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EXTRACTION OF SPAGHETTI SQUASH (*CUCURBITA PEPO* L.) BY USING SUPERCRITICAL CARBON DIOXIDE EXTRACTION METHOD

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Abstract

Spaghetti squashes are produced for the production of snacking seeds in significant amounts in the Central Anatolia region and Thrace region in Turkey. Spaghetti squash seeds are used as snacking seeds, but fruits are the agricultural by-products which are evaluated as animal feed.

In this research, carotenoids were extracted from by-product of spaghetti squash by using supercritical carbon dioxide extraction. Ethyl alcohol was used as co-solvent for extraction studies. Temperature and pressure kept constant during extraction process. Extraction experiments were carried out at 60 ^{0}C and 350 bar. Total extraction time was 180 minutes. Total carotenoids and cupric reducing antioxidant capacity (CUPRAC) analysis was conducted by taking samples from the extract at: 30th, 60th, 90th, 120th, 150th and 180th minutes during the extraction process.

The total carotenoid content of spaghetti squash is 1.81 g/100 g dry matter. The maximum carotenoid extraction occurred within the first 30 minutes. The amount of the extracted carotenoids at the 30th, 60th, 90th, 120th, 150th and 180th minutes were calculated as 0.293, 0.253, 0.246, 0.238, 0.232, 0.222 g/100 g dry matter respectively. The total amount of carotenoids extracted at the end of the extraction process is 1.484 g/100 g dry matter. The antioxidant capacity of spaghetti squash is 287.23 mg Trolox/100 g dry matter. Antioxidant capacity at each time interval is 92.78, 49.21, 32.08, 18.14, 14.48, 11.26 mg Trolox/100 g dry matter respectively. The total antioxidant capacity at the end of the extraction process is 217.97 mg Trolox /100 g dry matter.

As a result, it was observed that the amount of total carotenoid and antioxidant capacity gradually decreased as time elapsed. Cumulative total carotenoid content and antioxidant capacity are increased as the time progressed under constant temperature and pressure.

***Key words**: Supercritical carbon dioxide, Spaghetti squash, Carotenoids, CUPRAC.*

1. Introductıon

A growing interest and demand for healthy, environmentally safe, and cost-efficient products has driven the research and application of new technologies in the food, pharmaceutical, and cosmetic industries. (Shi *et al.,* [19]). Last decades, consumers have taken a negative attitude towards the use of synthetic additives. This has increased the orientation towards the production of various additives with extraction from natural sources. Especially since synthetic antioxidants and synthetic colorants are beginning to show negative effects on human health, the tendency to use natural antioxidants and colorants has increased (Özcan and Akgül, [1]; Moure *et al.,* [2]; Saldana *et al.,* [3]).

Nowadays, recovery of agricultural waste and by-products are of great economic and environmental importance and the awareness of this issue is increasing. An example of such wastes is the spaghetti squash which is the source of the snacking seeds production.

Spaghetti squash which belongs to *Cucurbitaceae* family, commonly grown all over the world. Cucurbit vegetables, as well as all over the world because of the suitability of ecological conditions are grown widely in Turkey. Spaghetti squashes are produced for the production of snacking seeds in significant amounts in the Central Anatolia region, especially in Thrace region in Turkey (Konca, [4]).

Spaghetti squash leaves a significant amount of waste after the kernels are separated. The total of crust, fruit and small seeds constitutes 92 - 95% of the whole flour (Konca, [4]). These wastes which are used as animal feed and fertilizer etc., contain a certain amount of bioactive component (especially carotenoid) (Church, [5]).

Carotenoid pigments are natural coloring agents that are typically extracted from seeds, fruits, flower petals, and so on for the food, cosmetic, and pharmaceutical industries. These components play a vital role in the human body and are the main source of vitamin A for humans.

Common carotenoid applications include animal feed, cosmetic products, and natural colorants for food products (Sabio *et al.,* [21]), Goncalves *et al*.,[13]).

Extraction processes are commonly used to enrich and detoxify food through the removal of targeted components from natural products. Organic solvents have been a popular choice in the extraction of biomolecules such as carotenoids from plant materials. However, alternative and reliable extraction techniques have been of great interest because organic solvents are costly, environmentally hazardous, and require expensive disposal procedures (Shi *et al*., [19]; Hartano et al., [20]).

