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Impact of culinary heat treatment on Lodosa Piquillo peppers (*Capsicum annuum L.*): Total phenolic content and antioxidant capacity

Firmado:

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Abstract

Introduction: An emerging concept of disease prevention is ground-breaking these days. Research has been done to examine the possibility that the most common diseases affecting people worldwide may be preventable by a healthy diet. Antioxidants have been the focus of major attention in the attempt for reducing morbidity and mortality from chronic diseases because of their capacity to delay or inhibit oxidative damage (the attack of oxygen-containing free radicals on biological molecules) to a target molecule, linked to various diseases. In the present study, jarred Lodosa Piquillo peppers (*Capsicum annuum* L.) have been brought into sharp focus, as they are one of the most traditional crops in the region of Navarre. Peppers are rich in antioxidants, but these ones are subjected to a jarring process before they are consumed. For that reason, the principal aim of this study was to examine the influence of different cooking techniques on the antioxidant properties of Lodosa Piquillo peppers. **Material and methods:** Extracts from jarred Lodosa Piquillo peppers (PDO) were examined. These vegetables were subjected to different cooking methods, microwave heating (1-minute heating at 750 W) and frying (6-minute heating at 90 ºC in a pan previously heated at 110 ºC for 5 minutes) and were compared to the raw sample. Total phenolic content was quantified following Folin-Ciocalteu methodology using a Spectrophotometer, and the antioxidant capacity was evaluated by DPPH and ABTS assays. **Results:** Total phenolic content and the antioxidant capacity of jarred Piquillo peppers was not significantly affected by additional thermal treatments in microwaved and fried samples, compared to raw jarred peppers. **Conclusion:** Additional cooking techniques do not significantly affect the antioxidant capacity of Lodosa Piquillo peppers once they are exposed to a jarring process. Therefore, the antioxidant capacity of the Piquillo peppers is similar independent of how they are consumed.

Key words: *Capsicum annuum* L.; Antioxidants; Heat treatment; Phenolics; Vegetables

Resumen

Introducción: La prevención de enfermedades es un concepto pionero que ha revolucionado el estudio de la nutrición estos últimos años. Se ha estudiado mucho la posibilidad de que una dieta saludable pueda prevenir enfermedades que afectan a la sociedad hoy en día. Así, los antioxidantes están siendo el principal foco dietético de estudio con el objetivo de reducir la morbilidad y mortalidad debida a estas enfermedades crónicas. Estos elementos de la dieta son capaces de inhibir o retrasar el daño oxidativo que se produce en las células, relacionado a varias enfermedades. En este estudio se ha examinado el pimiento del Piquillo de Lodosa (*Capsicum annuum* L.), una de las cosechas más tradicionales en la región de Navarra. Los pimientos son reconocidos por su alto contenido en antioxidantes, pero en este caso, los pimientos se someten a un proceso de embotado. Por ello, el principal objetivo de este estudio fue analizar el efecto del tratamiento térmico en las propiedades antioxidantes del pimiento del Piquillo. **Material y métodos:** Se analizaron extractos del pimiento del Piquillo de Lodosa (DOP). Estos pimientos fueron sometidos a dos diferentes métodos culinarios, calentamiento al microondas (1 minuto a 750 W) y fritura (6 minutos de cocción a 90 ºC en una sartén previamente calentada a 110 ºC por 5 minutos), y fueron comparados con la muestra en crudo. Se cuantificó el contenido fenólico total de las muestras mediante la técnica Folin-Ciocalteu usando un espectrofotómetro, así como la capacidad antioxidante de los vegetales siguiendo la metodología de DPPH y ABTS. **Resultados:** Tanto el contenido fenólico total como la capacidad antioxidante de los pimientos del Piquillo no sufrieron cambios significativos tras la cocción en microondas o mediante fritura, en comparación con los pimientos en conserva. **Conclusión**: Las propiedades antioxidantes de los pimientos del Piquillo de Lodosa son similares independientemente del modo de consumo.

Keywords: *Capsicum annuum* L.; Antioxidants; Heat treatment; Phenolics; Vegetables

1. Introduction

Humankind has always shown interest in human body, health and the cure of disease. Clear examples are the ancient Greeks, such as Hippocrates, Socrates, Plato and Aristotles, who in the 5th and 4th centuries BC already discussed the definition of health. Nevertheless, the viewpoint on human health developed in the following centuries. Nowadays an emerging concept of disease prevention is ground-breaking.

Therefore, some scientists have studied the possibility that the most common diseases affecting people worldwide may be preventable by a healthy diet (1,2). These conditions include non-communicable diseases (NCD), such as obesity, diabetes mellitus, cancer and cardiovascular diseases (CVD), and are the principal cause of chronic illness and an increased incidence of years lived with disability (YLD).

Up to now, a healthy diet does not follow a unique eating pattern, but it is widely assumed that a diet for being healthy needs to include fruits and vegetables. The Mediterranean Diet is one of those diets rich in fruits and vegetables (5 portions per day), well known to be beneficial for health due to its high content of essential nutrients and antioxidants (3).

Given their high presence in the Mediterranean Diet, antioxidants have been the focus of major attention in the attempt for reducing morbidity and mortality from chronic diseases (1). The most notable antioxidants are β-carotene, vitamin E and vitamin C, but there is a wide range of other compounds, such as polyphenols, to which beneficial health effects are attributed (4, 5). They all have in common the capacity to delay or inhibit oxidative damage (the attack of oxygen-containing free radicals on biological molecules) to a target molecule, which is linked to various diseases. There are many mechanisms by which this protection is possible; the scavenging of oxygen derived species, reduction in the formation of oxygen derived species, the binding to metal ions, repairment of damage target and destroying damaged molecules (4).

Naturally occurring oxidation reactions can produce reactive organic free radicals, which are highly reactive with molecular oxygen, and their reaction leads to the formation of reactive oxygen species (ROS). Ideally, the human organism copes with the creation of these compounds and no harm is caused in cellular tissues. Nevertheless, if defence mechanisms do not work properly, an excess of ROS can take place, causing cellular impairment in all major organs. Accordingly, cellular components such as polyunsaturated fatty acids, phospholipids, free cholesterol, DNA and proteins get

affected (1, 6). The accumulation of this damage over the time is the main cause of cancer development in the elderly, among some chronic diseases.

