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EFFECT OF COOKING METHODS ON ANTIOXIDANT LEVELS OF RED ONION VARIETIES IN SRI LANKA

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ABSTRACT

The neutralization via antioxidants of reactive oxygen species (ROS) formed due to exogenous factors and endogenous metabolic activities are imperative in order to maintain healthy cellular conditions within the body. This study is focused on analyzing the effects of home-like cooking techniques on the antioxidant levels of onions. Commercially bought big and small red onion varieties in Sri Lanka were subjected to the effects of the three cooking techniques; boiling, baking and frying, attested at varied time intervals 5, 10 and 15 minutes. To compare and contrast the effect of the cooking treatments; total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), the 2, 2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+) and ferric reducing antioxidant power (FRAP) assay were utilized. The findings between TFC, TPC and TAC quantification of three different cooking treatments were significant due to statistical evidence of $p<0.05$ by single factor ANOVA. The results were suggestive of the antioxidant capacity of small onion varieties as being affected the least by cooking. For both onion varieties in terms of cooking methods, antioxidant loss can be seen as boiling>frying>baking and the optimum time for highest antioxidant activity of cooked onions was mostly at 10 minutes. (196 words) Keywords: Total antioxidant capacity, total phenolic content of onions, total reducing power, percentage inhibition, cooking-methods

INTRODUCTION

Onions (*Allium Cepa* L.) of the genus *Allium* is the most cultivated of the genus which is widely known for its aromatic properties, used as a common ingredient in recipes and in identified for its medicinal properties (Liguori et al., 2017). Mainly used as food materials, the onion bulb can vary according to color (red, white and yellow), pungency, sugar content, bulb shape, size, seed stem formation and disease resistance (Kavalcová, et al., 2015). *Allium* species, is a rich source of flavonoids, organosulfur compounds, lectins, fructan, prostaglandin, vitamins B1, B2, B6, C, E, essential amino acids and nicotic (Corczo-Martinez et al., 2007).

Flavonoids is the most abundant phytonutrient component in onions, which makes up bulk of the high levels of phenolic compounds responsible for antioxidant properties. High concentrations of flavonol are present as two glycosides namely quercetin and kaempferol. In red onion varieties, anthocyanins of the anthocyanidins are also abundant along with the rich flavonol composition as yellow onions (Liguori et al., 2017; Santas, Almajano and Carbo 2010). The presence of phenolic compounds is of great interest in order to study the radical scavenging potential as food antioxidants. Additionally, other substances such as thiosulfinates and thiols are abundantly constituent in onions contributing to its antioxidative properties (Konciˇc and Jug, 2011).

The importance of antioxidants is associated with reduction of developing diseases associated with reactive oxygen species (ROS) damage. ROS are generated due to redox reactions in the cells whilst carrying out cellular metabolism reactions and or by exogenous environmental agents as well. Transition metals such as Fe^{2+} and Cu^{2+} are known to catalyze the production of ROS in cells (Prakash, Singh and Upadhyay, 2006). ROS are known to be the cause of many degenerative diseases due to the oxidation of nucleic acids, proteins, lipids or DNA leading to oxidative pathways (Kavalcová, et al., 2015). Repair mechanisms which amend deleterious or various types of adverse effects may fail at times in order to completely revert the damage. Accumulation of such ROS damage may result in diseases, for example; cancers, cardiovascular disease, diabetes and retinal disease (Masuda, Shimazawa and Hara, 2017; Phaniendra, Jestadi, Periyasamay, 2015; Nimse and Pal, 2015; Kabel, 2014). The role of phytonutrients or phytochemicals as antioxidants has been identified in reducing oxidative damage (Tiwari and Cummins, 2013). There are three types of phytochemicals described; polyphenols, carotenoids and glucosinolates where polyphenols the largest phytochemical group collectively represents a variety of antioxidative compounds present in onions such as flavonoids and anthocynins (Tsao, 2010).

The presence of antioxidants in natural samples has been evident in many studies where the characteristics and properties have been studied extensively. It has been reported that an attempt to prevent oxidation via synthetic antioxidants have caused external and internal bleeding in rats (Borneo *et al.*, 2009). Therefore, there is a need for extensive research in order to bridge the gap in ROS damage and discovery of safer natural antioxidant sources that are rich in bioactive flavonoids, which can be made affordable

to general public at a sufficient supply (Thaipong et al., 2006). However, on a day to day basis the ideal laboratory conditions for extraction of optimum levels of nutrients does not happen. Hence, the requirement for awareness of daily consumption of probable antioxidant values of food products requires further analysis.

House-hold preparation of most vegetables involves cooking prior to consumption. The importance of nutritional value in food choices in widely cited evidence shows that among the key influences of taste, cost, convenience and nutritional value, taste was the most influential followed by cost or convenience and nutritional value rated lower on the scale (Aggarwal et al., 2016; Glanz et al., 1998; Masrizal et al., 1997). Culinary traditions during household cooking (Table 1) incorporates variety of heating involved techniques, such as boiling, baking, frying, steaming, roasting and microwave heating. Induction of alterations in biological, chemical and physical properties via thermal processing results in improved palatability and appeal of food, as well as safety by microorganism destruction (van Boekel et al., 2010).

Table 1: Summary of cooking methods used for home like processing (Fabbri and Cosby, 2016).

Method	**Definition**
Boil	To cook foods in boiling liquid in a pot set on a hot burner.
Fry	To cook in a hot oil in a skillet on a hot burner.
Microwave	To cook by placing the food in the path of microwaves (the induced molecular friction in water molecules will to produce heat).
Pressure- cooking	To cook food using water or other liquid in a sealed pot, normally a pressure cooker or an autoclave (Laboratory simulation).
Roast	To cook foods in a pan in a hot oven.
Saute	To cook foods in a thin film of hot oil in a skillet set on a hot burner.
Simmer	To cook foods in liquid (below the boiling point in a pot set or hot burner).
Sous-vide	To cook in a vacuumed plastic pouches at precisely controlled temperatures.
Steam	To cook food that is suspended, generally in a basket, over simmering liquid in a covered pot set on the stovetop.
Stew	To sauté the food, and then simmer.

Evidently the loss of nutritional value of domestic food preparation has been investigated as well as the enhancement of nutrient availability through selection of appropriate cooking methods (Palermo, Pellegrini and Fogliano, 2013). The requirement for daily consumption of vegetables has been related with prevention of many chronic diseases due to the high fiber, vitamin, minerals, phenolics, low

glycemic index, proteins and antioxidants present (Mozaffarian, 2016; Fabbri and Cosby, 2016; Tiwari and Cummins, 2013, Boeing et al., 2012).

The aim of this research project is to identify the effect of home-like cooking methods on onions. This effect is measured in terms of antioxidant levels and antimicrobial activity shown by the onion samples. The cooking methods will not be homogenous thus the time and extent of thermal treatment will be altered to various degrees thus the aim of identifying an optimum level of thermal treatment for home-like processing maybe identified in order to prepare onions. Also the Sri Lankan onion variety to show the most antioxidative activity following heat treatment could be identified as well. Therefore, the study can be helpful in spreading awareness of suitable preparation methods in order to consume a commonly used spice/vegetable with conserved antioxidant levels.

The current study will utilize three cooking methods namely boiling, baking and frying at three different time periods (5, 10 and 15 minutes) in order to determine the type of commonly consumed onion variety either big or small onion variety as the best source of antioxidants following home-like processing.

METHODOLOGY

Sample selection

Two varieties of commonly consumed onions (big and small varieties) were purchased at the local grocery store in Wellawatte, Sri Lanka and used for analysis.

Chemicals and reagents

Methanol, Hydrochloric acid, Sodium carbonate, Sulphuric acid, Sodium sulphate, Ammonium molybdate, Ferric chloride, Sodium acetate, Glacial acid, Sodium nitrate, Aluminium Chloride, Sodium hydroxide, Ammonium persulphate, Folin-Ciocalteu phenol reagent, 2,4,6,-tri(2-pycridyl)-s-triazine reagent, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS•+)

Standards

Ascorbic acid standard, Gallic acid standard, Quercetin standard (See appendix section 9.4)

Apparatus

Consumables: Micropipettes, beakers, measuring cylinders, filter funnel, centrifuge tubes (15ml and 50ml), watch glasses, mortar and pestle, tripod, whatman no.1 filter paper, gauze, sterile pipette tips, sterile eppendorf tubes, cuvettes, spatula, foil

Equipment: Spectrophotometer (JENWAY 6305), food processor, hot air oven (meditry DHA-9053A), centrifuge, electronic balance, refrigerator

Sample preparation

General

The onion samples were prepared according to cooking methods used in households for daily consumption. In brief, the onions were peeled and the inedible parts were removed. The large onions were cut into ~2x2cm pieces and the small onion variety bulbs were halved. Portion sizes of 15g each was weighed and treated with the relative cooking method for separate time spans of 5,10 and 15 minutes each for all

cooking methods for both varieties. A separate untreated raw portion was separated for each variety as well. Following the cooking treatment each sample was cooled by immersing in an ice bath. Samples were broken into smaller pieces using a food processor and ground into a paste using a mortar and pestle. Thereafter the samples were used for extraction.

Cooking treatments

Boiling: A beaker was filled with 100ml of distilled water and allowed to reach boiling point. The 15g portion of onions was transferred into the boiling water and a timer was used to measure 5 minutes. The procedure was repeated for 10 minutes and 15 minutes in fresh samples of water.

Frying: A nonstick pan was kept over a high heat to a point where at the addition of a few drops of water would dissipate in a few seconds. 15g portions were added on to the pan and tossed around to prevent charring and few drops of water was also added occasionally to allow the onions to brown without charring. The procedure was repeated for 10 minutes and 15 minutes each after washing the pan.

Baking: The oven was preheated to reach a temperature of 180 °C. Each portion of 15g of onions were wrapped in foil and placed inside the oven. The samples were removed at 5, 10, 15 minutes respectively.

