

Full Length Research Paper

Impact of cooking and conservation for twelve days on total polyphenols content, antioxidant and anticholinesterase activities of red onion

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The objective of this work was to determine the impact of three cooking modes (boiling, steaming and microwaving) and the conservation of onion at 4°C for 12 days on total polyphenol, flavonoid, tannins, phenolic acids contents antioxidant and anticholinesterase activity of red onion: *Allium cepa*. The results showed that the three cooking modes caused an increase of the levels of total polyphenols, flavonoids and tannins, but decreased the levels of phenolic acids. Storage at 4°C for 12 days caused a decrease in levels of total polyphenols, flavonoids, tannins and phenolic acids. A decrease in anti-radical activity during storage was found. Three cooking modes resulted in a decrease in antioxidant activity. However, the use of microwave was more effective as' to higher polyphenol contents. The level of anticholinesterase activity steadily decreased during refrigerated storage and after 12 days it was 1/4 of the value found in the raw material. The raw onion showed a moderate activity which increased after most cooking treatments. The highest level of capacity was observed after microwaving.

Key words: Total polyphenol, flavonoids, tannins, phenolic acid, cooking, conservation, antioxidant activity, anticholinesterase activity, red onion.

INTRODUCTION

Onion (*Allium cepa*) is a versatile vegetable that is consumed fresh as well as in the form of processed products. More recently, there has been renewed attention given to the antioxidant content of onions, because many epidemiological studies suggested that regular consumption of onions in food is associated with a reduced risk of neurodegenerative disorders, many forms of cancer, cataract formation, ulcer development, reduction in symptoms associated with osteoporosis

(NOA), prevention of vascular and heart diseases by inhibition of lipid peroxidation (LPO) and lowering of low density lipoprotein (LDL) cholesterol levels (Kaneko and Baba, 1999; Kawaii et al., 1999; Sanderson et al., 1999; Shutenko et al., 1999; Singh et al., 2009). It is an important food because it supplies various activated phytochemicals such as phenolic acid, flavonoids, organosulfur compounds (OSCs), and anthocyanin (Slimestad et al., 2007).

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Similarly, protection against neuronal degeneration in Alzheimer's disease can be achieved using natural antioxidants. Acetylcholinesterase inhibitors (AChEI) are used for the treatment of Alzheimer's disease because they enhance neuromediator acetylcholine level (Grossberg, 2003). In contrast, polyphenols and flavonoids have shown certain stability when exposed to high temperatures, a quality that is reflected in the preservation of their antioxidant capacity (Vallejo et al., 2003). Studies performed on different vegetables after cooking or conservation showed that the total polyphenol content and antioxidant capacity could be either higher or lower in comparison to the fresh food (Lombard et al., 2005; Turkmen et al., 2005). The purpose of this study is to analyze differences in the antioxidant, anticholinesterase capacity, and polyphenol retention after preservation and simulated domestic processing (boiling, steaming and microwave).

METHODOLOGY

Samples

Onion samples (*A. cepa* L.) were collected in the region of Djenan el Anab, Beni bechir, 10 km from Skikda, Algeria and they were placed in plastic bags and taken to the laboratory for analysis.

Effect of preserving vegetables on the content of total polyphenols

The effect of preserving vegetables on the content of total polyphenols was studied. Conservation is stopped when a beginning of softening fabrics onion was seen.

Raw vegetables are stored (4°C) in the refrigerator in the laboratory for 12 days. Every two days, an aliquot of these vegetables was washed and cut into small pieces, cooked (in water, steam and microwave), ground and homogenized for analysis.

Sample preparation and cooking

Cooking and pretreatment procedures are reported by Turkmen (2005). Cooking conditions are optimized by preliminary experiments (Miglio et al., 2008). Vegetables (raw or stored) are washed and all inedible parts are removed manually or by using a steel knife. Then, they are cut into small pieces of uniform shapes. 900 g onions are reserved for cooking procedures, using 300 g per method applied. All culinary experiments were performed in triplicate, using 100 g of vegetable each time.

Boiling

Onion (100 g) is placed in a stainless steel pan with 150 ml of distilled water bouillante à 100°C. Cooking time varies between 16 and 18 min. After this procedure, the vegetables are drained to remove excess water and then cooled in a water bath.

Steaming

Onion (100 g) is placed in a stainless steel steam cooker which was covered with a lid and steamed, over boiling water. The baking time

varies between 15 and 20 min. After this step, the vegetables are cooled in a water bath in a steel container.

Microwave

Onion (100 g) is placed in a glass dish in a microwave oven Giant CE137NM (consumption: 220 to 240, operation 245 MHZ frequency, power 1200 W, External Dimensions: 262 mm (H) × 452 mm (W) × 330 mm (D), size of the cavity of the oven: 198 mm (H) × 315 mm (W) × 297 mm (D), oven capacity: 20L Net Weight Env. 10.5 kg). The vegetables are cooked in a microwave oven at full power and then cooled in a water bath, the cooking time is 4 min.

