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Effect of foam-mat drying condition on physical properties and rehydration behavior of mushroom powder

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Abstract

In this study the effect of drying conditions on physical and rehydration properties of foam-mat dried mushroom powder was investigated. Physical properties included moisture content, a_w, hygroscopicity, particle size, flowability and cohesiveness, angle of repose, and T_g. The results showed physical properties of mushroom powder significantly ($p<0.05$) affected by dry temperature. The water activity of mushroom powder was below 0.3, which leads to stable conditions. As decreasing drying temperature, the particle size of mushroom powder increased and led to the increase moisture content and a_w. The mushroom powder showed better flowability as increased drying temperature. T_g of mushroom powder ranged from 41.3- 55.6°C. An increase in drying temperature led to increasing wettability and dispersibility. The drying condition had no-significant effect ($P<0.05$) on the solubility of mushroom powder.

Keywords: Mushroom powder, Flowability, Wetability, Dispersibility, Solubility.

Introduction

The white button mushroom (*Agaricus bisporus*) is one of the most important edible mushrooms. This is due to the high nutritional value, medicinal attributes, and lower prices (Meng *et al.*, 2017; Qin *et al*., 2015). Due to the lack of cuticle, high water content, and respiration rate, the shelf life of mushrooms has been limited to a few days. With consideration of beneficial properties and short shelf life of mushrooms, it is necessary to use an appropriate preservation method to extend the shelf life (Gholami *et al*,. 2017). Different methods have been used to improve the shelf life of mushrooms, such as film packaging (Gholami *et al.*, 2017; Salamat *et al.*, 2020), coating (Nasiri *et al*., 2018), drying (Carrión et al., 2018), and frying et al., 2011).

Nowadays, demand for food powders have increased due to various advantages such as stability and usability for a long period (Bhandari, 2013). In the food industry, drying is a common way to produce powdered food. Drying methods include spray drying, freeze-drying, air drying, and foam-mat drying. Among the methods of drying foam-mat drying is an economical and simple method. The porous structure of foam leads to a higher heat transfer rate and reduces drying time (Hardy & Jideani, 2017). Powdered food obtained by foam-mat drying can be used in beverages, meat and bakery products, ice cream, instant foods, and pasta (Hamzeh *et al.*, 2019). Foam mat drying technique has been successfully applied to many foodstuffs such as jambolan juice (Tavares *et al.*, 2020), lime juice (Dehghannya *et al*., 2019), grape juice (Maria de Carvalho Tavares *et al.*, 2019), fig (Varhan, Elmas, & Koç, 2019), strawberry and banana pulps (Guazi *et al.*, 2019), yoghurt (Malik & Sharma, 2019), shrimp (Azizpour *et al.*, 2016; Hamzeh *et al.*, 2019), dates (Seerangurayar *et al*., 2017), cantaloupe pulp (Salahi *et al*., 2017), muskmelon (Asokapandian *et al.*, 2016), yacon juice (Franco *et al.*, 2016), and mushrooms (Pasban *et al.*, 2015).

Physical properties are used to define characteristics of powders and their behaviors

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during operation, transportation, and storage. Understanding these properties of powder can be useful to develop strategies for efficiency and reduces cost of powder processing (Jaya & Das, 2004). The physical properties of powders are divided into two groups: bulk and particle properties. Bulk properties of powder include flowability, bulk density, and mixture quality. Particle properties include size and shape, composition, and particle density (Fitzpatrick, 2013).

Rehydration is the critical quality of powders when focusing on standard benchmark for consumption. In most cases, food powders are dissolved in water or an aqueous system. The rehydration process of powder often includes three stages: wettability, dispersibility, and solubility. Among them, solubility is considered a rehydration quality of the powder (Hogekamp & Schubert, 2003).

Pasban (2012) investigated of foaming parameters of button mushroom. Mushroom puree: water ratio of 2:1 (w/w) and xanthan gum at a concentration of 0.17% (w/w) were selected to optimize foaming conditions for foam-mat drying of button mushroom. This study was done to investigate the effects of different levels of air temperature (50, 65, and 80°C), and foam thickness (3 and 5 mm) on physical and rehydration behavior of foam-mat dried mushroom powder.

Materials and methods

Button mushroom was purchased from a local market (Mashhad, Iran) and stored at 4°C. Xanthan gum as a foam stabilizer was purchased from Sigma-Aldrich Company.

Mushroom powder production

Sample preparation

The mushrooms were washed and cut into uniform pieces. To prevent browning, the pieces of mushroom were immersed in aqueous solution of sodium metabisulfite (2% w/w) for 10 minutes (Pasban, 2012). Then, they washed with water. After removing the excess water, the sliced mushrooms were homogenized by the kitchen blender (210 W; Tefal) at a maximum speed of 1500 rpm to obtain a homogenized puree. 100 mL of distilled water and 0.17 g of xanthan powder were mixed to obtain xanthan gum solution. The mixture stirred with a magnetic stirrer (Ray Noor Azma Company, Tehran, Iran) until a uniform solution was obtained. The solution was kept in the refrigerator at 4°C for 18– 24 h to make complete hydration.

Foam production

According to Pasban (2012), To prepare 100 g of foam, 33 g of xanthan gum solution with a concentration of 0.17% (w/w) and 67 g of mushroom puree were mixed. The mixture of gum and puree was stirred using a kitchen mixer (Sunny, SM88, maximum speed 1500 rpm) for 8 min.

Foam drying

Mushroom puree foam was poured into an aluminum plates of diameter 9 cm in a thin layer with a thickness of 3 to 5 mm. The plates were placed in a dryer (Pars Azma Company, Tehran, Iran) at a constant speed of 1.5 m/s at three temperatures of 50, 65, and 80°C. Drying of the samples was continued until reaching a constant weight. A certain amount of dried foam was removed from the dryer and pulverized by a kitchen miller. The samples were poured into glass containers after passing through a sieve (Mesh No.50) and stored at ambient temperature until further analysis.

properties

Moisture content

The moisture content of the powder was determined by using an oven (AOAC, 1995). The samples were dried at 105°C to a constant weight. Moisture content was calculated by the difference between the weight of powder before and after drying.

Water activity

A water activity meter was used to measure a_w of samples. Powder samples were poured into the device and the a_w was recorded.

Hygroscopicity

To measure the hygroscopicity of mushroom powder, 1 g of the powder samples was poured

into a glass plate. The plates were then placed in a desiccator containing a saturated solution of sodium chloride at 25°C. After a week, the plates were removed from the desiccator and weighed. Hygroscopicity was calculated by weight differences between samples (Tonon *et al.*, 2008).