Modern extraction techniques, which have been replacing conventional ones (such as solid-liquid extraction), include: supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). The major advantages of these techniques are the considerable reduction in the use of solvents and acceleration of the extraction process. Especially in the case of SFE, under supercritical conditions, the extraction process is suitable to decrease volatility and thermal degradation. Supercritical fluid extraction is ideal in the food and pharmaceutical industries because solvents such as CO_2 have low critical temperatures, thus facilitating and optimizing the extraction of thermally unstable compounds (Hartano *et al.,* [20]). Additionally, supercritical carbone dioxide has been extensively used as "green solvent" for many extraction processes (Sun and Temelli, [6]; Nyam *et al.,* [7]; Aguiar *et al.,* [8]). Solid-liquid extraction and supercritical carbone dioxide extraction (SCCO2) are the examples of the extraction method from *Cucurbita pepo* L. species (Muntean *et al.,* [9]). Especially, naturally occurring carotenoids extracted with SCCO2 should be in great demand for their use in food and medicine (Yu *et al.,* [10]).

In the recent years, researches were carried out in order to investigate effects of the extraction conditions such as temperature, pressure and time on the yield and amount of bioactive components. At the study which was done with *Spirulina pacifica algae*, temperatures were chosen 40 - 60 - 80 ^{0}C, pressure were chosen 150 - 250 - 350 bar, extraction time were chosen 40 - 70 - 100 minutes and ethanol percentage were chosen 5 - 20 - 25. Best experimental conditions for β- Carotene were determined as 60 ^{0}C, 350 mbar, 100 minutes and 15% ethanol. As a result, time was important parameter as much as temperature and pressure (Careri *et al.,* [22]).

Another study the effect of pressure (8 - 15 - 20 MPa), temperature (30 - 40^{0}C) and extraction time (40 - 70 - 100 - 120 min) on the yield and composition of the extracts of *T. zygis* subsp. *sylvestris* obtained by supercritical CO_2 extraction. The best extraction pressure was found 18 MPa and temperature was nearly 40 ^{0}C. Extraction time should not be longer than 60 minutes (Moldao-Martins *et al.,* [23]).

The study which was done with apricot puree for the obtain maximum amount of β-carotene by SCCO2. Extraction pressure were between 13.3 - 47.3 MPa, temperature were between 316 - 350 K and ethanol percentage were between 2 - 28%. Also effect of static extraction time (5 - 10 - 15 - 20 min) was investigated before the dynamic extraction. Total extraction time was 90 minutes. It was observed that static extraction time did not affect yield, but extractable β-carotene were increased by the time progress (Şanal *et al.,* [16]).

This paper describes the SCCO2 extraction of carotenoids from dried spaghetti squash. The main objective of this study was to determine the effect of time on total carotenoid content, antioxidant capacity during the supercritical carbone dioxide extraction process at the constant temperature and pressure. For this purpose, samples were taken at the different time interval.

2. Materıals and Methods

2.1 Plant material

Spaghetti squash was purchased from local farmer from Thrace Region in Turkey. Snacking seeds were separeted from fruit. Fruits without seeds were sliced into pieces of 0.5 mm thickness by choper (Crytopeerless, United Kingdom) and dried at tray dryer (Lab T2 Model) at 60 ^{0}C until they came to constant balance. Dried fruits milled with hummer mill and screened with 0.5 mm screen. Powder was used for the $SCCO_2$ extraction studies. Processes applied to the fresh spaghetti squash was shown at Figure 1.

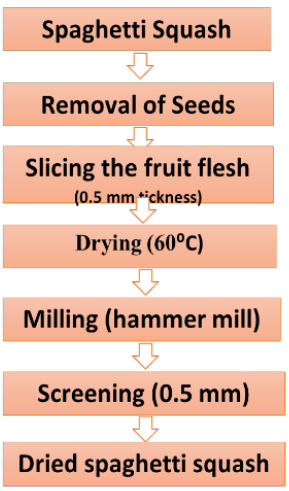

Figure 1. Operations applied to spaghetti squash

2.2 Supercritical carbone dioxide extraction ($SCCO_2$)

$SCCO_2$ extractions were performed on a Waters (Milford, Massachusetts, ABD) model supercritical fluid extractor with solvent modifier unit (Figure 2). Ethanol (96%) was used as cosolvent (flow-rate 6.0 mL/min).

