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INFLUENCE OF TEMPERATURE AND TIME OF APPLE DRYING ON PHENOLIC COMPOUNDS CONTENT AND THEIR ANTIOXIDANT ACTIVITY

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Key words: polyphenols, antioxidant activity, drying apple,

The purpose of this study was to compare the effects of the different temperatures and time of apple convective drying on the phenolic compounds content and their antioxidant activity. The apple were washed, peeling and cut into cubes of 1 cm before processing, and was dried at three different temperatures of 50, 60, 70°C during 14 h of convection drying method. Phenolic profiles determined by HPLC and antioxidant activity (ABTS, DPPH, FRAP) of apples were analyzed after 2, 7, 10 and 14 hr of drying.

The main phenolic compounds identified and quantified in dried apples was chlorogenic acid, *p*-coumaric acid, phloretin-2'-O-glucoside, phloretin-2'-O-xyloglucoside, (+)-catechin, (-)-epicatechin, procyanidins B1, B2, C1. The total phenolics content in fresh apple was 224.82 mg/100 g of dw. Procyanidins were the most predominant phenolic group and contributed to 73.68% of total phenolics content. The total content of procyanidins ranged from 165.64 mg/100 g dw in fresh apple to 67.51, 44.05, and 32.26 mg/100 g dw in dry apple after it was exposed to 14 h of hot-air drying process at 50°C, 60°C, and 70°C, respectively. *p*-Coumaric and chlorogenic acids during drying process were successively degraded. Dihydrochalcones content decreased with the rise of temperature and the time of processing. After 14 hours of drying at 60°C and 70°C, 100% loss of compounds belonging to dihydrochalcones occurred in apples. The antioxidant activity of tested dried apple samples was very strongly correlated with the content of phenolic compounds. The obtain results ranged from 5.51 μmol T/100 g dw for ABTS to 41.10 μmol T/100 g dw for DPPH and 28.56 μmol T/100 g dw for FRAP for fresh apples. Drying processes might destroy some of the phenol compounds what strongly correlated with antioxidant activity losses. The higher losses of antioxidant capacity, especially for DPPH and ABTS, was observed after 7 hours of drying process.

Our results indicate that the drying process together with the rise of temperature and processing time could diminish the phenolic compounds content and antioxidant activity of apples.

According to these results, it would seem clear that the most appropriate drying temperature in order to preserve polyphenols content and the antioxidant capacity of apple would be around 50°C or around 70°C provided that the drying time is short.

INTRODUCTION

Drying technique is one of the oldest and efficient methods for food preservation. The basic objective of drying food products is to remove free water to a level where microbial spoilage is reduced and shelf-stable and less perishable product is ensured. The degree of thermal damage resulting from food dehydration techniques is directly proportional to the temperature and duration of exposure [Lin *et al.,* 1998].

In many European countries, dehydrated fruits and vegetables are popularly used as ingredients in soups and are usually included in foods such as instant noodles. This means that a large market exists for dehydrated fruits especially apples, small berries and vegetables. Relatively cheaper hot air-drying is commonly used in food production but the longer drying usually results in inferior products quality. The high temperature and long time required in hot air-drying adversely affect texture, flavor and colour of products [Kwok *et al.,* 2004]. Generally, processed products have been considered to have lower nutritional value than their respective fresh commodities mainly due to the loss of nutritional compounds such as vitamin and polyphenols during processing.

Apple (*Malus domestica*) fruits and their products are a widely consumed and this sources contain considerable amounts of polyphenols which contribute significantly to total antioxidant activity [Khanizadeh *et al.,* 2007]. Five major polyphenolic groups have been found in apple: flavanols, hydroxycinnamates, dihydrochalcones, flavonols, and anthocyanins. The polyphenolic compound of apple may play an important role because apples are very significant part of the diet and epidemiological studies have shown an inverse correlation between the consumption of apples and various diseases. Apple intake has been negatively associated with lung cancer incidence [Knekt *et al.,* 1997; Le Marchand *et al.,* 2000] and with cardiovascular disease, coronary, and total mortality [Knekt *et al.,* 1996], risk of thrombotic stroke [Knekt *et al.,* 2000], symptoms of chronic obstructive pulmonary disease [Tabak *et al.,* 2001], and proliferation activities [Sun *et al.,* 2002]. Therefore the knowledge of changes that take place in apples during dehydration process is important with respect to the role of phenolic compounds and their antioxidant prop-

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erties. The purpose of this study was to compare the effects of the different temperatures and time of apple convective drying on the phenolic compounds content and their antioxidant activity.