Particle morphology and microstructural properties

The microstructure of the samples (i.e. size and shape) was evaluated by using a scanning electron microscope (SEM) model XL 30 (manufactured by Philips, Netherlands). The required amount of mushroom powder was covered with gold. Imaging was performed at two magnifications (500 and 1000) and particle size was calculated with ImageJ 1.51p software.

Bulk and tapped densities

To determine the bulk density, a quantity of mushroom powder was poured into a glass cylinder. Bulk density was calculated considered as the ratio of mass to volume according to equation 1. The glass cylinder was then tapped from a height of 15 cm (30 taps) to obtain a constant volume. Tapped density was calculated considered as the ratio of mass to the final volume according to equation 2.

$$\rho_b=\frac{m}{V} \quad (1)$$

$$\rho_t=\frac{m}{V_f} \quad (2)$$

m= quantity of mushroom powder (g)

V= volume of mushroom powder (cm^3)

V_f= final volume of mushroom powder after mechanically tapping (cm^3)

Flowability and Cohesiveness

Classification of flowability and cohesiveness of mushroom powder was done by using the Carr index (CI) and Hausner ratio (HR) according to equations (3, and 4) respectively.

$$CI=\frac{\rho_t-\rho_b}{\rho_t}\times 100 \quad (3)$$

$$HR=\frac{\rho_t}{\rho_b} \quad (4)$$

Angle of repose

A glass funnel and graph paper were used to measure the angle of repose. The funnel of diameter 10 cm and a base with 7 cm length and 1 cm diameter were held by a suspension clamp. The distance between the end of the funnel base and the graph paper was 3 cm. The powder was poured on the graph paper until it touched the end of the funnel base. According to the radius and height of the powder (3 cm), the angle of repose was calculated according to equation (5) (Alanazi, 2010).

$$\theta=\tan^{-1}\left(\frac{h}{r}\right) \quad (5)$$

h= Height of the powder

r= Radius of the powder

Glass transition temperature

The glass transition temperature was measured using a differential scanning calorimetry (DSC, OIT-500 Sanaf Electronic Co.). After calibration, about 10-12 mg of the powder was placed inside the sample pan; an empty aluminum pan was used as a reference. All experiments were performed with the same heating rate of 10°C/min in the temperature range of 25-150°C.

Rehydration behavior

Wettability

To measure the wettability, 100 ml of distilled water was poured into a 250 ml beaker. A glass funnel was placed on top of the beaker using a clamp. The distance between the end of the funnel bottom and the surface of the water was 10 cm. A test tube was inserted into the funnel to block its end. 1g of mushroom powder was poured around the test tube. Then the test tube was removed and the time was recorded using a stopwatch, simultaneously. The wettability is equal to the time of wetting of the powder particles (Jinapong *et al*., 2008).

Dispersibility

To measure the dispersibility, 10 ml of distilled water was poured into 50 ml beaker. 1g of powder was added to the beaker and the solution was stirred for 15 seconds by a spoon.

The solution was passed through a sieve (212 μm); after weighing, 1 ml of the solution dried in an oven at 105°C for 4 hours. The dispersibility was calculated according to equation (6) (Jinapong *et al*., 2008).

$$\text{Dispersibility}=\frac{(10+a)\times \%\ TS}{a\times\frac{100-b}{100}} \quad (6)$$

a= amount of powder (g)

b= moisture content in the powder

%TS= percentage of dry matter in the reconstituted mushroom powder after it has been passed through the sieve.

Solubility

To measure the solubility of the samples, 1 g of powder was poured into a 50 ml beaker. Ten ml of distilled water was added to the beaker. The solution was stirred by a magnetic stirrer for 60 seconds at a constant speed. Then, 1 ml of the solution was weighed (m_1) and dried in an oven at 105°C to a constant weight. After cooling inside the desiccator, the sample was reweighed (m_2). Solubility (%) was calculated according to equation (8) (Zhang *et al.*, 2013).

$$\text{Solubility}=\frac{10\times m_2}{m1}\times 100 \quad (8)$$

Statistical analysis

The effect of drying conditions on physical and dehydration properties of mushroom powder analyzed by the SPSS software (SPSS 22.0; IBM SPSS Statistics, Chicago, IL, USA). Duncan's test was used to establish the multiple comparisons of the mean values that were considered at 95% significance level ($P<0.05$).

Results and discussion

Moisture content

Moisture content is one of the important properties of the powder, which indicates the drying efficiency (Shrestha, Howes, Adhikari, & Bhandari, 2007). The average moisture content of mushroom powder ranged from 2.46- 6.04 g/100 g. As shown in Table 1, increasing drying temperature led to a significant effect ($p<0.05$) on moisture content. At higher drying temperature, the greater temperature difference between foam and drying air led to an increase in heat penetration into foam and accelerated removal of moisture (Hamzeh *et al.*, 2019). Moreover, at the same drying temperature, decreasing in foam thickness led to reduction in the moisture content of the mushroom powder. This might be due to the increase in drying time. Salahi et al. (2017) reported that with increasing foam thickness, drying time increases. With the increase in drying time, the foam structure decomposes and a weak structure is formed. Therefore, drying process is difficult and more water remains in the foam. This observation is in agreement with many studies using different materials such as lime juice, shrimp, fig, cantaloupe, and yacon juice. The moisture content values of lime juice, fig, cantaloupe, and yacon juice powders ranged from 1.02-9.55 g/100 g (Dehghannya *et al.*, 2019), 7.65-8.27 g/100 g (Varhan *et al*., 2019), 4.59-8.02 (Salahi *et al.*, 2017), and 4.33-4.91 g/100 g (Franco *et al*., 2016) respectively. Based on the results, the best drying condition in terms of storage and stability included combination of higher drying temperature and lower thickness (80°C- 3mm).

Table 1- Moisture content of foam-mat dried mushroom powder

Drying conditions	Moisture content (g/100g, wb)
3 mm- 50°C	5.34 ± 0.13^{b}
5 mm- 50°C	6.04 ± 0.12^{a}
3 mm- 65°C	4.09 ± 0.10^{d}
5 mm- 65°C	4.72 ± 0.10^{c}
3 mm- 80°C	2.46 ± 0.03^{f}
5 mm- 80°C	3.05 ± 0.05^{e}

Mean with different letters in the same column indicate significant differences at $p<0.05$.

Water activity

The water activity of mushroom powder is illustrated in Table 2. It was in the range of 0.12-0.28. The lowest water activity was obtained at 80°C- 3 mm, whereas the highest water activity was for sample prepared at 50°C-

5 mm. Powders with water activity less than 0.3, prevents the growth of microorganisms, reduces enzymatic and biochemical reactions and delays the non-enzymatic browning (Belitz *et al*., 2009). All powder samples have a_w below 0.3, which leads to stable conditions during storage. The a_w of foam-mat dried product such as shrimp, fig, cantaloupe, and yacon juice powders was reported as 0.13- 0.29, 0.21- 0.39, 0.14- 0.28, and 0.10- 0.22 by Hamzeh et al. (2019), Varhan et al. (2019), Salahi et al. (2017), and Franco et al. (2016) respectively.