Sample extraction for Antioxidant assays

The grounded paste of sample was mixed in 25ml of methanolic extract (methanol: HCl: water, 50:5:45, V: V: V) and the mixture was squeezed through gauze clothe to separate the large residues. The obtained liquid was centrifuged at 1200 rpm for 10 minutes and the supernatant was obtained. A Whatman no. 1 filter paper was used to decant the centrifuged liquid. Thus a clear solution was obtained and evaporated overnight in an oven at 40 °C. The residue was weighed and dissolved in 70% methanol to produce 2.5% sample solution and stored at -4 °C till analysis.

Determination of Total Phenolic Content (TPC)

The TPC of the samples was determined using the Folin-Ciocalteu (FC) method (El-Sohaimy *et al*., 2013). Briefly, to 50 µl of sample, 0.5ml of FC reagent and 1ml of deionized water was added. The sample was kept at room temperature for 3 minutes. A 2.5 ml of 20% sodium carbonate was added and incubated in the dark for 1hour at room temperature. Methanol was used to blank where the absorbance was measured at 765 nm. The concentration was expressed in mgGAE/100g.

Determination of Total Flavonoid Content (TFC)

The flavonoid content was measured via the aluminum chloride colorimetric technique (Chua *et al*., 2012). To 0.3ml of sample extract, 3.4ml of 30% methanol, 150µl of 0.5M sodium nitrate and 150 µl of 0.3M aluminum chloride was added and left in the dark for 5 minutes at room temperature. Hence, 1ml of 1M sodium hydroxide was added to the solution and the absorbance was measured at 506nm. A methanol blank was used for calibration. Flavonoid concentration was expressed in mgQE/100g.

Determination of Total Antioxidant Capacity (TAC)

The phosphomolybdate assay system was used to quantify the TAC of the samples

(Baskar *et al.*, 2011). To 1ml of reagent solution (0.6M sulfuric acid: 28mM sodium sulfate: 4mM ammonium molybdate, 1:1:1, V: V: V) 3ml of extract was added. The sample mixture was covered in foil and incubated at 90°C for 90 minutes in a hot air oven. A methanol blank was used to blank and the absorbance was measure at 695nm. Results were expressed in mgAAE/100g.

Determination of Ferric Reducing Antioxidant Potential (FRAP)

The ferric reducing potential of the onion samples was determined by the FRAP protocol (Benzie and Strain, 1999). The FRAP reagent was prepared by mixing 25ml of 300mM acetate buffer at pH 3.6 with 2.5ml of 10mM TPTZ solution in 40mM HCl and 2.5ml of 30mM ferric chloride. To 1.5ml of FRAP reagent 100µl of distil water and 100µl of sample was added. The solution mixture was incubated at room temperature for 4 minutes and absorbance readings were at 593nm with a methanol blank

Determination of ABTS•+ radical scavenging activity

The ABTS•+ radical solution was prepared by modifying the protocol described by Al-Jasass, Siddiq and Sogi, 2015. It was prepared by combining 5ml of 7mM ABTS•+ solution and 5ml of 2.45mM ammonium persulfate. The solution was kept in the dark for 16 hours at room temperature and diluted with 100ml of 3% methanol to prepare a working solution. The initial absorbance of the solution was recorded prior to the addition of sample extract. To 150µl of sample 2850µl of working solution was added and the scavenging activity was recorded.

DATA ANALYSIS

Total Antioxidant Capacity (TAC) results

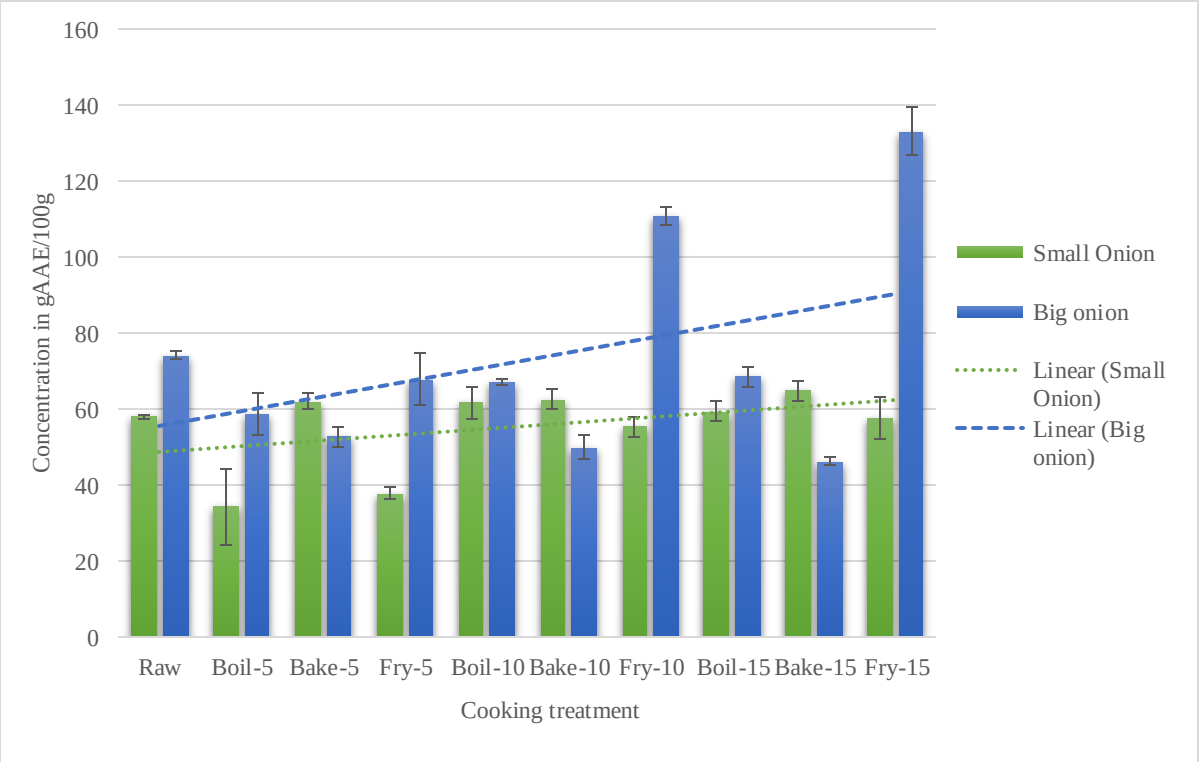

Figure 1: Mean concentrations of total antioxidant capacity of big and small onions samples with all cooking methods in ascorbic acid equivalents per 100g.

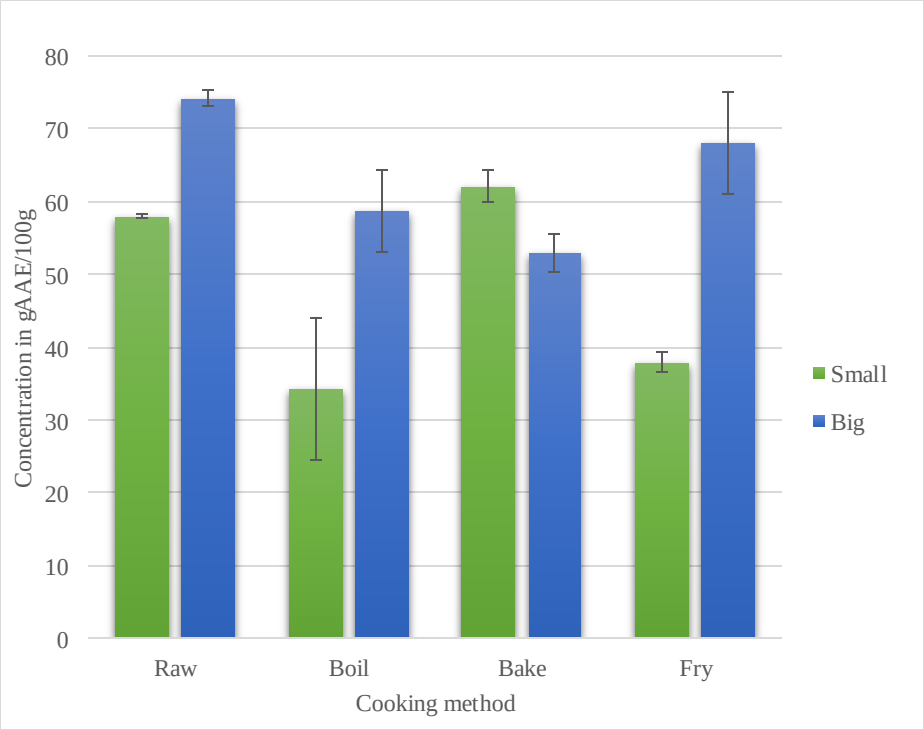

Figure 2: Mean concentration of total antioxidant capacity of big and small onions following 5 minutes via different cooking methods.

Table 2: ANOVA: single factor for TPC of big onion and small onion samples cooked for 5 minutes.