In this line, extensive studies have taken place in order to determine if antioxidants can be used in preventive, as well as in therapeutic medicine, in diseases like cancer, CVD and aging process (6). Antioxidant phytochemicals could be considered the potential agents to face chronic diseases. They possess a variety of biological activities and health benefits, such as antioxidant and free radical scavenging abilities, anti-inflammatory action, anticancer, anti-aging, and protective action for cardiovascular disease, diabetes mellitus, obesity and neurodegenerative diseases. According to Zhang et al. (2015) (2), antioxidant phytochemicals such as quercetin, resveratrol, cyanidin and kaempferol are significantly associated with reduced cancer risks. In accordance, diets rich in fruits and vegetables are associated with protection from breast, colon, prostate, lung, tongue, gastric, skin and other organ sites cancer. Additionally, dietary antioxidants such as flavonoids, have shown to protect the cardiovascular system not only from oxidative stress, but also from high blood pressure and inflammation. For instance, quercetin is related with the prevention of atherosclerosis, and allicin has been found to protect the cardiovascular system alleviating cardiac hypertrophy, angiogenesis, platelet aggregation, hyperlipidaemia, and hyperglycaemia. Fruit and plant extracts high in antioxidant phytochemicals have shown anti-diabetes and anti-obesity activities in vitro and in vivo. Finally, Zhang et al. (2015) (2) reports epidemiological evidence for a protective role of nutritional antioxidants in prevention of age-related cognitive dysfunction and inflammation. Therefore, it is potentially reasonable to find a daily recommendation of fruits and vegetables intake in most of the dietary guidelines. However, it is important to consider the fact that these foods are not always eaten raw. Hence, some researchers (7, 8, 9, 10) started to investigate the effect that heat treatments could have in the composition of these foods.

In the present study, jarred Lodosa Piquillo peppers (*Capsicum annuum* L.) have been brought into sharp focus, as they are one of the most traditional crops in the region of Navarre. Family farms from this area have provided this raw material to the industry since ancient times for the elaboration of Protected Designation of Origin Lodosa Piquillo peppers, grown in selected fields in the community of Navarre (11,12).

Fresh peppers are perceived to be good sources of vitamin C, as well as other elements such as polyphenols, especially the flavonoids, quercetin and luteolin. Furthermore, carotenoids are responsible for the characteristic red colour of these vegetables. The

most common ones are provitamin A and xanthophylls (7). All these compounds, because of their antioxidant capacity, have proven to be protective for health. Nonetheless, not many studies have been done on the effect of thermal treatments on the antioxidant capacity of jarred vegetables. For that reason, the principal aim of this study was to examine the influence of different cooking techniques on the antioxidant properties of jarred Lodosa Piquillo peppers. Additionally, the performance of this study intended to increase interest in the effects of thermal treatments on the antioxidant properties of cooked vegetables as a tool to improve dietary intake, and therefore, the health of the population, preventing nutrition related diseases by subsequent onset of new health strategies and dietary recommendations.

2. Material and methods

2.1. Chemical and reagents

Jarred Lodosa Piquillo peppers (PDO) were obtained from a local store. Ethanol, Methanol, Formic Acid, Folin – Ciocalteau reagent and Gallic Acid were purchased from Panreac (Barcelona, Spain). Trolox reagent (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2-azinobis diammonium salt) (3-ethylbenzoathiazonile-6-sulfoic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were acquired from Sigma Aldrich (Steinheim, Germany). Additionally, Na_2CO_3 and Potassium persulfate were used.

2.2. Sample preparation

All jarred peppers, including the liquid, were combined in a flask in order to reduce variability among different jars. Then, pepper samples were divided into three lots: one without applying any additional cooking method (*raw* sample), the second lot of peppers was fried in olive oil (*fried* sample), and the third lot was heated into the microwave (*microwave-heated* sample).

Raw sample

A total of 400 g of jarred Piquillo peppers and their liquid were put in a flask as reference sample in order to compare the "non-cooked" samples (omitting that all peppers went through roasting and jarring processes) with cooked samples (fried and microwaved).

Fried sample

An amount of 200 g of jarred Piquillo peppers was fried in 15 ml of olive oil in a pan, which was preheated for 5 minutes until it reached 110 ºC. The peppers were fried for 6 minutes at approximately 90 ºC. This frying process was duplicated, and final samples were mixed.

Microwaved sample

An amount of 200 g of jarred Piquillo peppers were placed in a plate covered with a microwavable cover and heated up at 750 W for 1 minute. This heating process was duplicated, and final products were combined.

Once the samples were taken through appropriate cooking methods, they were lyophilised. Firstly, all samples were stored at -20 ºC for 15 hours, and then at -80 ºC for at least 24 hours before they were lyophilised with a Telstar Cryodos freeze – drier. Finally, samples were stored at -18 ºC.

2.3. Vegetable extracts

Extracts of the three samples (raw, fried and microwaved) were obtained according to Siddiq et al. (2013) (13). First of all, a solution of methanol (80/20) was prepared with distilled water. Then, 0.5 ml of methanol (80/20) were added to 25 mg of each sample in an Eppendorf of 2 ml. In order to homogenise the content, Eppendorf bottles were mixed on a vortex and later ultrasonicated in a cold-water bath for 90 minutes. Subsequently, samples were centrifuged at 14000 rpm for 10 minutes. Supernatant from each Eppendorf was collected, and the residues were subjected to another extraction. For the second extraction 0.5 ml of methanol (80/20) were used. Samples were mixed on a vortex and then ultrasonicated for 25 minutes. Centrifugation was performed at 14000 rpm for 10 minutes. Finally, first and second supernatants were combined in an Eppendorf and diluted with methanol (80/20) to 2 ml. Extracts were duplicated for each of the samples.