Table 2- Water activity (a_w) of foam-mat dried mushroom powder

Drying conditions	Water activity (a_w)
3 mm- 50 °C	0.26 ± 0.00^{a}
5 mm- 50 °C	0.28 ± 0.00^{a}
3 mm- 65 °C	0.20 ± 0.00^{c}
5 mm- 65 °C	0.22 ± 0.00^{b}
3 mm- 80 °C	0.12 ± 0.00^{e}
5 mm- 80 °C	0.15 ± 0.00^{d}

Mean with different letters in the same column indicate significant differences at $p<0.05$.

Hygroscopicity

The hygroscopicity of mushroom powder is presented in Table 3. It was in the range of 3.94-6.77 g/100 g. At the same foam thickness, increasing the drying temperature had a significant effect ($P<0.05$) on hygroscopicity of samples. The highest hygroscopicity wasobtained at 80°C- 3 mm, whereas the lowest hygroscopicity was for sample prepared at 50°C- 5 mm. The results showed an increase in moisture content of the samples caused an increase in hygroscopicity. This is due to the difference in moisture content between the powder samples and the environment because the samples absorb water until they reach equilibrium with ambient humidity. Therefore, increasing the difference in moisture content between the powder samples and the environment led to an increase in moisture absorption (Tonon *et al*., 2008).

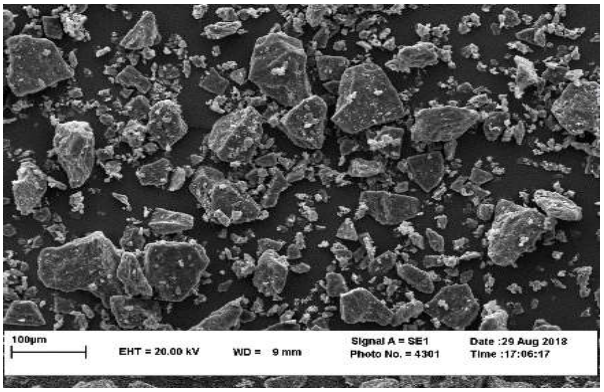
Our results are in agreement with the other studies (Franco *et al*., 2016; Moghbeli *et al*., 2020; Salahi *et al.*, 2017). The hygroscopicity of date, feijoa, cantaloupe, yacon juice, and acai powder was reported as 25- 29% (Moghbeli *et al.*, 2020), 19.8-27.2% (Henao-Ardila *et al.*, 2019), 16.73-20.76% (Salahi *et al.*, 2017), 15.24-22.31% (Franco *et al.*, 2016), and 12.54-15.79% (Tonon *et al.*, 2008).

The differences between our results and other studies could be related to the nature of mushroom. Mushrooms are low in sugar content (0.16-0.42 g/100 g) (Kalač, 2013). In food powders, sugar-rich products, due to the ability of the sugar hydroxyl group to create hydrogen bonds with water molecules, have a higher moisture absorption ability (Jaya & Das, 2004). Moreover, Our results are very close to ideal hygroscopicity for instant products which outlined by Jaya and Das (2004) (5.13-9.38 g/100 g). Powders with hygroscopicity below 20% are not very hygroscopic during storage (Henao-Ardila *et al.*, 2019), thus, the foam-mat dried mushroom powder can be considered quite stable during storage.

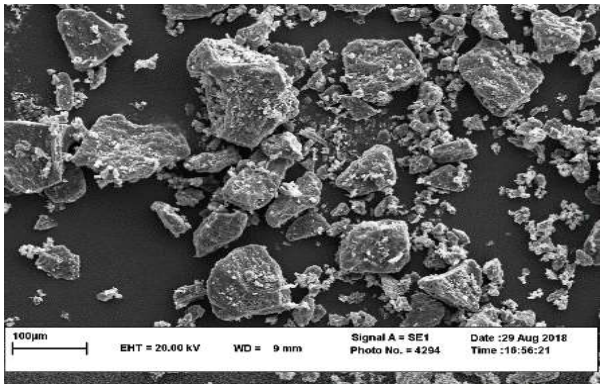
Table 3- Hygroscopicity of foam-mat dried mushroom powder

Drying conditions	Hygroscopicity (g/100g)
3 mm- 50°C	4.63 ± 0.08^{c}
5 mm- 50°C	3.94 ± 0.06^{d}
3 mm- 65°C	5.55 ± 0.08^{b}
5 mm- 65°C	5.16 ± 0.06^{b}
3 mm- 80°C	6.77 ± 0.12^{a}
5 mm- 80 °C	6.38 ± 0.12^{a}

Mean with different letters in the same column indicate significant differences at $p<0.05$.



3mm-50×500



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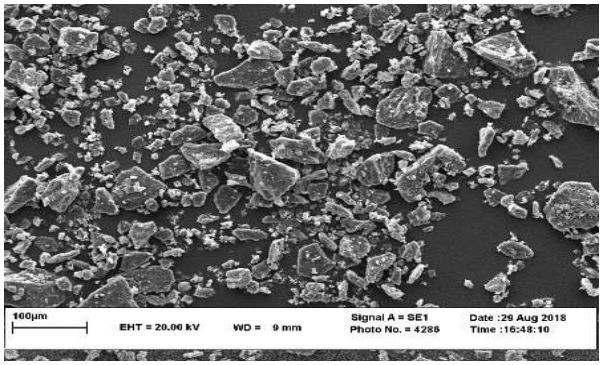

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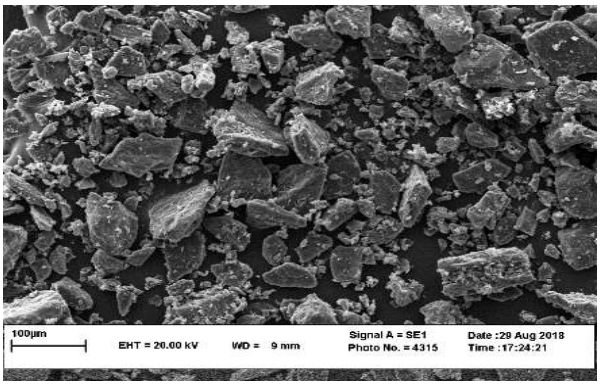