$SCCO_2$ system equipped with a 500 mL extraction cell was used. Experiments were conducted at temperature of 60 ^{0}C and pressure at 350 bar. The extraction temperature was monitored by a thermocouple immersed in the centre of the extractor. CO_2 was compressed to the desired pressure using a diaphragm compressor and the extraction pressure was controlled by a back-pressure regulator. CO_2 flow rate (30 g/min) was controlled by a micrometering valve. All controls were automatically made and monitored by the computer which is mounted to the system. A stainless steel basket was fitted into the extraction vessel for easy loading and unloading of the dried sample (10 g). Extraction time was continued for 180 minutes. Extract fractions were collected every 30 min in side-armed cyclone attached to the depressurization valve. Samples were taken from the extract 30th, 60th, 90th, 120th, 150th and 180th minutes during the extraction process. New collecting vessel was mounted to the cyclone at each time interval. Volume of the extract was measured 114 mL at each time interval. The samples were stored at -24 ^{0}C until analysis. At least two replicates were carried out for all the experiments.

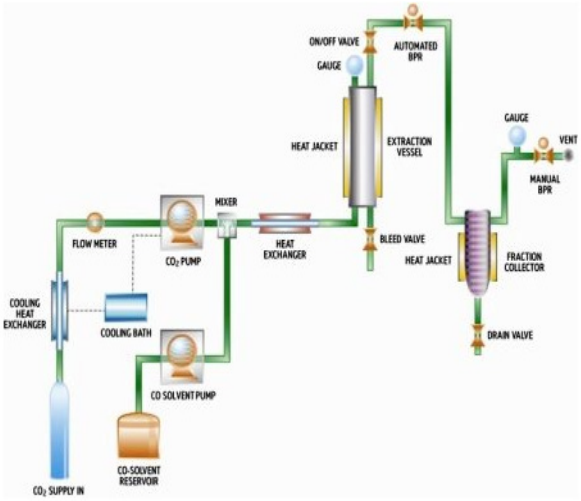

Figure 2. Schematical diagram of $SCCO_2$ system (Waters, [25])

2.3 Moisture content analysis

The moisture analysis of the samples was carried out in a vacuum oven (WiseVen WOW-30, Germany) at 65 ^{0}C (Uysal *et al.*, [24]).

2.4 Total carotenoids analysis

Total carotenoid determination was carried out by measuring the absorbance at 450 nanometer (nm) in a Agilent Technologies Cary 60 UV-VIS spectrophotometer (USA). Total carotenoids were calculated using an extinction coefficient of β-carotene, ε =2505 (Lee and Castle, [11]).

$$A = \frac{\varepsilon x b x c}{1000}$$

A: Absorbans.

ε: Extinction coefficient of β-carotene.

b: Optical path length (cm).

c: Carotenoid content (mg/g).

2.5 Total antioxidant capacity analyses

Antioxidant capacity analyses were carried out based on cupric reducing antioxidant capacity (CUPRAC) analysis (Apak *et. al.*, [12]). With this method, the copper(II) (or cupric) ion reducing ability of carotenoids is measured. The method comprises mixing of a copper(II) chloride solution, a neocuproine solution and an ammonium acetate aqueous buffer and samples which will be analysed. Absorbance of the mixture measured at 450 nm after 30 min. Standard calibration curve were drawn with Trolox concentrations of 0.01 - 0.10 mg/mL in isopropanol for lipophilic extracts.

2.6 Extraction efficinecy

Extraction efficiencies were calculated as Liu *et al.*,[26].

$$\text{Efficiency} = \frac{\text{Total carotenoid amount of extracts}}{\text{Total carotenoid amount of dried spaghetti squash flesh}} \text{x100}$$

2.7 Statistical analysis

Statistical analyses were carried out in duplicate and means were reported. Analysis of variance of results and multiple comprison tests (Duncan Test) were performed using SPSS 20 software.

3. Results and Discussion

3.1 Analyses of spaghetti squash flesh

Moisture content, total carotenoid content and antioxidant capacity analyses were done with the spaghetti squash flesh (Table 1).

Table 1. Analyses of spaghetti squash flesh

Sample	Analyse	Amount
Fresh fruit flesh	Moisture content	93.6 ± 2.2%
Dried fruit flesh	Moisture content	8.43 ± 0.02%
Dried fruit flesh	Total Carotenoid Content	1.81 ± 0.15 mg/100 g dry matter
Dried fruit flesh	Antioxidant Capacity	287.23 ± 42.12 mgTrolox/100g dry matter

3.2 Determination of extracted total carotenoid amount

Supercritical carbone dioxide extraction of carotenoids was carried out at constant temperature and pressure. Examination of the time effect on the carotenoid amount, samples were taken from the extract at different time intervals during the extraction process. Total carotenoid content and efficiency values of the extract at the different time intervals were shown at the Table 2.