SUMMARY

Groups	Count	Sum	Average	Variance
S-Raw	3	165.5314	55.17714	0.169796
S-Boil	3	89.64571	29.8819	0.161088
S-Bake	3	128.0457	42.6819	3.583129
S-Fry	3	101.1886	33.72952	1.754558
B-Raw	3	147.4743	49.1581	3.321905
B-Boil	3	135.5886	45.19619	0.226395
B-Bake	3	136.7314	45.57714	22.89633
B-Fry	3	120.7314	40.24381	3.348027

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3974.925	7	567.8465	23.94702	2.41E-07	2.657197
Within Groups	379.4019	16	23.71262			
Total	4354.327	23				

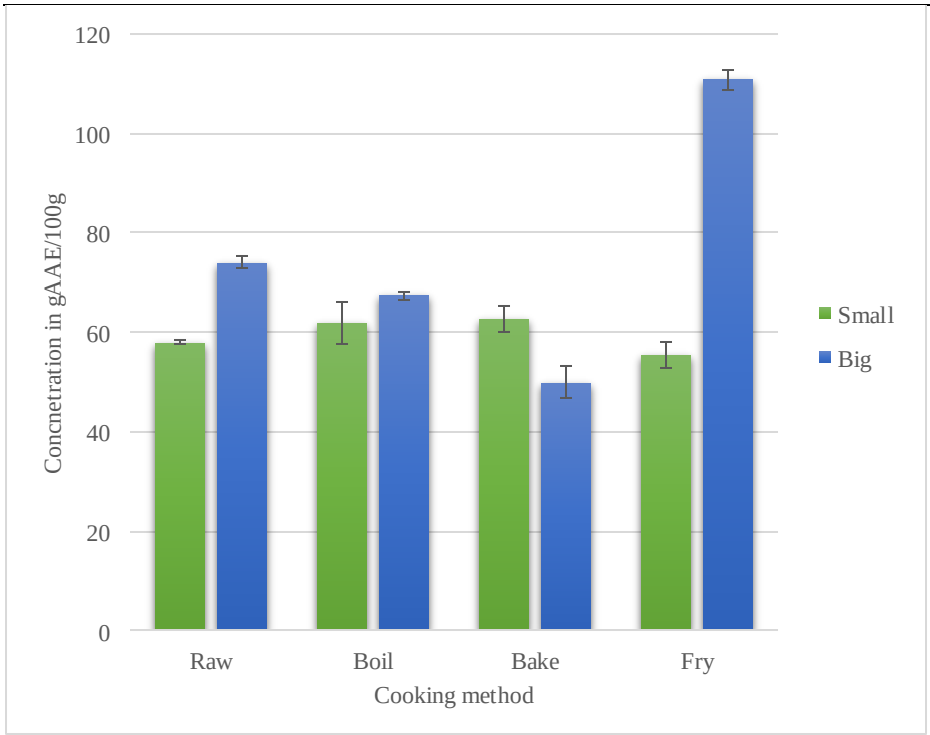

Figure 3: Mean concentration of total antioxidant capacity of big and small onions following 10 minutes via different cooking methods.

Table 3: ANOVA: single factor for TAC of big onion and small onion samples cooked for 10 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	173.9382	57.97939	0.122094
S-Boil	3	185.3927	61.79758	18.68342
S-Bake	3	187.9745	62.65818	6.760992
S-Fry	3	166.0597	55.35323	6.713461
B-Raw	3	222.4109	74.13697	1.250028
B-Boil	3	202.0473	67.34909	0.491901
B-Bake	3	149.8655	49.95515	10.22457
B-Fry	3	332.4182	110.8061	4.639118

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7564.366	7	1080.624	176.8413	6.07E-14	2.657197
Within Groups	97.77116	16	6.110698			
Total	7662.137	23				

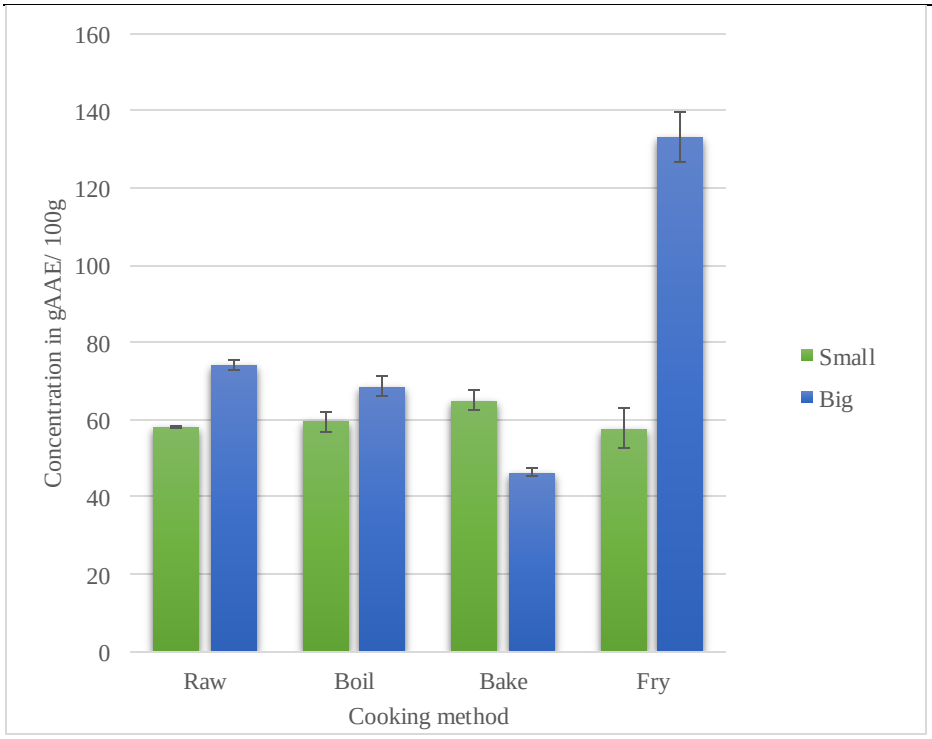

Figure 4: Mean concentration of total antioxidant capacity of big and small onion samples following 15 minutes via different cooking methods.

Table 4: ANOVA: single factor for TAC of big onion and small onion samples cooked for 15 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	173.9382	57.97939	0.122094
S-Boil	3	118.88	39.62667	1181.212
S-Bake	3	194.8109	64.93697	7.259945
S-Fry	3	173.1745	57.72485	28.21333
B-Raw	3	222.4109	74.13697	1.250028
B-Boil	3	137.1709	45.72364	1571.564
B-Bake	3	139.0291	46.34303	0.773994
B-Fry	3	399.5091	133.1697	41.8292

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18588.79	7	2655.541	7.500936	0.00044	2.657197
Within Groups	5664.448	16	354.028			
Total	24253.24	23				

Total Phenolic content (TPC) results

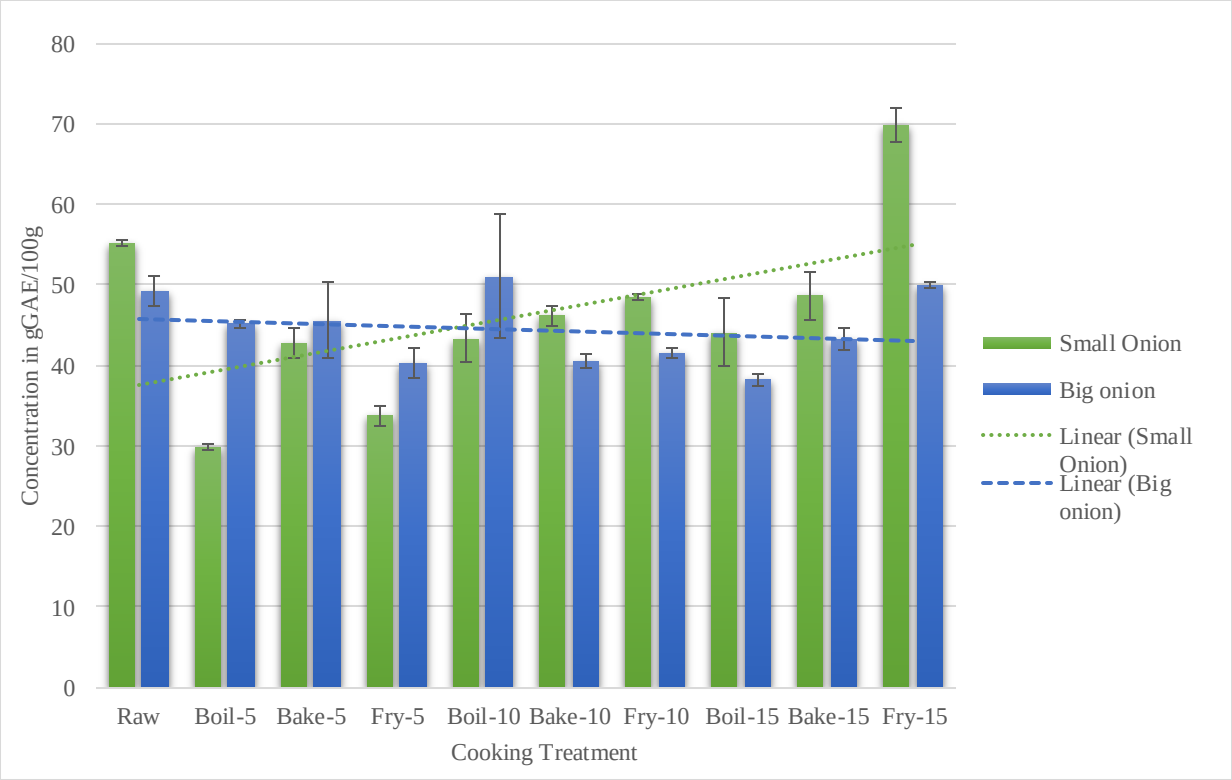

Figure 5: Mean values of the concentration of total phenolic content in big and small onion samples with all cooking methods in gallic acid equivalents per 100g.

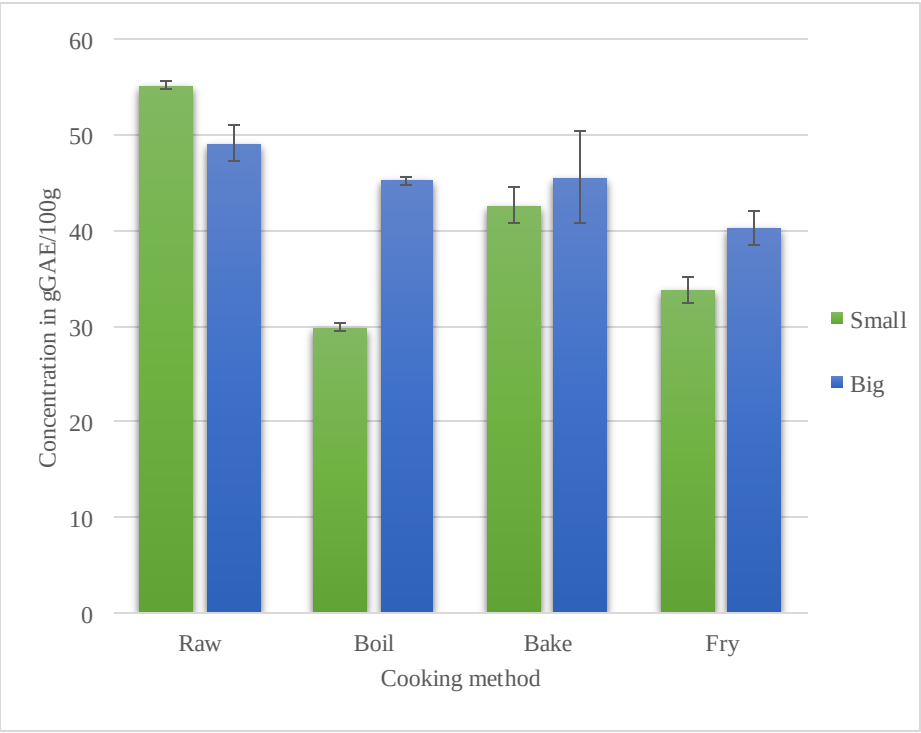

Figure 6: Mean concentration of Phenolic content of big and small onions following 5 minutes via different cooking methods.