2.4. Sample adaptation to analytical methods

Adapting samples to antioxidant capacity by DPPH and ABTS assays

Both methods consist of analysing the absorbance (capacity of a substance to absorb light of a specified wavelength) of the samples in order to examine their antioxidant capacity. Piquillo peppers are known to be rich in carotenoids, which are mainly responsible for the strong red colour of these vegetables. Therefore, vegetable extracts obtained for analysis showed an orange-red colour. This intense colouration of the samples was thought to be presumably interfering or disrupting the measurement of the antioxidant capacity of Piquillo peppers. As a result, two techniques were performed in order to prevent the mentioned dilemma: filtration and dilution.

a) Filtration

The aim of filtering the samples was to reduce their colour and avoid it from interfering with the absorbance measurement. This process was carried out by using small syringes, filtering the samples through a 0.45 μm pore filter.

b) Dilution

The aim of dissolving the samples was to reduce their colour and avoid it from interfering with the absorbance measurement, while maintaining the integrity of all the components that constitute the sample.

DPPH and ABTS assays, both techniques required different sample dilutions. Nevertheless, the steps followed for their execution were the same: First, the calibration curve was done with mother solutions. Secondly, different dilutions were made with a single sample extract, and were measured. Finally, the appropriate dilution was applied to all the samples in order fit the calibration curve.

2.5. Analytical methods

Total phenolic content

Total phenolic content (TPC) was measured by using the Folin-Ciocalteau reagent according to the Singleton and Rossi method (1965) (14), with some adjustments. This technique evaluates the antioxidant capacity of a sample due to the reduction of the Folin-Ciocalteau Reagent. This reagent is formed by phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$) combination, which is reduced by the phenolic action to tungsten blue oxides (W_8O_{23}) and molybdenum (Mo_8O_{23}) mixture.

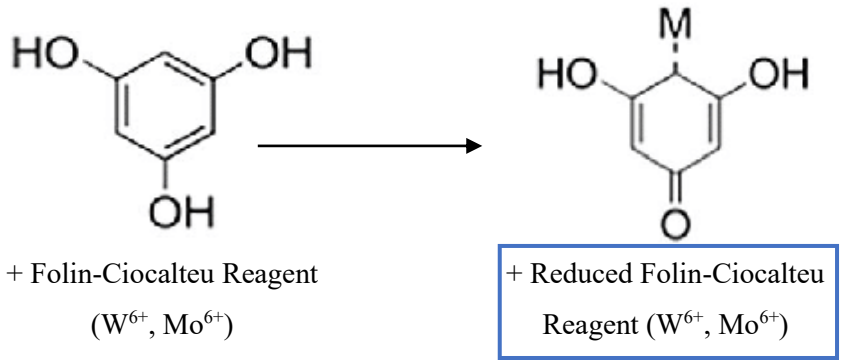

Figure 1: Chemical reaction involved in Folin-Ciocalteu reagent reaction with a phenol (15).

No dilutions were needed for the examination of the Piquillo pepper extracts.

A volume of 75 µl of Folin-Ciocalteu reagent was incorporated to a 15 µl of each pepper extract and 1185 µl of demineralized water mixture in an Eppendorf. 2 minutes after, 225 µl of a 25% of sodium carbonate solution was added and properly combined. Then, the mixture was stored in the darkness at room temperature for 2 hours. The absorbance of the sample was measured at 765 nm (Lambda 25 UV/VIS Spectrophotometer, Perkin Elmer Instruments, Madrid, Spain).

The quantification of total phenolic content of all the three samples was performed with a calibration curve (y = ax – b) of gallic acid. Different dilutions of gallic acid (5 – 2000 µg/mL) were prepared. Gallic acid was used as a reference, and the results were expressed as grams of gallic acid per 100 grams of Piquillo pepper sample (g GA / 100g of vegetable).

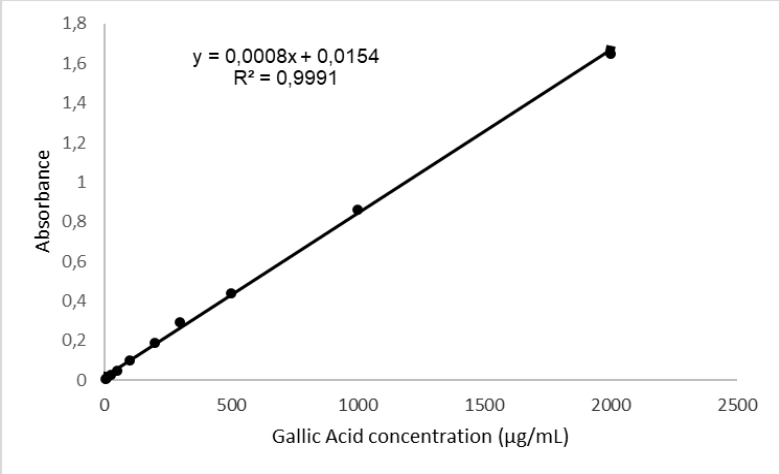

Figure 2: Calibration curve used for TPC.

Concentration (µg GA /mL)	Abs_{765nm}
5	0.004
25	0.024
50	0.048
100	0.097
200	0.190
300	0.290
500	0.438
1000	0.858
2000	1.6475

Antioxidant capacity by DPPH measurement

The antioxidant capacity of the three samples was measured using a free radical DPPH in a methanolic solution, following Brand – William et al. (1995) (16) methodology with some adjustments. The solution was prepared by diluting 2 mg of DPPH in 50 ml of methanol (80/20), whose final concentration was of 0.04 mg/ml. Afterwards, the solution was adjusted with methanol (80/20) until 200 µl reached an absorbance of 0.76±0.02 at 516 nm (Lambda 25 UV/VIS Spectrophotometer, Perkin Elmer Instruments, Madrid, Spain).

This violet-coloured radical solution is reduced by antioxidants, turning it into a yellowish – translucent solution, and consequently, decreasing its absorbance.

