

## Antioxidant activities of local oregano and sweet basil leaves and application in a natural antioxidant enriched ravioli

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### Abstract

Total phenolic contents (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of methanolic extracts of local oregano and sweet basil were appraised and the ratio of oregano:sweet basil at 50:50 was most suitable for incorporation into the ravioli padding. When stored at -18°C for 4 months, the ravioli test sample (added with 5% of the blended herbs) was more stable in terms of thiobarbituric acid reactive substances (TBARS) values in comparison with the control. Although the herb blend adversely affected colour values of the samples, it helped improve sensory properties of the ravioli. Results of this study indicate the potential use of occidental and oriental blended herbs in the pasta product with the dairy-based padding.

### Keywords

Antioxidant  
Oregano  
Phenolics  
Ravioli  
Sweet basil

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### Introduction

The oxidation of lipids in foodstuffs results in the development of off-flavour, rendering the product unacceptable for human consumption. During processing, the structure of food is changed and the naturally occurring antioxidant systems are impaired, making lipids more exposed to oxygen and the processed foods more susceptible to oxidation (Madsen and Bertelsen, 1995). Antioxidants are compounds that can delay or prohibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidising chain reactions (Velioglu *et al.*, 1998). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

Spices and herbs with their ability to enhance and complement the flavours of an extensive variety of foods are popular ingredients in every cuisine. With the growth in the use of spices and herbs, there has been continued research to explore the antioxidant properties of plants which are essential for preserving foods and providing health benefits (Zheng and Wang, 2001). Oregano (*Origanum vulgare* L.) is widely used as a condiment and its flavour and smell

are greatly appreciated throughout the world for culinary applications. Moreover, it has antioxidant and antimicrobial properties (Loizzo *et al.*, 2009). Basil (*Ocimum basilicum* L.) is aromatic herb that is used extensively for adding a distinctive aroma and flavour to food. The leaves can be used fresh or dried for use as spices (Lee *et al.*, 2005). At present, there is increasing interest both in the industry and in scientific research for spices and aromatic herbs due to their strong antioxidant properties. In Thailand, spices and herbs are extensively used in household-cooking of traditional Thai dishes. The ordinarily used ones include sweet basil, clove, nutmeg, coriander, fennel, garlic, shallot, ginger, bergamot, lemon grass and others. However, such examples from occidental origins as oregano, marjoram, rosemary, sage and thyme are not common for general Thai consumers. In addition, a study on antioxidative properties of the locally produced Thai and western spices or herbs and their application in food products is relatively scarce. Therefore, this study was conducted in order to i) determine antioxidant activities of western (oregano) and Thai (sweet basil) herbs, ii) apply the appropriate blend of the two herbs in formulation of the ravioli padding, and iii) investigate alterations of the product's properties during frozen storage.

## Materials and Methods

### Materials and chemicals

Oregano was supplied by the Highland Royal Agricultural Project (Chiangmai province) and sweet basil (also known as common basil and specified onwards as basil) was purchased from the local market in Bangkok. Materials for preparation of ravioli dough were as follows: all-purpose wheat flour (United Flour Mill, Co., Ltd., Samutprakarn province); butter (United Dairy Foods, Co., Ltd., Bangkok); salt (Purified Salt Industry, Co., Ltd., Nakornrajsima province) and whole chicken eggs (approximately 65-70g/each, Bangkok Agricultural Products, Co., Ltd., Bangkok). Materials for preparation of ravioli padding were as follows: mozzarella cheese (Minor Cheese, Co., Ltd., Nakornrajsima province, with 23.6% fat) and sour cream (Foremost Dairy Foods, Co., Ltd., Bangkok, with 23.2% fat).

The chemicals used for extraction and appraisal of antioxidant activities of the leaf extracts were: absolute methanol (99.9%) and Folin-Ciocalteu (Carlo Erba Reagenti, Rodano, Milan, Italy), 2,2-diphenyl-1-picrylhydrazyl (Fluka Analyticals, Seelze, Germany), sodium carbonate (Fisher, Springfield, NJ, USA) and gallic acid (Sigma-Aldrich Inc., St. Louis, MO, USA). The chemicals used for assessment of TBARS were of analytical grade or the highest grade available and were obtained from Sigma (Sigma-Aldrich Inc.) and Merck (Darmstadt, Germany).

