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Evaluation of Heat Processing Temperature and Time on Functional Properties of Garlic Juice

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Abstract

To develop a functional juice using garlic (*Allium sativum* L.), heated garlic juice was investigated using a central composite design set with variables of treatment temperature (110, 115, 120, 125, and 130°C) and time (1, 2, 3, 4, and 5 hr) using high temperature and pressure treatment apparatuses. Total soluble solid, total acidity, reducing sugar, total pholyphenol contents, electron donating activity (EDA), and ascorbic acid equivalent antioxidant capacity (AEAC) in heated garlic juice were increased with increasing heating temperature and time. The highest total soluble solid content was 17.81 °brix at 123.10°C for 1.10 hr. The highest total acidity was 1.43% at 127.35°C for 4.35 hr. The highest reducing sugar content was 86.67 mg/mL at 119.90°C for 4.35 hr. The highest total polyphenol content was 8.42 mg/mL at 127.75°C for 4.26 hr. The highest EDA and AEAC were 60.09%, and 7.40 mg AA eq/mL at 127.85°C for 4.23 hr, and 128.10°C for 4.18 hr, respectively

Key words: heated garlic juice, total soluble solid, total acidity, reducing sugar, antioxidant activity

INTRODUCTION

Garlic (*Allum sativum* L.) has been used as a valuable healing agent worldwide for thousands of years (1). Its consumption has become widely accepted as a general dietary component for promoting overall human health in Eastern Europe and Asia. Many beneficial health- related biological properties are attributed to garlic, including anticarcinogenic, antitumorigenic, antimutagenic, cardiovascular protective, antimicrobial, immunomodulatory and antioxidant effects (2-5), and much of the health-promoting and related biological activities of garlic have been ascribed to the organosulfur compounds (6). Thiosulfinate is generated only after the vegetable tissues are injured, and the enzymes react with their substrates. Naturally, heating at high temperatures decreases the antimicrobial activity of garlic (2) due to the inactivation of the enzyme alliinase by heat. There are published reports that heat has an effect on the bioactive compounds present in garlic. It has been shown that the antimicrobial activity of garlic decreased as the heating temperature increased. This fact suggests that alliinase may be the most critical factor for producing activity when garlic is heated (7).

Several studies have been performed to test the effect of heating on several garlic properties including the antioxidant properties. Boiling garlic cloves for 15 min significantly impairs their ability to inhibit cyclooxygenase activity (8) and thromboxane B2 synthesis (9). In addition, heating garlic cloves for 60 s in a microwave reduces their anticancer properties (10). Interestingly when microwave heating was applied 10 min after garlic crushing the anticancer properties were preserved, indicating that allinase activation is necessary to generate anticancer compounds which are thermostable (10,11). There are few processed foods available using garlic. Production techniques for garlic-processed foods such as bread, wheat-gluten, jam, soybean paste, pills, vinegar, bean curd, and tea have been developed, but they are rarely incorporated into commercial products. Considering garlic's various effects on physiological activity, there is a gross lack of garlic-processed foods that can be easily consumed.

In preceding studies, evaluating the preparation of garlic juice under high temperature and pressure, as temperature increased, various antioxidant substances also increased. However, preference showed a decreasing trend after 130°C, as did the acidity; possibly because acidity increased along with a sour taste with increases in heat temperature and time, also the effect of the burned taste that resulted from long term heat treatment

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decreased its likeability as a beverage (12). Therefore, this study aimed to increase the temperature increment by 5°C within the range of 110～130°C, heat treat for 1, 2, 3, 4, and 5 hr to measure total soluble solid, acidity, reducing sugar, total polyphenol, electron donating ability (EDA) and ascorbic acid equivalent antioxidant capacity (AEAC) to assess the possibility of high temperature and pressure treatment garlic juice.

MATERIALS AND METHODS

Sample preparation

Garlic, *Allium sativum* L., was purchased from the Chungbuk Agriculture and Marine Products Market in June 2006 and stored at -20°C. The garlic was placed into a sample bottle and sealed tightly. The sample bottle was inserted into the instrument and heat treatment was performed using high-pressure steam generated by a temperature and pressure controlling apparatus (model HR-8200, Jisico, Seoul, Korea) (13). The samples (100 g) were heated to temperatures of 110, 115, 120, 125, and 130°C for 1, 2, 3, 4, and 5 hr. The heated samples were homogenized in 100 mL of deionized water and centrifuged at 3,000 rpm for 10 min, and then supernatants filtered through a 0.45 μm syringe filter (Millipore, Billerica, MA, USA). Raw garlic was subjected to the same process as heated garlic, except for heating. The garlic juice was kept at -20°C until analysis.