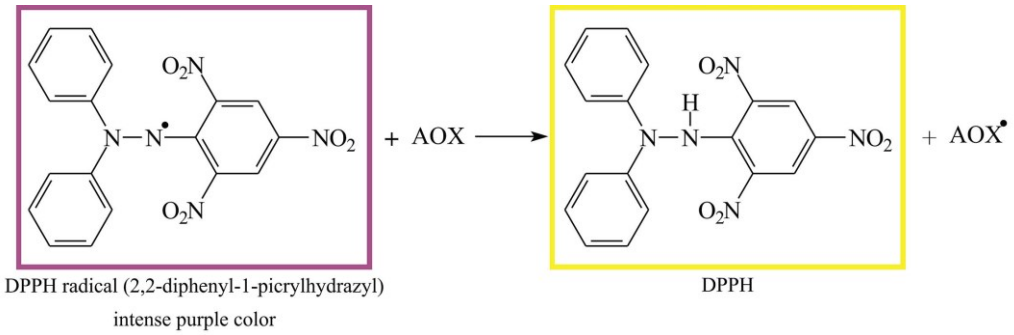

Figure 3: Chemical reaction involved in the DPPH spectrophotometric assay (17).

For the analysis, 40 µl from each extract was diluted with distilled water in a 2 ml volumetric flask prior to the examination to adjust the absorbance values to the calibration line. As a result, a solution diluted at 1:50 was obtained.

A volume of 350 µL of each extract was mixed with 350 µl of DPPH radical solution. After 30 min, the absorbance was measured at 516 nm. A blank was prepared by using methanol (80/20) instead DPPH. The final absorbance (Abs_{final}) was calculated by the difference between the absorbance of the sample with DPPH solution (Abs_{DPPH}) and the absorbance of the blank (Abs_0).

The evaluation of the antioxidant capacity of all samples was performed with a calibration curve (y= ax – b) using Trolox (a water – soluble vitamin E analog) as standard. Six dilutions were prepared from a 10 µg/mL Trolox solution. The antioxidant capacity was expressed as milligrams of Trolox equivalent per 100 grams of Piquillo Pepper (mg Trolox / 100 g of vegetable).

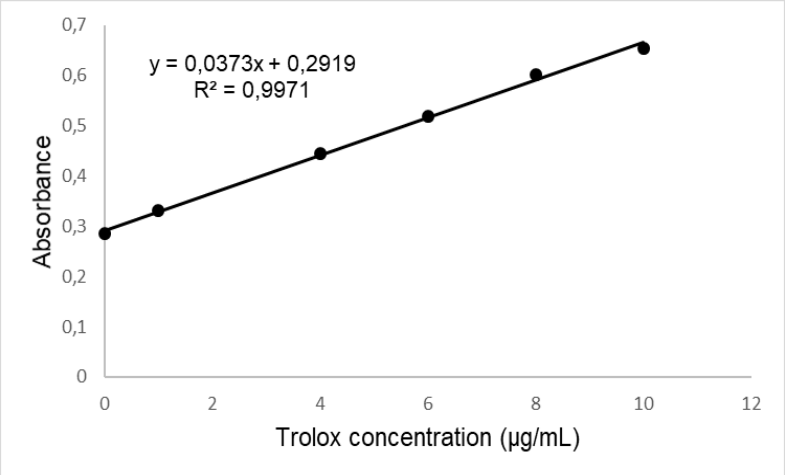

Figure 4: Calibration curve for the DPPH method.

Concentration (µg Trolox/mL)	Abs_{DPPH}-Abs_0
0	0.285
1	0.330
4	0.443
6	0.519
8	0.601
10	0.653

Antioxidant capacity by ABTS assay

The ABTS antioxidant capacity was performed according to the method of Re et al. (1999) (18) with some modifications. This technique is based on the reduction of ABTS radical by other antioxidant – containing compounds. In this study, Trolox (a vitamin E derivative) was used as a reference. First of all, a solution of potassium persulfate ($K_2S_2O_8$) 140 mM in 100 ml of water was prepared. Then, a tablet (35.6 mg) of ABTS was dissolved in the previous mentioned solution, resulting in a 2.45 mM concentrated mixture. This solution was stored in the dark for 16 hours in order to allow the formation of the ABTS+ radical.

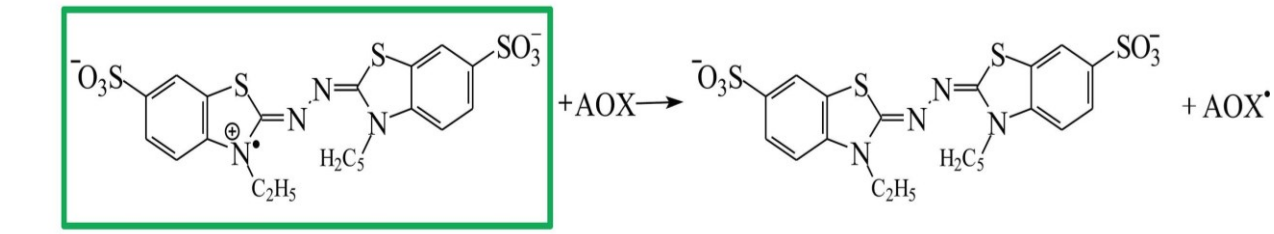

Figure 5: Chemicals reaction involved in the ABTS spectrophotometric assay (17).

The ABTS+ solution was adjusted with a Methanolic (80/20) solution to an absorbance of 0.7±0.2 at 741 nm (Lambda 25 UV/VIS Spectrophotometer, Perkin Elmer Instruments, Madrid, Spain).

In this study, 100 µl of each extract was diluted with distilled water in a 1 ml volumetric flask prior to the examination, in order to adjust the absorbance values to the calibration line. As a result, a solution diluted at 1:10 was obtained. Samples (90 µl) were added to 910 µl of ABTS+ solution. After 6 minutes, the absorbance was measured using the spectrophotometer at 741 nm. The final absorbance (Abs_{final}) values were calculated by the difference between the absorbance of the ABTS+ solution (Abs_{ABTS}) and the absorbance of the blank sample (Abs_0).

The antioxidant capacity of all samples was evaluated by a calibration line (y= ax – b), using a Vitamin E analog (Trolox) as standard. From this, 9 dilutions were accomplished with concentrations of 0 to 250 µg Trolox/mL. The antioxidant capacity

was expressed as milligrams of Trolox equivalent per 100 grams of Piquillo Pepper (mg Trolox / 100 g of vegetable).