### Preparation of the leaf extracts

Each kind of plant material was cleaned and drained off excess tap water. Only the leaves were separated manually from the stems, evenly distributed as a one layer and dried at 60°C for 10hr in a tray dryer (HA-100S, Yiao Heng Co., Ltd., Bangkok). The dried leaves were then pulverised in a blender (Philips, HR 1797, Jakarta, Indonesia) and sieved through 100-mesh screen. The leave powder was dried again at 60°C in the same tray dryer until a moisture content of 7% was obtained. The dried leave powder was packed in Al-laminated PE bags under vacuum (-100 kPa) using a packaging machine (Multivac AG-500, Sepphagenmüller KG, Wolfertschwenden, Germany) and stored at -18°C in a freezer (Sanyo SF-C95, Tokyo, Japan) until used.

Leaf powder (3 g), either as a single kind of each plant or as a blend, was extracted with 150 mL of methanol in an Erlenmeyer flask plugged with cotton wool and tightly wrapped with Al-foil. Methanol was used because it is the most effective solvent for extraction of antioxidants from plant materials

including mulberry leaves (Arabshahi-Delouee and Urooj, 2007). The flasks were kept in a shaking water bath at 30°C with a speed of 150 rpm for 8 hr. The content was then centrifuged at 10,000 x g (Hettich, Rotanta 460R, Tuttlingen, Germany) at ambient temperature (30°C) for 20 mins and filtered through a Whatman No.1 filter paper on a Buchner funnel. Methanol was then removed using a rotary vacuum evaporator (Eyela, N-N series, Rikakikai Co., Ltd., Tokyo, Japan) at 40°C. The content (in a round bottom flask) was then dried in a hot air oven (Memmert, Model 600, Memmert, Germany) at 105°C for 1hr and kept in a desiccator and the dried extract was weighed only to calculate its yield as:  $(W_1/W_2) \times 100\%$ , where  $W_1$  is the weight of the dried extract and  $W_2$  is the weight of the dried leaf sample. However, the combined filtrate was evaporated to dryness with the same manner and the dried extract was redissolved with methanol to a specific concentration and decanted into the brown bottle. All the leaf extract samples were assembled and kept at -18°C until analysed.

### Antioxidant activities of the crude extract of the blended herbs

Determination of TPC was achieved by following the method described by Maisuthisakul *et al.* (2007). The TPC in the leaf extract was expressed as mg of gallic equivalent (GAE) per g dry weight of leaf. DPPH radical scavenging activity was conducted according to the method of Tomaino *et al.* (2005). The percentage radical scavenging effect was calculated as:  $[(A_{\text{DPPH}} - A_s)/A_{\text{DPPH}}] \times 100\%$ , where  $A_{\text{DPPH}}$  is the absorbance of the control solution containing only DPPH• radical and  $A_s$  is the absorbance of the sample (with the leaf extract). Various values of % scavenging effect were plotted against corresponding concentrations of the extract to assess for the  $EC_{50}$ , i.e., the efficient concentration of the extract necessary to decrease DPPH• radical concentration by 50%.

The two analyses were conducted for the dried leaf ratio of oregano:basil at 100:0, 75:25, 50:50, 25:75 and 0:100, respectively. The experimental design was a completely randomised design (CRD) with 3 replicates. The data were analysed using analysis of variance (ANOVA) and the means were compared by Duncan's New Multiple Range Test (DNMRT) at  $P \leq 0.05$  (Cochran and Cox, 1992). The most suitable ratio (50:50), composing of both the occidental and the oriental herbs and had appropriate antioxidant activities, would be used in the next experiment.

### Application of the blend of herbs in ravioli padding

The selectively appropriate ratio (50:50) of the two herbs was used in preparation of the ravioli padding with basic formulation of 200 g of mozzarella cheese, 150 g of sour cream, 9.2 g each of finely chopped fresh oregano and basil leaves. These ingredients were mixed in a Kenwood mixer (KM800, Havant, UK) using a K-blade for 2 mins. The addition levels of the blended herbs were varied at 2.5, 5.0, 7.5 and 10.0% (by the total weight of the padding). It was intentionally decided to use the blended herbs instead of the methanolic extracts and this was due to toxicity of residual methanol which might possibly remain.

Sensory evaluation of the padding was performed with a group of thirty panelists recruited from the undergraduate and graduate students of Department of Food Technology. They had acuity for four basic tastes and were familiarised with testing of food products by different sensory methods. The samples were evaluated for colour, appearance, herb odour, herb flavour and cheese flavour using a ten-point structured scale modified from the method of Larmond (1982), where, 0 corresponded to component's characteristic of the lowest quality and 10 to component's characteristic of the highest quality. For example, a score of 0 means "very rancid" and a score of 10 means "not rancid at all". Scores higher than 7 were considered acceptable. Each sample (approximately 15 g) was coded with a three digits random number and served at ambient temperature (30°C).

