Effect of Different Cooking Methods on Natural Antioxidants in Pumpkin (*Cucurbita moschata*) Products

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ABSTRACT

The present study aimed to investigate the effects of various cooking methods on antioxidant content and radical scavenging activity of pumpkin. Three heat treatments were applied, namely,blanching, and stir frying for cubes(1.5×1.5×1.5 cm) and roasting for slices (0.5mm) to produce soup, juice and roasted pumpkin. The results indicated that carotenoids content ranged between 11,07 IU/100g (soup) and 892 IU/100g (juice).Roasted product exhibited significantly ($P \leq 0.05$) the least flavoniod content (12.09g/kg), while soup possessed significantly ($P \leq 0.05$) the highest content(17.96 g/Kg). Lycopene content (0.15 mg/100g) was found to be significantly($P \leq 0.05$)the least (in soup produced from cubes blanched for 4 mins). In contrast, the counterpart raw juice had significantly ($P \leq 0.05$) the highest lycopene content (0.70mg /100g). Total polyphenols content of stir fried product was significantly ($P \leq 0.05$) the least(406.25 g/kg), while roasted product exhibited significantly ($P \leq 0.05$) the highest polyphenols content (670.18g/kg). It was obvious that raw juice possessed significantly ($P \leq 0.05$) the highest DPPH (63.79%), on contrary to roasted product being significantly ($P \leq 0.05$) the least antioxidant activity as assessed by DPPH method (30.31 %).The H_2O_2 scavenging (2.85 %) was found to be significantly ($P \leq 0.05$) the highest (in soup produced from cubes blanched for 4 min) .While, the raw juice has significantly($P \leq 0.05$)the least H_2O_2 scavenging (0.32%).So, It is necessary to apply the most proper cooking conditions (temperature and cooking time) to avoid any degradation of the bioactive compounds present in pumpkin. The results revealed the superiority of blanching process since it maintained the bioactive compounds in pumpkin without any deteriorative effects.

Keywords: ***Natural antioxidants, antioxidant activity, cooked pumpkins, blanching, stir-frying, juicie,soup,roasting.***

INTRODUCTION

The tent "let food be the medicine and medicine be the food," espoused by Hippocrates nearly 2.500 years ago, is receiving renewed interest. It is worth to mention that there is a consumer interest in functional foods from the health point of view (Hasler, 1998, Anonymous, 2009). Antioxidants are compounds that protect cells against the damage effects of reactive oxygen species. It is worth to mention that some antioxidants are found in vegetables. In this respect, products high in vitamin C, vitamin E and carotene content (provitamin A) are believed to be the most beneficial antioxidants (Palace *et. al.* 1999).

Pumpkin (*Cucurbita moschata)* is defined as a fruit botanically. Flesh and seeds of pumpkin are commonly used for culinary and medicinal purposes. Carotenoids are responsible for the orange colour of pumpkin. Murkovic *et. al.* (2002) reported that three species of pumpkin *(Cucurbitapepo, C. maxima and C. moschata*) contained β-carotene (0.06-7.4 mg/100g), α-carotene (0-7.5 mg/100g) and lutein (0-17 mg/100g). Similarly, it was reported that pumpkin contains both β-carotene and lycopene. Pumpkin is cooked or pureed, and has numerous culinary uses either as a vegetable or as an ingredient in many food products. Fresh pumpkins are very sensitive to microbial spoilage, even at refrigerated conditions, and thereby they must be frozen or dried (Doymaz, 2007).

Food preparation at home, in particular, cooking is often the final step in food processing. Various cooking methods were found to affect content of phytochemicals, in particular, antioxidants present in the vegetables (Ismail *et. al.* 2004, Zhang & Hamauzu, 2004, Turkmen *et. al.* 2005). Notwithstanding, Podşedek (2007) suggested that both antioxidant levels and activities of processed vegetables were lower than those of the corresponding fresh samples. This is probably attributed to degradation