Table 5: ANOVA: single factor for TPC of big onion and small onion samples cooked for 5 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	165.5314	55.17714	0.169796
S-Boil	3	89.64571	29.8819	0.161088
S-Bake	3	128.0457	42.6819	3.583129
S-Fry	3	101.1886	33.72952	1.754558
B-Raw	3	147.4743	49.1581	3.321905
B-Boil	3	135.5886	45.19619	0.226395
B-Bake	3	136.7314	45.57714	22.89633
B-Fry	3	120.7314	40.24381	3.348027

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1388.103	7	198.3004	44.73628	2.48E-09	2.657197
Within Groups	70.92245	16	4.432653			
Total	1459.025	23				

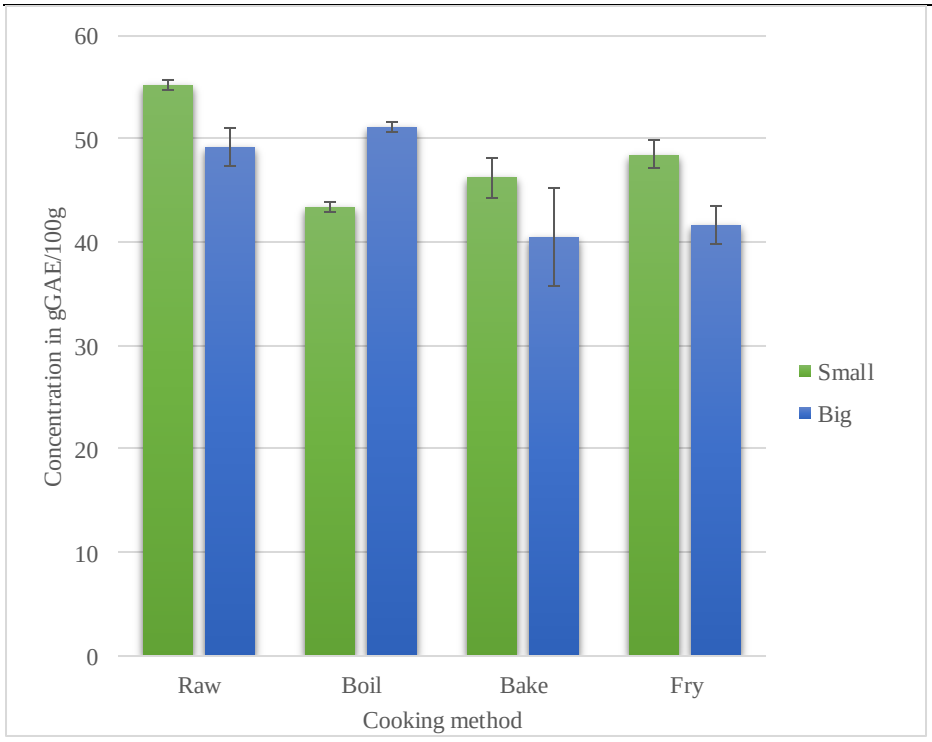

Figure 7: Mean concentration of Phenolic content of big and small onions following 10 minutes via different cooking methods.

Table 6: ANOVA: single factor for TPC of big onion and small onion samples cooked for 10 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	165.5314	55.17714	0.169796
S-Boil	3	130.1029	43.36762	8.650884
S-Bake	3	138.56	46.18667	1.441088
S-Fry	3	145.4486	48.48288	0.092046
B-Raw	3	147.4743	49.1581	3.321905
B-Boil	3	153.1886	51.06286	60.55184
B-Bake	3	121.5314	40.51048	0.748844
B-Fry	3	124.8457	41.61524	0.39619

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	525.5099	7	75.07284	7.968185	0.000312	2.657197
Within Groups	150.7452	16	9.421574			
Total	676.2551	23				

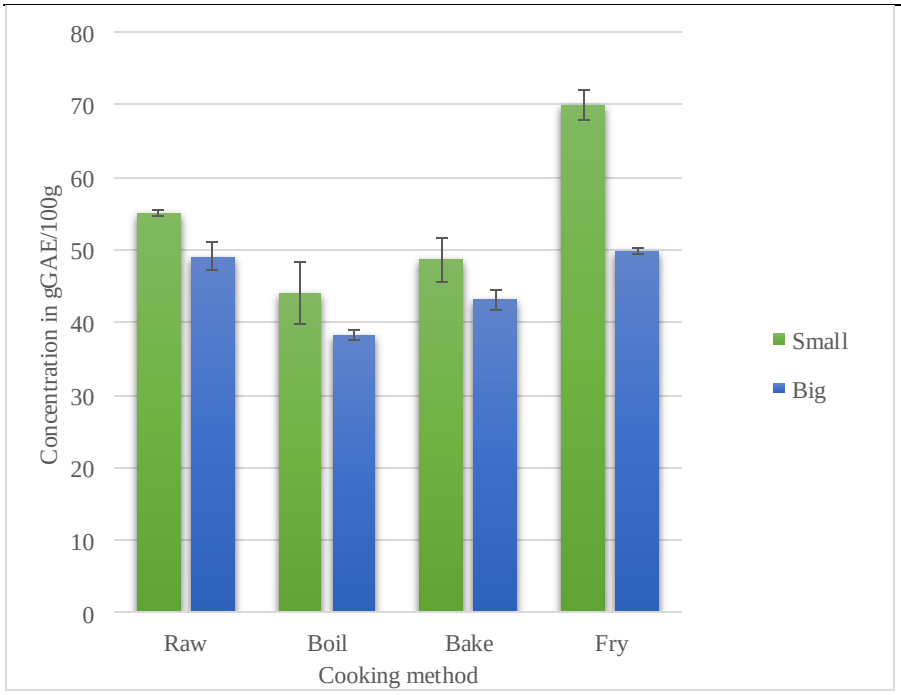

Figure 8 Mean concentration of Phenolic content of big and small onions following 15 minutes via different cooking methods.

Table 7: ANOVA: single factor for TPC of big onion and small onion samples cooked for 15 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	165.5314	55.17714	0.169796
S-Boil	3	132.3886	44.12952	18.83864
S-Bake	3	145.8743	48.62476	8.520272
S-Fry	3	209.76	69.92	4.231837
B-Raw	3	147.4743	49.1581	3.321905
B-Boil	3	114.6743	38.22476	0.474558
B-Bake	3	129.5314	43.17714	1.92
B-Fry	3	149.76	49.92	0.169796

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1936.613	7	276.659	58.79045	3.14E-10	2.657197
Within Groups	75.29361	16	4.70585			
Total	2011.907	23				

Total Flavonoid Content Results

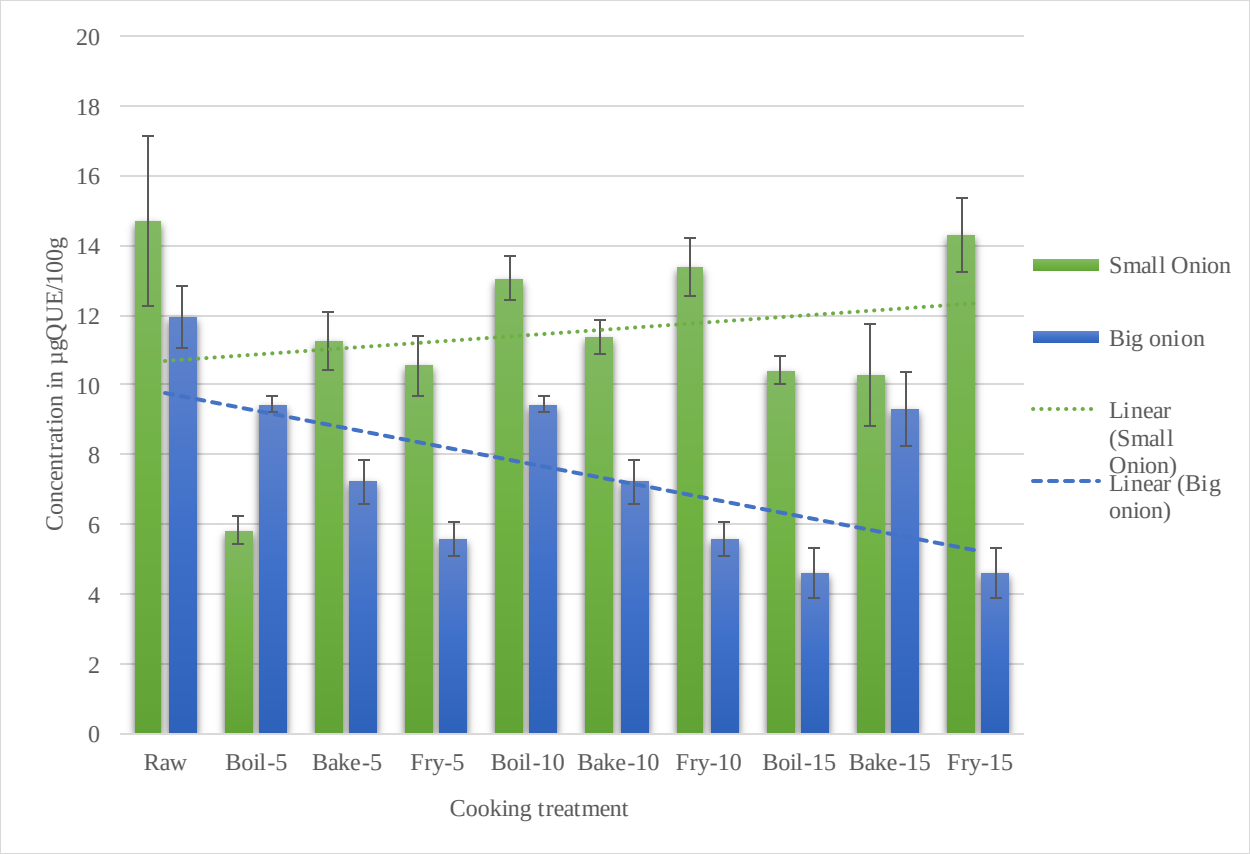

Figure 9: Mean values of the concentration of total flavonoid content in big and small onion samples with all cooking methods in quercetin acid equivalents per 100g