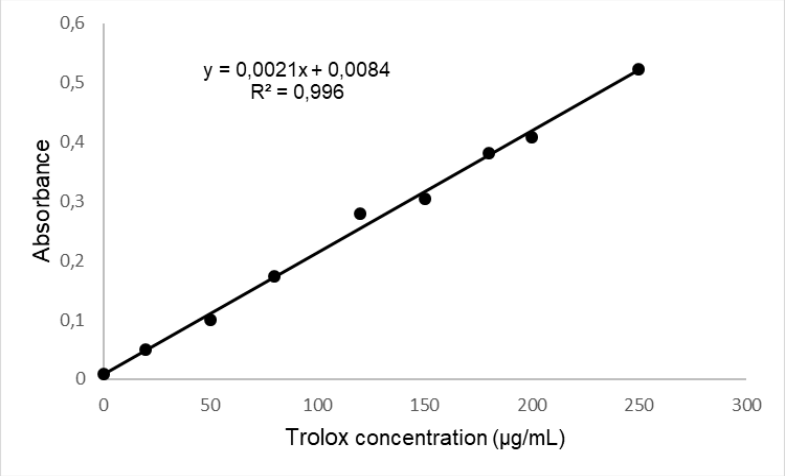

Figure 6: Calibration curve for the ABTS method.

Concentration (µl Trolox /mL)	Abs_{ABTS}-Abs_0
0	0.008
20	0.049
50	0.101
80	0.174
120	0.279
150	0.305
180	0.381
200	0.408
250	0.523

2.6. Statistical analysis

Each sample was analysed in quadruplicate for ABTS and total phenolic content, and in triplicate for DPPH assay. Results are presented as the mean ± standard deviation. One – way analysis of variance (ANOVA) was performed for all samples in order to study whether there was a statistically significant difference among samples. A significance level of $p<0.05$ was considered for all analyses. All statistical analyses were performed using the STATA v.15.0 software package.

3. Results

In the study at matter, jarred red Piquillo peppers were examined. These vegetables were subjected to different cooking methods, leading to microwave – heated and fried peppers. Some raw (non – cooked) jarred samples were also kept.

The samples were adapted for the analytical methods by filtration. The results were as expected; the colouration of the samples was paler and less intense. However, after analysing about the results of this technique, it was concluded that some of the filtered particles responsible for the colouration of the samples could also contribute to the antioxidant capacity thereof.

In this way, even though all samples could be filtered, and consequently, the loss of the mentioned antioxidant components would not impair the comparison between different cooking methods, samples went through a second approach to reduce the intensity of the colour and keep all the constituents of the samples was accomplished, dilution.

Afterwards, antioxidant capacity and total phenolic content were examined, comparing cooked peppers to the raw samples. The examination was accomplished with spectrophotometric techniques. The results obtained are the ones shown in **Table 1.**

Table 1. Total phenolic content and antioxidant capacity (DPPH and ABTS)

	RAW	MICROWAVED	FRIED
Total phenolic content (g GA/100g vegetable)	0.318 ± 0.017^{a}	0.337 ± 0.007^{a}	0.293 ± 0.001^{a}
DPPH (mg Trolox/100g vegetable)	0.256 ± 0.006^{a}	0.254 ± 0.011^{a}	0.236 ± 0.008^{a}
ABTS (mg Trolox/100g vegetable)	0.753 ± 0.003^{a}	0.759 ± 0.044^{a}	0.765 ± 0.003^{a}

In each raw, the same letter indicates not statistically significant differences among samples.

3.1. Total phenolic content

Figure 7 displays the result of the total phenolic content of Piquillo pepper samples in their raw, microwaved and fried conditions by Folin-Ciocalteu methodology. Results are shown in grams of Gallic Acid equivalent per 100 grams of Piquillo sample (g GA / 100 g of vegetable).

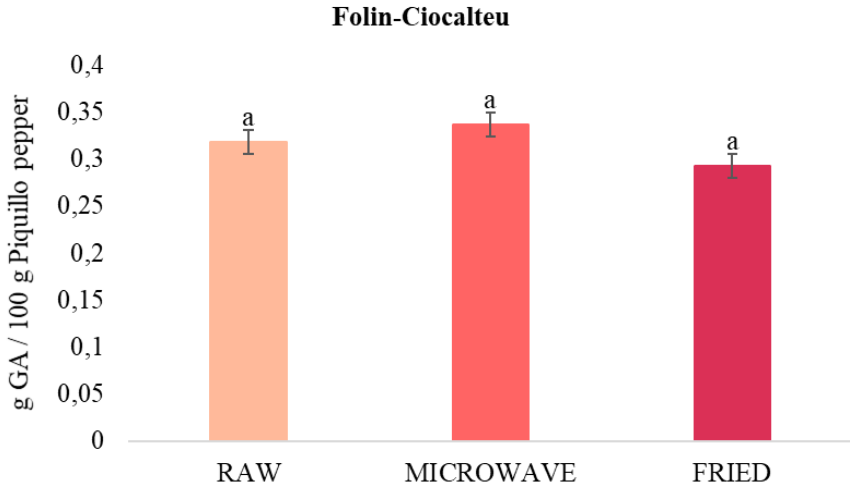

Figure 7: Total phenolic content of raw, microvawed and fried Piquillo peppers by Folin-Ciocalteu assay. The values for each cooking technique are the mean of quadruplicates. The same letter above all columns indicates a not statistically significant diference ($p>0.05$) among the three samples.

The results showed that the difference between the three samples was not statistically significant ($p>0.05$). Nonetheless, the sample that led to the highest total phenolic content was the microwaved sample (0.335 g GA/ 100 g vegetable). Raw (0.318 g GA/ 100g vegetable) and fried samples (0.293 g GA/ 100g vegetable) resulted in lower values in terms of total phenolic content.

3.2. Antioxidant capacity

a) DPPH

Figure 8 illustrates the result of the antioxidant capacity of Piquillo pepper samples in their raw, microwaved, and fried conditions using the DPPH methodology. Results are shown in milligrams of Trolox equivalent per 100 grams of Piquillo sample (mg Trolox / 100 g of vegetable).