of the bioactive compounds and adsorption of water during boiling, resulting in dilution of the active compounds. Moreover, a significant increase was reported in release of β-carotene and tocopherol in broccoli upon cooking. Antioxidants inhibit oxidation of lipids by transforming free radicals/ peroxy radicals into non-radicals by donating electron and hydrogen or by chelating transition metals (Frankel, 1998). Several synthetic antioxidants are available in the market. Due to their reported carcinogenic effects, use of such antioxidants is restricted. Moreover,synthetic antioxidants may cause kidney damage (Branen, 1975, Ito *et al.* 1983, Lindenschmidt *et al.,* 1986). Consequently,it is necessary to search for new natural antioxidants as an alternative to synthetic antioxidants to prevent lipid oxidation in foods (Farvin *et al.* 2014).Many epidemiological studies have indicated that the oxidative stress imposed by reactive oxygen stress species (ROS) plays an important role in many chronic and degenerative diseases, (Young & Wood, 2001, Azizova, 2002, Fu *et al.,* 2011., Zhang & Tsao, 2016)

The present study aimed at investigation the effect of various cooking methods (blanching, stir-frying and roasting) on the antioxidants content and potency of pumpkins

MATERIALS AND METHODS

Materials

Fresh pumpkin (*Cucurbita moschata*) of commercial maturity was obtained in July 2014. Representative sample was withdrawn from the Central Market of Vegetables and Fruits. Alexandria Governorate, Egypt. Other ingredients were purchased from Alexandria market, Egypt.

All chemicals and reagents used in the present study were purchased from El-Gomhoria Company for Chemicals, Alexandria, Egypt, except for DPPH reagent (1,1- dipheny1 -2 picry1 hydrazy1) which was obtained from Sigma Company, Germany.

Preparation of samples:

Pumpkin fruits were carefully washed with tap water, dried with a soft cloth and the skin was peeled. The seeds were removed from the pulp using knife, then the pulp was washed thoroughly with distilled water and cut into small pieces " cubes" (1.5×1.5×1.5 cm), and slices (0.5mm), One portion was retained raw while others were used to prepare different products (Juice, soup, fried and roasted).

Preparation of pumpkin products:-

Raw juice (RJ), boiled juice (BJ),boiled soups (BS), roasted (RO-P) and stir fried pumpkin (SF-P) were prepared according to the methods shown in Fig. (1).

Cooking methods

Three thermal treatments were used including boiling, stir- frying, roasting along with fresh samples as a control. For preparing soup, 2.800 Kg of pulp cubes were blanched in boiling water (100°C) for 2,4, 6 min. Meanwhile, traditional soup was prepared by boiling in water (100°C) for 20 mins. The chopped raw onion, garlic, carrots and celery were fried in corn oil until these ingredients have golden colour, and then the other ingredients were cooked for 8 min and added to blanched cubes to make soup. Ingredients used in preparation of pumpkin soup are shown in Table (1). Boiling was conducted for 4 and 2 min.

Analytical methods

Determination of lycopene

For extracting lycopene, one g of homogenized fresh or semi –dried pumpkin sample was weighed

