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EFFECT OF SOME PRESERVATION PROCESSES ON THE PHYTOCHEMICAL COMPOUNDS WITH ANTIOXIDANT ACTIVITIES OF BROCCOLI

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ABSTRACT

The effect of common aquathermal processes of broccoli using steam or immersion in hot water as traditional technology and storage conditions by either canning or freezing on the content of compounds with antioxidant properties, i.e. vitamin C, carotenoids, chlorophyll, polyphenol and flavonoid compounds parameters was investigated. Results show that the content of carotenoids was 11.41µg/g while the content of chlorophyll A and chlorophyll B was 30.66 and 9.79µg/g, respectively. The content of both carotenoids and total chlorophyll were decreased after being treated prior to different period storage for 3 and 6 month. Fresh broccoli had 40.45µg/g total chlorophyll this content decreased more significantly for the control (fresh broccoli) than for treated and different storage samples. The content of vitamin C was 124.24 mg/100g. Frozen broccoli after blanching by steam and immersion in hot water at 0 times decreased the percent of ascorbic to 83.85 and 75.89%, respectively. Jarred and canned broccoli with dill, coriander and parsley additives showed decrease in ascorbic acid to 45.98 and 40.55% meanwhile, jarred and canned broccoli without additives decreased ascorbic acid to 43.34 and 39.01%, respectively. Total polyphenols and flavonoids were 276.82 and 7.64 mg/100g on fresh weight. These contents were decreased after processing and different storage conditions. The present of antioxidant activity in fresh broccoli was 75.50%. The degradation of antioxidant activity was increased after 6 month period. The highest degradation observed with canned broccoli without additive while the lowest degradation showed with frozen broccoli blanched by steam which the percents of total antioxidant activities were14.84 and 29.65 %, respectively. The content of ferrous and zinc was 6.4 and 3.9 mg/100g on dry weight. Ferrous increased to 7.0 and 8.4 mg/100g with Jarred with dill, coriander and parsley additives and storage for 3 and 6 month broccoli and to 9.3 and 8.7 mg/100g with canned broccoli with dill, coriander and parsley additives.

INTRODUCTION

Broccoli is one of the major agricultural products widely considered to contain high level of phytochemicals including, flavonoids, vitamins, and minerals **(Cao *et al* 1996)**. While investigating health benefit effects of broccoli, many studies have indicated that consumption of broccoli is inversely related with the occurrence of cancer in human. The health benefits of vegetables in preventing cancer and cardiovascular diseases are mostly attributed to the quality and quantity of antioxidative components. The cancer preventing action of vegetables is supposed to reside in the fact that vegetables contain not only abundant nutritional antioxidants, such as vitamins C and E, and β-carotene, but also a great quantity of non-nutritional antioxidants, such as flavonoids, flavones, and other polyphenolic compounds **(Vinson *et al* 1998).**

The main antioxidative components present in broccoli are flavonoids and vitamins. Moreover, broccoli has been recognized as an important source of various biologically active compounds including polyphenols **(Moreno *et al* 2006).**

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Many people have no opportunity to eat fresh vegetables every day and frequently use frozen vegetables, mainly for convenience, time-saving and practical reasons **(Ninfali & Bacchiocca, 2003)** and like many other fruit or vegetables, broccoli is subjected to thermal processing before consumption. Traditionally, processing fruits and vegetables by thermal treatment is considered to decrease water-soluble and heat sensitive nutrients, such as vitamin C (**Murcia *et al* 2000).** Although vitamin C contribute to the antioxidant profile of many green vegetables, variation in antioxidant profile amongst various broccoli genotypes was not correlated with ascorbic acid content **(Kurilich *et al* 2002)**, indicating molecules other than vitamin C may contribute to the total antioxidant capacity of this agricultural product. In fact, natural variation in the polyphenol content is strongly correlated with the variation of antioxidant capacity amongst broccoli genotypes **(Eberhardt *et al* 2005).**

While investigating antioxidant capacity and active constituents such as carotenoids, ascorbic acid, and flavonoids, which are essential for preparing a food composition database many previous studies used raw broccoli **(Azuma *et al* 1999).** The fact that the concentration of nutrients or their activity may change through cooking and storage conditions such as frozen or jars and cans practices is now reflected in many recent studies. There has been growing research about both their importance to health and new techniques to protect the nutritional and sensory qualities definitely needed by consumers. Recently, **Gawlik-Dziki (2008)** reported that boiling of fresh broccoli significantly decreased polyphenol content. It is believed that steam blanching would protect broccoli from the loss of phytochemical in to water in comparison to seeping broccoli in boiling water. At present, steam blanching is the most commonly used method in the food industry. Steam blanching is relatively inexpensive and retains most minerals and water-soluble components over boiling water blanching. Steam-processing may release more bound phenolic acids from the breakdown of cellular constituents **(Roy *et al* 2009).**