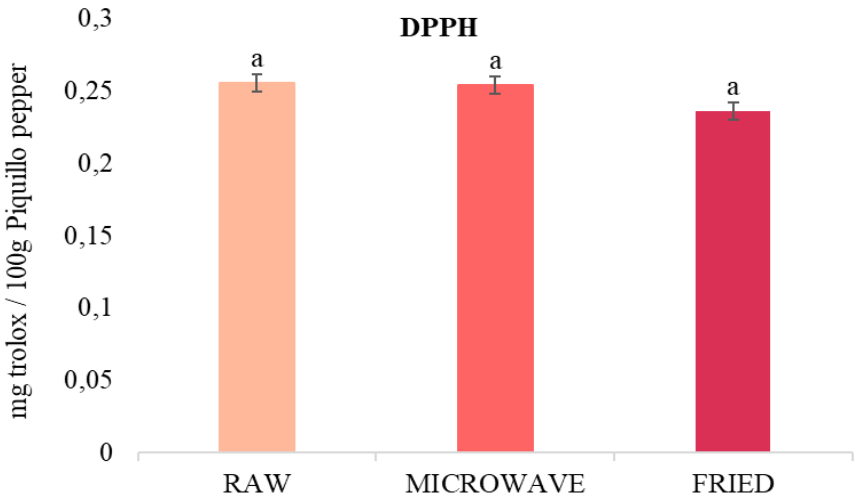

Figure 8: Antioxidant capacity of raw, microvawed and fried Piquillo peppers by DPPH assay. The values of each cooking technique are the means of triplicates. The same letter above all columns indicates a not statistically significant diference ($p>0.05$) among the three samples.

The antioxidant capacity measured by DPPH methodology resulted in no significant differences among the three samples ($p>0.05$). However, a slightly higher value for the raw sample (0.255 mg Trolox/100g of vegetable) was observed compared to the microwaved one (0.254 mg Trolox/100g vegetable). Fried samples led to the lowest values for antioxidant capacity (0.236 mg Trolox/100 g vegetable).

b) ABTS

Figure 9 represents the result of the antioxidant capacity of Piquillo pepper samples in their raw, microwaved and fried conditions using the ABTS technique. Results are shown in milligrams of Trolox equivalent per 100 grams of Piquillo sample (mg Trolox / 100 g of vegetable).

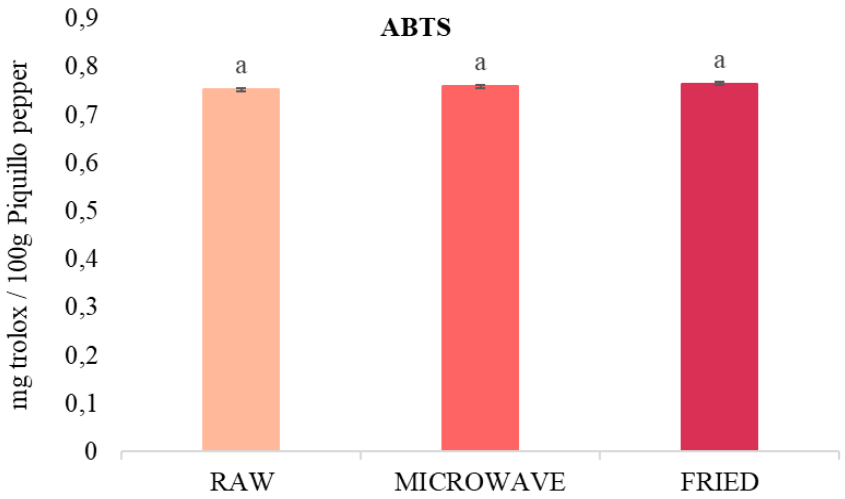

Figure 9: Antioxidant capacity of raw, microvawed and fried Piquillo peppers by ABTS assay. The values for each cooking technique are the mean of quadruplicates. The same letter above all columns indicates a not statistically significant diference ($p>0.05$) among the three samples.

The antioxidant capacity measured by ABTS methodology resulted in a not statistically significant ($p>0.05$) difference among the three samples. Additionally, very similar values for both, the raw (0.753 mg Trolox / 100 g of vegetable) and the microwaved samples (0.758 mg Trolox / 100g vegetable) were observed, and fried samples led to the highest values in terms of antioxidant capacity (0.765 mg Trolox / 100 g vegetable).

4. Discussion

Fruits and vegetables can be consumed in their natural form, but most of them are ingested after some thermal treatments (cooking procedures or preservation techniques). This processing induces biological, physical and chemical modifications in food, which can lead to sensorial, nutritional and textural changes (19).

A common perception is that foods that undergo thermal processes have lower nutritional value than fresh foods (19). Nevertheless, recent reports stated that cooking can be used to enhance the nutritional value of vegetables, increasing the bioaccessibility of health-promoting constituents (8,20).

In the present study, the vegetables examined have had already gone through some heating treatments. The elaboration of Protected Designation of Origin Lodosa Piquillo peppers starts with the roasting of the vegetables by direct flame. Afterwards, they are peeled, and seeds and the core are removed by hand. Once they undergo the jarring process, they are sterilized by an immersion in hot water or water steam, in order to destroy all the microorganisms that could have been present in the vegetables (12). Therefore, it is important to note that the present research did not take into consideration the effect that the previously mentioned processing could have had in the properties of the peppers. Moreover, the results obtained display the effect that additional frying in olive oil and microwave heating have on the jarred 'raw' Piquillo peppers. What is more, the outcome of the study is to evaluate what the consumer ingests, which is, in fact, a product that has undergone a heating process.

Total phenolic content (TPC)

Peppers are an excellent source of vitamin C, carotenoids, polyphenols, and other phytochemicals, which are powerful antioxidants that scavenge free radicals. Their concentration in vegetables depends on cultivar, maturity, growing conditions, and climate, as well as postharvest conditions (19), and can vary depending on the cooking processes (8).

As claimed by Minatel et al. (2017) (19) and Juániz et al. (2016) (10), heating affects the content of some polyphenols, such as flavonoids, by the disruption of the cell walls. This rupture can liberate soluble phenolic compounds from insoluble ester bonds (8), increasing their accessibility (10,19, 21). In addition, Lutz et al. (2011) (21) claimed

that heating can also deactivate oxidative enzymes (PPO), preventing enzymatic oxidation to cause loss of antioxidants in raw materials. This enzyme catalyses the oxidation of phenolics to quinones, and its deactivation can cause an increase in the extractability of phenolic compounds.

