

## Chemical, Rheological and Sensory Evaluation of Pate Stuffed with Broccoli (*Brassica oleraceae* L.)

Esmat A. Hassan<sup>1</sup>, Ahmed M.S. Hussein<sup>2,\*</sup>, Azza A.A. Hussein<sup>2</sup>

<sup>1</sup>Botany Department, Division of Agricultural and Biological Research, National Research Centre, Dokki, 12311, Giza, Egypt

<sup>2</sup>Food Technology Department, National Research Centre, Dokki, 12311, Giza, Egypt

Key words: baking quality, methionine-treated broccoli florets, characteristics, cancer, pate, reduced glutathione

This study aimed to produce a baked dietary product (pate) enriched with broccoli florets that were sprayed in the field with methionine. Treated broccoli florets were separately stuffed in pate at three levels (5, 10 and 15%, w:w) in the form of minced, steam blanched and fried broccoli florets. A significant increase in reduced glutathione content was observed in the methionine-treated broccoli florets compared to methionine-untreated broccoli florets during processing to minced, steam blanched or as fried with butter. The pate stuffed with methionine-treated broccoli florets at different levels had higher fiber and protein contents if compared to control sample (not stuffed pate). Stuffing the processed pate had no effect on the estimated rheological properties, color attributes, baking tests and organoleptic properties. Increasing the ratio of stuffed methionine-treated broccoli florets increased loaf weight and decreased crumb moisture. The results revealed that stuffing pate with methionine-treated broccoli florets had enriched the nutritive value and baking quality. Generally, pate stuffed with methionine-treated broccoli florets (5:15%) did not significantly affect technological, rheological, sensory quality of pate and improved its nutritional values.

### INTRODUCTION

Broccoli (*Brassica oleraceae* L.) is a cool season crucifer of Mediterranean origin, belonging to the plant Order Papaverales. Broccoli is well known for its high content of antioxidants, e.g. sulforaphane, beta-carotene, indole, quercetin, and glutathione. It also contains fat, protein, carbohydrates, fiber, water, iron, calcium, minerals, and various vitamins *i.e.* A, C, E, riboflavin, nicotinamide [Jeffery & Araya, 2009]. Sulforaphane is found in abundance in broccoli. It plays an important role in human health where it inhibits cancer cells [Li *et al.*, 2010]. The initial precursor in the synthesis of sulforaphane from broccoli (family *Cruciferaeae*) is methionine [Sarikamis *et al.*, 2006]. Therefore, to increase the production of sulforaphane it is possible to use methionine and broccoli seed extract as precursor in the growth media. Tilaar *et al.* [2012] found that the combination of 100 mg methionine with 1 g seed extract was the best combination with the highest sulforaphane content in adventive shoots (182.09 ng/g of shoot).

Yanaka *et al.* [2009] reported that a diet rich in broccoli reduced cancer risk. The protective effect of broccoli is attributed to its contents of the sulfuraphane compound (SF, R-1-isothiocyanate-4-methylsulfinylbutane), which is conjugated to reduced glutathione (GSH). Thus inducing an anti-inflammatory/anticarcinogenic effect [Kim *et al.*, 2009]. Numerous epidemiological studies indicate that Brassica

vegetables in general, and broccoli in particular, protect humans against cancer [Moreno *et al.*, 2006]. Borowski *et al.* [2008] analysis of 15 florets of broccoli cultivated in the same agricultural and climatic conditions showed wide individual variation in bioactive compound content and in antioxidant properties. From the consumer standpoint, this wide variation is an undesirable feature. Among glucosinolates, the greatest differences (CVs) were found for progoitrin (34.22%), 4-hydroxybrassicin (27.32%) and neoglucobrassicin (24.44%). Large variations were also observed for vitamin C (29.11%), including dehydroascorbic acid (26.72%) and OH• (25.76%) and DPPH• (21.77%) radical-scavenging activities. Glucoraphanin (14.84%) and phenolics (14.95%) showed lesser variation. Sanwal *et al.* [2006] found out that synthetic fertilizers had negative effects on the antioxidants of broccoli. On the other hand, the recovery of broccoli antioxidants, *i.e.* the sulfuraphane (SF) substance, from raw florets or sprouts when macerated or eaten reached 60–80 %. Mild cooking however, may realize 100% of the compound, whereas further cooking is found to secure a high percentage of the SF recovery *via* certain microbial enzyme in the colon [Juge *et al.*, 2007]. It was further noted that among other vegetables, steam cooking of broccoli significantly improved the *in vitro* bile acid binding which is related to lowering the risk of exposure to cancer [Kahlon *et al.*, 2008].

In the present work we aimed to process a bakery product (pate) stuffed with methionine-treated broccoli florets taken from plants cultivated under non-chemical conditions. The florets were subjected to different types of cooking for

\* Corresponding Author:  
E-mail: a\_said22220@yahoo.com (A.M.S. Hussein)

stuffing with variable percentages per weight of the pate unit. Reduced glutathione content was determined to detect the changes in relation to broccoli cooking type. Physical, nutritional value and other quality characteristics of the pate product were determined under the performed treatments of the pate components.

## MATERIAL AND METHODS

### Broccoli cultivation

Broccoli (*Brassica oleraceae* var. *italica* plenck L. cv. De-cathlon Hybrid) was cultivated by transplants on October 15th /2010 in the Experimental Station of the National Research Centre in Noharia, Alexandria Governorate, Egypt. Method of cultivation, agricultural practices and biofertilization were carried out as described by Badawi *et al.* [2005] and Abd EL-Rahman *et al.* [2010].