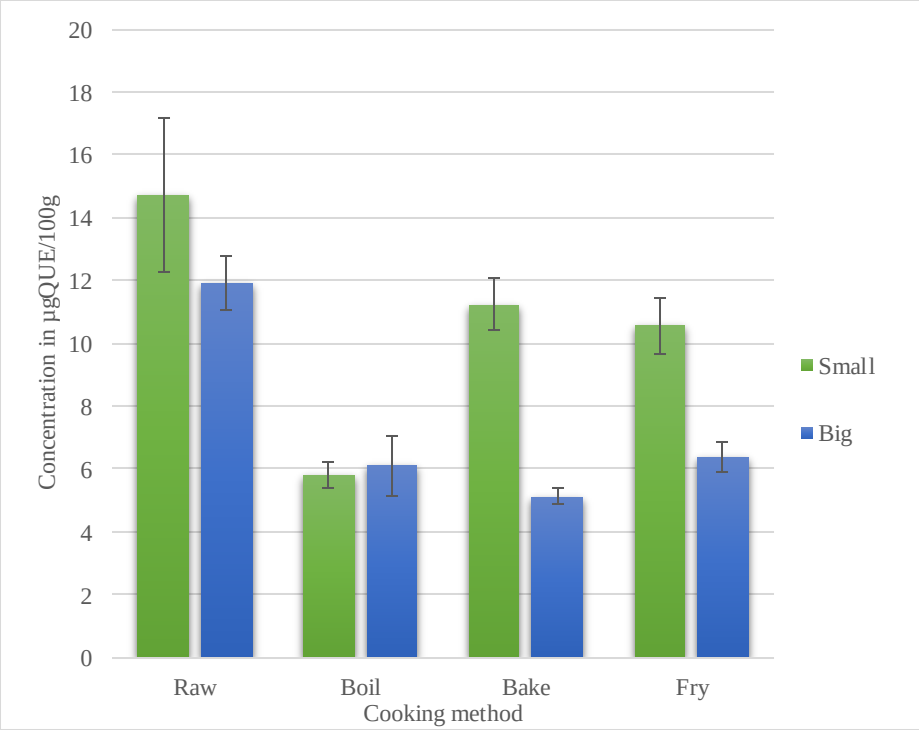

Figure 10: Mean concentration of Flavonoid content of big and small onions following 5 minutes via different cooking methods.

Table 8: ANOVA: single factor for TFC of big onion and small onion samples cooked for 5 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	44.16667	14.72222	5.960648
S-Boil	3	17.5	5.833333	0.173611
S-Bake	3	33.75	11.25	0.694444
S-Fry	3	31.66667	10.55556	0.752315
B-Raw	3	35.83333	11.94444	0.752315
B-Boil	3	18.33333	6.111111	0.925926
B-Bake	3	15.41667	5.138889	0.05787
B-Fry	3	19.16667	6.388889	0.231481

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	266.985	7	38.14071	31.95498	3.01E-08	2.657197
Within Groups	19.09722	16	1.193576			
Total	286.0822	23				

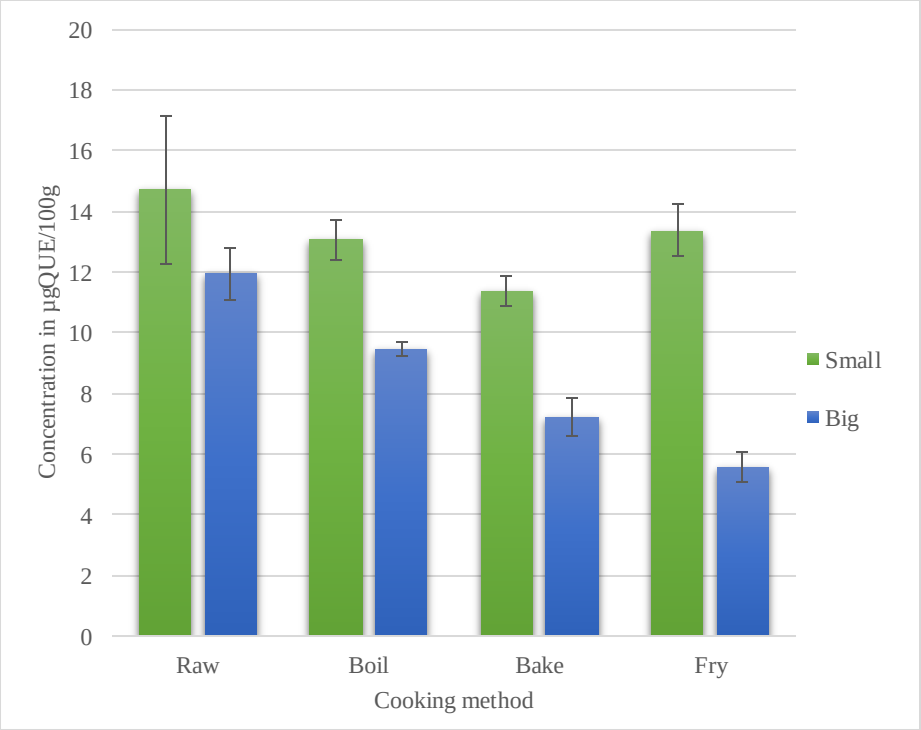

Figure 11: Mean concentration of Flavonoid content of big and small onions following 10 minutes via different cooking methods.

Table 9: ANOVA: single factor for TFC of big onion and small onion samples cooked for 10 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	44.16667	14.72222	5.960648
S-Boil	3	39.16667	13.05556	0.405093
S-Bake	3	34.16667	11.38889	0.231481
S-Fry	3	40.13483	13.37828	0.699134
B-Raw	3	35.83333	11.94444	0.752315
B-Boil	3	28.33333	9.444444	0.05787
B-Bake	3	21.66667	7.222222	0.405093
B-Fry	3	16.66667	5.555556	0.231481

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	212.7165	7	30.38807	27.80526	8.27E-08	2.657197
Within Groups	17.48623	16	1.092889			
Total	230.2027	23				

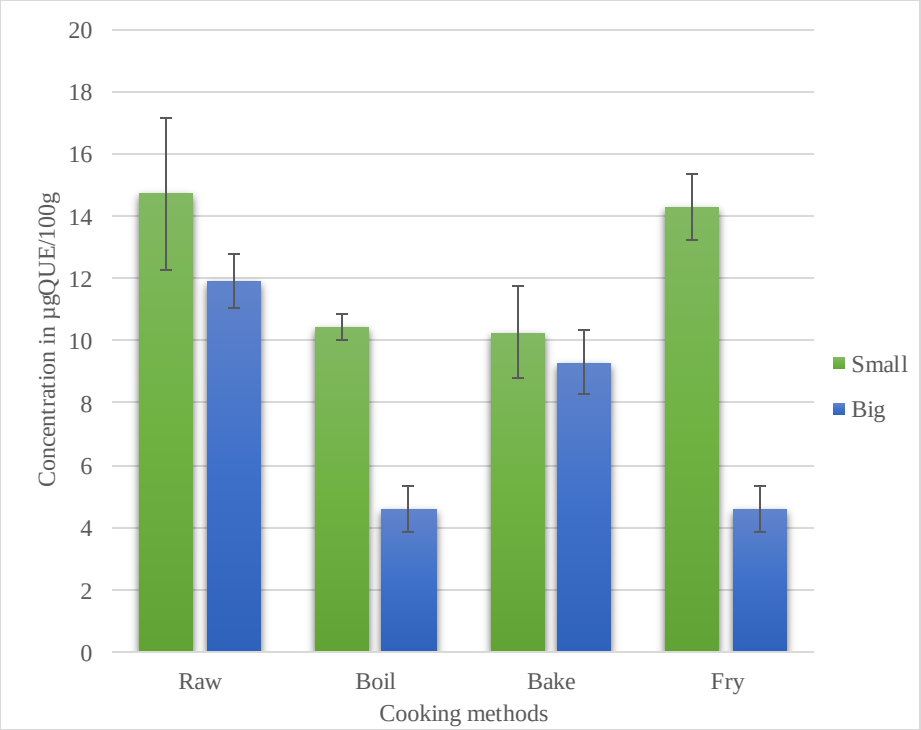

Figure 12: Mean concentration of Flavonoid content of big and small onions following 15 minutes via different cooking methods.

