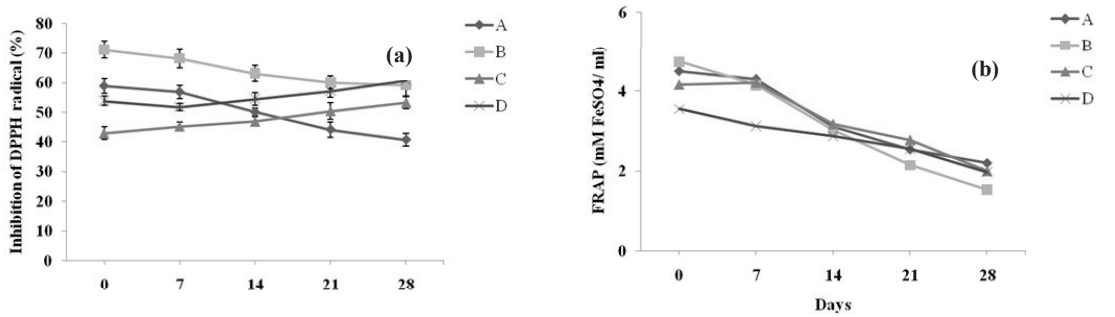

Figure 3. Antioxidant activity, DPPH assay (a) ; FRAP assay (b) of marigold juice at different storage times. (A: control ; B: marigold juice + vitamins C ; C: marigold juice + vitamins E and D: marigold juice + vitamins C and E).

3.5.2 Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+-TPTZ) complex, producing a coloured ferrous tripyridyltriazine (Fe2+-TPTZ) (Benzie & Strain, 1996). Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom (Gordon, 1990 ; Duh *et al.*, 1999). According to Benzie and Strain (1996), the reduction of Fe3+-TPTZ complex to blue colored Fe2+-TPTZ occurs at low pH. The ferric reducing power of marigold juice after different storage times, expressed as FRAP values (mmol FeSO4/ ml), is shown in Figure 3 (b). From the data presented by Gliszczynska-Swiglo and Tyrakowska (2003), it follows that storage of apple juice for 11 months at room temperature results in a decrease in the TEAC value by about 6-14%.

4. Conclusion and Suggestion

Effectiveness of storage time and vitamin added in stabilizing phenolic compound, carotenoids as well as their antioxidant activities were determined. The carotenoids (lycopene, β-carotene and lutein) and total flavonoids contents decreased during the storage period at 4 °C. Total phenolic contents increased during storage for three weeks storage at same condition while, declined at the end of storage. The phenolic acids found were ferulic acid sinapic acid the level of each increased during storage while, others phenolic acids and flavonoids were decrease during storage. The antioxidant activity, there was a slight increase in DPPH antioxidant capacity in marigold juice with added vitamin E and combination of vitamin C and vitamin E while, a decreased in marigold juice and marigold juice with added vitamin C. During storage, the stability of bioactive compounds in marigold juice is influenced by a complex interaction of storage time and chemical reaction. This study suggests that combined vitamin C and E improved quality and a more functional marigold juice to the consumer.

5. Acknowledgement

The authors gratefully thank the Office of the Higher Education Commission, Thailand (OHEC) for providing a research grant. We also thank Mahasarakham University for the laboratory facilities.

6. References

Abdel-Aal, E.M. & Rabalski, I. (2015). Composition of Lutein Ester Regioisomers in Marigold Flower, Dietary Supplement, and Herbal Tea. *Journal of Agricultural and Food Chemistry*, *63*, 44, 9740-9746.

Arena, E., Fallico, B. & Maccarone, E. (1999). The antioxidant activity of Pigmented oranges. In: Piga, A., Agabbio, M., Gambella, F., Nicoli, M.C. (Eds.), 2002. Retention of antioxidant activity in minimally processed Mandarin and Satsuma fruits. *Lebensmittel-Wissenschaft and Technologie*, *35*, 344-347.

Arts, I.C. & Hollman, P.C. (2005). Polyphenols and disease risk in Epidemiologic Studies. *American Journal of Clinical Nutrition, 81*, 317-325.

Bailey, C.A. & Chen, B.H. (1989). Chromatographic analyses of xanthophylls in egg yolks from laying hens fed turf Bermuda grass (*Cynodon dactylon*) meal. *Journal of Food Science, 54*, 584-586.

Baker, R. & Gunther, C. (2004). The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption. *Trends in Food Science and Technology, 15*, 484-488.

Bhattacharyya, S., Datta, S. Mallick, B., Dhar, P. & Ghosh, S. (2010). Lutein Content and *in Vitro* Antioxidant Activity of Different Cultivars of Indian Marigold Flower (*Tagetes patula* L.) Extracts. *Journal of Agricultural and Food Chemistry, 58*, 8259-8264.