### Treatment and harvest

At the age of 36 days the plants were sprayed with the amino acid methionine at 100 ppm concentration 3 times at 3 days intervals. Heads were collected and detached from the stems at the age of 45 days of the florets with a weight of ca. 500–600 g and height of 15–17 cm.

### Broccoli florets preparation for stuffing

Broccoli florets were divided into three parts and treated as follows: (a) minced fresh green in a blender; (b) steam blanched for 10 min at 100°C, minced in the blender with spices of cumin, salt and black pepper; and (c) amended with butter and exposed to light frying for 3 min with the addition of cumin, salt and black pepper and then smashed.

### Material for the control dough

Wheat flour (72% extraction) was obtained from South Cairo Mill Company, Giza, Egypt. Sugar, butter, oil, salt, eggs, bread improver and active dry yeast (*Saccharomyces cerevisiae*) from local market were used to make the control dough.

### Preparation of the dough for stuffing and baking

The dough was prepared as follows: to 1000 g wheat flour (72%) 10 g yeast was dissolved in warm water (35°C) and added together with 10 g NaCl, 100 g sugar, three eggs, 10 g bread improver and 100 g butter. The dough was then kneaded and covered with a plastic lid for 15 min. Melted warm 100 g butter kept in a warm place was added to the dough and kneading was repeated. The dough was left to complete its fermentation at 30°C and 80–85% relative

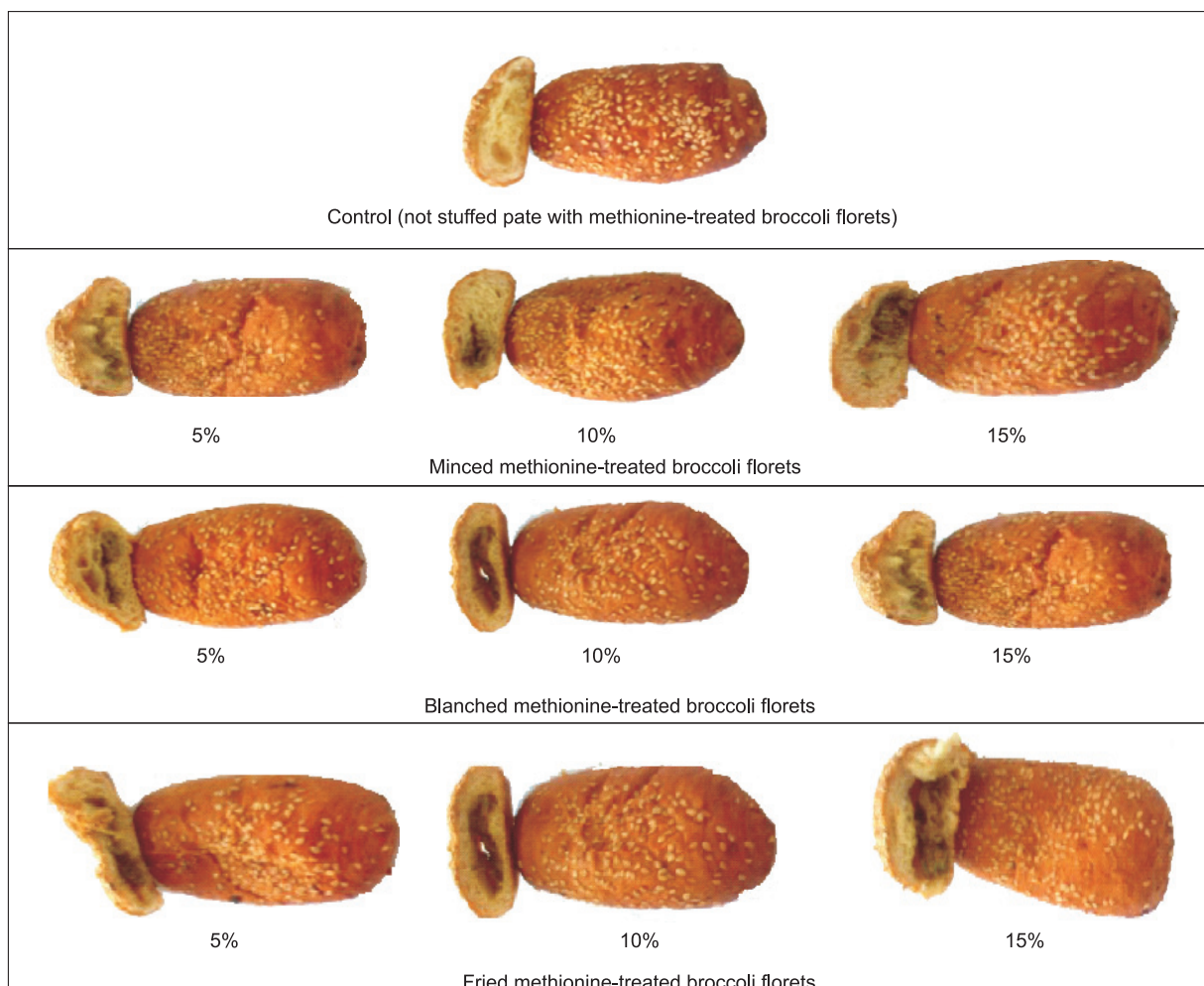


FIGURE 1. Photo of pate stuffed with methionine-treated broccoli florets.

humidity for 45 min in a fermentation cabinet. The dough was then sprinkled with flour to facilitate its shaping to reach a thickness of 1 cm before stuffing step.