Table 10: ANOVA: single factor for TFC of big onion and small onion samples cooked for 15 minutes.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
S-Raw	3	44.16667	14.72222	5.960648
S-Boil	3	31.25	10.41667	0.173611
S-Bake	3	30.83333	10.27778	2.141204
S-Fry	3	42.91667	14.30556	1.099537
B-Raw	3	35.83333	11.94444	0.752315
B-Boil	3	13.75	4.583333	0.520833
B-Bake	3	27.91667	9.305556	1.099537
B-Fry	3	13.75	4.583333	0.520833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	312.0877	7	44.58395	29.0721	5.99E-08	2.657197
Within Groups	24.53704	16	1.533565			
Total	336.6247	23				

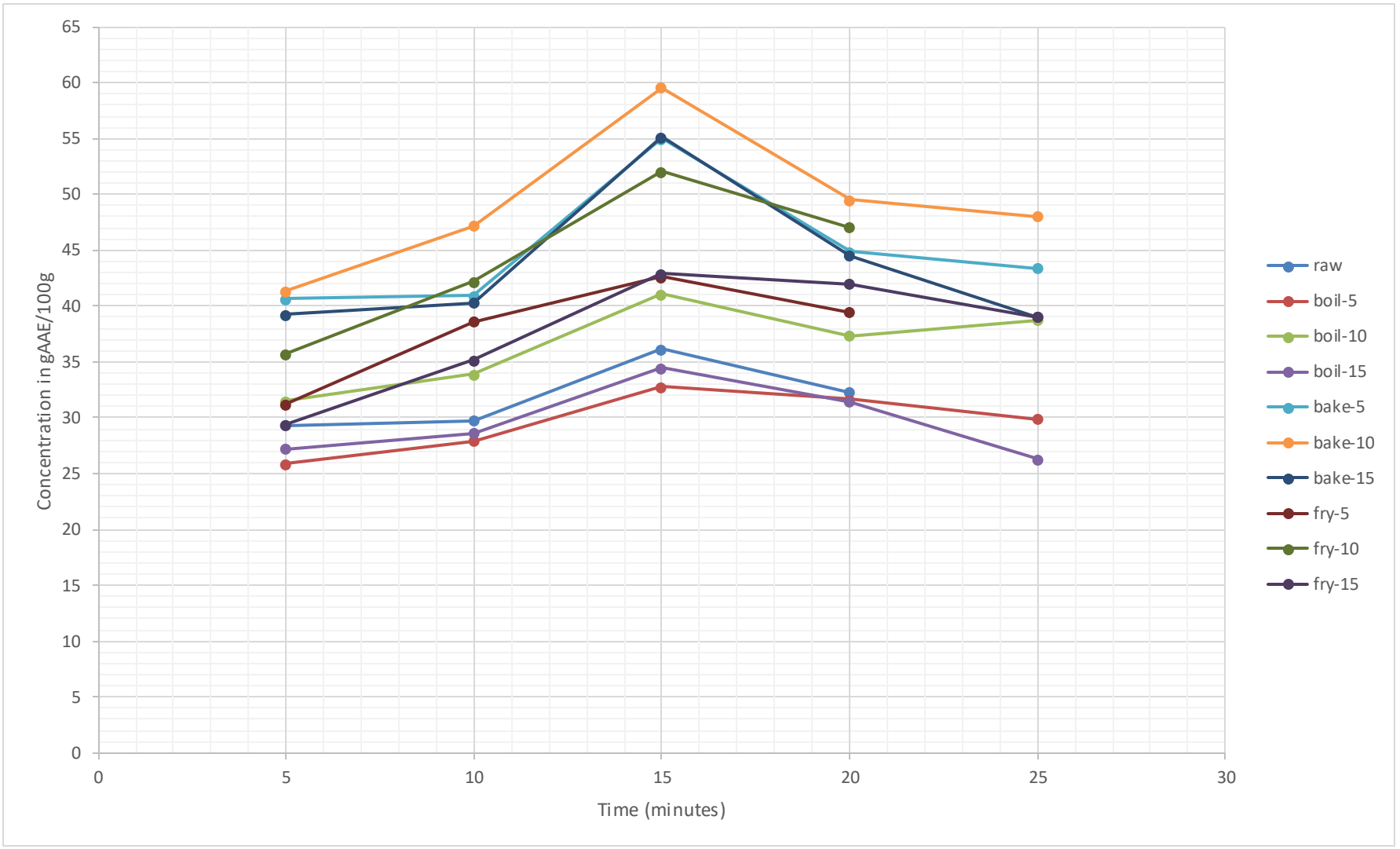

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Figure 13: Mean values of the FRAP assay concentration of small onion samples with all cooking methods in acetic acid equivalents per 100g.

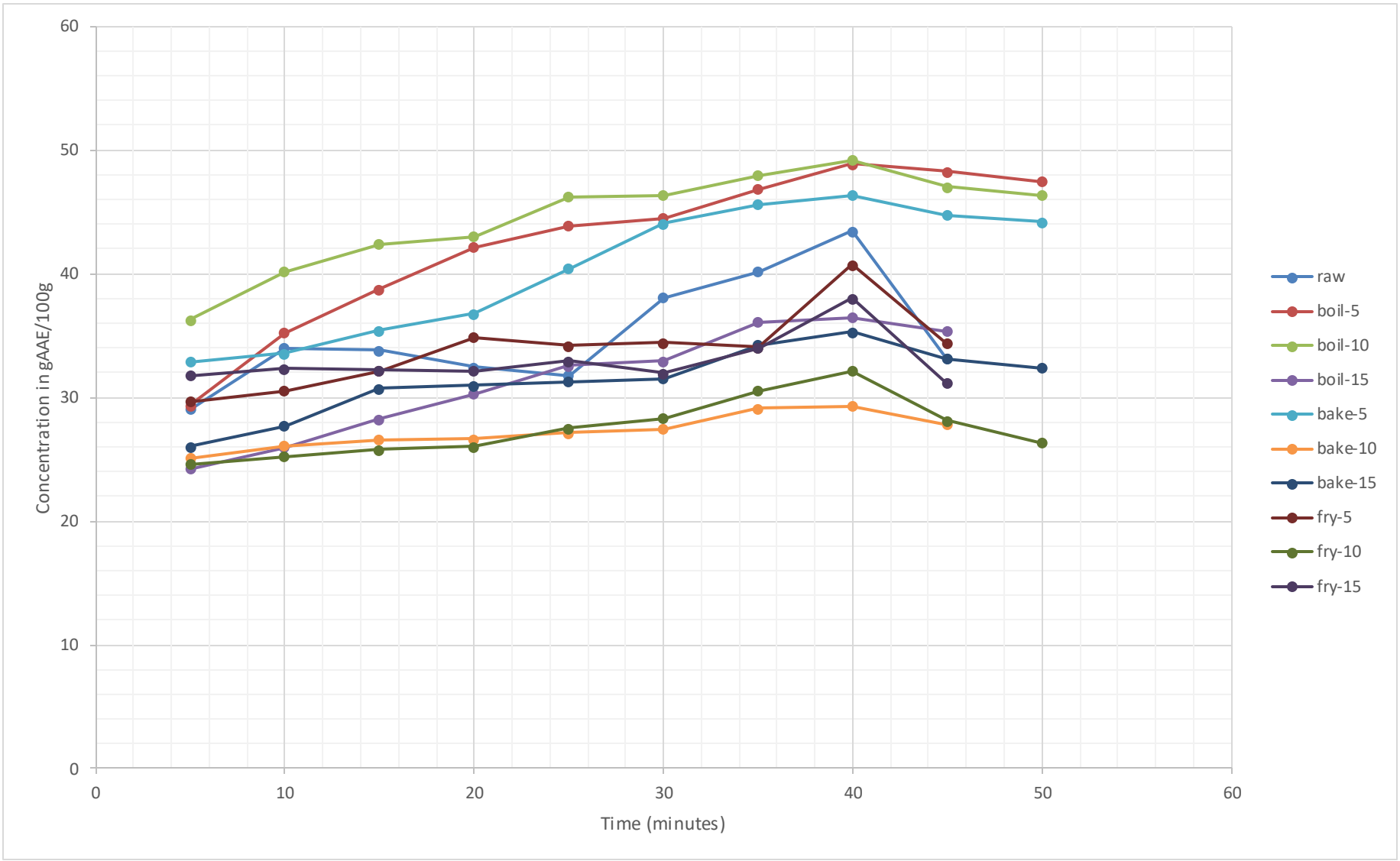
60
50
40
30
20
10
0
Concentration in gAAE/100g
0
10
20
30
40
50
60
Time (minutes)
raw
boil-5
boil-10
boil-15
bake-5
bake-10
bake-15
fry-5
fry-10
fry-15

Figure 14: Mean values of the FRAP assay concentration of big onion samples with all cooking methods in acetic acid equivalents per 100g.