Freezing is one of the most effective methods of preserving the nutritive constituents of raw materials for long time of storage. This method also permits a good preservation of initial sensory traits of the raw material and gives products well suited for further culinary processing. However, frozen vegetables prepared using traditional technology; where blanching is used for protection against enzymes mainly, need additional cooking before consumption.

Some modern cultures still consume wild plants as a normal spice and herb source, obtaining fairly good amounts of several nutrients, and it is widely accepted that herbs are significant nutritional sources of minerals. Furthermore, other nutrients, such as carotenoids and phenols, are found in larger quantities in these plants **(Guil *et al* 1997).** The nutritional and medicinal properties of these plants may be interlinked through phytochemicals, both nutrient and non-nutrient **(Ranhotra *et al* 1998).** Among of these plants dill, coriander and parsley which had a high content of ferrous and zinc **(Ozcan, 2004)**

The aim of this work is to characterise the content of compounds with antioxidant properties, i.e. vitamin C, carotenoids, chlorophyll, polyphenol and flavonoid compounds in broccoli, and the degree to which these substances are active as antioxidants. The effect of common aquathermal processes using steam or immersion in hot water as traditional technology and storage conditions by either canning or freezing on these parameters was investigated. Moreover, the effect of some foodstuffs rich in antioxidants were added such as dill, coriander and parsley on canned broccoli antioxidant activities.

MATERIALS AND METHODS

Samples: Fresh broccoli (*Brassica oleracea*) purchased from a local market prepared average laboratory sample of broccoli was cleaned from the leaves and inedible stems were removed subsequently, washed and dried at room temperature.

Blanching: One part of the broccoli was analysed without processing, while others were divided into portions which were pre-blanched by subjected to: 1- Immersion in hot water for about 3 min at a temperature 95± 2 °C, 2-Steaming was carried out in a closed vessel using a stainless steel steam insert with a single layer of the broccoli suspended above 500 g of boiling water for 5 min at a temperature about 85± 2°C. The blanched material was drained for 1 min, cooled in ice water for 2 min, finally drained for 1 min and packaged. Portion of blanched samples were frozen at (−20 °C) and other portions were prepared to be canned and jarred.

Preparation of canned and jarred solution: Solution was prepared by using sodium chloride 2%, calcium chloride 1%, citric acid 0.2% and ascorbic acid 0.2%; about 150 gm was packaged in cans and 180 gm in glass jars and added the optimum amount solution to packaged jars and cans.

Some aromatic and medicinal plants were used such as dill, coriander and parsley were using as antioxidant additive which chopped and added 9 gm to some jarred and canned samples and other samples were left without additives.

Sterilization of canned and jarred samples: Canned samples were sterilized at 121˚C for 30 min. while jars were sterilized at 100˚C for 30 min. cans were directly cooled by water at 40˚C and jars were left to cool down at room temperature. Frozen jarred and canned samples were chemically analyzed at 0 times and after 3 and 6 month.

Chlorophylls content: A spectrophotometric method adapted by **Vernon (1960).** was used to quantify chlorophylls a and b. Acetone was added with pure water to give a final solution which was 80% in acetone. Broccoli samples (2.5 gm) were cutting and homogenized into a Blender and 25 ml of acetone solution were added. The samples were homogenized for 3 min. The homogenate was filtered through filter paper Whatman no. 1, with a Büchner funnel under vacuum. The filter cake residues were washed with 80% acetone and the filtrate brought to a final volume of 50 ml with the same solution.

Determination of total polyphenolic content: The total polyphenolic content was estimated using the Folin–Ciocalteu assay, developed by **Velioglu *et al* (1998)**, with some modifications. Briefly, 125 µl of Folin–Ciocalteu reagent, 125 µl diluted sample, and 250 µl of distilled water were put into a test tube. The mixture was vortexes and allowed to stand for 5 min at room temperature. Then, 1.25 ml of sodium carbonate solution (7%) was added, followed by 1 ml of distilled water. The mixture was vortexes and allowed to stand at room temperature for 90 min. Total phenolic content was determined using a spectrophotometer (Jenway 6405 UV/VIS) at 760 nm. Gallic acid was used as standard, and total phenolic content was expressed as equivalents of gallic acid (GAE)/100 g of fresh broccoli.