MATERIALS AND METHODS

Champion variety of apple were used in the present study. The apple were washed, peel and cut into cubes of 1 cm before processing. The pieces of apple were dried at three different temperatures of 50, 60, 70°C during 14 hr of convection drying method. The air velocity was 1m s^{-1}. Phenolic profiles and antioxidant activity of apple were analyzed after 2, 7, 10 and 14 h of drying.

The HPLC analysis of polyphenols were carried out on a HPLC apparatus consisting of a Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). Separation was performed on a Synergi Fusion RP-80A 150x4.6 mm (4μm) Phenomenex (Torrance, CA USA) column. Column oven temperature was set to 30°C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 36 min 25% B, followed by washing and reconditioning the column. The flow rate was 1 mL min^{-1}, and the runs were monitored at the following wavelenghts: procyanidins at 280 nm, hydroxycinnamic acid at 320 nm, dihydrochalcones at 360 nm. Photo Diode Array (PAD) spectra were measured over the wavelength range 240--600 nm in steps of 2 nm. Retention times and spectra were compared to those of pure standards within 200-600 nm. The analyses were replicated (n=3), and the contents are given as mean values plus or minus the standard deviation. The results are expressed in milligrams of each compound per 100 g of dry weight (dw) of apple.

About 5 g of each apple dry were weighed into a test tube for antioxidant property analysis. A total of 25 mL of 80% aqueous methanol was added, and the suspension was stirred slightly. Tubes were sonificated twice for 15 min and one left at room temperature in dark (~ 20°C) for 24 h. The extract was centrifuged for 10 min (1500 *x g*), and supernatants were collected at 4°C until use within 24 h.

The DPPH radical scavenging activity of drying apples was determined according to the method of Yen *et al.* [1996]. The DPPH solution (1 mL) was added to 1 mL of centrifuged methanol extracts with 3 mL of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance was measured at 517 nm using a Shimadzu UV-2401 PC spectrophotometer. Ethanol was used to zero spectrophotometer. All determinations were performed in triplicate. The results were corrected for dilution and expressed in μmol Trolox per 100 g dry weight (dw).

The free-radical scavenging activity was determined by ABTS radical cation decolorization assay according to the method of Re *et al.* [1999]. ABTS was dissolved in water to a 7 mmol concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and following the mixture was left to stand in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than 2 days when stored in dark at room temperature. For the study of infusion samples the $ABTS^{\bullet+}$ solution was diluted with redistilled water to an absorbance of 0.700 (±0.02) at 734 nm. After addition of 30 μL of methanol extracts to 3.0 mL of diluted $ABTS^{\bullet+}$ solution (A734nm =0.700 (±0.02)) the absorbance was read exactly 6 min after initial mixing. All determinations were performed in triplicate. The results were corrected for dilution and expressed in μmol Trolox per 100 g dry weight (dw).

The total antioxidant potential of a sample was determined using a ferric reducing ability of plasma FRAP assay by Benzie *et al.* [1996] as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μmol, pH 3.6), a solution of 10 μmol TPTZ in 40 μmol HCl, and 20 μmol $FeCl_3$ at 10:1:1 (v/v/v). The reagent (300 μL) and sample solutions (10 μL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of Trolox. All solutions were used on the day of preparation. All determinations were performed in triplicate. The results were corrected for dilution and expressed in μmol Trolox per 100 g dry weight (dw).

Principal component analysis (PCA) were performed using XLSTAT (Addinisoft, France) was performed on mean values of 13 samples and 6 variables. The PCA is a multilinear modelling method given an interpretable overview of the main information in a multidimensional data table.