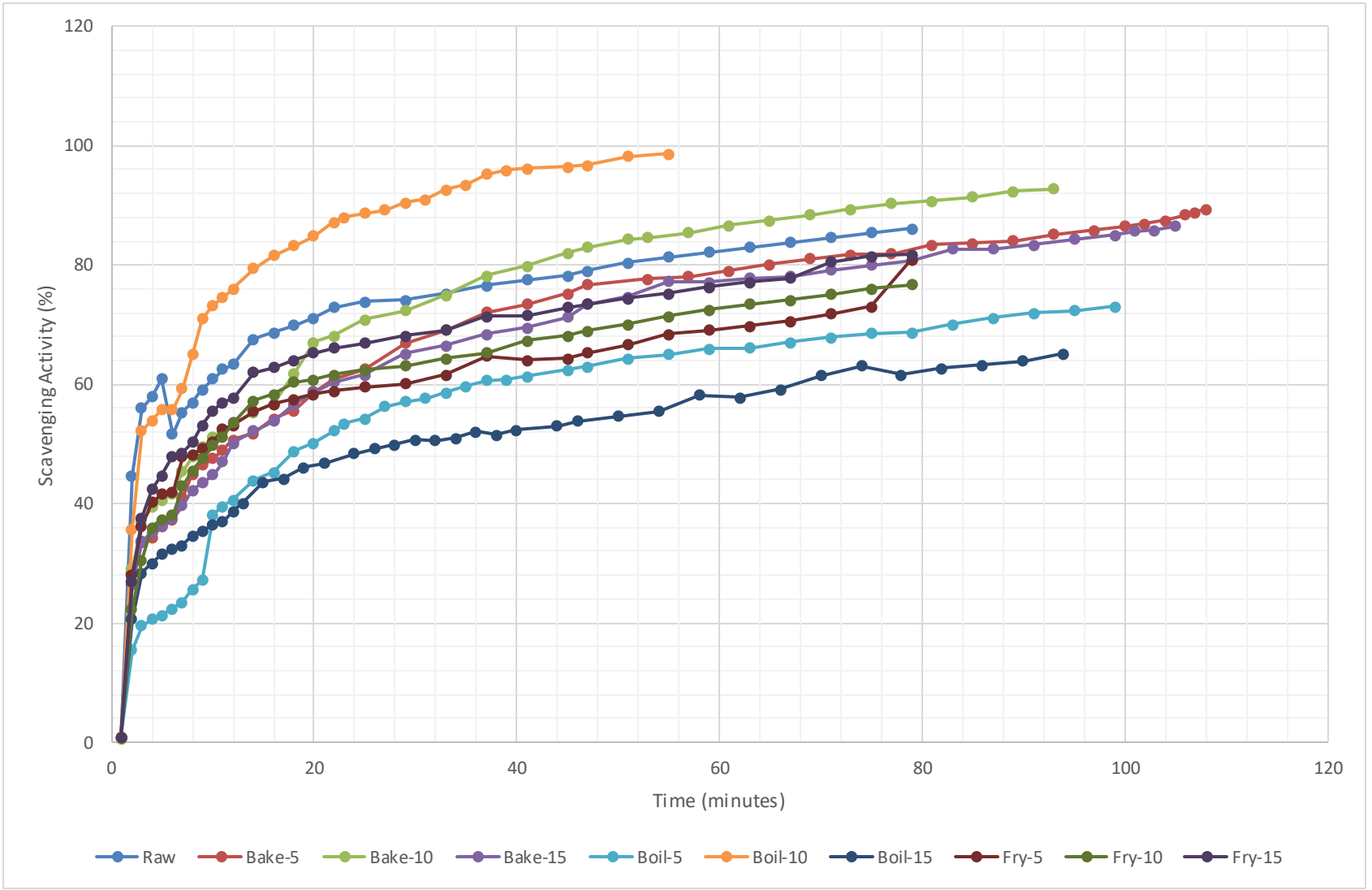

Figure 15: Percentage inhibition of ABTS•+ scavenging activity for big onions samples with all cooking methods in acetic acid equivalents per 100g.

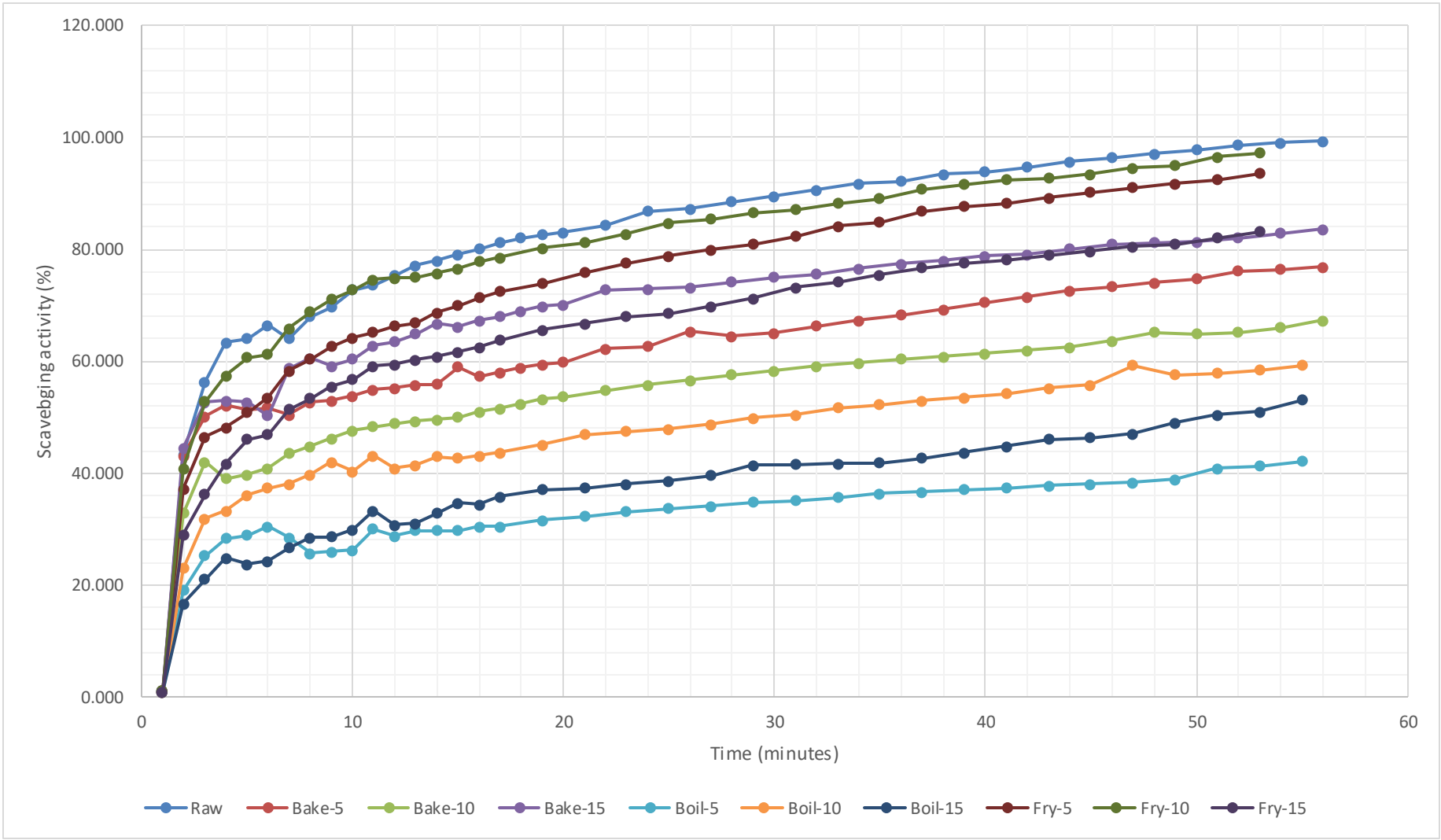

Figure 16: Percentage inhibition of ABTS•+ scavenging activity for small onions samples with all cooking methods in acetic acid equivalents per 100g.

DISCUSSION AND CONCLUSION

The progress in combating disease development due to oxidative stress caused by the imbalance of pro-oxidants and AO have led to numerous research avenues, where the role of onions of the *Allium* species have been evident (Akbarirad *et al.*, 2016). The role of the antioxidative compounds in raw onion have been demonstrated as well as the change in AO activity following processing of vegetables and spices have been researched as well. As example, a brief summary of findings on the effect of storage and processing on food products is shown below.

Table 11: Effect of storage and processing on phytochemical levels in vegetables (Modified from; Tiwari and Cummins, 2013).

Processing/storage	**Fruit, vegetables and food products**	**Effects**
Cooked for 30 min **Stored at 20 °C, 24 weeks**	Strawberry jam Raspberry jam	↓ Quercetin (15%) and Kaempferol (18%) Initial ↑ in free ellagic acid to 4.5 mg / 100 g than ↓ tob2.0 mg / 100 g
High hydrostatic pressure (450 MPa), **15 to 37 °C temperature - 5 min**	Strawberry puree/smoothies	Total phenolics slightly ↑ ~11%
Stored at 22 °C, 16 weeks	Strawberry puree/smoothies	↑ Ellagic acid concentration doubled (0.8 to 1.7 mg GAE / 100 g FW)

Stored at 8 °C, 11 weeks	Strawberry puree/smoothies	Total anthocyanins ↓~39%
Fresh to steaming - 7.5 min	Broccoli	Total phenolics ↑ 18%
Fresh to boiling - 5 min	Broccoli	Total phenolics ↓ 6.2%
Fresh to microwave cooking - 1.5 min	Broccoli	Total phenolics ↑ 25
Blanching - 3 min (96–98 °C)	Cauliflower	Total phenolics ↓ 10–21%; Total aliphatic and indole glucosinolates: ↓31% and 37%
Boiling - 10 to 15 min	Cauliflower - white or green	Total glucosinolates ↓ 36% or 43%
Blanching - 3 min at 80 °C	Cauliflower - white or green	Total glucosinolates ↓ 13% or 3%
Boiling - 60 min	Onion	Total flavonols ↓ 21%
Baking - 15 min	Onion	Quercetin glucosides ↓ 7–2 5%
Microwave cooking - 4 min	Onion	Quercetin glucosides ↓ 16–18%

Strong evidentiary support from studies have presented with possible reasons for the variation of AO levels of vegetables. A study by Jimenez-Monreal *et al.* (2009) stresses on factors such as cooking techniques, extent of temperature, bioavailability of phenols, synergistic activity and efficient utilization of assays in assessment. The effect of cutting, peeling chopping or physical maceration of samples has been reviewed as well (Tiwari and Cummins, 2013).

In terms of cooking methods and or effects of temperature, the change in total antioxidants, phenols and flavonoids in onions have been reported (Sharma *et al.*, 2015; Jimenez-Monreal *et al.*, 2009; Rodrigues *et al.*, 2009; Lee *et al.*, 2008; Ergoder *et al.*, 2007; Nemeth, Takacsova and

Piskula, 2003). Therefore, the effect of different methods of cooking on two commonly consumed onion varieties in Sri Lanka was comparable in terms of antioxidant capacities. Investigations in the current study was based on the radical-scavenging activity and phytochemical content analysis as to determine the antioxidant capacity of the samples.

The TAC value is determined due to the ability of the reducing species present in a sample are capable of producing Mo (V) from Mo (VI) present in the reagent to react with phosphate in order to form a green colored complex, which allows quantification of the reducing species by photometric methods (Prieto, Pineda and Aguilar, 1999).