The experiment was a randomised complete block design (RCBD) with two replicates. The data were analysed using ANOVA and the means separated by DNMR at the same significance level. The most suitable amount of the blended herbs was chosen for preparation of the ravioli, which was frozen by liquid nitrogen (LIN) and determined for alterations of its quality attributes during a frozen storage trial.

#### *Preparation of the ravioli and the frozen storage trial*

Firstly, the dough was prepared from wheat flour (300 g), whole egg (165 g), unsalted butter (24 g) and table salt (6 g). All these ingredients were added into a mixing bowl (Kenwood, KM800, Havant, UK) and kneaded with a ribbon-shaped blade except that only 55 g of the whole egg was added simultaneously at the beginning. When the dough was apparently formed, the rest (110 g) of the whole egg was added and the mixture was further kneaded until the dough texture was smooth enough, i.e., the whole kneading time being 5 mins. The dough was rested in a close container for 1hr and then rolled into a flat sheet of 0.85mm thickness. The flattened dough was cut into a circular shape of 6.5 cm diameter using a metal

mould. Two and a half grams of the padding (as described previously and mixed with 5.0% of the blended herbs at the most suitable ratio of 50:50) was placed on top of the dough sheet, which was wrapped and pressed manually to form ravioli. All the samples were cooked in a household steamer by placing 20 pieces separately on each perforated tray until the core temperature reached 80°C and held at this temperature for 5 mins. All the samples were packed in polyvinylidene chloride bags (Janjaras Chem Supply Co., Ltd., Bangkok, Thailand; 17 x 25 cm<sup>2</sup> with water vapour transmission rate of 4g m<sup>-2</sup> 24h<sup>-1</sup> and oxygen transmission rate of 15mL m<sup>-2</sup> 24h<sup>-1</sup>) and immediately sealed under atmospheric pressure using a packaging machine (Multivac AG-500). The packages were left in ambience for 5 mins and then frozen in a cryogenic freezer (Cryo-Test chamber, Model No. F831059E; Air Products and Chemicals Inc., Allentown, PA, USA) assembled with a LIN tank (Taylor-Wharton XL-55HP, Allentown, PA, USA) at a chamber temperature of -30, -50 and -70°C, respectively, until the internal temperature reached -18°C. This was performed in order to choose the most appropriate freezing conditions which were significantly different ( $P \leq 0.05$ ) as follows: 19.0 mins (at -30°C), 11.2 mins (at -50°C) and 5.0 mins (at -70°C), respectively. Therefore, the freezing temperature was chosen at -70°C since the central temperature of the samples reached -18°C with the very shortest time and should result in the thorough distribution of the smallest ice crystals within each ravioli piece and could help protect the integrity of the sample after thawing.

The ravioli test samples and the control (without any addition of the blended herbs) was prepared according to the method described previously. Nine pieces of the samples were packed in each polyvinylidene chloride (PVDC) bag under atmospheric pressure, frozen with LIN at -70°C and stored at -18°C for 4 months. At the regular time intervals of 2 weeks, the raviolis were randomly sampled, thawed in a refrigerator overnight and appraised for their colour values of CIELAB ( $L^*$ ,  $a^*$  and  $b^*$ ) system using a Minolta Chromameter (CR 300, Minolta, Tokyo, Japan) and TBARS values (of the padding) using the distillation method of Tarladgis *et al.* (1960). In addition, sensory evaluation of the stored and preheated (75°C) product were also conducted using ten-point structured scales for colour, herb odour, herb flavour, cheese flavour and rancid flavour.

The experiments were asymmetric factorial (2 x 9) in CRD with two replicates for an assessment of the physicochemical properties and asymmetric

Table 1. Yield and antioxidant activities of the methanolic extract of the blended herbs