Experimental design for response surface methodology

The experimental design for extraction conditions was a control composite design using SAS software for response surface methodology (RSM) analysis. Independent parameters, extraction temperature (110, 115, 120, 125, and 130°C, X_1) and extraction time (1, 2, 3, 4, and 5 hr, X_2) were assigned (-2, -1, 0, 1, and 2), and 10 intervals were set on the central composite design for the extraction experiment (Table 1). The response parameters (Yn): total soluble solid content, total acidity, reducing sugar, total polyphenol content, EDA, and AEAC as affected by the independent parameters were determined three times, and their average values were used for the regression analysis.

Physicochemical analysis

Total soluble solids were measured using an Atago digital refractometer (model RP-101, Tokyo, Japan). Refractive index was recorded and converted to °brix. Measurements were performed at 20.0±0.5°C. The refractometer prism was cleaned with distilled water after each analysis. Total acidity was measured by titrating with 0.1 N NaOH up to pH 8.2 and expressed as percentage of acetic acid (14). Reducing sugar content was measured at 546 nm with a UV/VIS spectrophotometer (UV-1601, Shimadzu, Tokyo, Japan) by dinitrosalicylic acid (DNS) method (15). Properly- diluted sample solution (1mL) was mixed with DNS solution (1 mL), heated in a boiling water bath for 10 min, frozen quickly, and then mixed with distilled water for measurement. The reducing sugar content was calculated from the glucose standard curve obtained by the above-mentioned methods.

Total polyphenol content

The amounts of total phenolic compounds in garlic juice were measured using a UV/VIS spectrophotometer at a wavelength of 700 nm, according to the modified Folin-Ciocalteu method (16). The gallic acid level was used as a reference, and the phenolic compound level was calculated based on a standard curve.

DPPH radical scavenging activity

The scavenging activity for the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical was evaluated using the method of Yen and Chen (17), at a wavelength of 517 nm with a UV/VIS spectrophotometer. The percentage inhibition of activity was calculated as $(A_0-A_1)/A_0\times 100$, where A_0 is the absorbance without the sample and A_1 is the absorbance with the sample.

ABTS radical scavenging activity

The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activites of the extracts and ascorbic acid as a control were determined according to Re et al. (18), with some modifications. The ABTS radical cation scavenging activity was expressed as the AEAC in milligrams of ascorbic acid equivalents per milliliter sample.

Statistical analysis

All data were expressed as mean±SD. Analysis of variance (ANOVA) and Duncan's multiple range tests (at $p<0.05$) were performed by using the SAS software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Total soluble solid contents

Table 1 shows the total solids content of garlic juice that was heated under each treatment condition. The total soluble solid content in raw garlic juice was 16.8 °brix. The soluble solid content according to extraction temperature increased gradually until 120°C for 3 hr, and the highest soluble solid content of 18.00 °brix occurred with heating at 120°C for 1 hr, but decreased after 130°C. The maximum and optimal conditions for increasing soluble solid content were heating at 123.10°C for 1.10

Table 1. Experimental data on total soluble solid content, total acidity, reducing sugar, polyphenol, EDA and AEAC of heat treated garlic juices by central composite design for response surface analysis