Nonetheless, according to Lutz et al. (2011) (21), TPC might decrease up to 50% due to antioxidant breakdown and leaking into water, as it might vary depending on the treatment applied to the vegetables. This occurrence is in line with the results of Ferracane et al. (2008) (20), which reported a decrease of TPC and water-soluble metabolites (polyphenols and phenolic acids) after cooking, in particular, a reduction of flavonoid concentration in frying samples.

However, in the present study, differences between samples were not statistically significant, even if peppers fried with olive oil presented a lower content of phenolic compounds (0.293 g of gallic acid/100 g of fresh vegetable) compared to raw (0.318 g of gallic acid/100 g of fresh vegetable) and microwave heated samples (0.337 g of gallic acid/100 g of fresh vegetable), representing a not significant decrease in phenolic content by 7.84%. Hwang et al. (2012) (8) found a decrease in TPC after boiling (35.8～54.9%), steaming (13.9～19.9%), roasting (4.1～4.9%) and stir-frying (1.84～4.8%). Nevertheless, the decrease caused by stir-frying and roasting was not significant, in other words, they basically had no impact on TPC (7, 8). The comparison of these values suggests that thermal treatments could not significantly affect the phenolic content of the peppers, regardless of being previously jarred of fresh. This result was also obtained by Chuah et al. (2008) (7), showing a not significant decline between raw, microwaved heating and stir-frying. Similar results were also found in other vegetables; Ewald et al. (1999) (22) concluded that boiling, microwaving, frying or further warm holding did not affect the levels of polyphenols, quercetin and kaempferol in onions, green beans and peas. Additionally, cooking had no deleterious effect on total antioxidant activity and total phenolic content in squash, peas and leek (20, 23). Others demonstrated that TPC may remain unchanged (6, 24, 25, 26).

In consequence, the explanation behind these results might be the cooking conditions (time and temperature). Omitting the fact that Piquillo peppers went through a sterilizing process before additional cooking methods, frying took longer than microwave heating, and, reached higher temperatures. This could have been the reason to find a lower concentration of TPC in fried peppers compared to the rest of the

samples. Nevertheless, the values obtained in this study were not statistically significant. As a result, can be said that neither microwave heating nor frying had an impact on the total phenolic content of the peppers in comparison to raw samples.

Antioxidant activity. DPPH and ABTS

In the present study, the antioxidant activity of the samples was examined using 2 assays, DPPH and ABTS. Both methods showed that there were no significant differences between raw and cooked samples. In the case of fried samples, values were contradictory in both assays; DPPH showed that frying in olive oil decreases antioxidant activity of the samples. On the other hand, ABTS assay showed a slight increase in antioxidant activity in those samples fried in olive oil. However, the results showed that cooking did not significantly affect the antioxidant activity of the Piquillo peppers. This outcome was presumably caused by the previous sterilization that the peppers suffered, with the subsequently scarce effect of thermal methods on the antioxidant capacity of the vegetables. In this manner, different mechanisms have been described to explain distinct results in the antioxidant capacity after cooking. Most of them justify this increase with molecular changes caused by heat. Ferracane et al. (2008) (20), reported that high temperatures could condition an increased antioxidant activity of neo-formed compounds as an outcome of the intramolecular transesterification of caffeoylquinic acids; the esterification of caffeic acid and isomerization of dicaffeoylquinic acid (21). These spatial rearrangements of phenolics into polyphenols could affect the antioxidant activity of the molecules (20, 21).

In accordance, recent reports (23, 27) showed that cooking increases antioxidant activities by liberating antioxidant compounds from insoluble portions of food (8). Same results were observed in tomatoes and other vegetables, where antioxidant compounds were liberated from insoluble portions of food, or that novel compounds from the Maillard reaction, which possess antioxidant activity (8). The increase in antioxidant capacity after cooking could also be explained by an increase in carotenoid concentration caused by thermal treatment. Ferracane et al. (2008) (20) and Miglio et al. (2008) (9) reported that small amounts of carotene precursors become detectable during cooking methods. Thermal disruption of the non-covalent association between carotenoids and proteins present in the cell chloroplasts or the dehydration of the food matrix could be enhancing the extractability of free carotenoids (7, 20, 28, 29).

However, this phenomenon can vary from one study to other, as thermal lability of carotenoids may be influenced by cooking conditions, food type and the nature of the food matrix (8).

Consequently, there are some authors that reveal a decrease in antioxidant activity after thermal treatment. For instance, Chuah et al. (2008) (7) observed a reduction in the ascorbic acid (AsA) of all peppers after cooking (by ABTS). Thermal treatment is known to accelerate the oxidation of ascorbic acid, leading to dehydroascorbic acid formation. This process might be followed by its hydrolysis to 2,3-diketogulonic acid, and eventually, the polymerization to other nutritionally inactive components (7, 29). In the same line, Juániz et al. (2016) (10) reported that both, DPPH and ABTS showed a decrease in antioxidant activity in red and green peppers fried in olive oil. Additionally, the losses on scavenging capacity in peppers were lower after frying or griddling because of the shorter time of exposition to thermal treatment (30). Same results were obtained by Hwang et al. (2012) (8), where a higher reduction in antioxidant capacity was reported for boiled and steamed red peppers, in comparison to the ones fried or roasted. Therefore, cooking time might also be a determinant factor in the variation and alteration of scavenging capacity.

However, not all studies observing the same effect as in this one were able to reach a conclusion. Hwang et al. (2012) (8) reported that the effects of cooking on antioxidant compounds such as polyphenols, carotenoids and vitamin C in fruits and vegetables were inconclusive. Most of the studies observed that antioxidant capacity remained unchanged after thermal treatment. Lutz et al. (2011) (21) showed that antioxidant capacity in vegetables such as squash, peas and leek after cooking remained the same (23). On the other hand, Chuah et al. (2008) (7) revealed that total carotenoid content, as well as ascorbic acid were reduced, but data was not significant. This result matches with the ones obtained in the present study.