Determination of total flavonoid content: Total flavonoid content was determined by modifying a colourimetric method described previously **(Dewanto *et al* 2002 & Eberhardt *et al* 2000)**. Briefly, 0.2 ml of the water-soluble extract, 0.8 ml of distilled water and 50 µl of a 5% NaNO2 solution were mixed in a test tube. After 6 min, 100 µl of a 10% $AlCl_3$ - $6H_2O$ solution was added and allowed to stand for another 5 min before 0.5 ml of 1 M NaOH was added. Then, 850 µl of distilled water was added to bring the mixture to 2.5 ml and mixed well. The absorbance was measured immediately against the blank at 510 nm using a spectrophotometer (Jenway 6405 UV/VIS) in comparison with the standards prepared similarly with known quercetin concentrations (10–800 µg/ml). The results are expressed as mean (micromoles of quercetin equivalents, QE/100 g of broccoli).

The ascorbic acid and carotenoids was determined by the method described by **AOAC (1990)**.

Perkin Elemer Atomic Absorption Spectrometer 3300 technique was used to estimate the content of Fe and Zn according to **AACC (1983).**

Determination of total antioxidant activity: Antioxidant activity was determined by the 2,2,-diphenyl- 2-picryl-hydrazyl (DPPH) method of **Zhang & Hamauzu (2004).** With some modifications. Broccoli content of the methanol extracts of fresh or processed broccoli were adjusted to 6 mg/ml (on dry basis), which was chosen as an appropriate concentration for assessing antioxidant activity after preliminary studies of the different concentrations. An aliquot of 1.5 ml of 0.1 mM DPPH radical in methanol was added to a test tube with 0.5 ml of broccoli extract, at 6 mg/ml. Instead of methanolic extract of broccoli, pure methanol was used as control. The reaction mixture was let to stand at room temperature in the dark for 60 min before the decrease in absorbance at 517 nm was measured. Pure methanol was used to calibrate the spectrophotometer. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

AA (%) = Abs control − Abs sample / Abs control × 100:

Statistical analysis: All data were recorded as means and analyzed by SPSS for Windows (ver.10.1.). One-way analysis of variance (ANOVA) and Duncan comparisons were tested any significant differences between raw and different treatment broccoli.

RESULTS AND DISCUSSION

The content of carotenoids and chlorophyll content in fresh and after different treatments prior to frozen, jarred and canned storage broccoli are shown in **Table (1).** Results explain that the content of carotenoids in fresh broccoli were 11.41µg/g while the content of chlorophyll A and chlorophyll B was 30.66 and 9.79 µg/g, respectively. Both carotenoids and total chlorophylls content were decreased after treated and different

Table 1. Carotenoids and chlorophyll content in fresh and different processed broccoli µg/g FW.

Period time	fresh and different processed	Carotenoids	Chlorophyll A	Chlorophyll B	Total chlorophyll
After processing Zero time	1	11.41 [a]	30.66 [a]	9.79 [a]	40.45 [a]
	2	10.59 [ab]	26.88 [bc]	9.20 [a]	36.08 [b]
	3	11.05[ab]	27.64[b]	9.53 [a]	37.17 [b]
	4	8.57 [cd]	25.73 [bc]	7.17 [bc]	32.90 [de]
	5	8.29 [cd]	27.03 [bc]	6.81 [c]	33.84 [cd]
	6	9.38 [bc]	24.45 [bc]	7.92 [b]	32.37 [de]
	7	8.27 [cd]	27.95 [ab]	7.29 [bc]	35.24 [bc]
	8	7.20 [d]	24.72 [c]	6.43 [c]	31.15 [e]
After 3 month storage	2	7.32 [c]	20.37 [cd]	7.12 [b]	27.49 [c]
	3	9.03 [b]	22.80 [bc]	7.60 [b]	30.40 [b]
	4	8.00 [bc]	19.54 [d]	5.69 [c]	25.23 [d]
	5	6.83 [c]	18.82 [d]	5.18 [c]	24.00 [d]
	6	8.57 [b]	22.85 [bc]	4.43 [d]	27.28 [c]
	7	7.10 [c]	23.47 [b]	6.88 [b]	30.35 [b]
	8	6.82 [c]	19.11[d]	5.65 [c]	24.76 [d]
After 6 month storage	2	6.39 [cde]	16.07 [e]	6.20 [c]	22.27 [cd]
	3	7.01 [bcd]	20.55 [bc]	6.94 [b]	27.49 [b]
	4	7.41[bc]	18.87 [cd]	3.71 [de]	22.58 [cd]
	5	6.12 [de]	12.06 [f]	3.18 [e]	15.24 [e]
	6	7.90 [b]	17.53 [de]	4.13 [d]	21.66 [d]
	7	8.13 [b]	21.42 [b]	5.79 [c]	27.21 [b]
	8	5.57 [e]	20.39 [bc]	3.48 [e]	23.87 [c]