Exp. No.	Independent variables		Response variables					
	Temp. (°C)	Time (hr)	Brix (%)	Acidity (%)	Reducing sugars (mg/mL)	Polyphenol (mg/mL)	EDA (%)	AEAC (mg AA eq/mL)
1	110 (−2)	3 (0)	16.90±0.69[1)bc2)]	0.47±0.07[ab]	28.73±1.24[c]	1.15±0.08[a]	7.79±1.24[c]	1.09±0.02[a]
2	115 (−1)	2 (−1)	16.80±0.69[b]	0.68±0.04[c]	29.27±1.27[c]	1.38±0.17[a]	4.16±0.45[b]	1.43±0.06[b]
3	115 (−1)	4 (+1)	17.89±0.18[cd]	0.70±0.04[c]	66.30±3.28[e]	2.46±0.18[b]	19.33±1.33[d]	2.41±0.16[c]
4	120 (0)	1 (−2)	18.00±0.30[d]	0.52±0.03[b]	19.70±0.07[b]	1.15±0.11[a]	8.13±2.46[c]	1.21±0.08[ab]
5	120 (0)	3 (0)	17.30±0.62[bcd]	0.81±0.10[cd]	79.06±0.68[fg]	2.92±0.30[b]	23.20±2.08[e]	3.23±0.20[d]
6	120 (0)	3 (0)	17.30±0.62[bcd]	0.81±0.10[cd]	79.06±0.68[fg]	2.92±0.30[b]	23.20±2.08[e]	3.23±0.20[d]
7	120 (0)	5 (+2)	16.50±0.52[ab]	0.94±0.10[de]	81.56±1.72[g]	5.44±0.12[c]	41.04±1.03[f]	5.16±0.04[e]
8	125 (+1)	2 (−1)	17.07±0.83[bcd]	0.78±0.06[cd]	76.31±1.82[f]	2.76±0.48[b]	22.64±0.40[e]	3.20±0.23[d]
9	125 (+1)	4 (+1)	16.67±0.46[ab]	1.36±0.14[f]	80.85±1.82[g]	6.47±0.39[d]	49.13±2.83[g]	5.70±0.33[f]
10	130 (+2)	3 (0)	15.70±0.46[a]	1.02±0.10[e]	44.27±1.80[d]	6.55±0.36[d]	48.53±1.30[g]	6.29±0.26[g]
Control			16.80±0.30[ab]	0.34±0.01[a]	11.36±0.21[a]	1.03±0.08[a]	1.37±0.02[a]	0.92±0.03[a]

[1)]Each value is mean±SD (n=3). [2)]Any means in the same column followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple range test.

hr (17.81 °brix), and 120.65°C for 2.01 hr (17.52 °brix), respectively. Although it showed that as heat-treatment temperature and time increases, the total solid content tends to gradually decrease; total solid content increased with short term heat-treatment at low temperatures. On the contrary, total solid content tends to decrease as heat-treatment time increased at high temperature (Fig. 1). This is considered to be due to an eventual decrease in macromolecule saccharides such as fructans which were degraded at high temperatures and gradually increased in number due to heat treatment (12). Jeong (19) reported that the soluble solid content according to extraction temperature increased gradually until 60°C and was highest at 70°C, but decreased after 80°C. As a whole F-value was affected by heat-treatment temperature more than time ($p<0.01$, Table 2). R^2 for the total solid content was appeared with 0.5216 (Table 3) and model (lack of fit) was suitable ($p>0.07$).

Total acidity

Table 1 shows the total acidity of garlic juice that was heated under each treatment condition. The total acid level of raw garlic juices is as low as 0.34%; but depending on heat treatment conditions, it was 0.47% up to a maximum of 1.36%. The total acid level of heated garlic juices tends to increase as heat-treatment temperature and time increase. The highest total acidity of 1.36% occurred with heating at 125°C for 4 hr, and was 4-fold higher than raw garlic juices. The maximum and minimum conditions determining total acidity were heating at 127.35°C for 4.35 hr (1.43%), and 120.80°C for 3.13 hr (0.92%), respectively. Increase in temperature and heat-treatment time tends to show an increase in acidic level as well (Fig. 2). This is considered to be a result of an increase in total acid level due to conversion of fructose and glucose in garlic into organic acid by heat treatment. Choi et al. (20) reported that

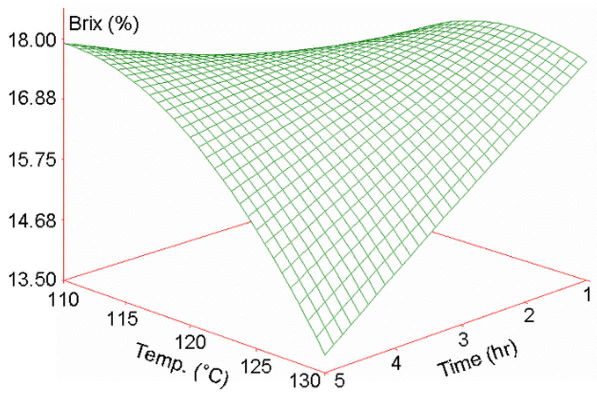

Fig. 1. Effects of temperature and time on the total soluble solid contents of the heated garlic juice.