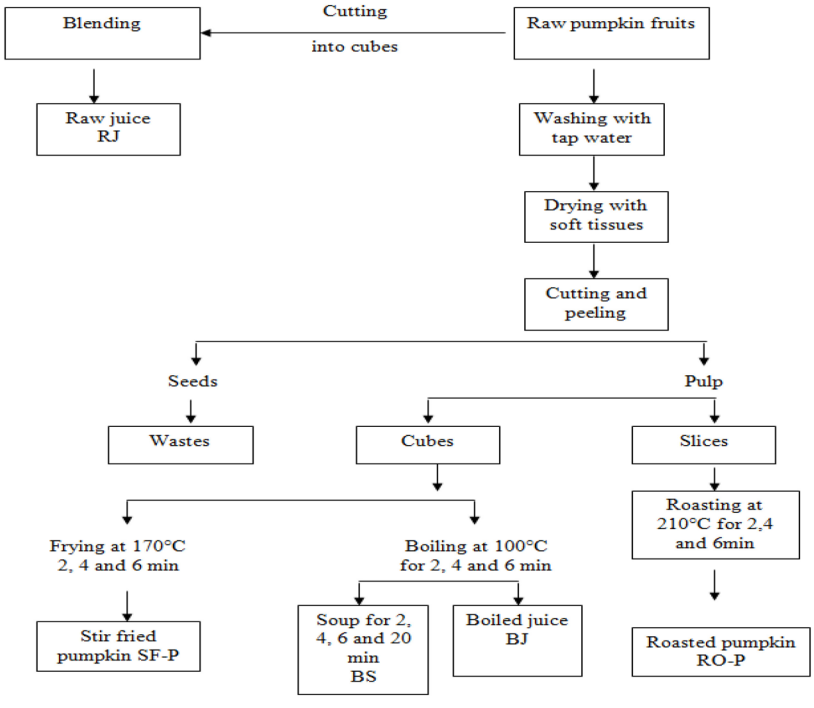

Fig. 1: Flow sheet of preparation methods of pumpkin products

Table 1 : Ingredients used in the preparation of pumpkin soup

Ingredient	Quantity (g)
Blanched cubes of pumpkins	700
Chopped raw carrots	27.30
Chopped raw onion	70.6
Cinnamon	0.1
Cloves	0.17
Chopped raw garlic	2.8
Black pepper	0.23
Ginger	0.3
Corn oil	7
Cumin	0.1
Food salt	6.5
Cardamom	0.5
Celery	92.5
Water	500 (ml)

into a screw-top tube, which was covered with aluminum foil to exclude light. The 1ycopene from the samples was extracted according to the method of Sadler *et al.* (1990) using 25 ml mixture of hexane – acetone- ethanol (2:1:1:,v:v:v) .

Lycopene was determind by Shimadzu spectrophotometer. The absorbance was measured at 503 nm and hexane was used as blank .Lycopene content was calculated using lycopene extinction coefficient (E% 3150) according to the method described by Chang & Liu (2007).

Determination of total corotenoids

Total carotenoids (mg 100g) were determined by a modified method of Ranganna (1997). Acetone and petroleum ether were used as extracting solvents and the absorbance was measured at 450nm.

Determination of total polyphenols

The method of Skerget *et al*. (2005) was applied to extract polyphenols with some modifications. Approximately 5g sample was extracted using 50 ml ethanol in 250 ml conical flask. The flask was placed in a water bath at 25°C for 1 hr. the crude extract was then filtered through a 0.4 um filter (Whatman, Maidstone, England) and the solvent was then evaporated under vacuum.The extraction yield was then determined as% weight. The extracts were kept at –20°C until analysis.

Total polyphenolics content was determined by-Folin- Ciocalteu method (AOAC. 1990).

Determination of total flavonoids compounds (TFC)

The method of Ordon *et.al*. (2006) was used to determine the total flavonoid content (TFC). A volume of 0.5ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of the supernatant. After 1hr of incubation at the room temperature, the absorbance was measured at 420 nm using Shimadzu spectrophotometer.

Determination of antioxidant activities

The DPPH radical scavenging activity

The method of Brand-Williams *et al.* (1995) with some modifications was used to measure free radical scavenging activity. The extracts were dissolved in 1.0 ml MeOH and the solutions were added to a 1.0 ml DPPH. The absorbance at 515 nm was measured using Shimadzu Spectrophotometer. The following equation was applied to calculate DPPH scavenging activity:

DPPH scavenging activity (%) = $[(A_0-A_t)/A_0]\times100$

Where A_0 is the absorbance of the control at t=0 min, and A_t is the absorbance of the antioxidant at t=15 min.

Hydrogen peroxide scavenging activity

The modified method of Ngonda (2013) was used to measure the scavenging activity of extract towards hydrogen peroxide radicals. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula:

Percentage scavenged $[H_2O_2]$ = 1- Abs (standard)/ Abs (control) × 100 where, Abs is the absorbance of the control (without extract) at 560 nm, Abs (sample) is the absorbance in the presence of the extract at 560 nm.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Kotz, *et al.* 2006 & Kirkpatrick and Feeney, 2013), with one way ANOVA.

RESULTS AND DISCUSSION

The present study was conducted on four pumpkin products namely,juice,soup, stir-fried and roasted. Such products were subjectively evaluated along with the control. The highest ranked treatments were investigated in the present study, including(1-Juice:boiling for 2 mins, 2-Soup: boil-

ing for 4 min, 3-Stir fried:stir fried for 2 min and 4- Roasted:roasting for 4 min.) as figured out in our previous study **(**Lotfy *et al***,** 2016).