RESULTS AND DISCUSSION

The main phenolic compounds identified and quantified in dried apples by HPLC analysis are shown in Table 1. Ten phenolic compounds found in apples can be categorized into three groups: chlorogenic and p-coumaric acid (hydroxycinnamic acids); phloretin-2'-O-glucoside and, phloretin-2'-O-xyloglucoside (dihydrochalcones); (+)-catechin, (-)-epicatechin, and procyanidins B1, B2, C1. Our results agree with those by Veberic *et al.* [2005], Vanzani *et al.* [2005] and Khanizadeh *et al.* [2007] with one exception. In this study we failed to detect compounds belonging to flavonols because during preparation for drying the skin has been removed from apples. Quercetin and its derivatives are located in peel; therefore, these compounds were removed during apple coring and peeling processes.

The total phenolics content in fresh apple was 224.82 mg/100 g of dw. Procyanidins were the most predominant phenolic group and contributed to 73.68% of total phenolics content. The total content of procyanidins ranged from 165.64 mg/100 g dw in fresh apple to 67.51, 44.05, and 32.26 mg/100 g dw in dry apple after it was exposed to 14 h of hot-air drying process at 50°C, 60°C, and 70°C, respectively. Among procyanidins, the procyanidins B1, B2, and (-)-epicatechin were the most abundant phenolic compounds found in

TABLE 1. The change of polyphenolic compounds (mg/100 g dw) content in raw apples and during the drying processing.

Sample	Chlorogenic acid	p-Coumaric acid	Phloretin-2'-O--glucoside	Phloretin-2'-O--xyloglucoside	(+)-Catechin	(-)-Epicatechin	Procyanidins B1	Procyanidins B2	Procyanidins C1	Total phenolic compounds
0	47.61±1.56b	6.44±1.06a	2.49±0.54b	2.63±0.11b	6.16±0.12b	37.43±1.84ab	62.48±2.11 a	34.55±1.56a	25.02±1.04a	224.82
A2	40.28±1.03d	5.03±0.32b	4.74±0.45 a	2.48±0.23b	7.92±0.34a	34.17±0.23b	2.84±0.23b	32.02±0.78ab	13.59±0.39c	142.34
A7	39.59±1.32d	4.17±1.02c	1.44±0.02 c	1.84±0.12c	2.64±0.12d	34.27±0.43b	2.78±0.43b	29.32±1.08b	11.74±0.34c	127.79
A10	33.31±1.41e	3.52±0.85d	1.20±0.12 c	1.14±0.34e	2.19±0.07de	34.96±0.56b	2.29±0.08d	28.73±0.34b	10.20±0.82cd	117.55
A14	27.49±1.00f	2.01±0.36e	0.57±0.00 f	0.51±0.01f	1.05±0.00g	30.93±0.79c	2.13±0.12d	23.57±1.67c	9.83±1.93cd	98.09
B2	44.01±1.30 c	5.99±0.43ab	2.96±0.12c	3.04±0.45a	2.12±0.09e	36.79±0.28ab	2.82±0.23b	33.97±1.73ab	25.02±0.67a	156.73
B7	45.82±1.32bc	5.50±1.04b	1.62±0.02c	1.51±0.12d	2.96±0.11d	28.93±0.58d	2.67±0.16c	22.62±0.39c	8.34±1.11d	119.96
B10	38.23±1.61d	3.00±0.23d	0.93±0.01e	0.45±0.05f	1.78±0.05f	23.73±0.19e	1.99±0.09e	20.56±0.95cd	5.48±0.49e	96.15
B14	22.53±1.04g	1.78±0.38 f	0.00±0.00f	0.00±0.00g	1.04±0.09g	21.24±1.83e	1.33±0.04f	17.06±1.17e	3.37±0.23f	68.36
C2	49.76±0.86a	6.18±0.28 a	3.54±0.12c	3.15±0.12a	4.50±0.28c	39.58±1.29a	2.73±0.05b	36.10±0.99a	16.07±1.01b	161.13
C7	36.01±1.03e	5.57±0.81b	0.74±0.05d	1.39±0.05d	2.72±0.19d	26.00±1.99d	2.30±0.12d	18.11±1.94e	6.43±1.1.02e	99.28
C10	15.09±1.00h	3.23±0.99d	0.12±0.00c	0.12±0.01f	1.87±0.07f	21.69±0.53e	2.07±0.10d	16.48±1.05f	5.26±0.74e	65.94
C14	9.45±0.56i	1.31±0.11f	0.00±0.00f	0.00±0.00g	0.79±0.04h	13.58±0.12f	0.88±0.03g	15.09±0.56f	1.91±0.21g	43.02

