Influence of Freezing Steps on Color Attributes, Phytochemical Contents and Antioxidant Capacity of Green Beans

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Abstract: Chlorophyll and phytochemical contents, antioxidant capacity and color attributes of green beans (*Phaseolus vulgaris* L.) after washing, blanching and freezing processes were evaluated. The hue angle values showed that the frozen beans had a bright yellow-greenish color. Chlorophyll *a* and *b*, total carotenoids, total phenolic and total flavonoid contents significantly decreased during processing steps (washing, blanching and freezing). The antioxidant capacity measured by ABTS method significantly decreased during freezing process steps ranged from 494.20 µmol trolox 100 g^{-1} of fresh green beans to 437.54 and 342.13 µmol trolox 100 g^{-1} (on dry weight basis) of blanched and frozen green beans, respectively. A plot of antioxidant capacity of green beans measured by DPPH or ABTS assay versus phytochemical compounds studied showed a strong positive correlation with total phenolics (R^2 = 0.9847 and 0.9609, respectively) and total flavonoids (R^2 = 0.9963 and 0.9636, respectively).

***Keywords*:** Green beans, freezing, blanching, antioxidant capacity, phenolics, flavonoids, chlorophylls, color

INTRODUCTION

Vegetables are important sources of exogenous antioxidants like phenolic compounds, carotenoids and vitamins C and E, which protect the cells from oxidative damage (Danesi and Bordoni, 2008). Epidemiological evidences suggest that the consumption of vegetables can prevent the degenerative diseases caused by oxidative stress (Sreeramulu and Raghunath, 2010). Green beans (*Phaseolus vulgaris* L.) are an annual or multi-annual plant cultivated for its edible pods or the seeds inside them. Green beans are rich in carotene, vitamins and minerals. Beans are a source of vitamins B and macro- and micronutrients (Butnariu and Butu, 2015).

Nowadays, consumers are aware of the need to consume a variety of fresh vegetables to get the most complete antioxidant support. However, many people cannot spend much time preparing fresh vegetable food every day and so they frequently use frozen vegetables that can be rapidly prepared. Consequently, the consumption of frozen food has increased in recent years (Danesi and Bordoni, 2008).

Freezing causes minimal destruction to phenolic compounds in fruits, with retention levels dependent on cultivar. For example, raspberries have been shown to loss up to 12% of phenolics in an early harvest cultivar, but a 12% gain of phenolics in a late harvest cultivar (Gonzalez *et al*., 2003). Late harvest raspberries have also been shown to contain higher levels of antioxidants in particular, total anthocyanins after freezing. Excellent antioxidants flavonoids can be well preserved during freezing of fruits. Even though they are rather delicate, there was no significant reduction found in their levels (Lohachoompol *et al.,* 2004). Rickman *et al.* (2007) showed that blanching prevented the degradation of phenolic antioxidants from oxidation during storage and increased bioavailability of those antioxidants. However, an extensive study of the effects of blanching/ freezing and long-time freezing storage on various bioactive compounds of more than 20 vegetables revealed strong plant dependence of these effects (Puupponen-Pimiä *et al.,* 2003) and expressive dropping (20–30%) of both antioxidant activity and total phenolics in many vegetables. Freezing fruits and vegetables did not cause any substantial loss of vitamin A and β-carotene. The B-group vitamins also remain unaffected. According to Shofian *et al.* (2011), freezing and even more drastic freeze-drying processes had little effect on some antioxidants in fresh fruits but could markedly affect others.

Accordingly, the objective of the present study was to evaluate the effect of freezing steps of green beans on their color attributes, phytochemicals and antioxidant capacity as well as the correlations between them.

MATERIALS AND METHODS

Materials:

Green beans

Fresh green beans (*Phaseolus vulgaris* L.) were sorted and trimmed, cut, washed and sampled prior entry into blancher and then the blanched green bean samples were taken. Final sampling was collected after freezing (-30 ºC/ 10 min) prior entry into storage place (-18 ºC).