Antioxidant activity and phytochemicals of pumpkin and its products:

Antioxidant activity

Pumpkin seems to be promising as antioxidants containing food. However, it was obvious that pumpkin and its products could serve as a good source of antioxidants. According to Moharram & Youssef (2014), antioxidant capacity should be evaluated by more than one method to trace the different mechanisms of free radicals scavenging. In the present study, DPPH- scavenging values of pumpkin products ranged from 30.31% (roasted pumpkin chips) to 63.79% (raw juice) (Table 2).The H_2O_2 scavenging values varied from 0.32% to 2.85% for the raw juice and soup boiled for 4 min, respectively, (Table 2). It was obvious that blanching process was superior in terms of maintaining the antioxidant potency of pumpkin as compared to both stir frying and roasting. Consequently, blanching is favourable method of cooking for pumpkin.

Phytochemicals:

Carotenoids

The carotenoids content of pumpkin and its products are presented in Table (3). The raw pumpkin juice (RJ-P) exhibited significantly ($P \leq 0.05$) the highest carotenoids content (1476 IU/ 100g). On the other hand, the least significantly ($P \leq 0.05$) carotenoids content was found in roasted pumpkin for 4 min (584.00 IU/ 100g). The other products could be ascendingly ordered in terms of their carotenoid content as follows: (IU/100 g): Raw-P (raw pumpkin) (1200), BS-P (soup boiling for 4 min) (1107), BJ-P (juice boiling for 2 min) (892), P-ST(boiling soup for 20 mins) (830) and SF-P (stirring fried for 2 min) (676).

Pumpkins are a good source of provitamin A. The high content of provitamin A in pumpkins can be utilized by availing the pumpkins to the markets worldwide, device cultivation methods for large production and determined conditions for long storage shelf life (Karanja *et.al.* 2012).

Carotenoids possess nutritional properties and health promoting effects. Pumpkins flesh has an intense yellow/ orange colour owing to the high level of carotenoids, mainly α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene and zeaxanthin (Durante *et al.* 2014). It is worth to mention that the total carotenoids content (on dry weight basis) of the pumpkins peel depends on variety being in a range of 12 to up to 175 mg/ kg (Kreck *et al.* 2006).

Table 2: Antioxidant activity of pumpkin and its products

Treatment	DPPH%	Scavenged H_2O_2%
Raw-P	61.69 ± 0.16 ^{b}	0.99 ± 0.84^{bc}
RJ	63.79 ± 0.47 ^{a}	0.32 ± 0.03^{c}
BJ	36.84 ± 0.33 ^{e}	2.30± 0.79^{ab}
BS	59.58 ± 0.72 ^{c}	2.85± 0.35^{a}
TS	37.89 ± 0.56 ^{e}	1.84 ± 0.33^{ab}
SF	46.44 ± 0.08 ^{d}	1.02 ± 0.74^{bc}
RO	30.31 ± 0.05 ^{f}	1.71 ± 0.65^{bc}
Mean	48.076	1.574
C.V	1.047	38.065
F	2192.32*	6.211*
P	<0.0001*	<0.001*

Values are expressed as means± SD.
Mean in a column not sharing the same superscript are significantly different at $P \leq 0.05$
Raw-P: control TS: boiling soup for 20 min
RJ: raw juice SF: stirring fried for 2 min
BJ: boiling juice for 2 min
RO: roasting pumpkin for 4 min
BS: boiling soup for 4 min

Lycopene content (mg/100g) ranged from 0.15 (soup boiled for 20 min) to 0.70 (raw pumpkin juice) as shown in Table (3). Significant differences ($P \leq 0.05$) could be traced in lycopene content of the different pumpkin products. It was obvious that boiling for 4 min, stir- frying for 2 min and roasting for 4 min significantly ($P \leq 0.05$) lowered the lycopene content of pumpkin (Table 3).

The β-carotene contents in pumpkin products investigated in the present study ranged from 98.59 to 6834.9 IU/ 100 gm. It is worth to mention that the high content of carotenoids in RJ-P may be attributed to increase the surface area as a result of disintegration that occurred in blending process. No β-carotene could be detected in both TS-P (soup boiled for 20 min) and SF-P (stirring fried for 2 mins), it was clear that heat applied in the preparation of these pumpkin products affected negatively the β-carotene content. The most pronounced effect was that for boiling for 20 min to prepare soup and stir frying for 2 min to prepare stir-fried pumpkin (Table 3).