Ratio of herbs	Yield (%) db)	Total	Total	DPPH radical	DPPH radical
		phenolics (mg GAE/g sample, db)	phenolics (mg GAE/g sample, wb)	scavenging activity (EC <sub>50</sub> , µg sample, db/µg DPPH)	scavenging activity (EC <sub>50</sub> , µg sample, wb/µg DPPH)
Oregano (100)	21.12 ± 0.71 <sup>a</sup>	116.8 ± 7.2 <sup>a</sup>	10.2 ± 0.6 <sup>a</sup>	3.37 ± 0.06 <sup>a</sup>	0.29 ± 0.01 <sup>a</sup>
Oregano:basil (75:25)	19.19 ± 0.49 <sup>a</sup>	69.6 ± 3.8 <sup>a</sup>	6.1 ± 0.3 <sup>a</sup>	3.91 ± 0.19 <sup>a</sup>	0.34 ± 0.02 <sup>a</sup>
Oregano:basil (50:50)	17.37 ± 0.18 <sup>a</sup>	59.1 ± 2.7 <sup>a</sup>	5.2 ± 0.2 <sup>a</sup>	4.45 ± 0.26 <sup>a</sup>	0.39 ± 0.02 <sup>a</sup>
Oregano:basil (25:75)	15.77 ± 0.18 <sup>a</sup>	36.9 ± 0.4 <sup>a</sup>	3.2 ± 0.0 <sup>a</sup>	8.38 ± 0.49 <sup>a</sup>	0.74 ± 0.04 <sup>a</sup>
Basil (100)	14.02 ± 0.25 <sup>a</sup>	12.1 ± 0.7 <sup>a</sup>	1.1 ± 0.1 <sup>a</sup>	39.70 ± 2.88 <sup>a</sup>	3.49 ± 0.25 <sup>a</sup>

a, b, ... Means ±SD within the same column bearing different superscripts are significantly different ( $P \leq 0.05$ ).

db: dry bulb; wb: wet bulb

factorial in RCBD for sensory evaluation. The data were analysed by ANOVA and the means were compared at  $P \leq 0.05$ .

## Results and Discussion

### Selection of the most appropriate ratio of the herbs

Ratios of the blended herbs affected significantly ( $P \leq 0.05$ ) all the %yields and TPCs of the methanolic extracts (Table 1). Both the TPC and DPPH radical scavenging activity were reported with the wet-bulb and dry-bulb basis due to possibly high variations in moisture content of the herbs used. For example, moisture content of fresh basil leaves is very high at 92.06%; wet basis (USDA, 2016). Moreover, distribution of phenolic compounds in plants at the tissue, cellular and subcellular levels is not uniform. Furthermore, their levels in plant sources are also dependent on such variables as cultivars, growing conditions, ripening process, cultivation techniques, as well as processing and storage conditions (Nacz and Shahidi, 2006). Therefore, the values with dry weight basis are also intentionally accompanied with the wet basis ones for extensive or crossed-reference comparison. As for the DPPH radical scavenging activity (as EC<sub>50</sub>), however, the values were not significantly different among the first three ratios but these values were significantly lower, i.e., reflecting the higher antioxidative capabilities, when compared to the other two ratios of 25:75 and 0:100 (oregano:basil). It was postulated that antioxidant activity was dependent mainly on the content of the phenolic compounds in each herb. Turkmen et al. (2006) also suggested that the higher the polarity of the solvent, the higher the DPPH radical scavenging

activity of the black and black mate tea which was closely related to the amount of polyphenols. A variety of herbs are known to be sources of phenolic compounds with antioxidant activities (Zheng and Wang, 2001; Yoo *et al.*, 2008). The %yield of the absolute methanol extract may be directly related to the overall contents of phenolic compounds present in each herb. The highest yield of pure oregano extract at 21.12% was comparable to those values of 26.2% (using methanol dispersion and incubation for 2 hr in an ultrasonic bath) and 25.4% (using water at 80°C for extraction) as reported by Škerget *et al.* (2005) and Boroski *et al.* (2012), respectively. The highest TPC of the sole oregano extract was in accordance with its highest %yield, and this was followed successively by the decreasing ratios of oregano:basil. This result suggested that there were higher quantity of total phenolic compounds in oregano leaves when compared to basil, and these chemicals were extracted more into methanol. Lin *et al.* (2005) also reported that a 100% mixture ratio of oregano extract powder contained higher amount of total phenolic (at 2.3 fold) than that of 100% cranberry and the major phenolic found in these extracts was rosmarinic acid.

Several methods have been used to determine antioxidant activities of plants. In this study, the DPPH radical-scavenging activity, based on both electron transfer and hydrogen atom transfer reactions (Prior *et al.*, 2005) was used to test for such activity of the extracts. It was clearly shown that the extract of oregano leaves had the lowest EC<sub>50</sub> value of 3.37 µg dry sample/µg DPPH, i.e., the highest antioxidant activity, whereas the extract of basil leaves had the highest value of 39.70 µg dry sample/µg DPPH. Interestingly, the EC<sub>50</sub> values were inverse to those