Benzie, I.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*, 70-76.

Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M. & Morelli, I. (2001). Antioxidant principles from *Bauhinia terapotensis*. *Journal of Natural Products, 64*, 892-895.

Butsat, S. & Siriamornpun, S. (2010). Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chemistry, 119*, 606-613.

Butsat, S., Weerapreeyakul, N. & Siriamornpun, S. (2009). Changes in phenolic acids and antioxidant activity in Thai rice husk at five growth stages during grain development. *Journal of Agricultural and Food Chemistry, 57* (11), 4566-4571.

Chen, H.E., Peng, H.Y. & Chen, B.H. (1996). Stability of carotenoids and vitamin A during storage of carrot juice. *Food chemistry, 57*, 497-503.

Chuchird, P. & Pattarathitiwat, P. (2021). Effect of Lotus Petal (Nymphaea Lotus) and Pollen (Nelumbo Nucifera) on Chemical and Physical Properties of Gummy Jelly Lotus Flavor. *Science Technology and Engineering Journal (STEJ), 7* (1), 32-39.

Del Caro, A., Piga, A., Vacca, V. & Agabbio, M. (2004). Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments ad juices during storage. *Food Chemistry, 84*, 99-105.

Duh, P.D., Du, P.C. and Yen, G.C. (1999). Action of methanolic extract of mung hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. *Food and Chemical Toxicology, 37*, 1055-1061.

Gliszczynska-Swigło, A. & Tyrakowska, B. (2003). Quality of commercial apple juices evaluated on the basis of the polyphenol content and the TEAC antioxidant activity. *Journal of Food Science, 68*, 1844-1849.

Gonzalez de Mejıa, E., Loarca-Pina, G. & Ramos-Gomez, M. (1997). Antimutagenicity of Xanthophylls present in aztec Marigold (*Tagetes erecta*) against 1-nitropyrene. *Mutation Research, 389*, 219-226.

Gordon, M. H. (1990). The mechanism of antioxidant action *in vitro*. In: Hudson, B.J.F. *Food Antioxidants* (pp. 1-15). Elsevier.

Gulluce, M., Sahin, F., Sokmen, M. (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* (L.) ssp. Longifolia. *Food Chemistry, 103*, 1449-1456.

Hamburger, M., Adler, S., Baumann, D., Forg, A. & Weinreich, B. (2003). Preparative purification of the major anti-inflammatory triterpenoid esters from marigold (*Calendula officinalis*), *Fitoterapia. 74*, 328-338.

Hojnik, M., Škerget, M. & Knez, Ž. (2008). Extraction of lutein from Marigold flower petals experimental kinetics and modeling. *LWT-Food Science and Technology, 41*, 2008-2016.

Imeh, U. & Khokhar, S. (2002). Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. *Journal of Agricultural and Food Chemistry, 50*, 6301-6306.

Jacobs, J.J.M.R., Engelberts, A., Croes, A.F. & Wullems, G.J. (1994). Thiophene synthesis and distribution in young developing plants of *T. patula* and *T. erecta*. *Journal of Experimental Botany, 45*, 1459-1466.

Koga, T. & Meydani, M. (2001). Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. *American Journal of Clinical Nutrition, 73*, 941-948.

Li, B.B., Smith, B. & Hossain, M.M. (2006). Extraction of phenolics from citrus peels I. Solvent extraction method. *Separation and Purification Technology, 48*, 182-188.

Lin, C.H. & Chen, B.H. (2005). Stability of carotenoids in tomato juice during storage. *Food Chemistry, 90*, 837-846.

Marx, M., Stuparic, M., Schieber, A. & Carle, R. (2003). Effects of thermal Processing on trans-cis-isomerization of β-carotene in carrot juices and carotene containing preparations. *Food Chemistry, 83*, 609-617.

Moussaid, M., Lacroix, M., Nketsia-Tabiri, J. & Boubekri, C. (2000). Phenolic compounds and color of oranges subjected to a combination treatment of waxing and irradiation. *Radiation Physics and Chemistry, 57*, 273-275.

Naczk, M. & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 1523-1542.

Naik, G.H., Priyadarsini, K.I., Satav, J.G., Banavalikar, M.M., Sohoni, P.P., Biyani, M.K. & Mohan, H. (2003). Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry, 63*, 97-104.

Narahari, D., Venugopal, K., Kothandaraman, P. & Kumararaj, R. (1981). Marigold flower meal as a source of xanthophylls for egg yolk pigmentation. *International Journal of Poultry Science, 16*, 409-411.