### Rheological properties of dough

Rheological properties of the dough were evaluated using Farinograph and Extensograph according to AACC [2000], Methods No. 54 -10 and 54 – 21 [2000], respectively.

### Pate stuffing

Pate pieces (50 g) made from the fermented pate dough were stuffed with the 3 types of the prepared methionine-treated broccoli florets described above, *i.e.* minced raw green fresh, steamed and butter amended fried ones at various levels of 5, 10 and 15% of the pate unit weight. This was done with florets taken from methionine-treated plants and from the control ones as well.

### Baking

The pate dough stuffed with methionine-treated broccoli florets was then placed in trays for one hour till complete fermentation in the fermentation room. Finally, it was covered with whipped egg and baked in an oven at 220°C for 10 min. The resulted products were cooled and packed.

### Determination of the reduced glutathione content in broccoli florets

Total reduced glutathione was estimated in the control and in the methionine-treated broccoli of the cooked florets. Spectrophotometric methods were adopted to this end as described in detail by Griffith [1980] and adopted by Anderson & Gronwald [1991]. Broccoli florets tissues were homogenized at 4°C in trichloroacetic acid (5% w/v) containing 10 mmol/L EDTA and centrifuged for 12,000×g for 10 min. Reduced GS (GSH) was determined in 100 mL phosphate buffer at pH 6.8, 10 mmol/L EDTA, and 1 mmol/L chloro-2,4-dinitrobenzene. The reaction was started by adding 1.0 U GST. Absorbance was recorded before starting the reaction and at completion. Calibration curve using GSH at variable concentrations was per-

formed. A control tube was prepared in the absence of GST to examine the possible non-enzymatic activity of GSH. Extracts were prepared by homogenizing plant tissue in a pre-chilled mortar in 20 mL chilled extraction buffer (pH 7.5). The extracts were then centrifuged at 6000 rpm for 20 min at 5°C. Enzyme assays were conducted immediately following this process.

### Determination of the basic chemical composition

Determination of the basic chemical composition of pate and its main ingredients such as wheat flour and broccoli florets from methionine-treated and non-treated broccoli included moisture, ash, crude protein, fat and crude fiber contents that were estimated following the AOAC [2000] methods 14.004, 14.006, 2.057, 14.018 and 7.065, respectively. Carbohydrates were then calculated

### Evaluation of the basic physical properties of pate stuffed with processed broccoli florets

Volume of cold pate was measured by rape seed displacement method. Specific volumes were calculated from loaf volume and loaf weight taken after one hour of baking [AACC, 2000]. Colour appearance was estimated by Spectra-Colorimeter (Tristimulus Color Machine) with CIF lab colour scale (Hunter, Lab Scan XE, Germany). Standardization was according to Sapers & Douglas [1987].

### Evaluation of the sensory properties of pate stuffed with processed broccoli florets

Evaluation of the baked pate product was performed by 10 trained panelists as described by Kulp *et al.* [1985] for symmetry of shape (5), crust colour (10), break & shred (10), crumb texture (15), crumb colour (10), aroma (20), taste (20) and mouth feel (10).

### Statistical analysis

Data obtained on reduced glutathione content were subjected to statistical analyses following the method described by Snedecor & Cochran [1980]. The least significant differenc-

TABLE 1. The basic chemical composition of wheat flour and unprocessed broccoli florets from treated and non-treated broccoli with methionine (on dry weight basis).

Sample	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	CHO (%)**
Wheat flour (72%)	12.9 <sup>c</sup> ±0.40	11.9 <sup>c</sup> ±0.04	1.7 <sup>c</sup> ±0.08	0.7 <sup>c</sup> ±0.07	0.6 <sup>c</sup> ±0.121	85.1 <sup>a</sup> ±0.70
Broccoli florets:						
Methionine untreated (raw)	89.1 <sup>a</sup> ±1.18	32.75 <sup>b</sup> ±0.08	2.73 <sup>b</sup> ±0.01	13.65 <sup>b</sup> ±0.12	10.01 <sup>b</sup> ±0.1	40.86 <sup>b</sup> ±0.72
Methionine treated (raw)	85.5 <sup>b</sup> ±1.26	36.6 <sup>a</sup> ±0.98	3.5 <sup>a</sup> ±0.52	15.3 <sup>a</sup> ±0.74	12.8 <sup>a</sup> ±0.68	31.8 <sup>c</sup> ±0.95
LSD at 0.05	0.204	0.735	0.165	0.238	0.163	0.180

CHO\*\*: carbohydrates,\*values are the average of 3 replicates determinations. Averages in row that have different letters mean there are significant differences between them.

TABLE 2. Reduced glutathione contents in processed broccoli florets untreated and treated with methionine (on dry weight basis).

	Broccoli florets						LSD at 5%
	Untreated (control)			Treated with methionine			
	Minced	Blanched	Butter	Minced	Blanched	Butter	
Glutathione content (µg/gm)	44.5 <sup>f</sup>	48.6 <sup>d</sup>	45.7 <sup>c</sup>	55.6 <sup>a</sup>	52.8 <sup>b</sup>	50.8 <sup>c</sup>	1.82

Value is the average of three replicates samples. Averages in row that have different letters mean there are significant differences between them.

TABLE 3. Rheological properties of dough.