1. Fresh broccoli, 2.frozen broccoli blanched by hot water, 3.frozen broccoli blanched by steam, 4.Jarred broccoli with dill, coriander and parsley additive, 5. Jarred broccoli without additive 6.Jarred broccoli blanched by steam, 7. Canned broccoli with dill, coriander and parsley additive and 8. Canned broccoli without additive.

storage conditions. Significant decreased was observed in both total carotenoids and chlorophyll contents after being blanched by either steam or immersion in hot water. Frozen after pre-blanched by steam for 5 min at 85± 2°C and hot water immersion for 3 min. at 95± 2°C decreased the content of carotenoids to 11.05 and 10.59 while total chlorophylls were 37.17 and 36.08 µg/g, respectively.

Jarred and canned broccoli showed significant decrease in both total carotenoids and chlorophyll content after blanched and sterilized treatments. The lowest carotenoids degradation observed with broccoli blanched prior to freezing while the highest degradation showed with canned treatment without additives.

Storage for 3 or 6 month showed fluctuation in carotenoids decreased which blanched by steam showed lowest degradation (9.03 µg/g) while canned without additives had the highest degradation. The lowest degradation were 20.86 % for frozen storage for 3 month with steam blanching and 28.75% for canned storage for 6 month with dill, coriander and parsley additives while, the highest degradation of carotenoids were 40.23 and 51.18 %, respectively for canned broccoli without additives.

Fresh broccoli had 40.45µg/g total chlorophylls this content more significantly decreased for the control (fresh broccoli) than for treated and different storage samples.

After different treatments and storage at zero times the content of total chlorophyll ranged from 31.15 to37.17 µg/g for canned broccoli without additives and frozen broccoli blanched by hot water, respectively. Jarred broccoli without additives gave the major lowering in the total chlorophylls content 24.00 and 15.24 µg/g after storage for 3 and 6 month, respectively while the lowering degradation showed with frozen broccoli blanched by steam. **Goncalves *et al* (2009)** concerning the thermal treatment induced a significant decrease in the level of the total chlorophylls.

The vitamin C content, total polyphenols, total flavonoids and total antioxidant activity in fresh and different processes and storage for 3 and 6 month of broccoli are shown in table 2. The fresh broccoli contained 124.24 mg/100g vitamin C. Frozen broccoli after blanching by steam and immersion in hot water at zero times decreased the percent of ascorbic to 83.85 and 75.89%, respectively. Jarred and canned broccoli with dill, coriander and parsley additives showed decrease in ascorbic acid to 45.98 and 40.55% meanwhile, jarred and canned broccoli without additives decreased ascorbic acid to 43.34 and 39.01%, respectively while jarred broccoli with steam blanching decreased ascorbic content to 47.83%. After storage broccoli by freezing or canned and jarred for 3 and 6 month ascorbic acid decreased. The highest decrease was storage by canned without additives while the lowest decease showed with storage by frozen broccoli blanched by steam. In comparison the losses of ascorbate caused by blanching or freezing were relatively small compared with canned and jarred vegetables which have been shown to be significantly higher ascorbate degradation **(Favell, 1998).**

Data on total polyphenols and flavonoids in fresh and processed broccoli was storage at different conditions reported that fresh broccoli contained 276.82 and 7.64 mg/100g on fresh weight. After processing and storage at zero times total polyphenols and flavonoids ranged from 261.65 and 4.91 for frozen broccoli blanched by hot water and 306.75 and 6.45 mg/100g for jarred broccoli blanched by steam, respectively. After storage for 3month polyphenols decreased but the content in jarred broccoli blanched by steam was still higher than that found in fresh broccoli. Flavonoids content ranged from 1.14 mg in canned broccoli without additive to 5.12 mg/100g in jarred broccoli blanched by steam. The content of polyphenols and flavonoids after 6 month ranged from 163.40 and 0.30 and 256.35 and 4.52 mg/100g, respectively. Results also explained the content of total polyphenols and flavonoids in broccoli with dill, coriander and parsley additive were higher than its content without additives. **Wachtel-Galor *et al* (2008).** Showed that during steaming, however, it may be that the temperature was lower than in the other methods and therefore did not affect the phenolic content as much.