Table 2. Carotenoid content of spaghetti squash extracts at different time interval and efficiency

Time interval (min.)	Total carotenoid content (mg/100 g dry matter)	Efficiency (%)
0-30*	0.29 ± 0.03^{a}	16.20
31-60*	0.25 ± 0.02^{ab}	13.96
61-90*	0.25 ± 0.02^{ab}	13.62
91-120*	0.24 ± 0.03^{ab}	13.15
121-150*	0.23 ± 0.03^{ab}	12.81
151-180*	0.22 ± 0.03^{b}	12.253

Legend: All data were the means ± SD.
a and b: Different letters in the same row indicate a significant difference ($p < 0.05$).
*Analyses were done at 30th 60th 90th 120 th 150th and180th minutes.

Extractable total carotenoid amount was measured 0.29 ± 0.03, 0.25 ± 0.02, 0.25 ± 0.02, 0.24 ± 0.03, 0.23 ± 0.03 and 0.22 ± 0.03 mg/100 g dry matter. Efficiencies were calculated as 16.20, 13.96, 13.62, 13.15, 12.81 and 12.25% respectively. There were no significance difference at the total carotenoid amount between second, third, fourth and fifth time intervals. In first thirty minutes and in last thirty minutes, there were significant difference at the total carotenoid amount ($p < 0.05$). Graphical representation of the total carotenoid amount was shown at Figure 3.

As shown in Figure 3, tendency was nearly lineer after first thirty minutes of extraction. Therefore the relationship between time and carotenoid content at each time interval exponential ($R^2 = 0.9665$).

The study that was done to determine of the optimum conditions for $SCCO_2$ extraction of β-carotene from apricot pomace. The effects of static extraction time (5 - 10 - 15 min) at the constant temperature (328 K) and constant pressure (30.3 MPa) on the amount of β -carotene extracted were studied. It was found that if static extraction time was increased, extractable carotenoids amount were increased (Şanal *et al.,* [16]).

Cumulative carotenoid content and efficiency values of the extract at the time as 30th, 60th, 90th, 120th, 150th and 180th minutes were shown at the Table 3.

Table 3. Carotenoid content of dried spaghetti squash and cumulative carotenoid content of spaghetti squash extract at the time progressing and efficiency

Time (min.)	Cumulative carotenoid content (mg/100 g dry matter)	Efficiency (%)
Dried Spaghetti Squash	**1.810 ± 0.145**	-
30th	0.29 ± 0.03^{a}	17.474
60th	0.55 ± 0.11^{a}	29.803
90th	0.79 ± 0.08^{b}	42.907
120th	1.03 ± 0.11^{c}	56.186
150th	1.26 ± 0.09^{d}	67.687
180th **1.48 ± 0.11**^{d} 78.811		

Legend: All data were the means ± SD.
a and b: Different letters in the same row indicate a significant difference ($p < 0.05$).

Cumulative carotenoid amounts were measured 0.29 ± 0.03, 0.55 ± 0.11, 0.79 ± 0.08, 1.03 ± 0.11, 1.26 ± 0.09 and 1.48 ± 0.11 mg/100 g dry matter. Efficiencies were

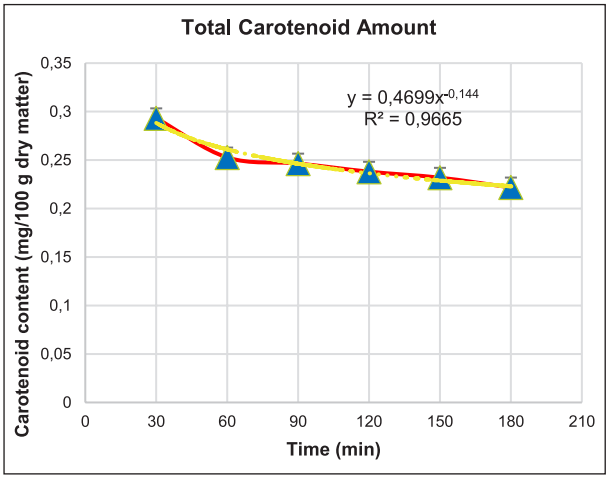

Figure 3. The experimental results of supercritical carbone dioxide extraction of carotenoids from spaghetti squash by-products