Polyphenol extraction

The samples of raw and cooked vegetables were homogenized using a commercial juice extractor obtaining a fluid vegetable extract (VE). Polyphenols extraction method was adapted from Vinson et al. (2001) and Faller and Fialho (2009).

Briefly, 1 ml of each VE was extracted using 500 µl of 50% methanol (methanol:water, 50:50, v/v) for the soluble polyphenols and 50% methanol (methanol:water, 50:50, v/v) acidified with 1.2 M HCl for the hydrolyzable polyphenols, in screw capped eppendorfs. The samples were then placed in a water bath at 90°C for 180 min with constant shaking. After 3 h, the samples were removed from water bath and cooled at room temperature (approximately 2 min). The volume was adjusted to 1 ml with 95% methanol and the eppendorfs were centrifuged at 12,000 g for 5 min. The supernatants, considered to be polyphenol extracts, were immediately used for the determination of polyphenol content.

Determination of total phenolic content

The total phenolic was determined according to the method of Veloglu et al. (1998) and Ismail et al. (2004) which used Folin-Ciocalteu reagent. Extract was prepared at a concentration of 1 mg/ml. 100 µl of extract was transferred into a test tube and 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) were added and mixed. The mixture was allowed to stand at room temperature for 5 min. 0.75 ml of 6% (w/v) sodium carbonate was added to the mixture and then mixed gently. After standing at room temperature for 90 min, the absorbance was read at 725 nm using a UV-Vis spectrophotometer. The standard calibration (0 to 1000 ppm) curve was plotted using gallic acid. The total phenolic content was expressed as gallic equivalents in microgram per 1 g vegetable extract.

Total flavonoid contents

The total flavonoid content was determined according as the aluminum chloride colorimetric method described by Chang et al. (2002) and Lin and Tang (2007). Briefly, aliquots of 1 g of onion sample were, respectively, dissolved in 1 ml deionized water. This solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate (AlCl₃), 0.1 ml of 1 M potassium acetate (CH₃COOK), and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against deionized water blank.

Quercetin was chosen as a standard. Using a seven point standard curve (0 to 1000 ppm), the levels of total flavonoid contents in fruits and vegetables were determined in triplicate, respectively. The data were expressed as microgram quercetin equivalents (QE)/1 g of fresh matter from onion analysed.

Tannin analysis

Quantitative estimation of tannins was carried out using the modified vanillin-HCl in methanol method described by Price et al. (1978). The method is based on the ability of condensed tannins to react with vanillin in the presence of mineral acid to produce a red color. Ground pulse samples (1 g) were extracted with 20 ml of 1% HCl in methanol for 20 min at 30°C in a water-bath. The samples were centrifuged at 2000 rpm for 4 min. The supernatant (1.0 ml) was reacted with 5 ml vanillin solution (0.5% vanillin + 2% HCl in methanol) for 20 min at 30°C. Blanks were run with 4% HCl in methanol in place of vanillin reagent. Absorbance was read at 500 nm on a UV/VIS spectrophotometer. A standard curve was prepared with catechin. Results were expressed in terms of catechin equivalents. Samples were analyzed in triplicate

Total phenolics acids content

Phenolics acids were determined according to the method of Netzel et al. (2006) and Sigh et al. (2012) with modification. Onion (1 g) were suspended in 0.3 M hydrochloric acid in 80% ethanol (2 ml) and placed in a sonicator for 20 min. The mixture was centrifuged at 2000 g for 10 min and the supernatant was transferred to a clean glass tube. The pellet was suspended in a second aliquot of 0.3 M hydrochloric acid in 80% ethanol (2 ml) and the process repeated. The combined supernatants were made up to 5 ml with 0.3 M hydrochloric acid in 80% ethanol and an aliquot (1 ml) was transferred to an Eppendorf tube and centrifuged at 15,700 g for 5 min, the absorbance was measured at 320 nm. The total phenolic acids, expressed as μg equivalents of chlorogenic acid per g of extract of fresh weight.

Total antioxidant capacity (DPPH)

The antioxidant capacity was measured by the DPPH radical method according to Kuskoski et al. (2006) and Faller and Fialho (2009). Briefly, a 100 μM DPPH solution was prepared with 80% methanol. In test tubes, 100 μl of each VE, fresh or after cooking, was placed, after which was added 3.9 ml of the DPPH solution (100 μM). The mixture was allowed to stand, in the absence of light, and the absorbance was measured at 60 min. The DPPH solution alone was measured before the addition of the samples (A0) and 80% methanol was used as blank. The antioxidant capacity was represented as the percentage radical scavenging capacity (%) remaining after 60 min according to the equation, which represents the absorbance of the DPPH solution alone measured, and the absorbance for each sample at 60 min after the addition of the DPPH solution at 517 nm.