Green bean samples from different processing steps and the final frozen products (Figure 1) were collected at an established frozen vegetables plant(Ismailia National Company for Food Industries, Foodico), Ismailia, Egypt during the 2014 season.

Chemicals and reagents

Folin-Ciocalteu's phenol reagent, anhydrous sodium carbonate, gallic acid, aluminum chloride and sodium hydroxide were purchased from Fluka (Fluka Chemie GmbH, Switzerland). Sodium nitrite, quercetin, 2.2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), potassium persulfate and 2,2´-azino-bis (3-ethylbenzothiazoline–6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, ethanol and acetone (analytical grade) were from Scharlab (Scharlab Chemies s. a., Barcelona, Spain).

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Methods:

Moisture content

The moisture content of all studied samples was determined by the oven at 70°C under vacuum until reaching constant weight according to the method described by AOAC (2002).

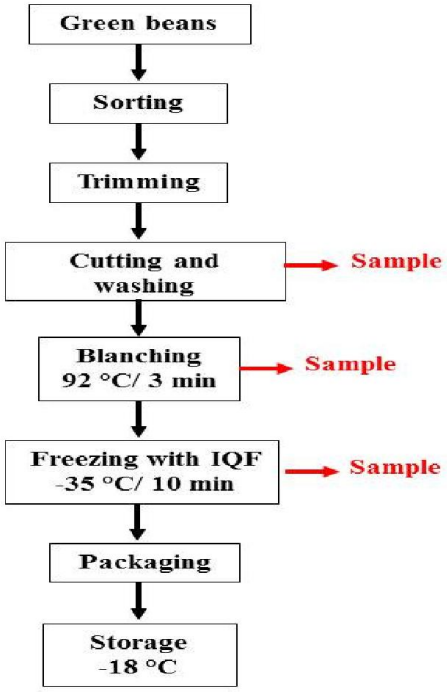

Figure (1): Frozen green beans production scheme (arrows outward indicate the sampling steps of the study).

Color measurements

Color attributes; lightness (L^*), redness (a^*) and yellowness (b^*) were performed using a Minolta Color Reader CR-10 (Minolta Co. Ltd., Osaka, Japan). The color intensity (C^*) was calculated as $C^* = (a^{*2} + b^{*2})^{0.5}$. Furthermore, the hue angle (h_{ab}) was calculated as $h_{ab} = \tan^{-1}(b^*/a^*)$, where h_{ab}= 0º for a red hue and h_{ab}= 90º for a yellow hue (RØrå and Einen, 2003). Whiteness Index (WI) was expressed as:

$WI = 100 - [(100-L^*)^2 + a^{*2} + b^{*2}]^{0.5}$ (Bolin and Huxsoll, 1991).

Determination of chlorophylls and total carotenoids

Chlorophylls *a* and *b* and total carotenoids contents were determined according to the method described by Wettestein (1957) as follows:

Five grams of the samples were mixed with 30 ml of 85% acetone in dark bottle and left to stand for 15 hours at room temperature. The sample was filtered on glass wool into a 100 ml volumetric flask and made up to volume by 85% acetone solution. The optical density of the sample was then measured using a spectrophotometer (6505 UV/ VIS, Jenway LTD, Felsted, Dunmow, UK) at 440, 644, and 662 nm. Acetone (85%) was used as a blank at each wavelength. The chlorophyll and total carotenoids were calculated according to the equation given by Wettestein (1957) as mg 100 g^{-1}.

Chlorophyll *a* = (9.784 **x** A_{662}) – (0.99 x A_{644})

Chlorophyll *b* =(21.426 x A_{644}) – (4.65 x A_{662})

Total carotenoids = (4.695 x A_{440}) – 0.268 (Chl. *a* + Chl. *b*)

Where A is the absorbance at the mentioned wave lengths.