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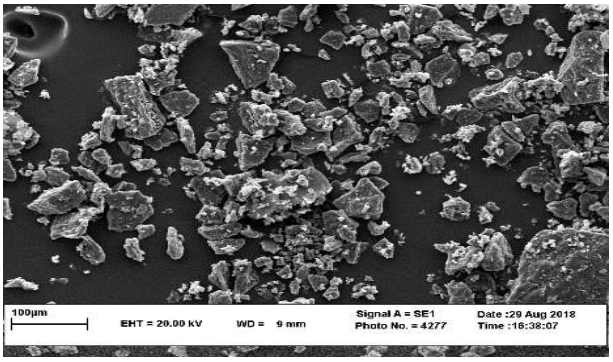
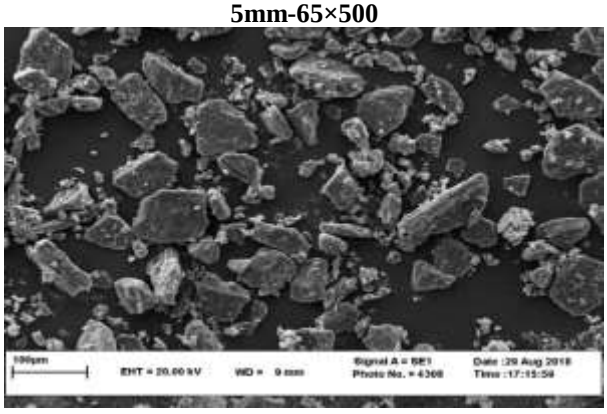
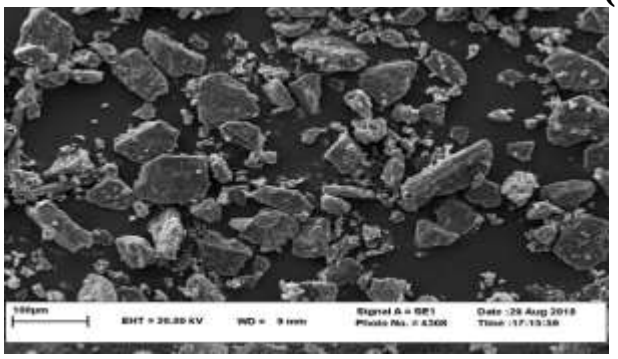


3mm-80×500



5mm-80×500

(a)



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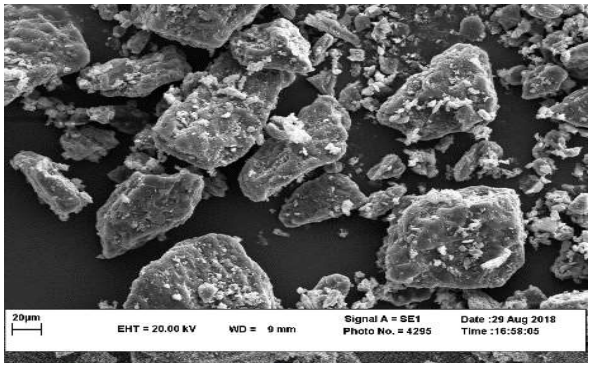
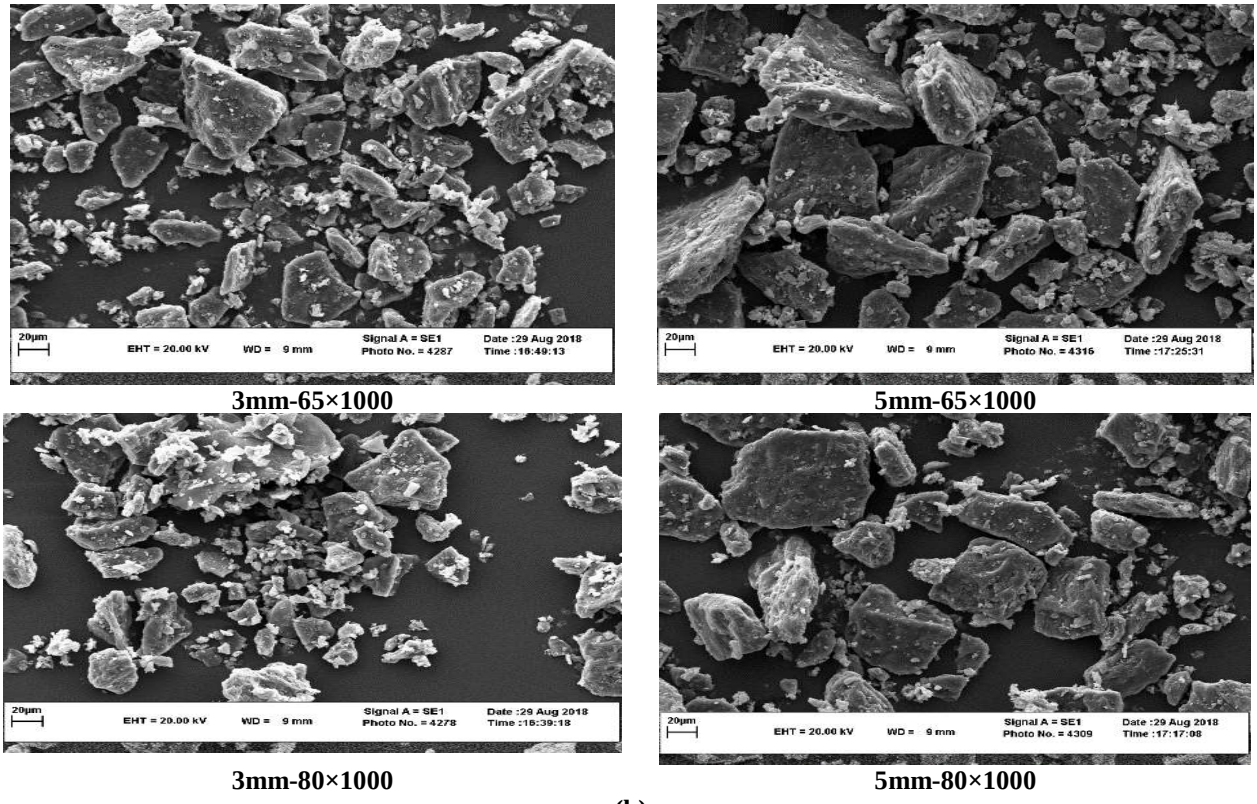


5mm-50×1000



(b)

Fig. 1. SEM images of mushroom powder at magnification of ×500 (a) and ×1000 (b)

Particle morphology and microstructural

Particle size is one of the important properties of food powder as it plays a role in determining the behavior and properties of powder such as density, flowability, rate of solubility, and food formulation (Fitzpatrick, 2013). The SEM images of the samples are shown in Fig. 1. According to Table 4, The lowest particle size was obtained at 80°C- 3 mm (120 µm), whereas the highest particle size was for sample prepared at 50°C-5 mm (282 µm). According to the results, particle size of samples decreased as increasing drying temperature. Moreover, at the same drying temperature, thinner foam layers produced powders with lower particle size. According to this study, particle size of samples was correlated with the moisture content.