$$\text{Antioxidant activity I\%} = (A_0 - A/A_0) \times 100$$

Anticholinesterase activity

A spectrophotometric method developed by Ellman et al. (1961) and Ertas et al. (2015) was established to indicate the acetylcholinesterase inhibitory effects. Aliquots of 150 μl of 100 mM sodium phosphate buffer (pH 8.0), 10 μl of sample solution and 20 μl of acetylcholinesterase (AChE) or butyrylcholinesterase (BChE) or solution were stirred and incubated for 15 min at 25°C, then 5,5-dithiobis-2-nitrobenzoic acid (DTNB, 10 μl) is added to mixture. In the next step, by the addition of acetylthiocholine iodide (10 μl) the reaction was started. At the end, final concentration of the tested solutions was 200 $\mu\text{g}/\text{ml}$. BioTek Power Wave XS at 412 nm was used to monitor the hydrolysis of these substrates. The experiments were carried out in triplicate. Galanthamine was used as a

reference compound. The percentages of inhibition were calculated by using the following equation:

DTNB (Ellman's reagent), AchE or butyrylcholinesterase BChE

$$\text{Inhibition (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Statistical analysis

The results of the antioxidant, anticholinesterase activities and total phenolic-flavonoid, tannins and phenolic acids contents were expressed as means \pm SEM.

RESULTS AND DISCUSSION

Effect of storage on the levels of total polyphenols and antioxidant activity

Effect of storage on the content of total polyphenols

The concentration values are read directly from the calibration curve established using the reference solution. The following equations were used to calculate total phenolic contents of the extracts: Absorbance = 0.048 gallic acid (μg) + 0.027 ($R^2 = 0.991$). Concentration of the sample is expressed in μg equivalent per gram of extract. The range is plotted for gallic acid concentrations between 0 and 1000 ppm. Onion contains a total polyphenol content of $90.88 \pm 0.4 \mu\text{g}$ EAG/g VE. This quantity decreases with increasing the shelf life of the onion. It appears that storage at 4°C to cause loss of total polyphenols. The levels of total polyphenols obtained after 12 days of storage was $70.22 \pm 1.11 \mu\text{g}$ EAG/g (Figure 1).

The flavonoid content is higher in fresh onion; it corresponds to EQ 70 $\mu\text{g}/\text{g}$ VE. The results showed decreased levels of flavonoids. Storage at 4°C causes a loss of flavonoids after 12 days, the rate of flavonoids was $49.25 \pm 0.6 \mu\text{g}$ EQ/g VE of onion (Figure 2). Conservation has a negative effect on the content of tannins. The results indicate that the decrease in tannin contents depends on the length of storage (Figure 3). Conservation at 4°C of onion causes phenolic acids decrease over time of conservation (Figure 4).

The results obtained show that storage (at 4°C) causes a decrease of the levels of total polyphenols, flavonoids, tannins and phenolic acids. This loss may be due to the effects of enzymatic browning. Polyphenols are converted by the action of the enzyme. They become inaccessible phenolic groups which cause the decrease of the content of total polyphenols, flavonoids, phenolic acids and tannins (Spagna et al., 2005). According Spagna et al. (2005), PPO retains 55% of its activity at 4°C, which is the cause of the decrease in phenolic content during storage in refrigerator. Different authors also observed the negative effect of the storage period on