of the TPC values, i.e., the highest TPC of the sole oregano extract being 116.8mg GAE/g dry sample and the lowest one of the sole basil extract being 12.1 mg GAE/g dry sample, respectively. Maisuthisakul *et al.* (2007) also found similar relationships between the TPCs, the total flavonoid contents and the DPPH radical-scavenging activity of Thai indigenous herbs and vegetables. Also, there were the similar results relative to the TPCs, the total flavonoid contents, the total antioxidant capacities and the DPPH radical-scavenging activity of various herb extracts available in Korea (Yoo *et al.*, 2008). In addition, Zheng and Wang (2001) also found, in a study with the extracts of various herbs collected from the National Herb Garden of the U.S. National Arboretum, that the Greek mountain oregano had the higher content of total phenolic compounds and the higher oxygen radical absorbance capacity (ORAC) value relative to the ones of the sweet basil.

Numerous researchers have identified different phenolic compounds in a variety of herbs and spices. As for oregano, derivatives of phenolic acids, flavonoids, tocopherol, rosmarinic acid and carvacrol were determined (Pizzale *et al.*, 2002). There are several varieties of basil grown in many regions of the oriental countries and the plants have the clove-like flavour that is generally stronger than that of western basil and the oxidative stability effect might be related to their phenolic content and structure (Juntachote *et al.*, 2007).

Our results revealed that oregano possessed higher antioxidant activity due to its higher total phenolic content. It was also disclosed that this parameter was correlated with antioxidant activity; higher concentrations were usually connected with better oxidation inhibition (Nadeem *et al.*, 2015). However, the DPPH radical scavenging activity values of the sole oregano, the 75:25 and 50:50 (oregano:basil) blends were not significantly different. This indicated the potential of using basil in the blend with oregano for the application in a variety of foods containing lipids with polyunsaturated fatty acids. In Thailand, oregano is cultivated and harvested only in the northern mountains where the weather is cool enough, whereas basil is grown all around the country. In addition, the price of oregano is highly expensive due to the scarce supply comparing to that of basil. Consequently, the ratio of 50:50 was the most appropriate due to the cost effectiveness, the more extensive use of both the locally grown western herb and popular Thai herb and, more importantly, the acceptable antioxidant activity of the blended extract.

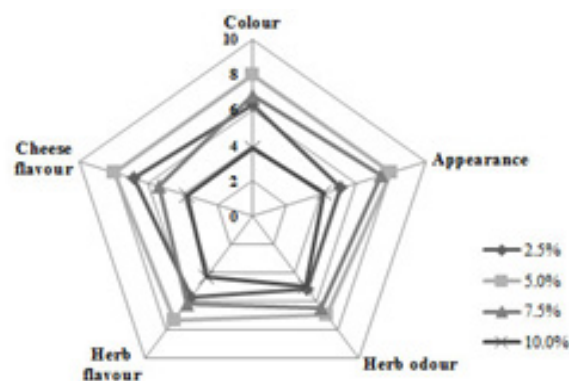


Figure 1. Sensory scores of the ravioli padding added with different amounts (% of the padding) of 50:50 (oregano:basil) leaves

#### Sensory evaluation of the padding

Four different quantities of the 50:50 ratio of oregano and basil leaves at 2.5, 5, 7.5 and 10% (based on the padding weight), respectively, were used in preparation of the ravioli paddings which were appraised for their sensory traits. The results are given in Figure 1.

The amount of the blended herbs affected significantly ( $P \leq 0.05$ ) all the sensory traits appraised and it seemed that the addition of the blended herbs enhanced sensory qualities of the padding. Hala *et al.* (2010) also reported, in a study on low fat UF-soft cheese supplemented with rosemary extract (RE) during cold storage ( $6 \pm 2^\circ\text{C}$ ), that cheese samples with more than 3% RE were highly acceptable and had significantly higher scores for flavour and total scores than the control either when fresh and throughout the storage of 30 days. When the data were analysed further, Pearson's correlation coefficients between overall preference and each sensory trait were: colour (0.97), appearance (0.89), herb odour (0.85), herb flavour (0.99) and cheese flavour (0.92). This reflected the overall sensory acuity of this group of panelists and it seemed that colour, herb flavour and cheese flavour dominated the overall preference. However, the most appropriate addition level was 5.0% which gave the sample with the highest sensory scores. Too low or too high amounts could possibly result in the padding with inferior sensory properties. Therefore, this amount was chosen for preparation of the ravioli which was very rapidly frozen and monitored for alterations of its physicochemical and sensory properties during storage.