Farinograph		Extensograph	
Parameters	Values	Parameters	Values
Water absorption (%)	65	Resistance to extension (R) (BU)	550
Arrival time (min)	1.25	Extensibility (E) (mm)	110
Dough development time (min)	3.0	Proportional number (R/E)	5.0
Dough stability (min)	8.0	Dough energy (cm <sup>2</sup> )	80
Mixing tolerance index (BU)	25		
Dough weakening (BU)	100		

es at 5% were then calculated. The results obtained in the processed / baked pate were subjected to the analysis of variance (ANOVA) test and the least significant difference (LSD) was calculated according to McClave & Benson [1991].

## RESULTS AND DISCUSSION

### The basic chemical nutrients composition of wheat flour and unprocessed broccoli florets from treated and non-treated broccoli with methionine

Data presented in Table 1 showed the chemical analyses of the 72% wheat flour, broccoli florets (control) and broccoli florets that were sprayed in the field with methionine. The obtained results showed that contents of protein, fat, fiber and ash were maximized in treated broccoli florets, where they reached 36.6, 3.5, 15.3 and 12.8%, respectively; and total carbohydrates were minimized to 31.8%. While protein, fat, fiber and ash were minimized in wheat flour; and total carbohydrate maximized to 85.1%.

From this table it is also noticed that broccoli florets are rich sources of nutrients. On the other hand, the florets taken from the methionine-treated broccoli plants showed a higher nutritive value in comparison to those taken from the control

untreated ones. The former had considerably higher estimated protein, fat, fiber and ash contents than these recorded in the control untreated ones. In support with our view herein, fiber included in a diet was found to reduce bowel cancer [Eastwood & Kritchevsky, 2005]. In addition, it was earlier reported that fibers in a diet have proved to be important for suppression of tumor formation in a rat model trial [McIntyre *et al.*, 1993]. In comparison to our results came the observations of Singh *et al.* [2004] that fiber contents of broccoli among other crucifer had ranged between 0.6 and 3.62, while carbohydrate ranged from 2.5 to 4.03 g/100 g fresh weight. From our results, one may suggest that in methionine-treated broccoli plants, the high fiber content was formed at the expense of carbohydrate accumulation as compared to the control. These values agreed with those reported by Hussein *et al.* [2010].

### Reduced glutathione contents in processed broccoli florets untreated and treated with methionine

Table 2 showed that reduced glutathione content was significantly increased in broccoli florets samples that were sprayed in the field with methionine and applied separately to mincing, steam blanching and frying when compared with the same samples that were not treated with methionine. This result is in agreement with findings of Pasakdee *et al.* [2007] and Sanwal *et al.* [2006] who explained that the methionine treatment with non-chemical growth conditions may improve the quality characteristics and the detoxification component action of the broccoli plant. In this respect, Kim *et al.* [2009] stated that the bioactive sulforaphane of broccoli is considered as a chemo-preventive agent (*in vivo* and *in vitro*) via the induction of certain enzymes causing detoxification of carcinogens. This observation was supported by Juge *et al.* [2007] and Yanaka *et al.* [2009].

### Pate dough rheological properties

Data presented in Table 3 showed the estimated rheological properties of the dough used for pate processing. From the same table it could be concluded that water absorption, arrival time, dough development time were 65%, 1.25 min

TABLE 4. Effect of differently processed broccoli florets from methionine-treated broccoli on the baking quality.

Sample	Weight (g)	Volume (cc)	Specific volume (cc/g)	Crumb moisture (%)
Control	50 <sup>c</sup> ±0.66	143 ±3.61	2.86 ±0.13	36.20±0.77
Mincing methionine-treated broccoli florets				
5%	55 <sup>bc</sup> ±0.76	144 ±2.33	2.62 ±0.19	36.00±0.26
10%	60 <sup>ab</sup> ±0.32	143.5±1.26	2.39 ±0.16	35.6±0.18
15%	65 <sup>a</sup> ±0.53	144±4.26	2.22 ±0.18	35.3±0.18
Blanching methionine-treated broccoli florets				
5%	55 <sup>c</sup> ±0.36	144 ±1.61	2.62 ±0.14	36.20±0.77
10%	60 <sup>ab</sup> ±0.32	144 ±3.31	2.4 ±0.11	36.00±0.26
15%	65 <sup>a</sup> ±0.43	143.5±1.17	2.21±0.11	35.6±0.18
Frying methionine-treated broccoli florets				
5%	55 <sup>bc</sup> ±0.23	144 ±3.61	2.62±0.16	36.01±0.18
10%	60 <sup>ab</sup> ±0.16	144.5 ±2.33	2.41 ±0.15	35.8±0.26
15%	65 <sup>a</sup> ±0.38	143.5±1.26	2.21 ±0.15	35.6±0.18
LSD at 0.05	6.94	N.S.	N.S	N.S

Averages in each column that have different letters mean there are significant differences between them. N.S = not significant.

TABLE 5. Hunter colour parameter of pate raw materials.

Samples	L	a	b
Wheat flour (72% extraction)	86.01 <sup>a</sup> ± 0.44	3.08 <sup>a</sup> ± 0.08	17.77 <sup>d</sup> ± 0.07
Minced broccoli florets*	37.40 <sup>d</sup> ± 0.21	-1.83 <sup>c</sup> ± 0.03	22.62 <sup>b</sup> ± 0.02
Blanched broccoli florets*	48.09 <sup>b</sup> ± 0.09	-3.4 <sup>d</sup> ± 0.40	30.13 <sup>a</sup> ± 0.03
Fried broccoli florets*	38.79 <sup>c</sup> ± 0.05	-0.03 <sup>b</sup> ± 0.01	21.40 <sup>c</sup> ± 0.02
LSD at 0.05	0.388	0.385	0.08

\* Broccoli were sprayed with methionine in the field. Value is the average of three replicates ± standard deviation. Averages in each column that have different letters mean there are significant differences between them. L = white (100) to black (-80); a = red (100) to green (-80); b = yellow (70) to blue (-80).

and 3.0 min, respectively. Stability reached 8.0 min whereas mixing tolerance index was 25 BU and dough weakening value was 100 BU. The results of extensograph were 110 mm, 550 BU and 80 cm<sup>2</sup> as estimated for extensibility, resistance to extension and dough energy, respectively. It is worth mentioning that stuffing wheat dough with broccoli florets (raw minced, steam blanched and fried) had no effect on the rheological properties, where broccoli florets were added after dough processing. Also, stuffing the dough with different forms and percentages of broccoli florets had no effects on gluten network formation, gas retention and final pate quality.