Table 4- Particle size of foam-mat dried mushroom powder

Drying conditions	Particle size (µm)
3 mm- 50°C	282.49± 8.19^{a}
5 mm- 50°C	279.20± 10.68^{a}
3 mm- 65°C	212.23± 9.84^{b}
5 mm- 65°C	262.41± 6.38^{ab}
3 mm- 80°C	120.20± 11.66^{c}
5 mm- 80°C	161.55± 8.43^{c}

Mean with different letters in the same column indicate significant differences at $p<0.05$

The lowest moisture content was obtained at 80°C-3 mm, whereas the highest moisture content was for sample prepared at 50°C-5 mm. The rate of heat transfer declines as the temperature decreases and the foam thickness increases, which increases the drying time. In this case, the foam moisture remains for a long time, as a result, the number of liquid bridges between the foam particles increases. The cohesion between the particles increases as the

number of bonds between the foam particles increases, then, larger particles produced. The same result were reported for cornmeal (Chinwan & Castell-Perez, 2019), jamun juice (Santhalakshmy, Don Bosco, Francis, and Sabeena, 2015), and acai powder (Tonan, 2008).

Bulk and tapped density

The bulk and tapped densities provide a perspective from packing and the compaction profile of a material (Asokapandian *et al.*, 2016). According to Table 5, increasing drying temperature led to a decrease in the density of mushroom powder. The bulk density of mushroom powder was in the range of 0.54-0.70 g/cm^3. Both densities of mushroom powder increased as an increase in the moisture content of powder samples. Due to the higher density of water than dry particles, bulking weight of powder increased as increasing moisture content (Chegini & Ghobadian, 2005). The bulk density of shrimp, fig, date, and muskmelon powder were reported as 0.56-0.79 g/cm^3, 0.36-0.50 g/cm, 0.56-0.70 g/cm^3, and 0.51-0.54 g/cm^3 by Hamzeh et al. (2019), Varhan et al. (2019), Seerangurayar et al. (2017), and Asokapandian et al. (2016) respectively.

Tapped density of samples ranged from 0.63-0.85 g/cm^3. The tapped density of mushroom powder was larger than bulk density due to make denser packing conditions during tapping, which is in agreement with other studies *(Seerangurayar et al., 2017; Varhan et al.*, 2019). The tapped density for foam-mat drying of fig, date, and muskmelon were reported as 0.57-.069 g/cm^3 (Varhan *et al.*, 2019), 0.75-0.90 g/cm^3 (Seerangurayar *et al.*, 2017), 0.54-0.64 g/cm^3 (Asokapandian *et al.*, 2016) respectively.

Table 5- Bulk and tapped density of foam-mat dried mushroom powder

Drying conditions	Bulk density (g/cm^3)	Tapped density (g/cm^3)
3 mm- 50°C	0.68± 0.00^a	0.91± 0.02^a
5 mm- 50°C	0.70± 0.00^a	0.95± 0.01^a
3 mm- 65°C	0.63± 0.01^b	0.77± 0.01^b
5 mm- 65°C	0.63± 0.00^{ab}	0.80± 0.01^b
3 mm- 80°C	0.54± 0.01^c	0.63± 0.02^d
5 mm- 80°C	0.55± 0.01^c	0.65± 0.02^c

Mean with different letters in the same column indicate significant differences at $p<0.05$.

Flowability and cohesiveness

Flowability is defined as a relative movement of particles between adjacent particles or along the wall surface of a container (Peleg, 1977). As illustrated in Table 7 the range of variations was 13.18-26.82% for the Carr index and 1.15-1.36 for the Hausner ratio. According to this study higher drying temperatures improved powder flowability. Moreover, increasing foam thickness led to increase in the Carr index and Hausner ratio, which indicating reduced flow ability. This is might be due to the reduced drying time at higher drying temperature which prevents foam collapse. The same results were reported for fig (Varhan *et al.*, 2019) and muskmelon (Asokapandian *et al.*, 2016). As shown in Table 2, drying conditions have a significant effect ($p<0.05$) on flowability and cohesiveness of mushroom powder.

Higher HR and CI values indicate reduced flowability. In general, the results showed that the Carr index and Hausner ratio increased as moisture content increased. This is probably due to the increase in the number of liquid bridges in the powder samples. Increasing moisture content causes an increase in the number of liquid bridges and reduces the flowability of powders (Kim *et al.*, 2005).

Based on Carr (1965) mushroom powder had fair flowability at 50°C and 65°C- 5 mm. The samples produced at 65°C-3 mm and 80°C-5 mm showed good flowability. The mushroom powder produced at 80°C- 3 mm showed very good flow ability. According to Hausner (1967) cohesiveness of mushroom powder produced at

80°C was low and the other sample was intermediate.

This study showed that the flowability of mushroom powder is inversely proportional to the particle size content of samples. The results showed that the larger particle size was worse in flowability. This is due to the increase in contact surface between powder particles due to the decrease in the inter-particle voids at smaller particle sizes, resulting in better flowability (Seerangurayar *et al*., 2017). The same results were reported for the other powders such as cornmeal (Chinwan & Castell-Perez, 2019), fig (Varhan *et al.*, 2019), and date (Seerangurayar *et al*., 2017).

Table 7- Flow ability, cohesiveness of mushroom powder obtained by foam-mat drying

Drying conditions	Carr Index (CI) (%)	Flowability	Hausner ratio (HR)	Cohesiveness
3mm- 50°C	24.5± 0.91[a]	fair	1.32±0.01[a]	Intermediate
5mm- 50°C	26.82± 0.37[a]	fair	1.36±0.00[a]	Intermediate
3mm- 65°C	18.90± 0.59[b]	good	1.23±0.00[b]	Intermediate
5mm- 65°C	21.02± 1.50[b]	fair	1.26±0.02[b]	Intermediate
3mm-80°C	13.18± 1.05[c]	Very good	1.15±0.00[c]	Low
5mm-80°C	15.24± 0.96[c]	good	1.18±0.01[c]	Low

Mean with different letters in the same column indicate significant differences at p<0.05.