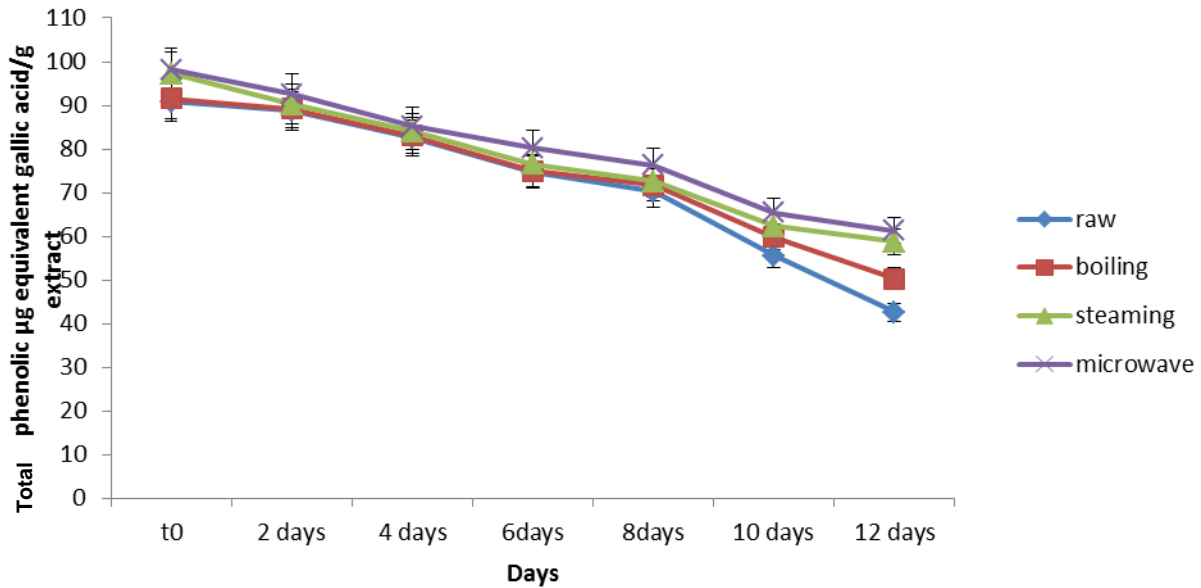


Figure 1. Effect of conservation and cooking on the levels of total polyphenols.

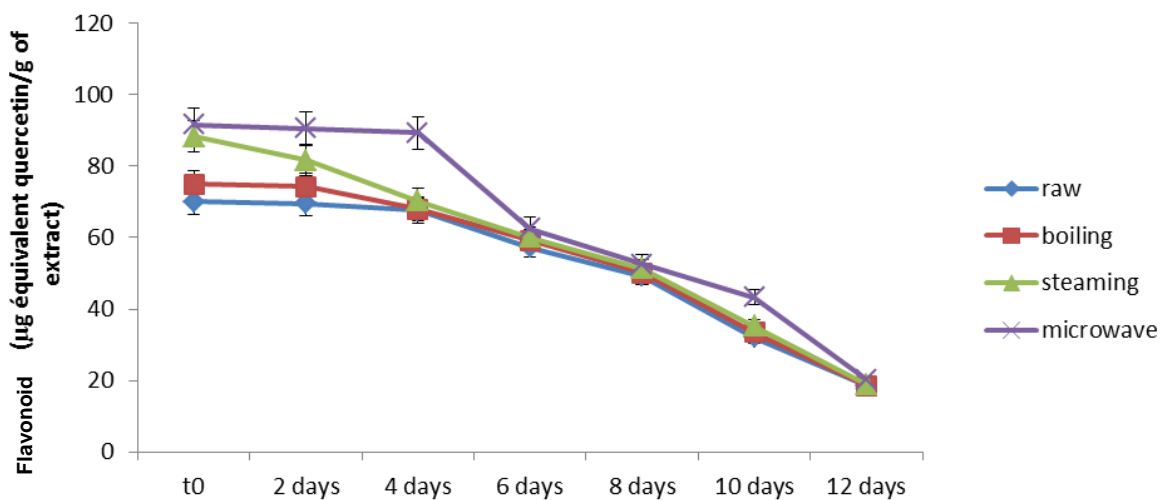


Figure 2. Effect of conservation and cooking on the levels of flavonoids.

the level of the analysed constituents in frozen vegetables.

Cisneros-Zevallos and Heredia (2009) showed that the conservation of onion reduces the levels of total polyphenols. Ferreres et al. (1996) studied the impact of storage content of flavonoids of onion. After 7 days, they found that the content of anthocyanins was significantly decreased. Ewald et al. (1999) showed that loss of the highest flavonoids of onion is obtained when the samples were subjected to a pretreatment before storage. According to Cheyner et al. (1998) and Spigno and De Faveri (2007), the conservation causes a decrease in tannins that could be due to hydrolysis of the polymers of

condensed tannins, or a condensation of tannins with anthocyanes. However, some studies have shown that conservation causes increase in the total polyphenol content in some vegetables. The amount of polyphenols increases spontaneously after 3 days. This is due to the release of tissue senescence (Rodriguez-Arcos et al., 2002).

Antioxidant activity and conservation

It appears that storage contributed to decrease of antioxidant activity (Figure 5). This is due to polyphenols of onion. The main role of these compounds as reducing