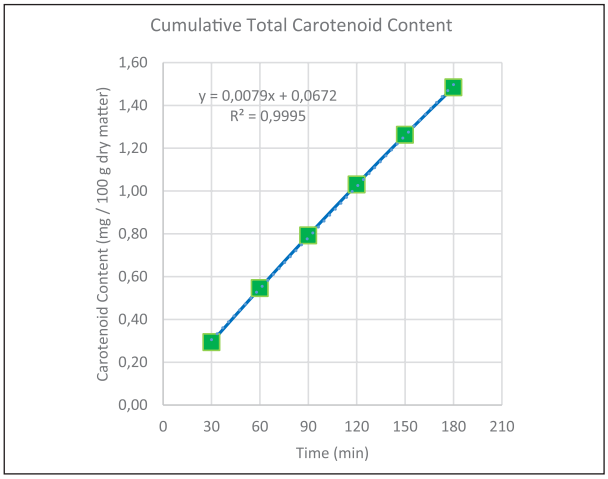

Figure 4. Cumulative carotenoid content of spaghetti squash extracts with the time progressing

calculated as 17.474, 29.803, 42.907, 56.186, 67.687 and 78.811 % respectively. There were significance difference at the cumulative carotenoid amount at each time ($p < 0.05$). Extractable total carotenoid amount was measured as 1.48 ± 0.11 mg /100 g dry matter at the end of the extraction process (180th). Graphical representation of the cumulative carotenoid amount was shown at Figure 4.

As shown in Figure 4, according to the cumulative approach, tendency was lineer at the extraction process. Therefore the relationship between time and carotenoid content with the time progress is lineer ($R^2 = 0.9995$).

Similar results were obtained the study which was done by Das *et al.,* [14]. β-carotene was extracted with solvent extraction method from carrot. Cumulative amount of the β-carotene increased with time progressed. The study which was done by supercritical carbone dioxide from carrot was showed that, cumulative amount of carotenoid was increased by the time progressed (Subra *et al.,* [17]). Xu *et al.,* [18] studied with sea buckthorn (*Hippophaë thamnoides* L.) oil. Results showed that time had a positive lineer effect on the extraction yield. The study was done with tomato waste for extraction of β-carotene. Time was shown polynomial effect on the β-carotene amount (Baysal *et al.,* [27]).

3.3 Determination of Total Antioxidant Capasity

Examination of the time effect on the total antioxidant capacity, samples were taken from the extract at different time intervals during the extraction process. Total antioxidant capacities values of the extracts at the different time intervals were shown at the Table 4.

Table 4. Antioxidant capacity of spaghetti squash extracts at different time interval

Time interval (min.)	Antioxidant capacity (mg Trolox/ 100 g dry matter)
0 - 30*	92.79 ± 4.42^{a}
31 - 60*	49.216 ± 9.26^{b}
61 - 90*	32.086 ± 4.48^{c}
91 - 120*	18.141 ± 2.77^{d}
121 - 150*	14.482 ± 2.71^{d}
151 - 180*	11.265 ± 2.49^{d}

Legend: All data were the means ± SD.
a, b, c and d: Different letters in the same row indicate a significant difference ($p < 0.05$).
*Analyses were done at 30th 60th 90th 120 th 150th and180th minutes.

Total antioxidant capacity values were measured 92.79 ± 4.42, 49.216 ± 9.26, 32.086 ± 4.48, 18.141 ± 2.77, 14.482 ± 2.71 and 11.265 ± 2.49 mg Trolox/100 g dry matter respectively. There were no significant difference at the antioxidant capacity at the 91 - 180 minutes time intervals ($p < 0.05$). In other words, after ninety minutes of the extraction process, antioxidant capacities were decreased at each time interval. Graphical representation of the total carotenoid amount was shown at Figure 5.

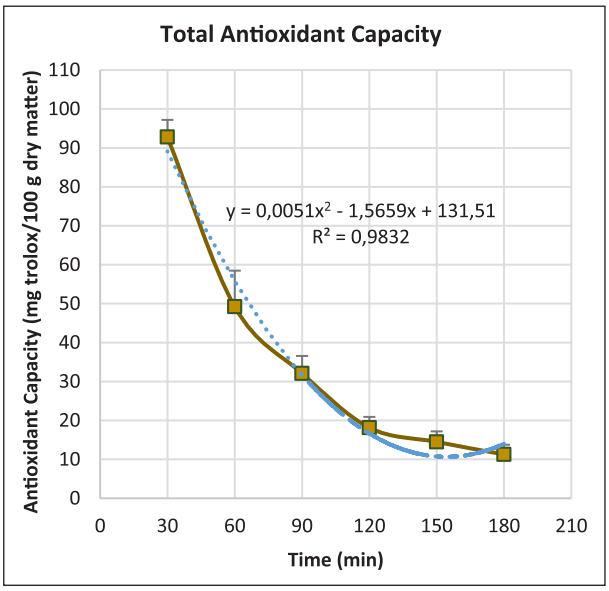

Figure 5. Antioxidant capacity of spaghetti squash extracts at different time interval

As shown in Figure 5, the relationship between time and total antioxidant capacity at the each time interval was polynomial ($R^2 = 0.9832$). Similar results were obtained with the study which was done with hydrodistillation extraction from rosemary. Antioxidant capacity (inhibition) were decreased at the time progress at each time interval (Carvalho Jr. *et al.,* [28]).