The mean TA concentration results of the all the samples derived from big and small onion varieties are seen in figure 1 and are expressed in g GAE/100g. The big onion variety of onions showed a higher content of AO than the small onion variety when at raw. The overall highest TAC was measured in big onions which were fried for 15 minutes. Also an overall increase in AO levels as the time period of the cooking treatment increased from 5 minutes to 15 minutes showed a linear increasing trend in both small and big onion varieties. In all the samples that were boiled or fried the TAC of big onion variety was higher than of smaller onions. However, the pattern was reversed in all three baked samples where the small onions showed high TAC concentrations than big onions.

Sample TAC analysis after 5 minutes of cooking either boiling, baking or frying revealed comparatively reduced amounts of AO in comparison with both raw samples (Figure 2). This was true for both varieties except for the baked sample and the raw sample of the small onion variety which seemed to overlap. The ANOVA output (Table 2) revealed a, $p < 0.05$ (P- 2.41E-07) and F-crit < F-value (F- crit-2.657197, F-23.94702) indicating a significant difference between all three cooking methods at 5 minutes. Hence frying big onions for 5 minutes showed the least loss in AO compared to raw sample levels, whilst the largest AO decrease was seen when boiling small onions for 5 minutes.

Analysis of samples cooked for 10 minutes (Figure 3) showed that, small onions had little or no decrease of TAC levels in comparison to raw sample. In big onions however a loss in AO was seen when boiling and cooking for 10 minutes and a noticeable increment in AO was seen in the fried sample. The statistical analysis values of, $p < 0.05$ (P- 6.07E-14) and F-crit < F-value (F-crit – 2.657197, F- 176.8413) implied that the results observed were significant between all the samples at 10 minutes (Table 3).

Samples cooked for 15 minutes (Figure 4) showed that, small onion variety has not shown little or slight decrement relative to TAC of raw sample, whilst baked and boiled samples of big onion variety showed decrement in TAC. Contrastingly frying for 15 minutes showed the largest increase in TAC. The statistical analysis values of $p<0.05$ (P-0.00044) and F-crit < F-value (F-crit – 2.657197, F- 7.500936) indicated that the findings were significant within all the cooking methods carried out on both varieties (Table 4).

The total phenolic content measurement via the FC reagent which in the presence of phenols forms a Mo (V) complex reflective of the concentration of phenols in the sample thus allowing the quantification of the phenol content (Everette *et al.*, 2010).

The figure 5 containing all samples showed TPC concentrations in mg GAE/ 100 g where, small onion variety of raw samples had higher concentration than big onion variety. The overall trend of small onion variety from 5 minutes of cooking to 15 minutes depicted an increase in

TPC values where small onions fried for 15 minutes reported the highest TPC concentration. Also the lowest TPC content was seen when boiling small onions for 5 minutes. Contrastingly the TPC values of samples of big onion variety showed a relative pattern of loss in phenolic content as the cooking time increased in comparison to raw big onion sample TPC concentration.

In comparison to raw samples, at 5 minutes of cooking (Figure 6) a general loss in phenolic content is seen in both varieties. The least TPC content was seen in small onions boiled for 5 minutes. Analysis of significance of various cooking methods for 5 minutes proved significant with $p<0.05$ (P-2.48E09) and F-crit<F-value (F-crit-2.657197, F-4473628) (Table 5).

Moreover, at samples cooked for 10 minutes (Figure 7), in comparison to the raw sample of small onion variety all three cooking methods showed a decrease in TPC. Nevertheless, the boiled big onion sample showed an increase in TPC relative to the raw TPC concentration. The findings was deemed significant as the ANOVA analysis showed $p<0.05$ (P-0.000312) and F-crit< F- value (F-crit- 2.657197, F-7.968185) for the TPC of all samples prepared using different cooking methods at 10 minutes (Table 6).

Samples cooked for 15 minutes (Figure 8) showed that in contrast to raw samples of both varieties boiling and baking revealed lower TPC concentrations. Whereas fried samples of both varieties showed a significant increase in TPC. The TPC results showed high significance with a $p<0.05$ (P-3.14E-10) and F-crit < F-value (F-crit-2.657197, F-58.79045) between all cooking methods at 15 minutes (Table 7).

Total flavonoid content measured via the aluminum chloride technique employs quantification of colored complex formation of flavonoid in sample with $AlCl_3$ provided via the reagent mixture. The formation of complexes in this technique are unique to flavonoids present after acid hydrolysis and extraction via organic solvent (Fernandes *et al*., 2012).

The TFC concentration was measured in µg QUE/100 g in all the samples (Figure 9). The raw sample of small onions contained the highest TFC concentration. Also the overall trend from 5 minutes of 15 minutes of cooking showed a slight elevation of TFC content in the small onion variety, whereas big onions showed an overall decrease in TFC content when cooked for longer periods of time using either technique in comparison with the raw TFC values. The TFC content in all cooking treatments was higher in small onions in comparison with big onions, where the boiling for 5 minutes' treatment was the only reversed exception.

At 5 minutes of cooking, (Figure 10) all samples showed a significant decrease with $p < 0.05$ (P-3.01E-08) and F- crit < F- value (F-crit-2.657197, F- 31.95498) in TFC values as indicated by statistical analysis (Table 8). The baking and frying methods showed relatively higher TFC content in small onions than big onions and contrastingly in boiled samples big onions showed marginally higher TFC content than small onions.

Whereas at 10 minutes (Figure 11) a similar decrease in comparison to the raw samples were seen, with a relatively lower loss of TFC in comparison to 5 minutes. In all cooking methods the TFC content was higher in small onion in comparison with big onions. The results were statistically significant where $p<0.05$ (P-8.27E-08) and F-crit< F-value (F-crit-

2.657197, F-27.80526) between all TFC measurements of different cooking methods at 10 minutes (Table 9).

The TFC content after cooking the samples for 15 minutes (Figure 12) showed a slight increase in concentration in baked big onion samples, nevertheless an overall decrease in TFC in comparison to raw big onion sample. Boiling and baking of small onions also showed a loss in TFC and no decrease in TFC for the fried sample. The findings were statistically significant with $p<0.05$ (P-5.99E-08) and F-crit< F- value (F-crit-2.657197, F-29.0721) within all cooking methods for 15 minutes using both onion varieties (Table 10).

The AO compounds efficacy was monitored via the FRAP assay (Figures 13, 14) which were expressed in AAE / 100g. The concentration of AO present in the sample is reflected by the Fe^{3+} to Fe^{2+} transformation in the presence of TPTZ ligand to form Fe^{2+} - TPTZ which can be analyzed by photometry (Apak *et al.*, 2016). In the small onion samples, the peak activity absorbance was seen at 15 minutes in all ten samples subjected to various cooking treatments. The sample with the highest concentration was the bake-10 sample, which was considered to be the maximum ferric reducing activity from the small onion samples. For the samples made from big onions all the samples showed peak activity at 40 minutes except for the samples, boil-15 and bake-10 which peaked at 20 minutes. Thereby the highest activity for big onions was reported from the sample boiled for 10 minutes at forty minutes.

The ability of radical scavenging of AO present in the sample is assessed by using cation radicals of ABTS•+ as colorimetric probes. The potential of the AO compound to decolorize the ABTS•+ solution by preventing production or reacting with existing radicals is acknowledged as the AO capacity (Apak *et al.*, 2016).

The ABTS•+ radical percentage inhibition was measured to compare and contrast the AO potential of the samples (Figures 15, 16). The most efficacious activity was seen in the sample boil-10 of big onions where the highest percentage inhibition of 100% at was reported at 55 minutes. In the small onion samples, the raw sample reported the highest activity by 97% inhibition at 50 minutes. The results depict the ability of the hydrogen donating ability of the samples over time, thus depicting high antioxidant capacity.

In general, increases in phenolic content following boiling can be attributed to the inactivation of oxidative enzymes. Also the reduction of flavonoid content after 5 minutes of boiling (Lombard *et al.*, 2005) was reported where the loss of TFC when boiling was attributed to the leaching of quercetin to cooked water rather than chemical breakdown of compounds.

Increases in AO levels have been related with factors such as; release of AO due to destruction of cell wall and relevant sub-cellular compartments and formation of nonnutrient antioxidants in AO reactions (Jimenez-Monreal, *et al.*, 2009).

Quercetin the main flavonol concerned has showed gains and losses in previous studies. Frying or sautéing for 5 minutes have shown considerable decrease in TFC (Ewal *et al.*, 1999; Crozier *et al.*, 1997) and decrease in flavonoids after 15 minutes was also reported (Price *et al.*, 1997). These findings are considerably seen in the current study as well. Several studies report the process of frying for over 15 minutes has a considerable decrease in TFC but reports high levels of TAC due to the color change by browning effect (Lombard *et al.*, 2005). The loss of flavonoids in terms of cooking methods, show that most studies report a

greater loss of TFC by boiling rather than baking (Palermo, Pellegrini and Fogliano, 2013).

Hence the combined analysis of the assays showed evidence of considerable variation of TPC, TFC and TAC between small and big onion varieties. Although the TAC of big onions was considerably high, the active AO compound, quecertin was more concentrated in samples of small onion which was evident from the high levels of TFC reported. Hence the radical scavenging ability deduced via ABTS•+ and FRAP assays which showed high activity by small onion samples correlates with the high concentration of TFC present in small onions in comparison to big onions.

In addition, when considering the cooking methods tested the current study correlated with most previous literature, where boiling showed the highest AO loss followed by frying and the least AO loss via baking methods. When considering boiling the optimum time period for big and small onion varieties was at 10 minutes. The exact effect of frying needs further analysis by quantification of active AO compounds as the actual results showed evidence of masking due to browning effect. The overall cooking time however shows a linear increment with increased cooking time in TFC, TAC and TPC levels for small onion samples. Although the big onion variety showed increasing TAC levels, the pattern was not replicated in TFC and TPC levels.

It can be concluded that consuming small onions is most efficacious in providing active AO compounds such as quercetin in high concentrations in comparison with big onion variety. In terms of cooking method AO loss can be seen as boiling>frying>baking when comparing current results with previous literature and the optimum time for highest AO activity of cooked compounds remains around 10 minutes. This experimental data is suggestive of the ability of onions in radical scavenging as an AO source in order to assist in the prevention of free-radical mediated diseases.

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