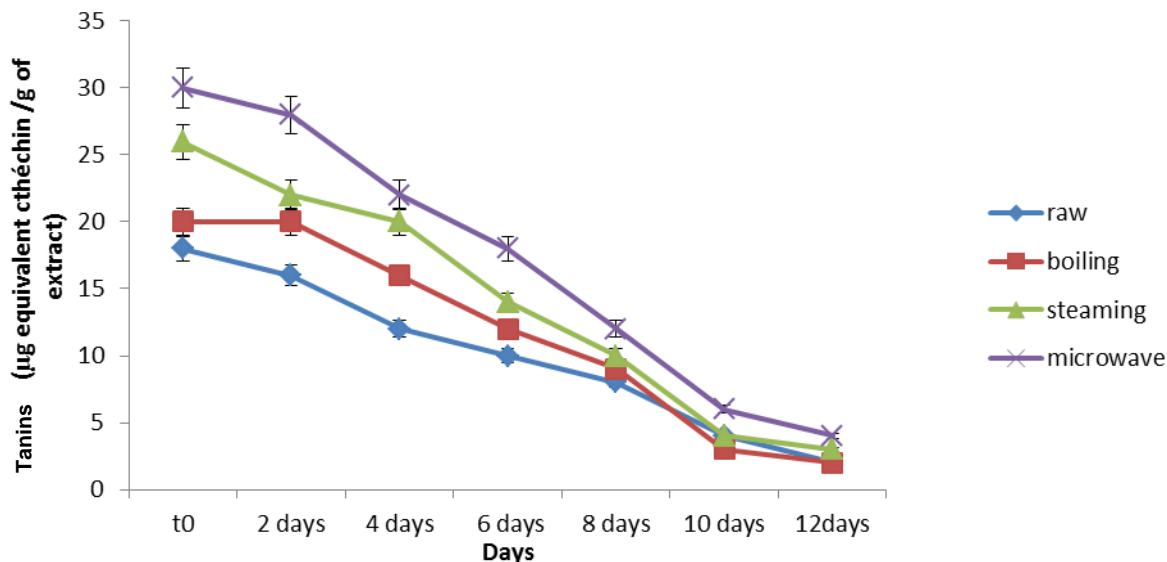


Figure 3. Effect of conservation and cooking on tanins content.

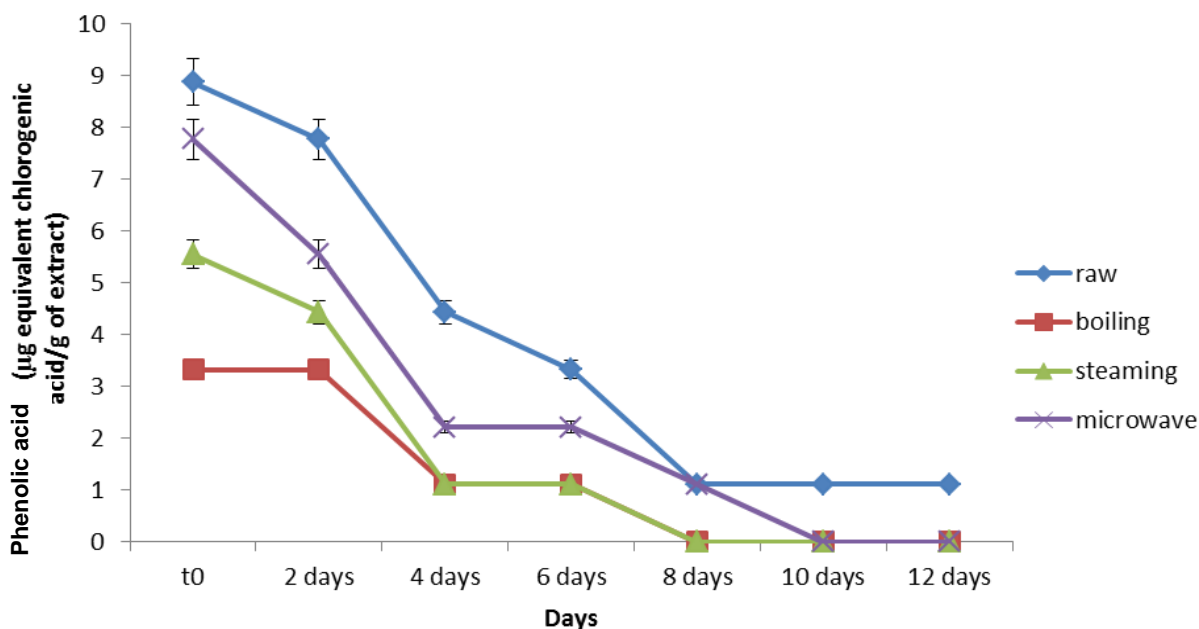


Figure 4. Effect of cooking on the levels of phenolic acids of raw and stored samples.

free radicals is emphasized in several reports. These results are consistent with those obtained by Ewald et al. (1999), Villano et al. (2007), and Cisneros-Zevallos (2009) who found that the conservation of onion causes a decrease in antioxidant capacity.

Effect of cooking on the content of total polyphenols

Figure 1 summarizes the changes in the content of phenolic compounds during the cooking process.

Cooking increases the total polyphenol content for both lots tested (raw and stored onion). However, the use of microwave and steam were more effective as' to higher polyphenol contents.