#### Baking quality of pate stuffed with processed broccoli

These estimations were restricted to the pate stuffed with broccoli florets that were sprayed in the field with methionine. Baking quality of pate stuffed with broccoli florets (minced,

steam blanched and fried) at different addition levels (5, 10 and 15%) were evaluated as shown in Table 4. Pate stuffed with different forms of broccoli florets at 15% (w/w) addition level increased both sample weight and loaf volume and decreased specific volume by 30%, 0.6–1%, 22.3–22.7%, respectively if they referred to control sample (unstuffed pate). Also, crumb moisture of all pates stuffed with broccoli florets forms was decreased at the same 15% (w/w) addition level by 1.65–2.48% when compared with control sample. These results are in agreement with those found by Shogren *et al.* [2003] and Mohamed *et al.* [2006].

#### Colour attributes of the baked pate as affected by different broccoli florets fillings

The colour parameters (L, a and b) of pate raw materials (wheat flour and minced, blanched and fried broccoli florets) were evaluated and presented in Table 5. Wheat flour was characterised by higher lightness and redness, while the lightness and yellowness of broccoli florets was maximized in steam blanched broccoli florets when compared with minced or fried broccoli florets.

Also, the colour parameters of the stuffed pate with different forms of broccoli florets that were sprayed with methionine in the field were evaluated as shown in Table 6. Broccoli stuffing had no effect on the crust and crumb colour of the baked pate, while the colour of crust and crumb of the pate stuffed with broccoli showed slight lightness (higher “L” value) and higher redness (higher “a” values) than those of the control samples (not stuffed pate). On the other hand, the addition of broccoli at all levels (% of the pate unit weight) changed the crumb colour to a significant yellow range (higher “b” values).

Since coloured pigments are ubiquitous in the tissues of plants, the yellow colour appearance of the baked pate is suggested to be a result of the inclusion of broccoli florets. The green

TABLE 6. Mean Hunter colour values of pate stuffed with differently processed methionine-treated broccoli florets at 5, 10 and 15 % (w/w) of each pate unit.

Sample	Crust			Crumb		
	L	a	b	L	a	b
Not stuffed pate	49.7f±0.21	13.12 <sup>f</sup> ±0.15	26.55 <sup>g</sup> ±0.72	49.39 <sup>h</sup> ±0.02	18.99±0.09	36.57±0.17
Stuffed pate with minced methionine-treated broccoli florets						
5%	56.21 <sup>a</sup> ±0.02	15.13 <sup>d</sup> ±0.03	32.62 <sup>c</sup> ±0.86	56.35 <sup>b</sup> ±0.18	17.79±0.01	39.05±0.01
10%	52.31 <sup>c</sup> ±0.05	16.21 <sup>b</sup> ±0.11	34.58 <sup>b</sup> ±0.02	54.31 <sup>c</sup> ±0.11	17.76±0.03	38.55±0.06
15%	51.11 <sup>cd</sup> ±0.07	14.76 <sup>f</sup> ±0.17	30.88 <sup>c</sup> ±0.04	48.11 <sup>h</sup> ±0.09	17.84±0.11	35.87±0.02
Stuffed pate with blanched methionine-treated broccoli florets						
5%	49.92 <sup>e</sup> ±0.15	14.56 <sup>e</sup> ±0.05	31.90 <sup>d</sup> ±0.35	58.99 <sup>a</sup> ±0.23	17.49±0.17	37.47±0.15
10%	54.16 <sup>b</sup> ±0.92	15.85 <sup>c</sup> ±0.08	35.56 <sup>a</sup> ±0.22	58.49 <sup>a</sup> ±0.56	17.43±0.25	38.50±0.11
15%	51.44 <sup>e</sup> ±0.03	13.33 <sup>i</sup> ±0.09	29.83 <sup>f</sup> ±0.27	49.74 <sup>f</sup> ±0.32	18.19±0.31	36.21±0.09
Stuffed pate with fried methionine-treated broccoli florets						
5%	46.96 <sup>f</sup> ±0.06	17.94 <sup>a</sup> ±0.07	32.07 <sup>d</sup> ±0.012	48.94 <sup>b</sup> ±0.07	19.15±0.56	34.72±0.03
10%	52.48 <sup>c</sup> ±0.02	13.51 <sup>b</sup> ±0.01	32.22 <sup>cd</sup> ±0.07	52.45 <sup>d</sup> ±0.12	14.05±0.44	34.67±0.02
15%	50.49 <sup>e</sup> ±0.08	14.90 <sup>a</sup> ±0.03	29.71 <sup>f</sup> ±0.14	51.36 <sup>e</sup> ±0.17	16.59±0.21	35.94±0.26
LSD at 0.05	1.11	0.044	0.339	0.765	NS	NS

Value is the average of three replicates ± standard deviation. Averages in each column that have different letters mean there are significant differences between them. N.S = not significant.