Ojeda, O.M.A., Avila, G.E. & Tirado, A.F.J. (1983). Waste pulp from whole marigold (*Tagestes erecta*) flowers in diets for poultry. *Veterinarian Mexico, 14*, 145-149.

Pesek, C.A., Warthesen, J.J. & Taoukis, P.S. (1990). A kinetic model for equilibration of isomeric β-carotene. *Journal of Agricultural and Food Chemistry, 38*, 41-45.

Piccagliy, R., Marotti, M. & Grandi, S. (1998). Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. *Industrial Crops and Products, 8*, 45-51.

Piga, A., Agabbio, M., Gambella, F. & Nicoli, M.C. (2002). Retention of antioxidant activity in minimally processed Mandarin and Satsuma fruits. *Lebensmittel-Wissenschaft and Technologies, 35*, 344-347.

Prasad, N.K., Yang, B., Dong, X., Jiang, G., Zhang, H., Xie, H. & Jiang, Y. (2009). Flavonoid contents and antioxidant activities from Cinnamomum species. *Innovative Food Science and Emerging Technologies, 10*, 627-632.

Rapisarda, P., Bianco, M.L., Pannuzzo, P. & Timpanaro, N. (2008). Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes (*Citrus sinensis* (L.) Osbeck). *Postharvest Biology and Technology, 49*, 348-354.

Rodriguez-Amaya, D.B. (1999). Changes in carotenoids during processing and storage of foods. *Archivos Latinoamericanos de Nutricion, 49* (1), 38s-47s

Sakanaka, S. Tachibana, Y. & Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry, 89*, 569-575.

Sharma, S.K. & Le Magure, M. (1996). Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. *Food Research International, 29*, 309-315.

Shimoji, Y., Tamura, Y., Nakamura, Y., Nanda, K., Nishidai, S., Nishikawa, Y., Ishihara, N., Uenakai, K. & Ohigashi, H. (2002). Isolation and identification of DPPH radical scavenging compounds in kurosu (Japanese unpolished rice vinegar). *Journal of Agricultural and Food Chemistry, 50*, 6501-6503.

Siriamornpun, S., Kaisoona, O. & Meeso, N. (2012). Changes in colour, antioxidant activities and carotenoids (lycopene, *β*-carotene, lutein) of marigold flower (*Tagetes erecta* L.) resulting from different drying processes. *Journal of functional foods, 4*, 757-766.

Slattery, M.L., Benson, J., Curtin, K., Ma, K.N., Schaeffer, D. and Potter, J.D. (2000). Carotenoids and colon cancer. *American Journal of Clinical Nutrition, 71*, 575-582.

Snodderly, D.M. (1995). Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *American Journal of Clinical Nutrition, 62*, 1448-1461.

Spanos, G.A., Wrolstad, R.E. & Heatherbell, D.A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural and Food Chemistry, 38*, 1572-1579.

Srisaikham, S. & Rupitak, Q. (2021). Growth of *Caesalpinia sappan* L. by using different growing media and evaluation of antioxidant activity of foliage. *Science Technology and Engineering Journal (STEJ), 6* (2), 50-61.

Toor, R.K. & Savage, G.P. (2006). Changes in major antioxidant components of tomatoes during post-harvest storage. *Food Chemistry, 99*, 724-727.

Uzelac, D.V., Pospisil, J., Levaj, B. & Delonga, K. (2005). The study of Phenolic profiles of raw apricots and apples and their purees by HPLC for the evaluation of apricot nectars and jams authenticity. *Food Chemistry, 91*, 373-383.

Van Buren, J., de Vos, L. & Pilnik, W. (1976). Polyphenols in golden delicious Apple juice in relation to method of preparation. *Journal of Agricultural and Food Chemistry, 24*, 448-451.

Vasquez-Caicedo, A.L., Schilling, S., Carle, R. & Neidhart, S. (2007). Effects of thermal processing and fruit matrix on β-arotene stability and enzyme inactivation during transformation of mangoes into purée and nectar. *Food Chemistry, 102*, 1172-1186.

Wanyo, P., Kaewseejan, N., Meeso, N., & Siriamornpun, S. (2015). Marigold flower-powder exhibits significant potential to inhibit lipid oxidation in rice bran tea. *Food and function, 6* (6), 1808-1817.

Xianquan, S., Shi, J., Kakuda, Y. & Yueming, J. (2005). Stability of Lycopene during food processing and storage. *Journal of Medicinal Food, 8* (4), 413-422.

Zanoni, B., Peri, C., Nani, R. & Lavelli, V. (1999). Oxidative heat damage of tomato halves as affected by drying. *Food Research International, 31*, 395-401.