Dewanto et al. (2002) attributed this increase in phenolics to the embrittlement of tissues with heat cooking which facilitates extraction of these compounds. This release would offset any loss by thermal degradation. The results indicate that the three cooking modes have positive impact on the flavonoid content of

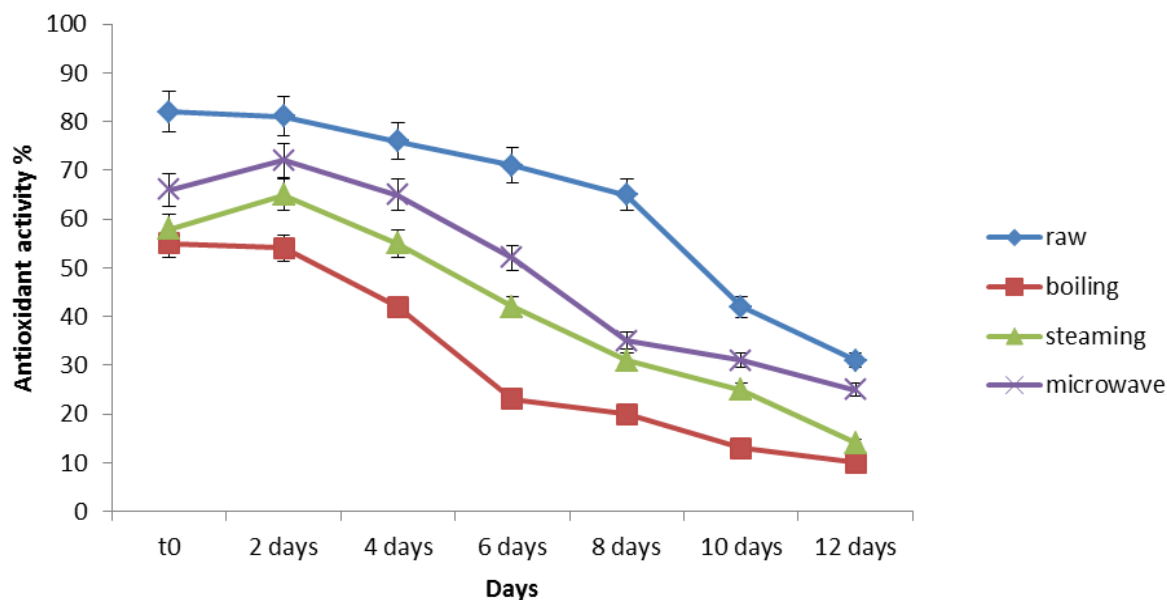


Figure 5. Effect cooking on the antioxidant activity of raw and stored samples.

samples stored at 4°C. However, the use of microwave and steam were more effective. Flavonoids exist as glycoside in food; one or more hydroxyl groups are combined with sugars. The presence of this glycoside fraction makes flavonoids very soluble in water (Figure 2). Increase in the content of tannins is observed for the three cooking modes of raw or stored samples (Figure 3). However, the use of microwave in cooking onion has proved relatively effective in comparison with the other two methods: steaming and boiling water

The results show that the three cooking modes cause an increase in the levels of total polyphenols, flavonoids and tannins, but decrease the levels of phenolic acids (Figure 4). However, the use of microwave and steam were the most effective. Increased levels of total phenolic compounds could be explained by the ease with which they are extracted following certainly a strong weakening of the cell walls of onion by heat (Gahler et al., 2003). Cooking in boiling water is less effective as increase in phenolic compounds; this is due to the solubilization of polyphenols in the cooking water during this type of thermal treatment (Price et al., 1997; Makris and Rossiter, 2001). However, two other cooking methods (by steam and microwave) allow a better retention of soluble polyphenols in plant tissues.

Effect of cooking on the antioxidant activity

Three cooking modes cause a decrease in antioxidant activity of the two samples tested onion (raw and stored) (Figure 5). This result is consistent with that of Fialho and Faller (2009) which showed that cooking in water, steam or microwave decreased antioxidant activity. This activity

is dependent on the mobility of the hydrogen of the hydroxyl group of the phenolic compounds atom. However, there is no relationship between the polyphenol content and antioxidant activity. A small amount of polyphenols can generate strong antioxidant activity (Makris and Rossiter, 2001). Cooking leads to the modification of the amount of polyphenols, but it can also change the structure of polyphenols, which may affect the antioxidant capacity (Makris and Rossiter, 2001). This may explain the decrease in antioxidant activity despite the increase of total polyphenols, flavonoids and tannins.

In addition, each phenolic compound has a degree of affinity (high or low) for free radical DPPH which could affect the antioxidant variations observed (Heim et al., 2002).

Effect of storage and cooking on anticholinesterase activity

The level of anticholinesterase activity steadily decreased during refrigerated storage and after 12 days it was 1/4 of the value found in the raw material (Table 1). The influence of home cooking methods (boiling, microwaving and steaming) on the anticholinesterase activity of raw and stored onion has been evaluated. The raw onion showed a moderate activity which increased after most cooking treatments. The highest level of capacity was observed after microwaving (Table 1). This result indicates that extract of onion contain an acetylcholinesterase inhibitors, which are selective organophosphorus anticholinesterases. The mode of action of these compounds is to block the action of the acetylcholinesterase enzyme, leading to the excessive

Table 1. Anticholinesterase activity of the extracts and standards.