Table 2. Regression analysis for regression model of physiochemical properties of heat treated garlic juices

Variable	F-value					
	Total soluble solid content	Total acidity	Reducing sugar content	Total polyphenol content	EDA	AEAC
Heating temperature (°C)	6.90**	4.85***	51.84***	347.00***	266.78***	509.76***
Heating time (hr)	2.69	3.45***	60.86***	238.01***	181.91***	278.93***

**$p<0.01$, ***$p<0.001$.

Table 3. Regression coefficients of the second order polynomials for total soluble solids content, total acidity, reducing sugar, polyphenol, EDA and AEAC with heat treatment conditions

Parameter[1)]	Estimate					
	Total soluble solid content	Total acidity	Reducing sugar	Total polyphenol	EDA	AEAC
Intercept	17.34***	0.88***	80.13***	2.93***	22.64***	3.14***
X_1	-0.28*	0.15***	7.72***	1.35***	10.81***	1.29***
X_2	-0.19	0.12***	13.77***	1.11***	8.96***	0.95***
$X_1 \times X_1$	-0.25**	-0.02	-10.77***	0.23***	1.31***	0.13***
$X_1 \times X_2$	-0.37*	0.15***	-8.12***	0.66***	2.83***	0.38***
$X_2 \times X_2$	-0.02	-0.03	-7.24***	0.09*	0.42	-0.00
R-square	0.5216	0.8556	0.9216	0.9859	0.9822	0.9898
Lack of fit	$p>0.07$	$p<0.01$	$p<0.001$	$p>0.6$	$p<0.001$	$p>0.07$

[1)] X_1: temperature (°C), X_2: Time (hr). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

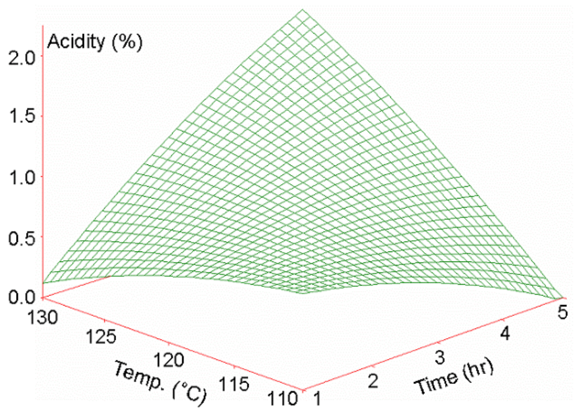

Fig. 2. Effects of temperature and time on the total acidity of the heated garlic juices.

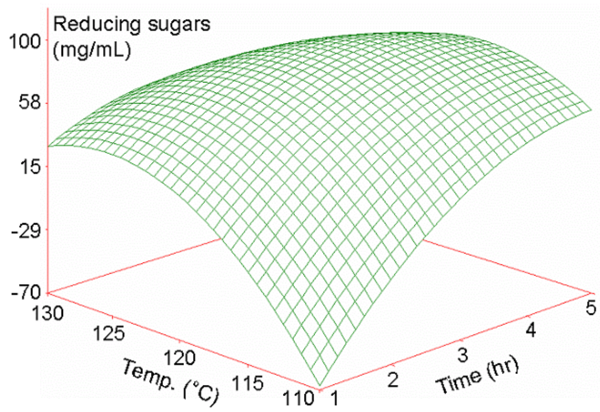

Fig. 3. Effects of temperature and time on the reducing sugar contents of the heated garlic juices.

the total acidity of heated pear juices increased at 100°C for 30 min. Akinyele et al. (21) also observed an increase in the total acidity after orange juice pasteurization, but Zarate and Ortega (22) did not find variations in total acidity of PEF-treated apple juice when compared with an untreated sample. As a whole F-value was affected by heat-treatment temperature more than heat-treatment time ($p<0.001$, Table 2). R^2 for the total acidity was 0.8556 (Table 3) and the model was not suitable ($p<0.01$), and was affected by the heat-treatment temperature more than heat-treatment time ($p<0.001$,Table 3).