Table 3: Total carotenoids, lycopene and β carotene contents of the products prepared from pumpkins

Sample	Total carotenoids (IU/100g)	Lycopene (mg/100g)	β-carotene* (IU/100g)
Raw-P	1200 ± 0.27 [b]	0.65 ± 0.14[a]	845.30
RJ	1476 ± 0.08 [a]	0.70 ± 0.07[a]	6834.94
BJ	892 ± 0.56 [d]	0.18 ± 0.02[bc]	98.59
BS	1107 ± 0.47 [c]	0.54 ± 0.08[a]	602.04
TS	830 ± 0.27 [e]	0.15 ± 0.06[c]	Not detected
SF	676 ± 0.05 [f]	0.35 ± 0.03[b]	Not detected
RO	584 ± 0.74 [g]	0.17 ± 0.05[bc]	566.86
Mean	967.025	0.3959	
C.V	0.435	19.19	
F	1656948*	30.20*	
P	<0.0001*	<0.0001*	

Values are expressed as means± SD.
Means in column not sharing the same superscript are significantly different at $P \leq 0.05$.
Raw-P: control
RJ: raw juice
BJ: boiling juice for 2 min
BS: boiling soup for 4 min
TS: boiling soup for 20 min
SF: stirring fried for 2 min
RO: roasting pumpkin for 4 min

The data presented here regarding the effect of boiling and stir- frying on carotenoids content of pumpkins are not in agreement with data of Azizah *et al.* (2009). They found that the aforementioned treatments elevated the contents of both β-Carotene (2 to 4 times) and lycopene (17 to 40 times) of pumpkin after cooking for 2, 4 and 6 min.It is well known that numerous factors affect the stability of carotenes in food. Temperature, oxygen and duration of heat treatment are the main factors in this respect. Consequently, cooking of pumpkins by different methods (blanching, stir-frying and roasting) vary in terms of their effects on the stability of carotenes.

Flavonoides and phenolic compounds

Table (4) shows that each of Raw -P, RJ-P and P-ST possessed significantly ($P \leq 0.05$) the highest content of flavonoids. The other pumpkin products had flavonoids contents ranged from 12.09 to 14.64 g/kg.

Data in Table (4) show that raw - Pand RJ-P exhibited significantly ($P \leq 0.05$) the highest polyphenolies content being 670.18 g/kg, followed by Raw-P (530.32, RO-P (518.22) TS-P (440.86),

Table 4: Flavonoids and phenolies contents of pumpkins and its products

Treatment	Flavonoids (g/kg)	Phenols (g/kg)
Raw-P	17.24 ± 0.46 [a]	530.32±0.10 [b]
RJ	17.96 ± 0.76 [a]	670.18± 0.61 [a]
BJ	13.53 ± 0.78 [bc]	430.26 ± 0.40 [e]
BS	17.18 ± 0.17[a]	411.25 ± 0.44 [f]
TS	14.64 ± 0.85 [b]	440.86±0.10 [d]
SF	13.59 ± 0.86 [bc]	406.25 ± 0.85[g]
RO	12.09 ±0.61[c]	518.22± 0.69 [c]
Mean	15.177	486.76
C.V	4.502	0.108
F	33.15*	97051.59*
P	<0.0001*	<0.0001*

Values are expressed as means± SD.
Mean in a column not sharing the same superscript are significantly different at $P \leq 0.05$
Raw-P: control
TS: boiling soup for 20 min
RJ: raw juice
BJ: boiling juice for 2 min
BS: boiling soup for 4 min
SF: stirring fried for 2 min
RO: roasting pumpkin for 4 min

BJ-P (430.26), BS-P (411.25) and SF-P (406.25) g/kg. It is well known that several phenols, i.e. non-flavonoids mainly drived from cinamic acid, are part of the plant antioxidant system. Most common hydroxycinnamates are *p*-coumaric, caffeic, ferulic as well as caffeoylquinic acid and chlorogenic acid.

Data presented here regarding the effect of boiling and stir- frying on total phenols of pumpkins are in accordance with data published by Azizah *et.al.* (2009), who found that the aforementioned treatments resulted in 18 to 54% lossess of total phenolics content of the pumpkins.