Preparation of total phenolics, total flavonoids and antioxidants extract

The antioxidants extract was prepared according to the method described by Barros *et al.* (2011) with some modifications as follows: one half gram of the sample was stirred with 25 ml of methanol at 100 rpm on Orbital Shaker (LAB-LINE Instruments, Inc., USA) for one hour at room temperature (33 ± 1°C) and then filtered through filter paper No. 102. The residue was re-extracted with 25 ml of methanol. The methanol extracts were combined and stored at 4°C until further analyses.

Determination of total phenolic contents

Total phenolic contents were determined in the methanolic extracts, according to the Folin-Ciocalteu assay with slight modifications (Barros *et al.*, 2011). A half ml aliquot of the extract was mixed with 5 ml of Folin–Ciocalteu phenol reagent (diluted with water 1:10 v/v) and 4 ml of sodium carbonate (75 g/L). The tubes were vortexed for 30 s and allowed to stand for 60 min at room temperature (33 ± 1°C) for color development. The absorbance was measured at 765 nm by spectrophotometer. A calibration curve (R^2= 0.9986) of gallic acid (0 – 0.10 mg/ml) was prepared and treated in similar conditions. The results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW).

Determination of total flavonoid contents

Total flavonoid content was determined by a technique modified by Dewanto *et al.* (2002). Briefly, a half ml aliquot of the extract was mixed with 2 ml of distilled water followed by addition of 0.15 ml of $NaNO_2$ (5%) solution. After 6 min, 0.15 ml of $AlCl_3$ solution (10%) was added and allowed to stand for another 6 min before 2 ml of NaOH solution (4%) were included. The mixture was brought to 5 ml with distilled water. Then, the mixture was mixed well and allowed to stand for 15 min then the absorbance was measured at 510 nm. A calibration curve of quercetin was prepared and total flavonoids content was determined from the linear regression equation (R^2 = 0.9976) of the calibration curve. The results were expressed as mg quercetin equivalents per 100 g of dry sample.

Determination of DPPH radical–scavenging activity

The antioxidant activity of the extract was assessed by DPPH method described by Lee *et al.* (2003) and modified by Ravichandran *et al.* (2013). Shortly, 0.1 ml of the methanol extracts was mixed for 30 s with 3.9 ml of DPPH solution (6 x 10^{-5} M), and left to react for 30 min, after which the absorbance of the mixture was measured at 515 nm. The DPPH solution without extract was analyzed as blank. The antioxidant activity was calculated as follows, on dry weight basis:

DPPH radical–scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

Where A is the absorbance at 515 nm.

$ABTS^{\bullet+}$ assay (trolox equivalent antioxidant capacity, TEAC)

The method modified by Rufino *et al.* (2010) was used. Briefly, $ABTS^{\bullet+}$ radical cations were produced by reacting 7 mM ABTS stock solution with 145 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.800±0.020 at 734 nm. After addition of 30 µl of the sample extract or trolox standard to 4 ml of diluted $ABTS^{\bullet+}$ solution, absorbance was recorded after 6 min of mixing. Ethanolic solutions of known trolox concentrations (0-10 µg per ml) were used for calibration (R^2 = 0.9988) and results were expressed as µmoltrolox per 100 g dry sample.

Statistical analysis

Each experiment was done in six replicates. The results were expressed as mean ± standard deviation and were analyzed by SPSS (version 17.0 SPSS Inc). One–way analysis of variance was performed using ANOVA procedures. Significant differences between the means were determined by Duncan's Multiple Range test. $P \leq 0.05$ was considered as a level of significance.

RESULTS AND DISCUSSION

Influence of freezing steps on the color attributes of green beans

Frozen vegetables are subjected to color modifications which take place during blanching and/ or during frozen storage. Chlorophylls are mainly responsible for the color in green beans. The negative values of chromaticity (a^*) showed a tendency to the greenish color. The chromaticity (b^*) resulted in yellowness color due to positive values. Chroma (C^*) showed a similar behavior in relation to b^* values (Table 1).