Reducing sugar

Table 1 shows the effects on reducing sugar of heating the garlic juice. The content of reducing sugar of heated garlic juices tends to increase as heat-treatment temperature and time increases. The reducing sugar of garlic juice heated at 120°C for 5 hr was highest at 81.56 mg/mL, which was 7-fold higher than raw garlic juices. The maximum and minimum heating effects on reducing sugar content were at 117.55°C for 3.00 hr (80.96 mg/mL), and 119.90°C for 4.35 hr (86.67 mg/mL), respectively. As heat-treatment temperature increases, content of reducing sugar sharply increased (Fig. 3), and this can be interpreted as that the polysaccharides in garlic were broken down by the heat which, in turn, liberates reducing sugars such as glucose, fructose and maltose (20). Among them, it is considered that fructan, a plentiful fructose polymer in garlic, was been broken down by the heat, increasing the content of fructose (12). As a whole, the F-value was affected by heat-treatment time more than heat-treatment temperature ($p<0.001$, Table 2). R^2 for the reducing sugar was 0.9216 (Table 3) and model was not suitable ($p<0.001$), and was affected by the heat-treatment time more than temperature ($p<0.001$, Table 3).

Total polyphenol contents

Phenolic compounds are secondary metabolic products that occur throughout the plant kingdom. They contain the phenolic hydroxyl group, which has an antioxidative effect via interactions with the phenol ring and its resonance stabilization effect (23). Table 1 shows the total polyphenol contents of garlic juice that was heated under different conditions. The total polyphenol content in raw garlic juices was 1.03 mg/mL. The highest total polyphenol content of 6.55 mg/mL occurred with heating at 130°C for 3 hr. The maximum and minimum heating effects on total polyphenol content were heating at 127.75°C for 4.26 hr (8.42 mg/mL), and 120.75°C for 3.13 hr (3.30 mg/mL). The optimal condition was heating at 124.65°C for 3.76 hr (5.75 mg/mL). Dewanto et al. (24) suggested that there was a significant increase in soluble phenolic compounds in sweet corn after heating. Stewart et al. (25) reported that heat treatment increased the level of free flavonols because heating released bound phenolic compounds through the breakdown of cellular constituents. The total polyphenol content of heated garlic juices increased significantly with increased heat-treatment temperature and time (Fig. 4). As a whole, the F-value was affected by heat- treatment temperature more than heat-treatment time ($p<0.001$, Table 2). R^2 for the polyphenol content was 0.9859 (Table 3) and the model was suitable ($p>0.6$), and was affected by the heat-treatment temperature more than heat-treatment time ($p<0.001$, Table 3).

DPPH radical scavenging activity

Antioxidants decrease the absorbance of the DPPH radical by scavenging the radicals by hydrogen donation; this is a visible change from purple to colorless (26). Table 1 shows the DPPH radical scavenging activity of garlic juice that was heated at each temperature and time. Raw garlic exhibited no radical scavenging activity at 5 mg/mL. Heated garlic juices in 5 mg/mL concentrations showed high scavenging activities of 49.13% for juice heated at 125°C for 4 hr and 48.53% at 130°C for 3 hr. The maximum and optimal condition for DPPH radical scavenging activity were heating at 127.85°C for 4.23 hr (60.09%) and 124.70°C for 3.75 hr (42.86). Kwon et al. (12) reported that the DPPH radical scavenging activity (IC_{50}) with heating at 120°C for 2 hr (1.19 mg/mL). Several studies have reported the effects of heating on the antioxidant activity of various foods. For example, the antioxidant activity of tomato (25), Shiitake mushroom (*Lentinus edodes*) (27), sweet corn (24), and citrus peels (23) increased, depending on the heating temperature and time. Halvorsen et al. (28) reported large increases in antioxidant activity for several vegetables after microwaving, steaming and boiling. The DPPH radical scavenging activity of heated garlic juices increased significantly with increased heat-treatment temperature and time (Fig. 5). As a whole, the F-value was affected by heat-treatment temperature more than heat-treatment time ($p<0.001$, Table 2). R^2 for the DPPH radical scavenging activity was 0.9822 (Table 3) and the model was not suitable ($p<0.001$), and was affected by the heat-treatment temperature more than time ($p<0.001$, Table 3).