Angle of repose

The mean angle of repose of mushroom powder ranged from 23.13- 39.23θ°. The mushroom powder produced at 80°C showed lower angle of repose followed by samples produced at 65°C and 50°C. The lowest angle of repose was shown at 80°C- 3 mm, whereas the highest angle of repose was shown at 50°C- 5 mm. Variation of angle of repose could be related to microstructure (size and shape) of particles of powder samples which shown at Fig1. Kim et al. (2005) reported that flow properties of a powder mainly depends on the size and shape of powder particles. According to our results, angle of repose is directly proportional to the particle size of mushroom powder. Decreasing particle size led to surface contacts between powder particles, resulting in lower angle of repose. Similar observation was reported by Seerangurayar et al. (2017).

Flow properties of the powder are inversely proportional to the cohesiveness of powder particles such that an increase in the angle of repose leads to reduced flowability *(Geldart et al*., 2009). Based on Carr (1976), samples showed fairly cohesive at 50°C and the other samples showed free-flow ability. According to our results, the angle of repose increased as moisture content increased, indicating reduced flowability. This is might be due to the increasing the cohesiveness of particles which caused by increasing liquid bridges. The same observation was reported by other researchers (Chinwan & Castell-Perez, 2019; Suleiman *et al*., 2019; Vashishth *et al.*, 2020).

Table 8-Angle of repose of mushroom powder obtained by foam-mat drying

Drying conditions	Angle of repose (θ°)	Flowability
3mm- 50°C	37.30± 0.73[a]	fairly cohesive
5mm- 50°C	39.23± 0.61[a]	fairly cohesive
3mm- 65°C	33.03± 1.59[b]	free-flow
5mm- 65°C	35.53± 0.93[b]	free-flow
3mm-80°C	23.13± 1.15[c]	free-flow
5mm-80°C	25.13± 1.30[c]	free-flow

Mean with different letters in the same column indicate significant differences at p<0.05.

Glass transition temperature

The mean glass transition temperature of mushroom powder is presented in Table 9. It was in the range of 41.67-55.33°C. As shown in Table 9, glass transition temperature increased as drying temperature increased. Tg is mainly

related to moisture content and the chemical structure of food material (Hamzeh *et al.*, 2019). According to our results, the glass transition temperature of mushroom powder increased as moisture content decreased. As the increasing moisture content of powder, water acts as a plasticizer and leads to decreasing Tg of powder (Jaya & Das, 2004).

Roos (2003) pointed out that structural and physicochemical properties are proportional to Tg. These properties include stickiness, crispness, collapse, and amorphous-to-crystalline transformations. Food materials are shelf stable when stored below Tg due to reduction in chemical and microbial reactions, thus, the foam-mat dried mushroom powder can be stable from production to consumption.

Caparino et al. (2012) reported that Tg of mango powder ranged from 18-26°C. The high glass transition temperature of mushroom powder might be due to the low sugar content of mushrooms. The amount of sugar compounds is one of the main factors affecting the glass transition temperature. These compounds are one of the most important hygroscopic compounds that reduce the Tg and cause adhesion by absorbing water (Roos, 2003).

Table 9- Glass transition of mushroom powder obtained by foam-mat drying

Drying conditions	T_g (°C)
3mm- 50°C	44.00 ± 1.15^{bc}
5mm- 50°C	41.67 ± 0.57^{c}
3mm- 65°C	50.33 ± 0.88^{b}
5mm- 65°C	44.33 ± 0.88^{bc}
3mm-80°C	55.33 ± 1.76^{a}
5mm-80°C	53.00 ± 0.83^{a}

Mean with different letters in the same column indicate significant differences at $p<0.05$.

Rehydration properties

Wettability

The mean wettability of mushroom powder is presented in Table 10. It was in the range of 317.33– 412.33 s. Wettability of powder samples is significantly ($p<0.05$) affected by drying temperature. The variation of wettability of mushroom powder might be due to the microstructure of powder particles. According to the results, the wettability of mushroom powder decreased as increasing powder particle size. Increasing the particle size enhances the free space between the particles, therefore, water penetrated faster into powder particles, and the time required for wetting the powder particles reduced. Similar results were reported for date (Seerangurayar *et al*., 2018), and fig powder (Varhan *et al*., 2019).

Fitzpatrick et al. (2016) investigated the wettability of twelve different powders. Their results showed that the powder structure, especially its surface compounds, play an important role in the wettability of a powder. High wettability of mushroom powder might be due to the lower sugar content of mushroom. Seerangurayar et al. (2018) studied foam-mat drying of date at three ripening stages include khalal, rutab and tamr. The tamr powder was shown lower wettability. They conclude that it might be due to the variation of sugar content at ripening stages of date, since tamr had higher sugar content compare to the other ripening stages. The same result was reported for cocoa powder (Shittu & Lawal, 2007).

Hogekamp and Schubert (2003) reported that powder particles larger than 250 μm are usually have shown low wettability. Samples produced at 50°C and 65°C showed particles larger than 250 μm. Our results are different from Hogekamp and Schubert (Hogekamp & Schubert, 2003). This is might be due to the irregular and non-uniform of mushroom powder particles which caused an increase in mechanical interlocking and cohesiveness of powder.

Table 10- Wettability of mushroom powder obtained by foam-mat drying

Drying conditions	Wettability (s)
3mm- 50°C	327.00± 9.82^{c}
5mm- 50°C	317.33± 13.69^{c}
3mm- 65°C	394.00± 6.40^{ab}
5mm- 65°C	382.66± 7.80^{b}
3mm- 80°C	412.33± 8.21^{a}
5mm- 80°C	402.00± 5.68^{a}

Mean with different letters in the same column indicate significant differences at p<0.05.

Dispersibility

The mean dispersibility of mushroom powder is presented in Table 11. It was in the range of 78.53- 93.76%. The dispersibility of powder samples is significantly (p<0.05) affected by drying temperature. Higher dispersibility was shown by a combination of higher foam thickness and lower drying temperature. As shown in Table 3 the highest dispersibility was obtained at 50°C- 5 mm and the lowest dispersibility was obtained at 80°C-3 mm.

Table 11- Dispersibility of mushroom powder obtained by foam-mat drying

Drying conditions	Dispersibility (%)
3mm- 50°C	91.20± 0.55^{a}
5mm- 50°C	93.76± 0.47^{a}
3mm- 65°C	84.83± 0.79^{b}
5mm- 65°C	86.10± 0.60^{b}
3mm- 80°C	78.53± 0.92^{c}
5mm- 80°C	80.00± 0.45^{c}

Mean with different letters in the same column indicate significant differences at p<0.05.

Table 12- Solubility of mushroom powder obtained by foam-mat drying

Drying conditions	Solubility (%)
3mm- 50°C	72.00± 0.90^{a}
5mm- 50°C	72.06± 0.72^{a}
3mm- 65°C	73.36± 1.43^{a}
5mm- 65°C	72.67± 1.24^{a}
3mm- 80°C	74.06± 1.24^{a}
5mm- 80°C	74.23± 1.26^{a}

Mean with different letters in the same column indicate significant differences at p<0.05.