Mean values from the same row having letters differ statistically significantly at p≤0.05; A- 50°C, B-60°C, C-70°C; 0, 2, 7, 10, 14 h of drying

fresh apple. However, only procyanidins B2 and (-)-epicatechin were dominant in dry apple since procyanidin B1 and (+)-catechin degraded very quickly (Table 1). The procyanidins content in dried apple slices is very important because they play the main role as radical scavenging compounds [Lu *et al.,* 2000].

The second largest class of hydroxycinnamates, was represented by chlorogenic and *p*-coumaric acid, which amounted to about 24% of total phenolics compounds, whereas dihydrochalcones constituted only 2.28% of total phenolic compounds in fresh apple. Chlorogenic acid was the major compound of hydroxycinnamic acids. The concentration of this acid in fresh apple is 47.61 mg/100 g dw. However, after the samples were dried at 50°C, 60°C, and 70°C they contained only 57.75%, 47.33%, and 19.85% of chlorogenic acid, respectively. Chlorogenic acid also had an important antioxidative capacity, comparable to ascorbic acid [Lee *et al.,* 2003]. The concentrations of hydroxycinnamic acids were significantly reduced during drying especially in samples dried at 70°C. *p*-Coumaric acid amounted to only 6.44 mg/100 g dw, and during drying process this acid was successively degraded.

Dichydrochalcones, including phloretin-2'-O-glucoside and phloretin-2'-O-xyloglucoside were found in fresh apple. As it was found, in all samples, even after drying process, there was more phloretin-2'-O-xyloglucoside than phloretin-2'-O-glucoside. Trace amount of phloretin-2'-O-glucoside was found in fresh apples (1.05 mg/100 g dw). Dihydrochalcones content decreased with the rise of temperature and the time of processing. After 14 h of drying at 60°C and 70°C, 100% loss of compounds belonging to dihydrochalcones occurred in apples.

In general, we observed that after 2 h of drying, regardless of the temperature used in drying process, in some samples the total content of some polyphenols slightly increased, *i.e.* chlorogenic acid, phloretin-2'-O-glucoside, phloretin-2'O-xyloglucoside, and flavan-3-ol. It is possible due to the liberation of phenolic compounds from the matrix during the process. Chism *et al.* [1996] has mentioned that fruits and vegetables normally have high contents of phenolic compounds in their outer parts, as they are the metabolic intermediates and usually accumulate in the vacuoles. Probably, food processes might accelerate the release of more bound phenolic compounds that are released due to the breakdown of cellular constituents. Although, disruption of cell walls may also trigger the release of oxidative and hydrolytic enzymes that would destroy the antioxidant activity in apples; however, high temperature of hot-air drying process would deactivated these enzymes and prevent the loss of phenolic compounds and therefore lead to the increase of antioxidant activity.

The antioxidant activity of tested dried apples samples was very strongly correlated with the content of phenolic compounds. The antioxidant capacity of dehydrated apples measured by DPPH, ABTS free radical scavenging and FRAP assays and it is shown in Table 2. The results ranged 5.51 μmol Trolox/100 g dw for ABTS, 39.26 μmol Trolox/100 g dw for DPPH and 28.56 μmol Trolox/100 g dw for FRAP for fresh apples. In all samples during drying processing

TABLE 2. The change of antioxidant activity (μmol Trolox/100 g dw) in raw apples and during the drying processing.

Sample	ABTS	DPPH	FRAP
0	5.51±0.02g	39.26±0.07a	28.56±0.04a
A2	6.20±0.11a	41.10±0.13a	26.70±0.03a
A7	5.29±0.09bc	34.58±0.04c	19.51±0.10b
A10	3.50±0.11d	26.18±0.02d	18.48±0.05b
A14	1.93±0.03 f	12.67±0.09f	17.03±0.01b
B2	5.9±0.12 b	40.43±0.10a	28.57±0.00a
B7	3.85±0.06 d	21.90±0.08e	17.45±0.05b
B10	3.01±0.06 e	19.39±0.02e	16.23±0.03c
B14	1.79±0.02f	9.03±0.00g	14.78±0.11c
C2	6.58±0.10 a	37.12±0.08b	25.65±0.06a
C7	3.66±0.00 d	14.76±0.02f	13.76±0.03d
C10	1.63±0.09 f	8.01±0.06g	10.63±0.09d
C14	1.07±0.11 g	3.71±0.10h	6.83±0.00e