Cumulative antioxidant capacity values of the extract at the time as 30th, 60th, 90th, 120th, 150th and 180th minutes were shown at the Table 5.

Table 5. Cumulative antioxidant capacity values of the extracts with the time progress

Time (min.)	Antioxidant Capacity (mg Trolox/ 100 g dry matter)
Dried Spaghetti Squash	287.23 ± 42.12
30	92.79 ± 13.86a
60	142.00 ± 8.83ab
90	174.09 ± 12.18ab
120	192.23 ± 5.96c
150	206.71 ± 12.93d
180	217.98 ± 20.433e

Legend: All data were the means ± SD.
a, b, c, d and e: Different letters in the same row indicate a significant difference ($p < 0.05$).

Cumulative antioxidant capacities were measured 92.79 ± 13.86, 142.00 ± 8.83, 174.09 ± 12.18, 192.23 ± 5.96, 206.71 ± 12.93 and 217.98 ± 20.433 mg Trolox /100 g dry matter respectively. There were significant difference at the cumulative antioxidant capacities at each time ($p < 0.05$). Graphical representation of the cumulative carotenoid amount was shown at Figure 6.

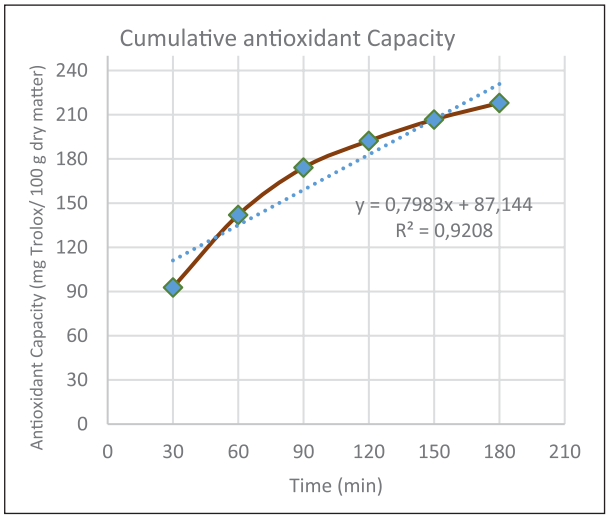

Figure 6. Cumulative antioxidant capacity values with the time progress

Cumulative antioxidant capacity values increased with the time progress. As it can be seen at Figure 6, according to the cumulative approach, tendency was nearly lineer at the extraction process. Therefore the relationship between time and carotenoid content with the time progress is lineer ($R^2 = 0.9208$). The minimum antioxidant capacity were occurred at the beginning of the extraction process and the maximum antioxidant capacity were occurred at the end of the extraction process. Total antioksidant capacity at the end of the extraction process were calculated as 267.991 ± 20.433 mg Trolox/100 g dry base. The study was done for the extraction of gingerols from ginger by Zancan *et al.,* [29]. It was found that cumulative antioxidant capacity increased by the time progress. The study which was done with black and red grape marc to measure the effect of extraction time and solvent type on the cumulative antioxidant capacity shows that, time increased antioxidant capasity increased (Lapornik *et al.,* [15]).

4. Conclusıons

- Carotenoid content of the spaghetti squash flesh which was the by-product of the snacking seed production was 1.81 mg/100 g dry matter. Nearly equal portions of carotenoids were extracted at each time interval. The majority of the carotenoids (78%) was extracted at the end of 180 minutes extraction process.

- Total antioxidant capacity of the spaghetti squash flesh was 287.23 ± 42.12 mg Trolox/100 g dry matter. Antioxidant capacity was measured substantially during the first 90 minutes (174.09±12.18 mg Trolox/100 g dry matter).

- At constant temperature (60 ^{0}C) and pressure (350 bar), for yielding higher extraction efficiencies, an extraction time of longer than 180 minutes is required.

Acknowledgments

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