The variation of dispersibility might be due to the difference in the particle size of samples. Particle size and morphology are two most important factors affecting dispersibility (Ding *et al.*, 2020). The largest particle size of mushroom powder was observed at 50°C- 5 mm, whereas the smallest particle size of mushroom powder was observed at 80 °C-3 mm. According to our results, powder dispersibility is inversely related to particle size.

The dispersibility of food powder depends on the ability of the powder dispersed in the solution. The rate of dispersion indicates whether a food powder can be categorized as an instant powder (Hogekamp & Schubert, 2003). Ding et al. (2020) reported that the optimal powder particle size for the best dispersibility in the medium range (180 μm< diameter< 355 μm). So, the mushroom powder produced at 50 and 65°C has a good potential to explore instant properties.

Solubility

The mean solubility of mushroom powder is given in Table 12. It was in the range of 72-74%. As shown in Table 3 the drying condition had no-significant effect (P<0.05) on the solubility of samples. This can be due to the fact that solubility is affected by physicochemical

properties of food powdered rather than processing conditions (Fitzpatrick, 2013). Similar observations were reported for yacon juice and cantaloupe powder (Franco *et al*., 2016; Salahi *et al.*, 2017).

In other studies, the solubility of date, lime juice, cantaloupe, and yacon powders ranged from 66- 89%, 66- 69%, 81- 82%, and 80- 84%, respectively.

Conclusions

In this study, the effects of three temperatures of 50, 65 and 80°C with two thicknesses of 3 and 5 mm on physical and rehydration properties of foam-mat dried mushroom power were investigated using a hot air dryer. At constant foam thickness, moisture content, and a_w of mushroom powder decreased as increasing drying temperature. However, their hygroscopicity and particle size increased significantly. Based on this study, moisture content, a_w, and hygroscopicity of foam-mat dried mushroom powder were decreased to a stable condition to prevent chemical and microbiological reactions. By increasing drying temperature, the flowability of mushroom powder improved. The mushroom powder produced at 80°C- 3 mm showed very good flowability with high stability. Powders produced at higher drying temperatures had lower wettability and higher dispersibility. Thus, lower drying temperature could be more desirable for production mushroom powder with instant food application.

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اثر شرایط خشک کردن کف‌پوشی بر خصوصیات فیزیکی و رفتار بازجذب آب پودر قارچ

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چکیده

این پژوهش با هدف بررسی شرایط خشک کردن بر خصوصیات فیزیکی و بازجذب آب پودر قارچ دکمه‌ای که با روش خشک کردن کف‌پوشی انجام شده است خصوصیات فیزیکی پودر قارچ شامل: مقدار رطوبت، فعالیت آبی، قابلیت جذب آب، اندازه ذرات، جریان‌پذیری و پیوستگی، زاویه ریپوز و دمای گذار شیشه‌ای بررسی گردید. دمای خشک کردن اثر معناداری ($P<۰/۰۵$) بر اکثریت خصوصیات فیزیکی پودر قارچ داشت. نمونه‌های پودر دارای فعالیت آبی کمتر از ۰/۳ بودند که منجر به ایجاد شرایط پایدار می‌شود. کاهش دمای خشک کردن منجر به افزایش رطوبت و تشکیل ذرات بزرگتر گردید. پودر قارچ تولید شده در دمای بالاتر دارای جریان‌پذیری بهتری بود. دمای گذار شیشه‌ای در محدوده ۵۵/۶-۴۱/۳ درجه سانتی‌گراد بود. افزایش دمای خشک کردن موجب افزایش ترشوندگی و پخش‌شوندگی گردید.

واژه‌های کلیدی: پودر قارچ، جریان‌پذیری، ترشوندگی، پخش‌شوندگی، حلالیت.

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Research Full Papers

Evaluation of antioxidant potential and antimicrobial activity of Mocheh (*Lepidium draba*) extract "*in vitro*"

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Abstract

Usage of natural products like herbals, provide unlimited opportunities for novel and suitable additives. Mocheh can be used in fresh form or as an ingredient in soup and salad. This study was aimed to determine the antimicrobial and antioxidant activities of Mocheh (*Lepidium draba*) extract. The antimicrobial activity of Mocheh extract was tested against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus niger*. The extract showed a strong antimicrobial activity with a concentration dependence and a broad antimicrobial spectrum for all tested microorganism species. The results showed that MIC of leaf extract of *Lepidium draba* on *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* was 128, 128, 128 and 128 mg/mL respectively. The results showed that MBC/MFC of leaf extract of Mocheh on the examined microorganisms was 256, 256, 256 and 256 mg/mL respectively. The values obtained for total flavonoid content and total phenolic content were 22.13 µg/mL and 18.88 mg/mL, respectively. Measured value in the radical scavenging activity was IC_{50}= 168/21 µL/mL. The results showed that Mocheh leaf aqueous extract as a novel source of natural antimicrobial and antioxidant agents for the food and pharmaceutical industries.

Keywords: Mocheh, Extract, Gram-positive and Gram-negative bacteria, Antioxidant activity.

Introduction

Foodborne disease and food poisoning are still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and toxic microorganisms in foods (Shan *et al.*, 2007). Antimicrobial agents are defined as chemical compounds that presented or added to foods for retarding microbial growth or inducing microbial death. In the last decades, there has been particular interest in the use of abundant naturally occurring antimicrobials (herbs, spices and plants) (Klančnik *et al.*, 2010).

On the other hand, antibiotic resistance has become a global concern. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Tchinda *et al.*, 2017). This has driven scientists to search for new antimicrobial substances from various sources like the medicinal plants (Al-Marzoqi *et al.*, 2015). Natural products, either pure compounds or standardized plant extracts, provide unlimited opportunities for novel and suitable additives and drug treatments due to their unmatched range of chemical diversity and may offer a new source of antibacterial agents (Brantner *et al.*, 1994; Klančnik *et al.*, 2010).

The medicinal plants are those which contain substances in their organs that can be used for therapeutic purpose or as a precursor

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for the synthesis of other useful medicines. It was well established that the plants which are naturally rich in a wide variety of secondary metabolites, such as alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties (Chyad, 2017).