Considering that there are many different factors that can determine the antioxidant capacity of vegetables, including the cultivar, maturity, climate, postharvest conditions, processing methods, cooking conditions (duration and temperature) and nature of the food matrix, it is not surprising the variation of results that can be found among different studies.

Conclusion

In summary, the present inquiry shows that cooking techniques do not significantly affect the antioxidant capacity of Lodosa Piquillo peppers once they are exposed to a jarring process. Consequently, can be stated that independently of the additional heat treatment applied to jarred Piquillo peppers, they provide polyphenols and antioxidant capacity. Hence, because jarring is considered an excellent tool to preserve food for a long time, allowing food availability for several months, the culinary technique applied to heat jarred Piquillo peppers can be mainly chosen by hedonic, nor by antioxidant related factors.

The execution of the present study brought to light the need for more investigation to analyse jarred fruits and vegetables, and the impact that this processing has on their antioxidant activity.

Understanding the influence that different cooking techniques have on antioxidant properties of vegetables could help develop new public health strategies and reformulate dietary guidelines. Hence, focusing health approaches on disease prevention to improve the health and quality of life of the general population.

Reference list

1. Garewal H. Antioxidants and disease prevention. Boca Raton, Florida: CRC Press, 1997.
2. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015; 20, 21138–21156.
3. Fundación Dieta Mediterránea. ¿Qué es la Dieta Mediterránea? Available at: https://dietamediterranea.com/nutricion-saludable-ejercicio-fisico/ [Accessed 1 March 2020].
4. Halliwell B. Antioxidants in human health and disease. Annu. Rev. Nutr. 1996; 16, 33–50.
5. Quiñones M, Miguel M, Aleixandre A. Los polifenoles, compuestos de origen natural con efectos saludables sobre el sistema cardiovascular. Nutr Hosp. 2012; 27(1), 76-89.
6. Madhavi D, Deshpande S, Salunkhe D. Food Antioxidants. Technological, toxicological, and health perspectives. New York: Marcel Dekker, 1996.
7. Chuah A, Lee Y, Yamaguchi T, Tamakura H, Yin L, Matoba T. Effect of cooking on the antioxidant properties of coloured peppers. Food Chem. 2008; 111(1):20-28.
8. Hwang IG, Shin YJ, Lee S, Lee J, Yoo SM. Effects of different cooking methods on the antioxidant properties of red pepper (Capsicum annuum L.). Prev Nutr Food Sci. 2012; 17(4):286–292.
9. Miglio C, Chiavaro E, Visconti A, Fogliano V, Pellegrini N. Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. J Agric Food Chem. 2008; 56(1):139-147.
10. Juániz I, Ludwig I, Huarte E, Pereira-Caro G, Moreno-Rojas J, Cid C, De Peña M. Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables. Food Chem. 2016; 197(Pt A):466-473.
11. Parlamento Europeo y del Consejo. Reglamento (UE) nº 1151/2012 sobre los regímenes de calidad de los productos agrícolas y alimenticios. Diario Oficial de la Unión Europea, DO L 343: 15-16.
12. Ministerio de Agricultura, Pesca y Alimentación. Orden de 8 de mayo de 1987, por el que se ratifica el Reglamento de la Denominación de Origen "Pimiento

del piquillo de Lodosa" y de su Consejo Regulador. Boletín Oficial del Estado nº 123: 15139-15143.

13. Siddiq M, Roidoung S, Sogi DS, Dolan KD. Total phenolics, antioxidant properties and quality of fresh-cut onions (Allium cepa L.) treated with mild-heat. Food Chem. 2013; 136(2), 803–806.
14. Singleton V, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965; 16(3):144-158.
15. Ford L, Theodoridou K, Sheldrake GN, Walsh PJ. A critical review of analytical methods used for the chemical characterisation and quantification of phlorotannin compounds in brown seaweeds. Phytochem Anal. 2019; 30(6):587-599.
16. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995; 28(1), 25–30.
17. Becker M, Nunes G, Ribeiro D, Silva F, Catanante G, Marty J. Determination of the antioxidant capacity of red fruits by miniaturized spectrophotometry assays. J Braz Chem Soc. 2019; 30:2.
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999; 26(9–10), 1231–1237.
19. Minatel OI, Borges CV, Ferreira MI, Gomez HAG, Chen O, Pace G et al. Phenolic compounds: functional properties, impact of processing and bioavailability. In: Phenolic compounds – biological activity. Mexico: Intech, 2017; 1: 1-24.
20. Ferracane R, Pellegrini N, Visconti A, Graziani G, Chiavaro E, Miglio C, Fogliano V. Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. J Agric Food Chem. 2008; 56: 8601–8608.
21. Lutz M, Henríquez C, Escobar M. Chemical composition and antioxidant properties of mature and baby artichokes (Cynara scolymus L.), raw and cooked. J Food Compos Anal. 2011; 24: 49–54.
22. Ewald C, Fjelkner-Modig S, Johnsson K, Sjoholm I, Akesson B. Effect of processing on major flavonoids in processed onions, green beans and peas. Food Chem. 1999; 64: 231–235.

23. Turkmen N, Sari F, Velioglu YS. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem. 2005; 93: 713-718.
24. Oboh G. Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. LWT–Food Sci Technol. 2005; 38: 513–517.
25. Sultana B, Anwar F, Iqbal S. Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan. Int J Food Sci Technol. 2008; 43: 560–567.
26. Pellegrini N, Miglio C, Del Rio D, Salvatore S, Serafini M, Brighenti F. Effect of domestic cooking methods on the total antioxidant capacity of vegetables. Int J Food Sci Nutr. 2009; 60: 12–22.
27. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem. 2002; 50: 3010-3014.
28. Hart DJ, Scott J. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed on the UK. Food Chem. 1995; 54: 101-111.
29. Mazzea T, N'Dri D, Chiavaro E, Visconti A, Fogliano V, Pellegrini N. Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and colour of selected frozen vegetables. Food Chem. 2011; 128: 627-633.
30. Jiménez-Monreal AM., García-Diaz L, Martínez-Tomé M, Mariscal M, Murcia MA. Influence of cooking methods on antioxidant activity of vegetables. J Food Sci. 2009; 74:97-103.