Mean values from the same row having letters differ statistically significantly at p≤0.05; A- 50°C, B-60°C, C-70°C; 0, 2, 7, 10, 14 hour of drying

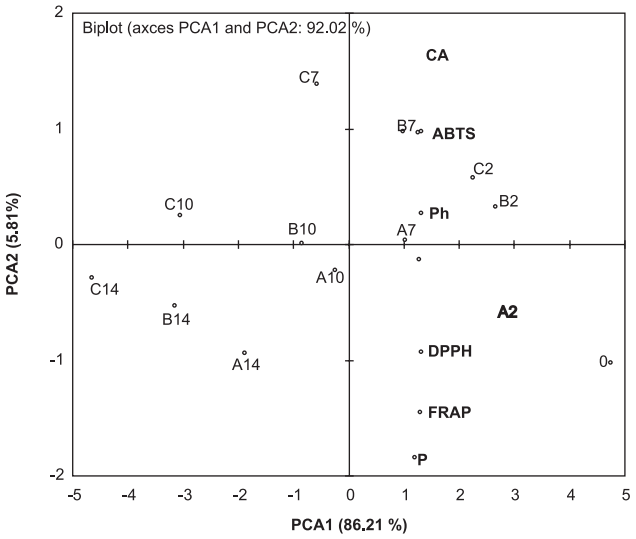

FIGURE 1. Principal component analysis of drying apple in different temperatures (A- 50°C; B- 60°C; C- 70°C) and time (0, 2, 7, 10, 14 h). The measured variables (phenolics: P-procyanidins; Ph-dihydrochalcones. CA-hydroxycinnamic acid; antioxidant capacity: ABTS, DPPH and FRAP) are shown on the same plot.

was observed decrease antioxidant activity. Higher decrease of antioxidant capacity, especially for DPPH and ABTS, was observed after 7 h of drying process. After 14 h, apples dried at 70°C contained only 19.42%, 9.45%, 23.91% of total antioxidant capacity for ABTS, DPPH, and FRAP, respectively. Therefore, during the same time of drying at 50°C, the dried apples contained 35.03%, 32.27%, and 59.63% of total antioxidant capacity, respectively. The high content of phenolics in apples dried at 50°C was found to affect the capability of free radical deactivation much stronger than in samples dried at other temperatures. The content of polyphenolic compounds in samples was strongly correlated with antioxidant activity [Kim *et al.,* 2004]. Drying processes and, in particular, high temperatures (*i.e.* 60°C and 70°C) and long time of drying, might destroy some of the phenol compounds and in affect antioxidant activity was losses. This dependency also, was observed in samples dried for 2 hours (Table 2) because this sample have the higher content of polyphenol compounds and the high level of antioxidant capacity ($p<0.05$). According to our results and those reported earlier Lee *et al*. [2003] (-)-epicatechin and procyanidin B2 were the main contributors of the higher total antioxidant activity. During all drying processes we observed that procyanidins were more stable than the other phenolic components. *o*-Dihydroxy (catechol) structure in B-ring, 2,3-double bond in conjunction with a 4-oxo function and the additional presence of both 3- and 5-OH groups constituted the three structural groups that were important for maximal radical-scavenging potential. Lu *et al.,* [2000] described that DPPH-scavenging activity decreased in the following order: quercetin glycosides ≥ procyanidins >> chlorogenic acid ≈ 3-hydroxyphloridzin >> phloridzin. Tsao *et al.* [2005] indicate that, the antioxidant activity measured by FRAP method, of the five polyphenolic groups of apples were in the following decreasing order: cyaniding-3-galactoside > procyanidins > quercetin glycosides > chlorogenic acid > phloridzin.