Antioxidant compounds work together as one unit which activation or inhibition related to cooperation these compounds with them. Results in **Table (2)** and **Figs**. (**1, 2 and 3**) shows that the present of antioxidant activity in fresh broccoli was 75.50% this percent was decreased after different treatments and storage periods. The highest percents of antioxidant activity showed with canned and jarred broccoli with dill, coriander and parsley additive 63.40 and 57.31%, respectively. These percents were decreased to50.38 and 41.94% after storage for 3 month. The lowest percent was 27.50% in frozen broccoli blanched by hot water decreased to 22.70 and 17.86%, respectively. The degradation of antioxidant activity was increased after 6 month period. The highest degradation observed with canned broccoli without additive14.84% while the lowest degradation showed with frozen broccoli blanched by steam 29.65 %.**Sun *et al* (2007)** concerning antioxidant properties of broccoli this effect on total antioxidant activity are neglected.

The content of ferrous and zinc in fresh broccoli and the effect of different processing and storage conditions on its broccoli content is represented in **Table (3).** The content of ferrous and zinc was 6.4 and 3.9 mg/100g on dry weight. Ferrous increased from 6.7 to 7.0 and 8.4 mg/100g with Jarred broccoli with dill, coriander and parsley additive and from 7.50 to 9.3 and 8.7 mg/100g with canned broccoli with dill, coriander and parsley additives these results due to the high content of ferrous in dill and parsley **Ozcan, (2004)** and may transfer to broccoli by solution medium. Also results indicated that the contents of zinc ranged from 3.2 to 3.6 mg/100g after storage for 3 month and this content was decreased to 2.6 and 3.3 mg/100g after 6 month of storage period.

Table 2. Content of natural antioxidants and antioxidant activity of fresh, processed and different storage conditions of broccoli (mg/100 g of FW)

Period time	fresh and different processed	Vitamin C mg/100gm of FW	Total polyphenols mg/100gm of FW	Total flavonoids mg/100gm of FW	Total antioxidant activity 5g/100ml
After processing Zero time	1	124.24 ^{a}	276.82 ^{abc}	7.64 ^{a}	75.5
	2	94.29 ^{c}	261.65 ^{c}	4.91 ^{d}	27.50
	3	104.18 ^{b}	278.81 ^{abc}	6.10 ^{bc}	40.69
	4	57.13 ^{de}	290.24 ^{abc}	5.86 ^{bcd}	57.31
	5	53.85 ^{de}	267.60 ^{c}	4.97 ^{d}	48.79
	6	59.42 ^{d}	306.75 ^{a}	6.45 ^{b}	40.53
	7	50.38 ^{e}	298.60 ^{ab}	5.81 ^{bcd}	63.40
	8	48.46 ^{e}	276.11 ^{bc}	5.21 ^{cd}	56.92
After 3 month Storage	2	62.15 ^{c}	225.36 ^{de}	3.79 ^{c}	22.70
	3	77.03 ^{b}	239.44 ^{cd}	4.32 ^{c}	34.89
	4	44.62 ^{d}	259.15 ^{bc}	3.83 ^{c}	41.94
	5	41.54 ^{de}	250.70 ^{c}	0.92 ^{d}	24.73
	6	36.15 ^{ef}	290.15 ^{a}	5.12 ^{b}	33.50
	7	31.54 ^{fg}	242.32 ^{cd}	3.72 ^{c}	50.38
	8	26.15 ^{g}	214.10 ^{e}	1.14 ^{d}	38.92
After 6 month Storage	2	39.50 ^{c}	205.66 ^{e}	2.24 ^{c}	17.86
	3	51.34 ^{b}	225.07 ^{cd}	2.58 ^{c}	29.95
	4	21.54 ^{e}	247.90 ^{b}	1.48 ^{d}	28.02
	5	20.05 ^{e}	239.44 ^{bc}	0.18 ^{e}	15.38
	6	28.46 ^{d}	256.35 ^{b}	4.52 ^{b}	22.80
	7	22.00 ^{e}	216.84 ^{de}	1.78 ^{d}	21.42
	8	18.23 ^{e}	163.40 ^{f}	0.30 ^{e}	14.84