Parameter	Inhibition % against AChE						
	t0	2 Days	4 Days	6 Days	8 Days	10 Days	12 Days
Raw	55.21±0.25	44.5±0.85	41.22±1.1	31.5±0.75	22.5±0.15	22.32±0.65	14.26±0.12
Boiling	56.98±0.95	42.5±1.06	35.25±0.25	32.6±0.26	25.02±0.25	13±0.26	8.08±0.75
Steaming	59.48±1.01	49±0.45	32.02±0.46	22.25±0.13	19±0.89	18.55±0.98	18.12±0.65
Microwave	66.25±0.32	55±0.94	44.13±0.25	29.78±09	23.08±0.49	21.77±0.29	20.33±0.35
Galanthamine	88.02±0.22	-	-	-	-	-	-

Values are means ± standard deviation (SD), n = 3.

build-up of the neurotransmitter acetylcholine.

Phenolic compounds are involved in the protective effect of neurodegenerative diseases (Ramassamy, 2006). In addition, previous reports demonstrated the potential of phenolic compounds to inhibit acetylcholinesterase activity (Williams et al., 2011). Because phenolic compounds have different intracellular targets, they can become an efficient approach to reduce the incidence of AD (Ramassamy, 2006).

Literature suggests that vegetables have neuroprotection effects. A prospective cohort study of 3718 human subjects aged 65 years and older based on food frequency questionnaire assessed cognitive functions at baseline and 3- and 6-year follow-ups. The rates of cognitive decline among persons in the fourth and fifth quintiles of vegetable intake were slower by 0.019 ($P=0.01$) and 0.018 ($P=0.02$) standardized units per year compared with that among persons in the lowest quintile; the overall mean change per year was a decline of 0.04 standardized units (Morris et al., 2006). Extracts of these fruits and vegetable fed to 19-month-old Fischer 344 rats for 8 weeks reversed age-related deficits in several neuronal and behavioral parameters. Blueberry particularly improves motor function that relies on balance and coordination (Joseph et al., 1999).

Conclusion

The effect of three modes of cooking and conservation at 4°C on total phenolic, flavonoid, tannins, phenolic acid content, antioxidant and anticholinesterase activities of extract of red onion were analyzed. Results indicated that red onion were rich sources of polyphenols and flavonoids and showed the promising antioxidant and free radical scavenging activities. Storage at 4°C reduced the levels of total polyphenols, flavonoids, phenolic acids and tannins. Decreased antioxidant activity was observed during storage temperature 4°C. The three modes of cooking used (water, steam and microwave) increased the levels of total polyphenols, flavonoids and tannins, but decreased the content of phenolic acids.

However, there was a decrease in antioxidant activity after using the three modes of cooking. The microwave

cooking mode has proven most effective as the increase of the polyphenols. The level of anticholinesterase activity decreased during refrigerated storage and after 12 days, it was 1/4 of the value found in the raw material. The raw onion showed a moderate activity which increased after most cooking treatments. The highest level of capacity was observed after microwaving. These results suggest that some common cooking treatments can be used to enhance the nutritional value of vegetables, increasing bioaccessibility of health-promoting constituents.

Conflict of interest

The authors have not declared any conflict of interest

REFERENCES

- Chang CC, Yang MH, Wen HM, Chern JC (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10:178-182.
- Cheyrier W, Souquet JM, Fulcrand H, Sarni P, Moutounet M (1998). Stabilisation Tanins-Anthocyanines: Données Générales. IPV-INRA. Extrait « les composés phénoliques. *J. Tech.* pp. 1-10
- Cisneros-Zevallos L (2009). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chem.* 101:1254-1262.
- Dewanto V, Wu X, Adom KK, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* 50:3010-3014.
- Ellman GL, Courtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharm.* 7:88-95.
- Ertas A, Boga M, Yilmaz MA, Yesil Y, Tel G, Temel H, Hasimi N, Gazioglu I, Ozturk ME, Ugurlu P (2015). Detailed study on the chemical and biological profiles of essential oil and methanol extract of *Thymus nummularius* (Anzer tea): Rosmarinic acid. *Ind. Crops Prod.* 67:336-345.
- Ewald K, Fjellner-Modig S, Johansson K, Sjöholm I, Akesson B (1999). Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chem.* 64:231-235.
- Faller ALK, Fialho E (2009). The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. *Food Res. Int.* 4:210-215.
- Ferreres F, Gil MI, Tomás-Barberán FA (1996). Anthocyanins and flavonoids from shredded red onion and changes during storage in perforated films. *Food Res. Int.* 29:389-395.
- Gahler S, Otto K, Böhm V (2003). Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. *J. Agric. Food Chem.* 51:7962-7968.