TABLE 7. Chemical composition of pate stuffed with methionine-treated broccoli florets (on dry weight basis).

Sample	Protein	Fat	Ash	Fiber	CHO
Not stuffed pate	12.20±0.29	7.5 <sup>e</sup> ±0.11	1.3 <sup>c</sup> ±0.29	1.2 <sup>f</sup> ±0.09	77.8 <sup>a</sup> ±1.35
Stuffed pate with minced methionine-treated broccoli florets					
5%	13.20 <sup>d</sup> ±0.11	7.64 <sup>fe</sup> ±0.17	1.75 <sup>cd</sup> ±0.21	1.92 <sup>e</sup> ±0.11	75.49 <sup>b</sup> ±1.03
10%	14.12 <sup>c</sup> ±0.13	7.85 <sup>de</sup> ±0.25	2.03 <sup>b</sup> ±0.03	2.72 <sup>d</sup> ±0.18	73.28 <sup>cd</sup> ±0.85
15%	15.05 <sup>a</sup> ±0.50	8.12 <sup>c</sup> ±0.16	2.65 <sup>a</sup> ±0.52	3.15 <sup>ab</sup> ±0.13	71.03 <sup>e</sup> ±1.12
Stuffed pate with blanched methionine-treated broccoli florets					
5%	13.11 <sup>d</sup> ±0.11	7.58 <sup>fe</sup> ±0.13	1.70 <sup>d</sup> ±0.02	1.90 <sup>e</sup> ±0.16	75.71 <sup>b</sup> ±1.13
10%	14.17 <sup>c</sup> ±0.15	7.75 <sup>ef</sup> ±0.01	1.96 <sup>bc</sup> ±0.12	2.65 <sup>d</sup> ±0.09	73.47 <sup>c</sup> ±0.78
15%	14.95 <sup>b</sup> ±0.07	8.09 <sup>c</sup> ±0.15	2.52 <sup>a</sup> ±0.16	3.08 <sup>b</sup> ±0.15	71.36 <sup>e</sup> ±0.65
Stuffed pate with fried methionine-treated broccoli florets					
5%	13.00 <sup>d</sup> ±0.31	7.95 <sup>cd</sup> ±0.49	1.80 <sup>cd</sup> ±0.09	1.85 <sup>e</sup> ±0.09	75.40 <sup>b</sup> ±1.25
10%	13.92 <sup>c</sup> ±0.04	8.6 <sup>b</sup> ±0.19	2.16 <sup>b</sup> ±0.14	2.85 <sup>c</sup> ±0.11	72.47 <sup>d</sup> ±1.06
15%	14.95 <sup>b</sup> ±0.38	9.2 <sup>a</sup> ±0.20	2.70 <sup>a</sup> ±0.19	3.22 <sup>a</sup> ±0.18	69.93 <sup>f</sup> ±0.18
LSD at 0.05	0.433	0.199	0.213	0.099	0.653

TABLE 8. Sensory evaluation of pate units stuffed with different percentages of prepared methionine-treated broccoli florets.

Samples	Taste (20)	Aroma (20)	Mouth feel (10)	Crumb texture (15)	Crumb colour (10)	Break and shred (10)	Crust colour (10)	Symmetry shape (5)
Not stuffed pate	18.4 <sup>a</sup> ±1.074	17.7 <sup>a</sup> ±2.869	9.2 <sup>a</sup> ±0.632	13.5 <sup>ab</sup> ±1.715	9.1 <sup>a</sup> ±0.316	8.8±0.632	9.0±0.001	4.6 <sup>a</sup> ±0.516
Minced methionine-treated broccoli florets								
5%	13.4 <sup>c</sup> ±3.178	15.1 <sup>cd</sup> ±2.131	8.5 <sup>bcd</sup> ±0.972	13 <sup>b</sup> ±2.054	8.7 <sup>ab</sup> ±0.823	8.7±0.823	8.7±0.674	4.8 <sup>a</sup> ±0.421
10%	11.7 <sup>c</sup> ±2.9907	15.4 <sup>bcd</sup> ±2.011	9.2 <sup>a</sup> ±0.632	13.6 <sup>ab</sup> ±1.074	8.7 <sup>ab</sup> ±0.823	8.8±0.632	8.6±0.516	4.1 <sup>b</sup> ±0.316
15%	12.5 <sup>c</sup> ±1.007	14.1 <sup>d</sup> ±2.378	8.8 <sup>abc</sup> ±0.632	13.6 <sup>ab</sup> ±1.265	8.6 <sup>ab</sup> ±0.843	8.9±0.737	8.7±0.483	4.3 <sup>ab</sup> ±0.923
Blanched methionine-treated broccoli florets.								
5%	16.2 <sup>b</sup> ±0.097	16.6 <sup>abc</sup> ±1.505	7.5 <sup>f</sup> ±1.080	14 <sup>ab</sup> ±0.943	8.7 <sup>ab</sup> ±0.674	8.9±3.147	8.6±0.516	4.5 <sup>ab</sup> ±0.527
10%	15.7 <sup>b</sup> ±1.678	15.3 <sup>bcd</sup> ±2.626	8.3 <sup>cd</sup> ±0.674	13.5 <sup>ab</sup> ±0.527	8.7 <sup>ab</sup> ±0.674	8.7±0.483	8.9±0.497	4.5 <sup>ab</sup> ±0.527
15%	16.7 <sup>ab</sup> ±1.654	16.5 <sup>abc</sup> ±1.35	8.2 <sup>d</sup> ±0.632	13.6 <sup>ab</sup> ±0.843	8.5 <sup>b</sup> ±0.707	8.6±0.516	8.1±0.567	4.3 <sup>ab</sup> ±0.483
Fried methionine-treated broccoli florets								
5%	16.2 <sup>b</sup> ±1.467	16.5 <sup>abc</sup> ±1.269	8.1 <sup>df</sup> ±0.001	13.6 <sup>ab</sup> ±0.843	8.8 <sup>ab</sup> ±0.632	8.8±0.423	9.0±0.666	4.8 <sup>a</sup> ±0.001
10%	17.3 <sup>ab</sup> ±1.494	17.7 <sup>a</sup> ±1.567	9.0 <sup>ab</sup> ±0.001	13.8 <sup>ab</sup> ±0.788	9.0 <sup>ab</sup> ±0.001	9.0±0.471	8.9±0.567	4.6 <sup>a</sup> ±0.516
15%	17.3 <sup>ab</sup> ±1.022	17.1 <sup>ab</sup> ±1.524	9.0 <sup>ab</sup> ±0.001	14.1 <sup>a</sup> ±0.567	9.0 <sup>ab</sup> ±0.001	9.3±0.483	9.0±.001	4.6 <sup>a</sup> ±0.516
LSD at 0.05	1.885	1.823	0.5894	1.0593	0.579	NS	NS	0.480