Table (1): Influence of freezing steps on the color attributes (mean ± SD, *n*= 6) of green beans

Color attribute	Sampling after		
	Washing process	Blanching process	Freezing process
L^*	58.60^{a}±6.80	54.67^{a}±0.64	45.60^{b}±1.76
a^*	-5.63^{a}±1.25	-6.00^{a}±0.77	-5.57^{a}±1.27
b^*	15.70^{a}±2.19	12.73^{b}±3.21	10.30^{c}±1.25
C^*	16.68^{a}±1.21	14.07^{b}±1.55	11.71^{c}±1.40
h_{ab}	-70.27^{a}	-64.76^{b}	-61.60^{c}
WI	55.37^{a}	52.54^{b}	44.35^{c}

SD= Standard deviation *n*= number of samples
There is no significant difference ($p \leq 0.05$) between means within the same row have the same superscript letter

The washed green beans cuts showed lightness (L^*) values higher than the frozen ones. The hue angle values displayed that the frozen beans had a bright yellow-greenish color (Table 1). The non significant decrease of a^* value and significant decrease in b^* values of green beans that observed during processing steps (washing, blanching and freezing) may be due to the decomposition of pigments such as chlorophylls and carotenoids, in addition to the presence of non-enzymatic reactions which caused a significant decrease in whiteness index (WI) values (Table 1).

Influence of freezing steps on some phytochemical contents and antioxidant capacity of green beans

Calculated chlorophyll *a* and *b* contents of washed green beans were found to be 6.54 and 3.92 mg 100 g^{-1} DW, respectively, with an initial chlorophyll *a*/ chlorophyll *b* ratio of 1.67 (Table 2). Steam blanching of green beans decreased the Chlorophyll *a* and *b* contents to 6.25 and 2.83 mg 100 g^{-1} DW, respectively with a chlorophyll *a*/ chlorophyll *b* ratio of 2.21. Whereas frozen green beans cuts had 5.60 and 2.53 mg 100 g^{-1} DW of chlorophyll *a* and *b*, respectively. The decrease in chlorophyll *a* and *b* contents was significantly differed between washed and frozen ones. The loss percents of chlorophyll *a* were 4.43 and 14.37, and of chlorophyll *b* were 27.81 and 35.46 after blanching and freezing steps, respectively (Table 2). Furthermore, the loss percents of chlorophyll *b* were higher than those of chlorophyll *a*. Chlorophyll *a* and *b* contents of green beans were affected by blanching time and temperature with the conversion of chlorophylls into corresponding epimers and pheophytins (Bahçeci *et al.,* 2005).

Results in Table (2) show the effect of freezing steps on the total carotenoid contents of green beans cuts. These results revealed that fresh green beans contained 19.34 mg/100 g^{-1} of total carotenoids (on dry weight basis). Blanching and freezing processes caused significant decrease of total carotenoids to 18.32 and 15.53 mg/100 g^{-1} of samples, respectively. The loss percents were 5.27 and 19.70, respectively. Yuan *et al.* (2009) and Vallejo *et al.* (2002) reported that boiling of broccoli resulted in significant decrease in carotenoids content.

The degradation of chlorophylls *a* and *b* and total carotenoids during freezing process of green beans well correlated with Hunter color readings. Figure (2) illustrates that, the correlation between the chlorophyll *a* content and Hunter color readings during processing was linear, whereas that between chlorophyll *b* and total carotenoids contents with color attributes were polynomial regressions (3rd and 2nd grade, respectively. The best correlations were between chlorophyll *a* contents and L^* (R^2= 0.9369), b^* (R^2= 0.9147) and a^* (R^2= 0.9105) values. Also chlorophyll *b* content had well correlation with L^* values (R^2= 0.9706), b^* values (R^2= 0.9216) and, a^* values (R^2= 0.8751). Figure (2) also illustrates a high correlation between total carotenoids content with a^* values (R^2= 0.9386), b^* values (R^2= 0.9280) and L^* values (R^2= 0.8401).