PCA is applicable when all of the variables collectively characterize each, and all, of the samples. In this study, PCA is used to see if the samples are characterized differently due to the methods of drying (Figure 1) and in content of polyphenols and antioxidant method (ABTS, DPPH, FRAP). The principal components explained 92.02% of the variance of the data. There is a strong positive correlation between procyanidins (P), FRAP, and DPPH (Person's correlation = 0.832 and 0.876, respectively). On the other hand, the content of hydroxycinnamates and dichydrochalcones has a strong correlation with ABTS (Person's correlation = 0.938 and 0.932, respectively). The second PCA (PCA2 = 5.81%) in drying in the samples, the samples with high phenolic compounds content laying in the right part of the PCA biplot. It is also possible to observe some samples: 0, A2, B2, C2, A7, B7 balanced in terms of polyphenols and antioxidant activity ABTS, DPPH and FRAP. In this case other samples in the PCA could not be distinguished.

CONCLUSION

Our results indicate that the drying process together with the rise of temperature and processing time could diminish the phenolic compounds content and antioxidant activity of apples. Drying process and, in particular, high temperatures (especially 70°C) destroy some of the phenol compounds the most. According to these results, it would seem clear that the most appropriate drying temperature in order to preserve polyphenols content and the antioxidant capacity of apple would be around 50°C or around 70°C provided that the drying time is short.

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WPŁYW TEMPERATURY I CZASU SUSZENIA JABŁEK NA ZAWARTOŚĆ ZWIĄZKÓW FENOLOWYCH I ICH AKTYWNOŚĆ PRZECIWUTLENIAJĄCĄ

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Celem pracy było określenie wpływu temperatury i czasu suszenia jabłek na zawartość związków fenolowych i aktywność przeciwutleniającą.

Jabłka obierane ze skórki i pocięte na 1 cm kostkę suszono metodą konwekcyjną w różnych temperaturach: 50, 60, 70°C przez 14 godzin. Zawartość związków fenolowych określono za pomocą wysokosprawnej chromatografii cieczowej po 2, 7, 10 i 14 godzinach suszenia. Aktywność przeciwutleniającą zmierzono metodami z użyciem syntetycznych rodników ABTS i DPPH oraz FRAP.

Główne związki fenolowe zidentyfikowane w suszu jabłkowym to: kwas chlorogenowy i *p*-kumarowy, florentyna-2'-O-glukozyd i florentyna-2'-O-ksyloglukozyd, (+)-katechina, (-)-epikatechina, procyjanidyny B1, B2, C1. Zawartość związków fenolowych w świeżych jabłkach wynosiła 224,82 mg/100 g w przeliczeniu na sucha masę (sm). Procyjanidyny, które były dominującą grupą stanowiły 73,68% z ogólnej ilości polifenoli. Zawartość procyjanidyn dla świeżego jabłka wynosiła 165,64 mg/100 g sm, podczas gdy dla jabłek suszonych 14 godzin w 50°C, 60°C i 70°C wynosiła odpowiednio: 67,51, 44,05, and 32,26 mg/100 g sm. Kwasy: *p*-kumarowy i chlorogenowy podczas całego procesu suszenia sukcesywnie ulegały degradacji. Zawartość dihydrochalkonów wraz ze wzrostem temperatury malała czego wynikiem była całkowita degradacja tych związków w wyższych temperaturach. Aktywność przeciwutleniająca suszonych jabłek była silnie skorelowana z zawartością związków fenolowych. Zdolność wychwytywania rodników ABTS przez świeże jabłka wynosiła 5. 51 μmol Trolox/100 g sm, dla DPPH 41,10 μmol Trolox/100 g sm, i dla FRAP 28,56 μmol Trolox/100 g sm. Najwyższą utratę zdolności przeciwutleniającej, w szczególności dla ABTS i DPPH zaobserwowano po 7 godzinach procesu suszenia. Przeprowadzone badania jednoznacznie wskazują, że temperatura i czas procesu suszenia istotnie wpływa na zawartość związków fenolowych, co znajduje swoje odzwierciedlenie w aktywności przeciwutleniającej. Na podstawie uzyskanych wyników w celu zachowana najwyższej zawartości związków fenolowych oraz aktywności przeciwutleniającej zaleca się przeprowadzać proces suszenia w 50°C bądź w 70°C lecz przez krótki okres czasu.