#### Changes in various properties of ravioli during frozen storage

As for colour, the extended storage resulted in variation of lightness (Figure 2). The control sample was lighter because it was not affected by the dark green colour of the blended herbs, i.e., the green

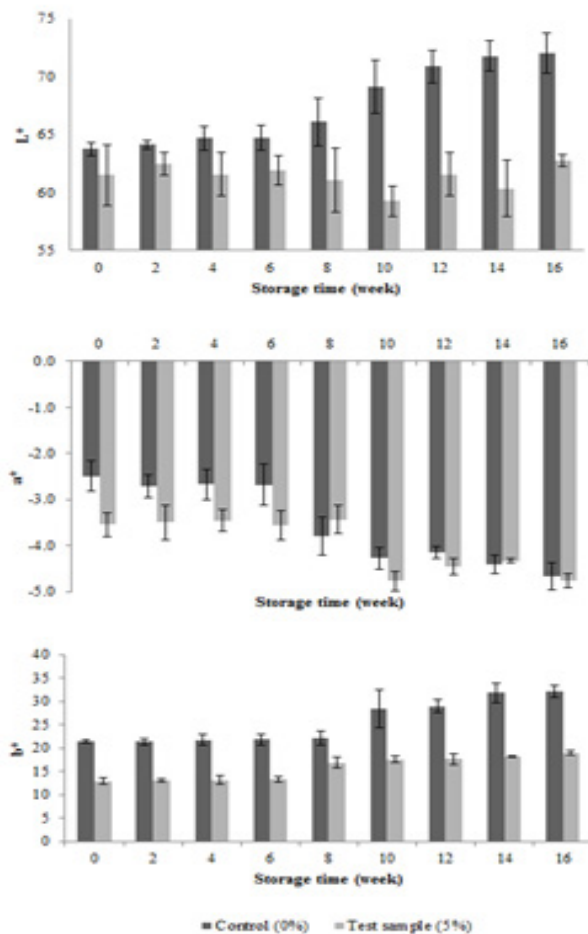


Figure 2. Effects of the addition level of the blended herbs and storage time on colour of the frozen ravioli;  $L^*$  (lightness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness)

colour of the control also being less than that of the test sample. Yellowness increased successively whereas redness tended to decrease with longer storage time. These results could be explained by the non-enzymatic Maillard reaction between carbonyl groups of glucose especially in egg white (used in dough formulation) and amino groups within amino acids of the proteins in ravioli dough (Fennema, 1996). The control had significantly higher  $b^*$ -values than the test sample. This might be due to the bright yellow colour of the control given by the whole egg (used at 33% of the dough weight). Contrastingly, the more disguising effect of the dark green colour of the two herbs was observed in the test sample.

There have been reports that herb extracts were utilised for preserving various kinds of foods such as meat, poultry, fish, lard, soybean oil and etc. from spoiling by rancidity (Bandyopadhyay *et al.*, 2007; Boroski *et al.*, 2012), however, the use in dairy products was scarce. In the present study, the blended oregano and basil leaves could also help improve the oxidative stability of the ravioli padding by lowering

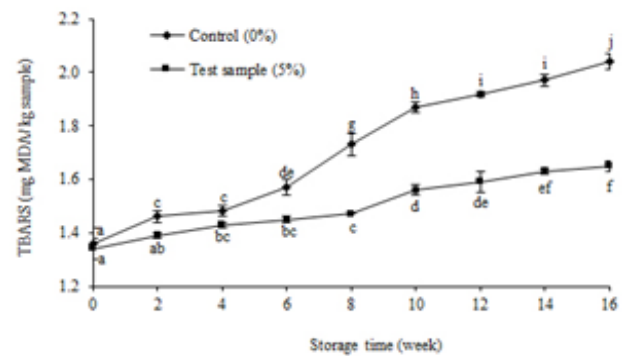


Figure 3. TBARS values of the padding from the frozen ravioli stored at  $-18^{\circ}\text{C}$  for 4 months

the TBARS values upon the frozen storage of 4 months. (Figure 3). This could be accounted for by the action of polyphenolic compounds existing in the two plants. Boroski *et al.* (2012), in a study on the use of oregano extract (OE) and oregano essential oil as antioxidants in functional dairy beverage formulations, found that the OE showed a high potential to inhibit the DPPH radical due to such compounds as rosmarinic acid, caffeic acid, coumaric acid, quercetin, and carvacrol (Exarchou *et al.* 2002) and the authors concluded that the OE may act as an antioxidant in dairy beverages enriched with omega-3 fatty acids. In addition, Merai *et al.* (2003) reported that water insoluble fraction of Tulsi (*Ocimum sanctum* L.) leaves possessed good antioxygenic properties and phenolic compounds present in the leaves were the main factors in extending the oxidative stability of ghee.