Table (2): Influence of freezing steps on some phytochemical contents (mg 100 g^{-1} DW) and antioxidant capacity of green beans

Item	Sampling after		
	Washing process	**Blanching process**	**Freezing process**
Chlorophyll *a*	$6.54^{a} \pm 0.45$	$6.25^{a} \pm 0.22$	$5.60^{b} \pm 0.41$
Loss %		*4.43*	*14.37*
Chlorophyll *b*	$3.92^{a} \pm 1.25$	$2.83^{b} \pm 0.18$	$2.53^{b} \pm 0.32$
Loss %		*27.81*	*35.46*
Total carotenoids	$19.34^{a} \pm 0.77$	$18.32^{a} \pm 1.34$	$15.53^{b} \pm 0.70$
Loss %		*5.27*	*19.70*
Total phenolics	$112.35^{a} \pm 13.38$	$106.03^{a} \pm 10.55$	$89.16^{b} \pm 1.62$
Loss %		*5.63*	*20.64*
Total flavonoids	$394.89^{a} \pm 50.89$	$354.24^{ab} \pm 57.25$	$300.20^{b} \pm 19.34$
Loss %		*10.29*	*23.98*
DPPH radical-scavenging activity (%, DW)	$13.13^{a} \pm 0.10$	$11.59^{b} \pm 0.35$	$11.15^{b} \pm 0.89$
Loss %		*11.73*	*15.08*
$ABTS^{\bullet+}$ scavenging capacity (µmol trolox 100 g^{-1}, DW)	$494.20^{a} \pm 62.87$	$437.54^{b} \pm 0.79$	$342.13^{c} \pm 22.05$
Loss %		*11.46*	*30.77*

DW= dry weight basis
Means ± Standard deviation of 6 replicates
There is no significant difference ($p \leq 0.05$) between means within the same row have the same superscript letter