ABTS radical scavenging activity

Table 2 shows the ABTS radical scavenging activity of garlic juice that was heated at each treatment condition. The ABTS radical scavenging activity of raw garlic juice was 0.92 mg AA eq/mL. The highest ABTS radical scavenging activity of 6.29 mg AA eq/mL occurred with heating at 130°C for 3 hr. The maximum, minimum and optimal condition on ABTS radical scavenging activity were heating at 128.10°C for 4.18 hr (7.40 mg AA eq/mL), 120.96°C for 3.12 hr (3.47 mg AA eq/mL), and 124.85°C for 3.71 hr (5.44 mg AA eq/mL). Several studies have reported the effects of heating on the antioxidant activity of various foods. For example, the antioxidant activity of licorice (29), Korean pear (30), and garlic (31) increased, depending on the heating temperature and time. The ABTS radical scavenging activity of heated garlic juices increased significantly with increased heat-treatment temperature and

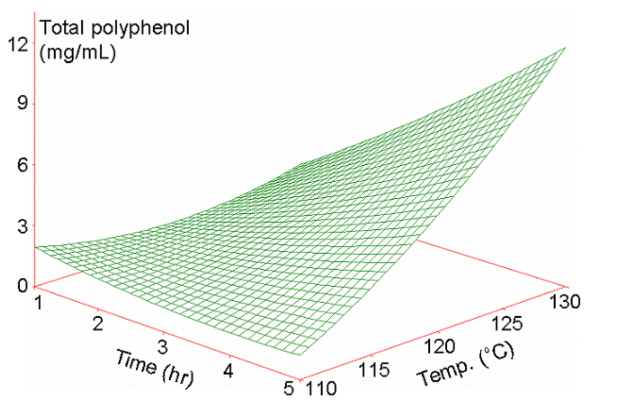

Fig. 4. Effects of temperature and time on the total polyphenol contents of the heated garlic juices.

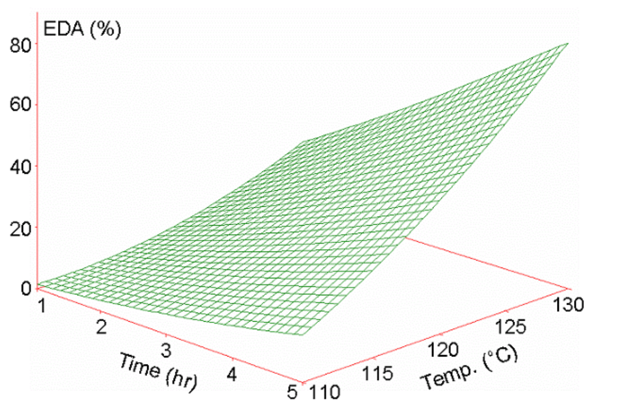

Fig. 5. Effects of temperature and time on the EDA (%) by DPPH of the heated garlic juices.

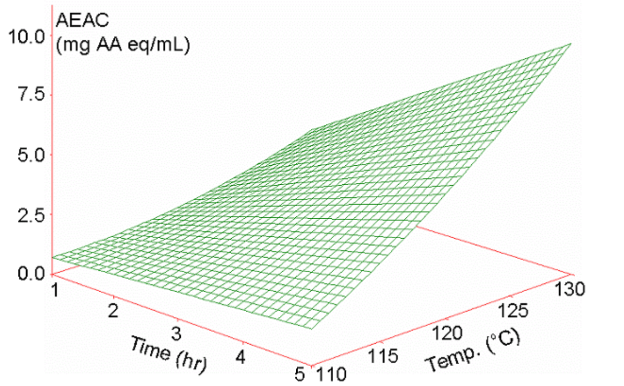

Fig. 6. Effects of temperature and time on the AEAC by ABTS cation decolorization assay of the heated garlic juices.

time (Fig. 6). As a whole, the F-value was affected by heat-treatment temperature more than heat-treatment time ($p<0.001$, Table 2). R^2 for the ABTS radical scavenging activity was 0.9898 (Table 3) and the model was suitable ($p>0.07$), and was affected by the heat- treatment temperature more than time ($p<0.001$, Table 3).

In summary, it is possible that the release of phenolic compounds and browning formation of melanodin in garlic by heat treatment could increase its antioxidant activity. Garlic increased its physiologically activated materials after heating; in this regard, heated garlic juice could be used as biological material for the manufacture of health foods and health supplements. The present study suggests that heated garlic juices are useful nutritional antioxidants for the nutraceutical industry.

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