Although the advantageous result was attained by incorporation of the two herbs, the resultant differences of TBARS values between the control and the test sample were not substantially pronounced specially during the first six weeks. Beyond the eighth weeks, the differences were more distinguished. In general, TBA-reactive compounds are produced in substantial amounts only from fatty acids containing three or more double bonds (Nawar, 1996). Furthermore, Swaisgood (1996) explained that, based on all of the fatty acids identified in milk, saturated fatty acids accounted for 62.38% of the total and monoenoic acids, dienoic acids, polyenoic acids, monobranch acids, multibranch acids and miscellaneous acids for 30.75, 2.97, 0.85, 1.36, 0.83 and 0.40%, respectively. Besides, milk itself acts as a good source of antioxidants including urate (Chen *et al.*, 2003), vitamins E and C, carotenoids and different forms of antioxidant proteins such as caseins (Cervato *et al.*, 1999). These constituents might furnish the antioxidant activities surpassing those exerted by the phenolic compounds present in the two herbs and also gave the similar effect both on the control and the

Table 2. Sensory scores of the frozen ravioli samples

Addition level (% of the padding)	Storage time (wk.)	Colour	Herb odour	Herb flavour	Cheese flavour	Rancid flavour
0	0	7.90 ± 0.99 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	8.10 ± 1.28 <sup>ab</sup>	9.07 ± 0.94 <sup>a</sup>
	2	8.57 ± 0.85 <sup>i</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	8.20 ± 0.92 <sup>b</sup>	8.63 ± 0.96 <sup>b</sup>
	4	8.43 ± 0.85 <sup>ii</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	8.27 ± 0.94 <sup>b</sup>	8.23 ± 0.72 <sup>cd</sup>
	6	8.23 ± 0.85 <sup>iii</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	8.20 ± 0.84 <sup>b</sup>	8.03 ± 0.66 <sup>cd</sup>
	8	8.10 ± 0.71 <sup>iv</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	8.23 ± 0.77 <sup>b</sup>	7.73 ± 0.63 <sup>d</sup>
	10	7.57 ± 0.67 <sup>v</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	7.77 ± 0.62 <sup>cd</sup>	7.43 ± 0.72 <sup>d</sup>
	12	7.07 ± 0.69 <sup>e</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	7.53 ± 0.72 <sup>cd</sup>	7.30 ± 0.74 <sup>d</sup>
	14	7.07 ± 0.78 <sup>e</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	7.40 ± 0.67 <sup>cd</sup>	7.27 ± 0.78 <sup>d</sup>
	16	6.97 ± 0.67 <sup>de</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	7.27 ± 0.74 <sup>cd</sup>	7.20 ± 0.76 <sup>d</sup>
5	0	5.70 ± 1.13 <sup>a</sup>	7.70 ± 1.41 <sup>a</sup>	8.13 ± 1.03 <sup>a</sup>	6.70 ± 1.90 <sup>a</sup>	9.12 ± 0.92 <sup>a</sup>
	2	6.63 ± 0.99 <sup>bc</sup>	7.97 ± 1.09 <sup>ab</sup>	7.77 ± 1.06 <sup>ab</sup>	6.93 ± 1.19 <sup>ab</sup>	8.50 ± 1.19 <sup>b</sup>
	4	6.40 ± 0.85 <sup>c</sup>	8.13 ± 1.00 <sup>a</sup>	7.50 ± 0.93 <sup>bc</sup>	7.23 ± 1.09 <sup>cd</sup>	8.47 ± 0.89 <sup>bc</sup>
	6	6.73 ± 0.78 <sup>cd</sup>	8.20 ± 0.92 <sup>a</sup>	7.83 ± 0.59 <sup>b</sup>	7.70 ± 0.87 <sup>bc</sup>	8.13 ± 0.60 <sup>c</sup>
	8	6.73 ± 0.73 <sup>cd</sup>	7.77 ± 0.79 <sup>cd</sup>	7.60 ± 0.72 <sup>cd</sup>	8.03 ± 0.71 <sup>bc</sup>	8.47 ± 0.68 <sup>bc</sup>
	10	5.90 ± 0.88 <sup>a</sup>	7.13 ± 0.72 <sup>d</sup>	7.27 ± 0.63 <sup>d</sup>	7.20 ± 0.71 <sup>cd</sup>	8.17 ± 0.64 <sup>cd</sup>
	12	5.77 ± 0.72 <sup>a</sup>	7.00 ± 0.82 <sup>d</sup>	7.33 ± 0.60 <sup>cd</sup>	7.13 ± 0.68 <sup>cd</sup>	7.97 ± 0.66 <sup>cd</sup>
	14	5.92 ± 0.61 <sup>a</sup>	7.10 ± 0.71 <sup>d</sup>	7.43 ± 0.77 <sup>cd</sup>	7.40 ± 0.76 <sup>cd</sup>	7.93 ± 0.69 <sup>cd</sup>
	16	5.67 ± 0.71 <sup>a</sup>	6.93 ± 0.78 <sup>d</sup>	7.37 ± 0.85 <sup>cd</sup>	7.47 ± 0.73 <sup>cd</sup>	7.93 ± 0.58 <sup>cd</sup>