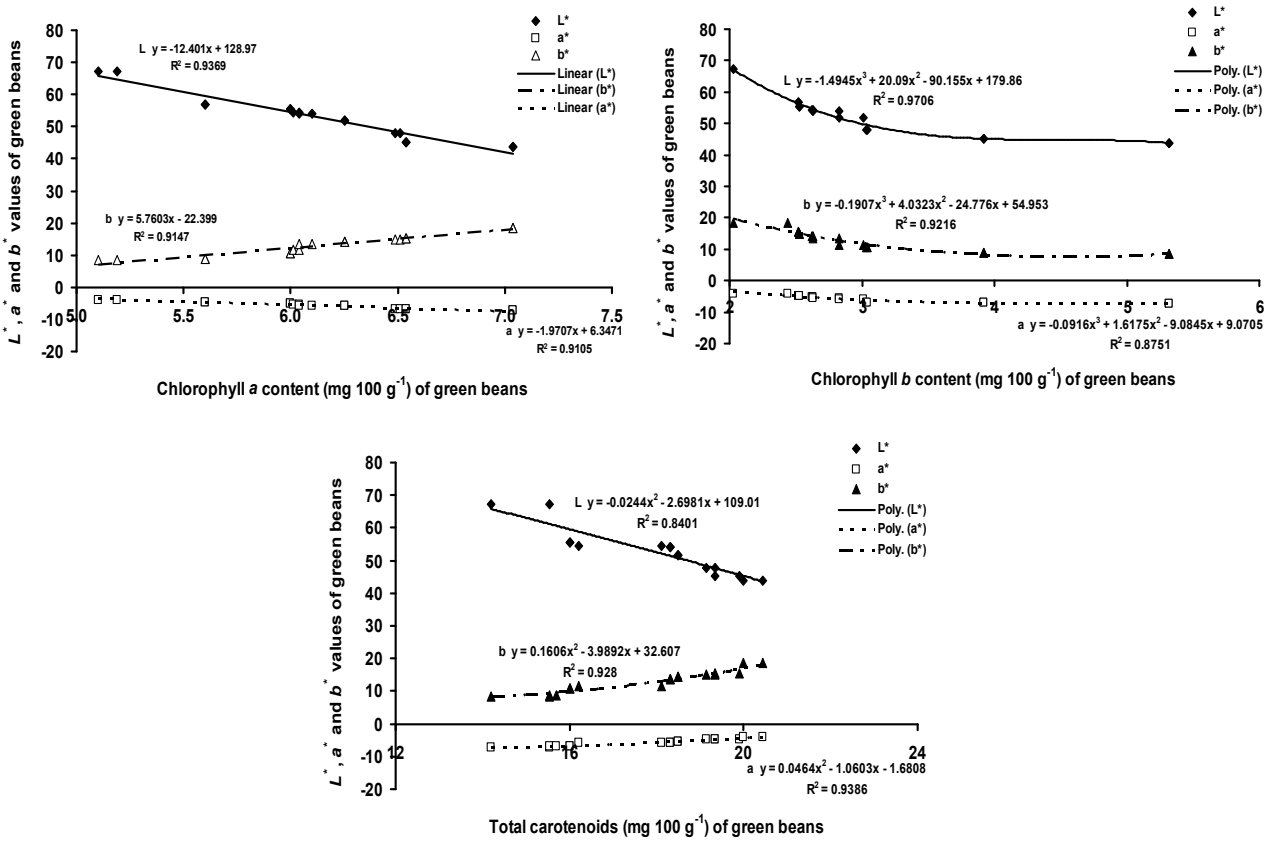

Figure (2): The relationships between the Hunter color values with chlorophylls *a*, *b* and total carotenoid contents of green beans during freezing process steps

Table (2) exhibits the effect of processing steps on the total phenolic contents of green beans. The initial level of total phenolics in washed samples was 112.35 mg 100 g^{-1} (on dry weight basis). Blanching and freezing treatments caused significant losses (5.63 and 20.64%, respectively) of total phenolic compounds. Phenolic compounds in vegetables are presented in both soluble forms and combined with cell-wall complexes. Thus, increased surface area of tissues in contact with steam blanching and cooling water, high blanching temperature and lengthy freezing time are all likely to have resulted disruption of cell walls and breakdown of phenolic compounds (Francisco *et al.,* 2010). Ahmed and Ali (2013) reported that steam-blanched, steam-boiled and microwaved cauliflower had significantly losses in phenolics content by 16.6, 17.53 and 18.30%, respectively.

Regarding the total flavonoids content, results showed that, it was significantly decreased from 394.89 mg 100 g^{-1} (on dry weight basis) to 354.24 and 300.20 mg 100 g^{-1}, DW after blanching and freezing treatments, respectively (Table 2). The highest reduction was noted after freezing (23.98%) followed by steam blanching (10.29%). Porter (2012) documented that boiling for 5 min resulted in a 49.55% reduction in flavonoids in purple-sprouting broccoli.

Several *in vitro* techniques have been developed in order to determine the antioxidant capacity of food matrices (Brand-Williams *et al.,* 1995; Sánchez-Moreno *et al.,* 1998; Prior *et al.,* 2005). Among them, one of the most popular is the method employing the stable 2,2-diphenyl-1-picrylhy-drazyl radical (DPPH), which is based on the colorimetric properties of the radical that bears a deep purple color at around 515 nm. When the DPPH reacts with hydrogen/ electron donor (an antioxidant), this radical loses its typical color and the measurement of this change, with an UV–VIS spectrophotometer, allows estimating the ability of a com-pound or a compound mixture to scavenge free radicals.