- Grossberg GT (2003). Cholinesterase inhibitors for the treatment of Alzheimer's disease: getting on and staying on. *Curr. Ther. Res.* 64:216-235.
- Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoids antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* 13:572-584.
- Ismail A, Marjan ZM, Foong CW (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chem.* 87:581-586.
- Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ (1999). Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J. Neurosci.* 19:8114-21.
- Kaneko T, Baba N (1999). Protective effect of flavonoids on endothelial cells against linoleic acid hydroperoxide-induced toxicity. *Biosci. Biotechnol. Biochem.* 63(2):323-328
- Kawai S, Tomono Y, Katase E, Ogawa K, Yano M (1999). Antiproliferative activity of flavonoids on several cancer cell lines. *Biosci. Biotechnol. Biochem.* 63(5):896-899.
- Kuskoski EM, Asuero AG, Morales MT, Fett R (2006). Frutos tropicais silvestres polpas de frutas congeladas: Atividade antioxidante, polifenólica e antocianinas. *Cienc Rural* 36:1283-287.
- Lin J, Tang C (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* 101:140-147.
- Lombard K, Peffley E, Geoffriau E, Thompson L, Herring A (2005). Quercetin in onion (*Allium cepa* L.) after heat-treatment. Simulating home preparation. *J. Food Compos. Anal.* 18:571-581.
- Makris DP, Rossiter JT (2001) Domestic processing of onion bulbs (*Allium cepa*) and Asparagus spears (*Asparagus officinalis*): effect on flavonol content and antioxidant status. *J. Agric. Food Chem.* 49:3216-3222.
- Miglio C, Chiavaro E, Visconti A, Fogliano V, Pellegrini N (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *J. Agric. Food Chem.* 56(1):139-147.
- Morris MC, Evans DA, Tangney CC, Bienias JL, Wilson RS (2006). Associations of vegetable and fruit consumption with age-related cognitive change. *Neurology* 67:1370-1376.
- Netzel M, Netzel G, Tian QG, Schwartz S, Konczak I (2006). Sources of antioxidant activity in Australian native fruits: Identification and quantification of anthocyanins. *J. Agric. Food Chem.* 54(26):9820-9826.
- Price KR, Bacon JR, Rhodes MJC (1997). Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). *J. Agric. Food Chem.* 45:938-942.
- Price KR, Vansoyoc S, Butler LG (1978). A critical evaluation of the vanillin reaction – An assay for tannins and sorghum grain. *J. Agric. Food Chem.* 26:1214-1218.
- Ramassamy C (2006). Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. *Eur. J. Pharmacol.* 545:51-64.
- Rodriguez-Arcos RC, Smith AC, Waldron KW (2002). Effect of storage on wallbound phenolics in green asparagus. *J. Agric. Food Chem.* 50(11):3197-203.
- Sanderson J, Mclauchlin W, Williamson G (1999). Quercetin inhibits hydrogen peroxide-induced oxidization of the rat lens. *Free Radic. Biol. Med.* 26(5/6):639-645.
- Shutenko Z, Henry Y, Pinard E, Seylaz J, Potier P, Berthet F, Girard P, Sercombe R (1999). Influence of the antioxidant quercetin in vivo on the level of nitric oxide determined by electron paramagnetic resonance in rat brain during global ischemia and reperfusion. *Biochem. Pharmacol.* 57(2):199-208.
- Singh BN, Singh BR, Singh L, Prakash D, Singh DP, Sarma BK, Upadhyay G, Singh B (2009). Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. *Food Chem. Toxicol.* 47:1161-1167.
- Singh DP, Beloy J, McInerney JK, Li D (2012). Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots (*Daucus carota*). *Food Chem.* 132:1161-1170.
- Slimestad R, Fossen T, Vagen IM (2007). Onions: a source of unique dietary flavonoids. *J. Agric. Food Chem.* 55:10067-10080.
- Spagna G, Barbagallo RN, Chisari M, Branca F (2005). Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content. *J. Agric. Food Chem.* 53(6):2032-2038.
- Spigno G, De Faveri DM (2007). Antioxidants from grape stalks and marc: influence of extraction procedure on yield, purity and antioxidant power of the extracts. *J. Food Eng.* 78:793-801.
- Turkmen N, Sari F, Velioglu YS (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chem.* 93:713-718.
- Vallejo F, Garcia-Viguera C, Tomas-Barberan FA (2003). Changes in broccoli (*Brassica oleracea* L. var. *italica*) health-promoting compounds with inflorescence development. *J. Agric. Food Chem.* 51:3776-3782.
- Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* 46:4113-4117
- Villano D, Fernandez-Pachon MS, Moya MI, Troncoso AM, Garcia-Parrila MC (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta* 71:230-235.
- Vinson JA, Su X, Zubik L, Bose P (2001). Phenol antioxidant quantity and quality in foods: Fruits. *J. Agric. Food Chem.* 49:5315-5321.
- Williams P, Sorribas A, Howes MJR (2011). Natural products as a source of Alzheimer's drug leads. *Nat. Prod. Rep.* 28:48-77.