CONCLUSIONS

Pumpkin pulp can be prepared in many forms (juice, soup, stir-fried cubes and roasted chips). It was obvious that all these aforementioned forms were well accepted by panelists. Due to presence of bioactive compounds mainly dietary fibers and antioxidants, it is advisable to promote and encourage consumption of pumpkin dishes in Egypt.

Recommendations

Based on the results of the present work, the

following recommendations are suggested:

1– It is necessary to apply the most proper cooking conditions (temperature and cooking time) to avoid any degradation of the bioactive compounds present in pumpkins. Study reveal the superiority of blanching since it maintained the bioactive compounds in pumpkins without deteriorative effects.

2– Promotion and encourage the consumption of different pumpkin products in Egypt, since these products are considered as functional foods and thereby protect against many degenerative diseases.

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تأثير طرق الطهو المختلفة على مضادات الأكسدة الطبيعية فى منتجات القرع العسلي

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في هذه الدراسة تم تقطيع ثمار القرع العسلي علي شكل مكعبات ذات أبعاد (١,٥X١,٥X١,٥ سم).وأجريت معاملتان حراريتان هما السلق والتحمير مع التقليب ، كذلك تم التقطيع علي شكل شرائح (بسمك ٠,٥)من القرع العسلي لمعاملة الشى .تم تقييم بعض المنتجات(عصير – شوربة- المكعبات المحمره – شرائح مشويه) وتم استخدام مكعبات القرع سابقة السلق في إعداد كل من عصير وحساء . تبين أن محتوي الكارتينيودات (وحدة دولية / ١٠٠جم) تراوحت بين ١١,٠٧ للحساء و٨٩٢ للعصير. أما بالنسبة لمحتوي المنتجات من البيتا كاروتين فكانت القيمة الأعلى معنوياً ٦٨٣٤,٩٤ وحدة دولية /١٠٠ جم في العصير الخام في حين احتوي العصير الذي تم إعداده باستخدام مكعبات سابقة السلق على ٩٨,٥٩ وحدة دولية /١٠٠جم.

أوضحت النتائج أن الشرائح المشويه قد احتوت علي الفلافونيدات بنسبه ١٢,٠٩ جم/كجم. بينما بلغ محتوي الليكوبين في الحساء الذي تم إعداده من مكعبات سابقة السلق لمدة ٤ دقائق علي التركيز الأقل معنويا (٠,١٥ ملجم /١٠٠جم)، علي عكس الحساء الذي تم اعداده بالطريقة التقليدية (الغليان لمدة ٢٠ دقيقة) حيث احتوى علي التركيز الأعلي معنويا من الليكوبين (٠,٧٠٣ ملجم /١٠٠جم). أوضحت النتائج أن المكعبات المحمره احتوت معنويا علي أقل تركيز من الفينولات (٤٠٦,٢٥ ملجم /كجم).علي عكس الشرائح المشويه التي احتوت علي التركيز الأعلي معنويا (٦٧٠,١٨ جم /كجم) .

تم قياس النشاط المضاد للأكسدة للمنتجات موضع الدراسة بطريقتي كسح كل من الـ DPPH وفوق أكسيد الهيدروجين، وتبين أن العصير الخام كان الأعلى معنوياً بالنسبة للكسح بالـ DPPH (٦٣,٧٩٪) على عكس الناتج المشوي والذي كان الأقل معنوياً (٣٠,٣١٪) في حين كانت قيمة كسح فوق أكسيد الهيدروجين هي الأعلى معنوياً (٢,٨٥٪) لناتج الحساء الذي تم تحضيره من مكعبات سلقت لمدة ٤ دقائق على عكس العصير الخام الذي أعطى القيمة الأقل معنويا لكسح فوق أكسيد الهيدروجين (٠,٣٢٪). ومن ثم فإنه من الضروري استخدام طريقة الطهو الأكثر مواءمة من حيث درجة حرارة وزمن الطهو وذلك للحيلولة دون حدوث تكسير للمنتجات النشطة حيوياً والموجودة في القرع العسلي. ولقد أوضحت نتائج هذه الدراسة تفوق معاملة السلق على المعاملات الأخرى وذلك لأنها تحافظ على المركبات النشطة حيوياً في القرع العسلي دونما أضرار.