Antioxidant activities of washed, steamed and frozen green beans, as evaluated by the DPPH radical scavenging method, are shown in Table (2). DPPH radical-scavenging activity expressed in inhibition percent of the washed, steamed and frozen green beans were 13.13, 11.59 and 11.15, respectively. Kenny and O'Beirne (2009) indicated that the loss of antioxidant activity was relative to the contact area between vegetables and water as well as processing time. It was clear that the contact areas in steaming and stir-frying processes were much smaller than that in boiling, so their antioxidant substances lost relatively very little (Zhang *et al.,* 2011). During vegetable cooking, qualitative changes, antioxidant breakdown and their leaching into surrounding water may influence the antioxidant activity of the vegetables (Podsedek, 2007).

Also, the antioxidant capacity of green beans measured by ABTS method significantly decreased during freezing process steps. Its values decreased from 494.20 µmol trolox 100 g^{-1}, DW of fresh green beans to 437.54 and 342.13 µmoltrolox 100 g^{-1}, DW of blanched and frozen green beans, respectively.

Many studies have reported on the relationships between phytochemicals, especially phenolics and antioxidant activity. A plot of antioxidant activity measured by DPPH (Figure 3) or ABTS (Figure 4) assay versus phytochemical compounds studied showed a strong positive correlation with total phenolics (R^2= 0.9847 and 0.9609, respectively) and total flavonoids (R^2= 0.9963 and 0.9636, respectively).

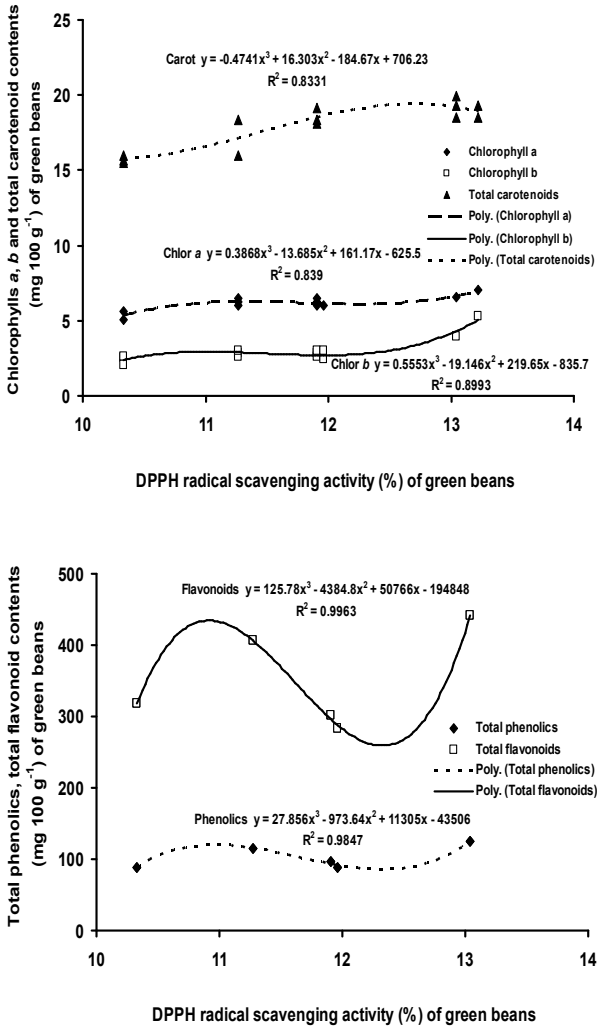

Figure (3): The polynomial regressions of the chlorophylls *a*, *b*, total carotenoids, total phenolics and total flavonoid contents with the DPPH radical-scavenging activity of green beans during freezing process steps

CONCLUSION

Blanching and freezing processes had significant effects on the antioxidant activity and phytochemical compounds of the frozen green beans. They led to decrease the chlorophylls and phytochemical contents, which well correlated with Hunter color readings and the antioxidant capacity. Optimizing these processes is important to maintaining green beans desirable properties.