1. Fresh broccoli, 2.frozen broccoli blanched by hot water, 3.frozen broccoli blanched by steam, 4.Jarred broccoli with dill, coriander and parsley additive, 5. Jarred broccoli without additive 6.Jarred broccoli blanched by steam, 7. Canned broccoli with dill, coriander and parsley additive and 8. Canned broccoli without additive

Table 3. Ferrous and zinc content in fresh and different processed broccoli mg/100g (on dry weight)

Storage	Fresh		Jarred without additives		Jarred with additives*		Jarred with steam blanched		Canned without additives*		Canned with additives	
	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
	6.4 ^{cd}	3.9 ^{ab}										
After processed at zero time			6.3 ^{d}	3.8 ^{ab}	6.7 ^{b}	3.2 ^{c}	6.4 ^{cd}	3.7 ^{b}	6.6 ^{bc}	3.8 ^{ab}	7.5 ^{a}	4.0 ^{a}
	6.4 ^{c}	3.9 ^{a}										
After 3 month storage			6.4 ^{c}	3.4 ^{bc}	7.0 ^{b}	3.5 ^{b}	6.7 ^{bc}	3.2 ^{c}	6.9 ^{b}	3.2 ^{c}	9.3 ^{a}	3.6 ^{b}
	6.4 ^{c}	3.9 ^{a}										
After 6 month storage			6.5 ^{c}	2.9 ^{c}	8.4 ^{b}	3.3 ^{b}	6.4 ^{c}	2.8 ^{cd}	6.5 ^{c}	2.7 ^{de}	8.7 ^{a}	2.6 ^{e}

Dill, coriander and parsley additives*

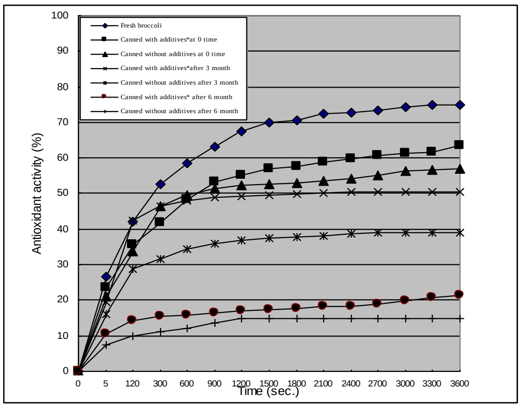

Fig.1. Effect of canned process at different period time with dill, coriander and parsley additives on the antioxidant activity in broccoli
*** Dill, coriander and parsley additives**

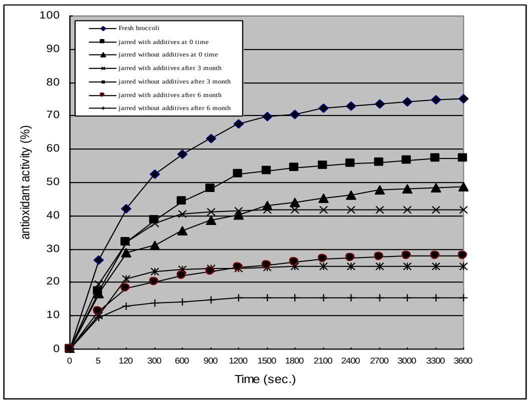

Fig. 2. Effect of Jarred process at different period time with dill, coriander and parsley additives on the antioxidant activity in broccoli
*** Dill, coriander and parsley additives**

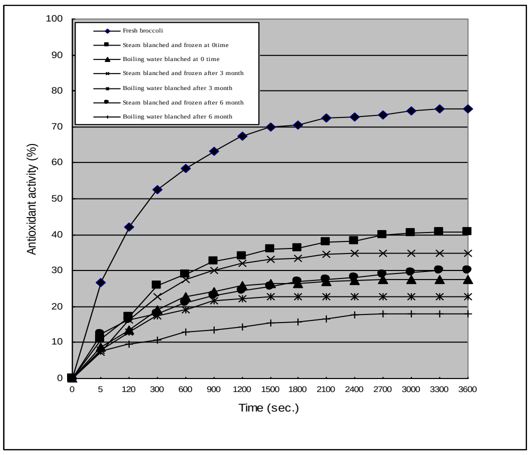

Fig. 3. Effect of blanched process at different period time on the antioxidant activity in broccoli

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