a, b... Means ± SD within the same column bearing different superscripts are significantly different ( $P \leq 0.05$ ).

test sample at the first stage of the storage yielding not-too-much different TBARS values. Although the TBA test is an old and popular colourimetric method used to measure rancidity in some foods, the reaction itself is not specific and is produced by a large number of secondary oxidation products developed lately during the course of lipid peroxidation (Frankel, 2005), thus, antioxidant activity given by the blended herbs was more effectively pronounced during the later storage. Skrede *et al.* (2004) observed that antioxidant capacity of milk products could be strengthened by the inclusion of fruit preparations, and the antioxidant capacity was well maintained in the products during storage under various conditions. Ozkan *et al.* (2007) also found that the essential oil of *Satureja cilicica* L., a well-known aromatic plant used to produce essential oil and aromatic water in the mountainous regions of the Mediterranean part of Turkey, exhibited a strong antioxidant activity by remarkably decreasing the peroxide values of butter (stored either at 4°C or 20°C) especially when used at higher concentrations. In our study, the differences between the TBARS values of the test sample and the control were increasingly observed during the frozen storage at -18°C. For example, the values were 0.02, 0.26 and 0.39 mg MDA/ kg sample at 0, 8 and 16 weeks, respectively.

Although the natural antioxidants are advantageous for protection of foods from chemical deterioration, evaluation of their impact on sensory properties is needed before these ingredients are

successfully used in food product development. Therefore, sensory properties of the oregano-basil enriched ravioli were also appraised during the frozen storage (Table 2).

The addition of oregano:basil blend helped improve specially the rancid flavour score of the raviolis especially during the early storage, while the test sample had better scores of rancid flavour in comparison with those of the control. Evidently, this phenomenon was resulted from antioxidant activities exerted by phenolic compounds existing in both herbs. Nevertheless, their resultant activities naturally diminished with extended storage time which also caused deteriorative effects on all sensory traits appraised. However, all the sensory scores decreased with the extended storage. It was also found that juiciness of the stored samples decreased significantly with time (data not shown). Hui (2006) stated that freezing of cheeses help maintain their quality attributes especially with those to be used in pizzas and food dishes prepared by ordinary cooking or baking. However, there is a tendency of crumbliness which can happen if the cheeses are frozen and thawed before use. This is due to migration and loss of moisture content caused by difference in vapour pressure (Sun, 2006). Colour and cheese flavour scores of the test sample were lower than those of the control. This was accordingly due to the presence or absence of the two blended herbs. If present, however, the two herbs added pleasant odour and flavour to the product. Usually, herbs and their



derivatives have long been used due to culinary and medicinal purposes and added in the food to prevent the formation of undesirable oxidation products. Not only are many of the flavouring and aromatic compounds distinctively present, but they can also be used to conceal off-flavour and odour (Ozkan *et al.*, 2007). This evidence could also be proved in our study. According to sensory results, the ravioli containing oregano and basil was more superior to the control especially if kept longer. This also confirms the necessity of using the two kinds of herb in developing such a pasta product stuffed with cheeses.

## Conclusion

It was demonstrated that the locally grown oregano and sweet basil leaves possess good antioxidant activities. If used in a ratio of 50:50, the two blended herbs at 5% helped improve sensory characteristics of the ravioli padding. During a frozen storage trial (at -18°C for 4 months), these two herbs also maintained essential quality attributes. Considering all the physicochemical and sensory properties, it is conclusive that the herbal ravioli is not only a value added but also a better preserved food. Also, the blend between occidental and oriental herbs can be potential source of natural antioxidants, not only in dairy-based products but also in other foods containing considerable amounts of highly unsaturated fat. Nevertheless, the qualitative and quantitative identification of specific phenolic compounds presenting in the appropriate blended extract of these two herbs should be further investigated.

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