a, b, c, d, e, f, g: Mean values in each row followed by a different letter are significantly different ( $p \leq 0.05$ ).

chlorophyll and/or xanthophylls pigments could be chemically changed to yellow carotenes during the exposure to heat during baking [Funamoto *et al.*, 2003; Buchert *et al.*, 2011].

However, while such carotene pigments existence in the produced pate appeared to affect the crumb colour, this is not considered as a disadvantage to the consumer. On the contrary it could be an advantage to achieve our objective aiming to introduce a carotene-rich product to be taken for cancer avoidance [Nichino *et al.*, 2002; Vainio & Weiderpass, 2006].

#### Chemical composition of pate stuffed with broccoli

Table 7 shows the effect of stuffing processed pate with broccoli florets that were sprayed in the field with methionine

on the chemical composition of stuffed pate. The obtained results showed that increasing the proportion of broccoli florets in pate increased the percentage of protein, fat, fiber and ash; and it is accompanied by decreasing its carbohydrate content. This result could be explained by an increment of these components in broccoli florets and it was lower in wheat flour (Table 1), which is composed mainly from carbohydrates. These results are in agreement with Singh *et al.* [2004] and Hussein *et al.* [2010].

#### Sensory evaluation of the processed pate stuffed with broccoli florets

Sensory properties of pate that was stuffed with minced, blanched and fried broccoli florets (treated with methio-

nine) were evaluated and presented in Table 8. The obtained sensorial scores showed that the not-stuffed pate (control) was more acceptable in all sensorial properties, but the data showed no significant difference between control sample and all stuffed pates in crumb texture, crumb colour, break and shred, crust colour and symmetry shape. It was noticed also that the pate with the highest addition level (15%) of blanched and fried broccoli showed no significant differences in taste, aroma and mouth feel. Furthermore, sensorial properties of colour and symmetry shape of pate samples were confirmed by Photo 1; and break and shred could be noticed in the cross-section of pate in the same photo. Results and photos for pate with minced (5%) and steamed (15%) florets and photos for steamed (10%) and fried with butter (10%) florets depended on the concentration of broccoli stuffing.

## CONCLUSION

From the obtained results, it could be concluded that broccoli florets which are sprayed in the field with methionine to minimize their glutathione content, can be processed and stuffed into pate to produce stuffed pate characterised with its good sensorial properties, higher nutritive value (protein, fat, fiber and ash) and lower glutathione content.