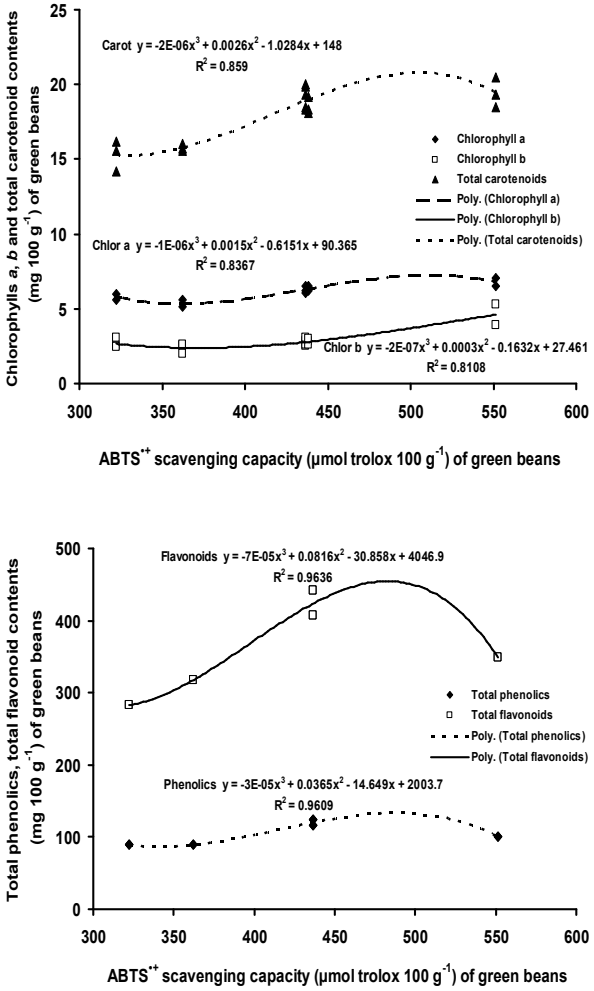

Figure (4): The polynomial regressions of the chlorophylls *a*, *b*, total carotenoids, total phenolics and total flavonoid contents with the $ABTS^{\bullet+}$ scavenging capacity of green beans during freezing process steps

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تأثير خطوات صناعة التجميد علي خصائص اللون، محتوى المركبات الفعالة والنشاط المضاد للأكسدة في الفاصوليا الخضراء

عبدالله صالح السنباني – خالد محمد يوسف – عادل ابو بكر شطا – صلاح كامل السماحي

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تم تقدير كل من محتوى الكلوروفيللات، المركبات الفعالة، النشاط المضاد للأكسدة وخصائص اللون في الفاصوليا الخضراء بعد عمليات الغسيل، السلق والتجميد. أوضحت النتائج أن الفاصوليا الخضراء المجمدة ذات لون أخضر مصفر لامع. انخفضت محتويات الكلوروفيل (أ) و (ب)، الكاروتينيدات، الفينولات والفلافونيدات الكلية معنويا أثناء خطوات التصنيع. انخفض أيضا معنويا النشاط المضاد للأكسدة مقدرا بطريقة مكافئ الترولوكس من ٤٩٢.٢٠ ميكرومول ترولوكس لكل ١٠٠ جرام في الفاصوليا الطازجة إلي ٤٣٧.٥٤ و ٣٤٢.١٣ميكرومول ترولوكس لكل ١٠٠ جرام في الفاصوليا الخضراء بعد عملية السلق والتجميد علي الترتيب. أوضحت النتائج أيضا وجود علاقة قوية بين محتوى الفينولات والفلافونيدات الكلية للفاصوليا الخضراء مع النشاط المضاد للأكسدة لها.