## REFERENCES

1. A.A.C.C., Approved Methods of AACC. 10th ed., American Association of Cereal Chemists, INC. St., Paul, 2000. Minnesota, USA.
2. A.O.A.C., Official Methods of Analysis of A.O.A.C. International, Published by A.O.A.C. 2000. International suite 400 2200. Wilson Boulevard Arlington, Virginia 22201-3301, USA.
3. Abd El-Rahman H.A., Zaki M.F., El- Behairy U.A., Abou El-Magd M.M., Effect of planting dates on productivity and head quality of some broccoli cultivars under sandy soil conditions. *Egypt J. Appl. Sci.*, 2010, 25, 52–56.
4. Anderson M.P., Gronwald G.W., Atrazine resistance in velvetleaf (*Abutilon theophrasti*) biotype due to enhanced glutathione S-transferase activity. *Plant Physiol.*, 1991, 96, 107–109.
5. Badawi M.A., Abou El-Magd M.M., Hassan H.A., El-Shakry M.F., Effect of biofertilization, nitrogen sources, nitrogen levels and their interactions on vegetative growth, chemical content and oil yield of sweet fennel. *Egypt. J. Appl. Sci.*, 2005, 20, 567–591.
6. Borowski J., Szajdek A., Borowska E.J., Ciska E., Zielinski H., Content of selected bioactive components and antioxidant properties of broccoli (*Brassica oleracea* L.). *Eur. Food Res. Technol.*, 2008, 226, 459–465.
7. Buchert A.M., Civello P.M., Martinez G.A., Chlorophyllase versus pheophytinase as candidates for chlorophyll dephytylation during senescence of broccoli. *J. Plant Physiol.*, 2011, 168, 337–343.
8. Eastwood M., Kritchevsky S., Dietary fiber: how did we get where we are? *Ann. Rev. Nutr.*, 2005, 25, 1–8.
9. Funamoto Y., Yamauchi N., Shigyo M., Involvement of peroxidase in chlorophyll degradation in stored broccoli (*Brassica oleracea* L.) and inhibition of activity by heat treatment. *Postharv. Biol. Technol.*, 2003, 28, 39–46.
10. Griffith O.W., Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (S-N butyl homosysteine sulfoximine). *J. Bio. Chem.*, 1980, 254, 7558–7560.
11. Hussein A.M.S., Saleh Z.A., Hegazy N.A., Physicochemical, sensory and biological properties of wheat-doum fruit flour composite cakes. *Pol. J. Food Nutr. Sci.*, 2010, 60, 239–244.
12. Jeffery E.H., Araya M., Physiological effects of broccoli consumption. *Phytochem. Rev.*, 2009, 8, 283–298.
13. Juge N., Mithen R.F., Traka M., Molecular basis for chemoprevention by sulforaphane: a comprehensive review. *Cell Mol. Life Sci.*, 2007, 64, 1105–1127.
14. Kahlon T.S., Chiu M.C., Chapman M.H., Steam cooking significantly improves *in vitro* bile acid binding of collard green kale, mustard greens, broccoli, green bell pepper and cabbage. *Nutr. Res.*, 2008, 28, 351–357.
15. Kim Y.S., Young M.R., Bobe G., Colburn N.H., Milner J.A., Bioactive food components, inflammatory targets, and cancer prevention. *J. Canc. Prev. Res.*, 2009, 2, 200–208.
16. Kulp K., Chung H., Martinez-Anaya M.A., Doerry W., Fermentation of water ferments and bread quality. *Cereal Chem.*, 1985, 32, 55–59.
17. Li Y., Zhang T., Korkaya H., Liu S., Lee H.F., Newman B., Yu Y., Cluthien S.G., Schwartz S.J., Wicha M.S., Sun D., Sulforaphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells. *Clin. Cancer Res.*, 2010, 16, 2580–2590.
18. McClave J.T., Benson P.G., Statistical for Business and Economics. 1991, Max Well Macmillan International Editions, Dellen Publishing Co.: USA, pp. 272–295.
19. McIntyre A., Gibson B.R., Young G.P., Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *GUT – Int. J. Gastroent. Hepatol.*, 1993, 34, 386–391.
20. Mohamed A.A., Rayas-Duarte P., Shogren R.L., Sessa D.J., Low carbohydrates bread: Formulation, processing and sensory quality. *Food Chem.*, 2006, 99, 686–692.
21. Moreno D.A., Carvajal M., Lopez-Berenguer C., Garcia-Viguera C., Chemical and biological characterisation of nutraceutical compounds of broccoli. *J. Pharm. Biomed. Anal.*, 2006, 41, 1508–1522.
22. Nishino H., Murakoshi M., Ii T., Takemura M., Kuchide M., Kanazawa X.Y., Masuda M., Ohsaka Y., Yogosawa S., Satomi Y., Ginno K., Carotenoids in cancer chemoprevention. *Cancer Metast. Rev.*, 2002, 21, 257–264.
23. Pasakdee S., Banuelos G., Shennan C., Cheng W., Organic N fertilizers and irrigation influence on broccoli production in two regions of California. *J. Veg. Sci.*, 2007, 12, 4, 27–46.
24. Sanwal S.K., Laxminaryana D.S., Yadav D.S., Rai N., Yadav R.K., Growth, yield, and dietary antioxidants of broccoli as affected by fertilizer type. *J. Veg. Sci.*, 2006, 12, 2, 13–26.
25. Sapers G., Douglas F., Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *J. Food Sci.*, 1987, 52, 1258–1262, 1285.
26. Sarikamis G., Marquez J., MacCormack R., Bennett R.N., Roberts J., Mithen R., High glucosinolate broccoli: a delivery system for sulforaphane. *Mol. Breeding*, 2006, 18, 219–228.
27. Shogren R.L., Mohamed A.A., Carriere C.J., Sensory analysis of whole wheat/soy flour breads. *J. Food Sci.*, 2003, 68, 2141–2145.
28. Singh J., Upadhyay A.K., Bahadur A., Singh K.P., Dietary antioxidants and minerals in crucifers. *J. Veg. Crop Prod.*, 2004, 10, 2, 33–41.

29. Snedecor G.W., Cochran W.G., Statistical Methods. 1980, 7th ed., . Iowa University Press Ames, Iowa, USA, p. 507.
30. Tilaar W., Ashari S., Yanuwadi B., Polii-Mandang J., Synthesis of sulforaphane during the formation of plantlets from broccoli (*Brassica oleracea* L. var *italica*) *in vitro*. Int. J. Eng. Technol., IJET-IJENS, 2012, 12, 3, 1–5.
31. Vainio H., Weiderpass E., Fruit and vegetables in cancer prevention. Nutr. Canc., 2006, 54, SI, 111–142
32. Yanaka A., Fahey J.W., Fokumoto A., Nakayama M., Inoue S., Zhang S., Tauchi M., Suzuki H., Hyodo I., Yamamoto M., Dietary sulfurafane-rich broccoli sprouts reduce colonization and attenuate gastric *Helicobacter pylori*-infected mice and humans. Canc. Prev. Res., 2009, 2, 353–360.

Submitted: 30 May 2011. Revised: 12 December 2011, 19 April 2012, 18 July 2012. Accepted: 4 September 2012. Published on-line: 24 October 2013.