Several antimicrobial compounds are extracted from easily available sources, such as agricultural and horticultural crops (e.g. grapevine, citrus, hops, berries, tea leaves etc.), or medicinal plants such as pine, sage, rosemary, and many others (Klančnik *et al.*, 2010). The Mocheh (*Lepidium draba*) was named as a class have a place under the family Brassicaceae, privately known as white top or hoary cress. The plant is local to western Asia including Iran and Eastern Europe and also as an intrusive species in North America (Chyad, 2017; Radonić *et al.*, 2011). It is a perennial herb with initial spoon like leaves and rectangular stem leaves, white flowers and grips like flat egg like or heart like fruit. It could be used in rice as cooked plant. Infusion of its leaves and seeds has purgative and expectorant effects. In the local dialect is called Mocheh (Haghighi *et al.*, 2011). Brassicaceae plant kingdom harbors an inexhaustible source of active ingredients valuable in the management of many intractable diseases. Numerous studies have identified compounds within herbal plants, which are effective antibiotics and antioxidants (Hussein, 2016).

The explore of biologically active components from plants has always been great interest to scientists looking for new promising sources of practical for herb- based medicines, food supplements, pharmaceuticals and health products (Hussein, 2016).

The aim of this study was assessing the *in vitro* the possible effects of antimicrobial activity of aqueous extract of Mocheh upon food born pathogenic microorganisms including *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus niger* and to evaluate its antioxidant properties.

Material and methods

Collection of plant materials and extract preparation

The leaves of *Lepidium draba* L. were collected at the beginning of the vegetative period (summer), from the rangelands of the Zagros Mountains, Shahrekord City, Chaharmahal Bakhtiari Province, Iran. A voucher example of the plant was stored to be recognized and validated at Ferdowsi University of Mashhad, Institute of Plant Sciences. The plant leaves were dried in the shade for a few days at room temperature and afterward pounded as powder and weighed (Chyad, 2017). Plants were extracted as described by Sigstedt et al., 2008 with some modifications. Fifty grams of plant powder were then macerated in 250 mL of distilled water. Extract was prepared using the maceration process for 72 h under constant shaking and filtered with Whatman No. 1 filter paper. The extract was evaporated to dryness under reduced pressure using a rotary evaporator (Heidolph laborota 400, Heidolph Instruments, Germany). The yield of the extract (w/w) was calculated according to Bazzaz et al., 2003.

Preparation of inoculum

The strains of *Bacillus subtilis* PTCC 1023, *Pseudomonas aeruginosa* PTCC 1707, *Staphylococcus aureus* PTCC 1337 and *Aspergillus niger* PTCC 5010were purchased from the laboratory of industrial microbiology, faculty of agriculture, Ferdowsi University of Mashhad (FUM). Microbial strains were cultured 24 h at 37ºC for bacteria and 72 h at 25ºC for fungal strains before the antimicrobial tests were performed. A 0.5 McFarland standard of microbial strains was used estimate their concentratin, which was equivalent to 1.5×10^8 colony forming unit (CFU)/mL (Amin Mir *et al*., 2016).

Antimicrobial activity

Determination of minimum inhibitory concentration (MIC)

Dilutions of Mocheh extract (512, 256, 128, 64, 32, 16, 8, 4, 2, 1 mg/mL) were prepared in

sterile Muller Hinton Broth (MHB) (Sigma-Aldrich), Potato Dextrose Broth (PDB) (Sigma-Aldrich) for bacterial and fungal strains, respectively.Ten µL of microbial suspensions (at 1.5× 10^8 CFU/mL) was added to each dilution at the 96-well microplates. The inoculated microplates were then incubated at 37˚C for 24 h and those inoculated with fungal strains were incubated at 25˚C for 72 h. The lowest concentration that prevented visible growth determined as MICs (Baba *et al*., 2015).

Determination of minimum bactericidal/ fungicidal concentration (MBC/MFC)

Ten µL of the culture from each well in which the red color was not observed, was streaked on MHA for bacterial strains and PDA for fungal strains. The plates were then incubated at 37°C for 24 h for bacterial strains or 25°C for 72 h for fungal strains and MBC or MFC was defined as the lowest concentration at which no colony of microorganism was observed (Wendakoon *et al*., 2012).

Determination of inhibitory zone by well diffusion agar method

A 20 mL of sterile MHA and PDA media was poured into plates and allowed to set. The plates were then seeded with 10 µL suspensions of microorganisms with concentration adjusted to approximately 1.5× 10^8 CFU/mL and were kept for drying. Wells were made on the plates with sterile whole puncture (6 mm diameter). Sixty µL of aqueous leaf extract of Mocheh (100, 200, 300 and 400 mg/mL) was poured into each respective wells. The plates were then incubated at 37°C for 24 h and those spread with fungal stains were incubated at 25°C for 72 h. The antibacterial activity of the plant extract was then assessed by an inhibition zone surrounding the well and zone of inhibition (ZOI) was measured (Sohail *et al.*, 2014).

Antioxidant activity

Free radical scavenging capability

DPPH (1,1-diphenyl-2-picryl hydrazine) free radical scavenging capability of *Mocheh* water extract was evaluated by the method of Bursal and Ekrem (2011) with a slight modification. Briefly, different concentrations (150, 200 and 250 µg/mL) of *Mocheh* water extract was prepared and the volume was then adjusted to 3 mL with ethanol. One milliliter of 0.1 mM alcoholic DPPH solution was added to each sample. These samples were vortexed and incubated in a dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank samples. Decreased absorbance of the sample indicates the DPPH free radical scavenging capability (Bursal *et al*., 2011). The control samples contained all the reagents except the extract. The percentage of inhibition was calculated using the following equation:

% inhibition=100× (A of control−A of sample)/A of control (Baba *et al.*, 2015).

Reducing power

Two and half mL of the extract and butylated hydroxyl toluene (BHT) were mixed with 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and were incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 200 g for 10 min. 2.5 mL of the supernatant was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance at 700 nm was measured against sodium phosphate buffer as a blank. The increased absorbance of the reaction mixture correlates with greater reducing power (Ardestani *et al*., 2007).

Determination of total flavonoid content

Total flavonoid content in water extract of Mocheh was estimated by a colorimetric assay. At first, 1 mg water extract Mocheh was pipetted into a test tube. Then, 0.1 mL CH_3COOK (1.0 M) and 0.1 mL of 10% Al $(NO_3)_3$ in 4.3 mL ethanol solution were added. The samples were vortexed and then left to stand at room temperature for 40 min. Absorbance measurements were recorded at 415 nm. Distilled water was used as blank and also instead of sample, distilled water was used for control. A calibration curve of quercetin was plotted, and flavonoid contents were determined from regression equation of the calibration curve. The results were reported as

quercetin equivalents per milligram extract (Kossah *et al*., 2011).

Determination of total phenolic content

A calibration curve of Gallic acid in methanol was constructed in concentration range of 0.04-0.7 mg/mL. The solutions for the spectrophotometric analysis were performed as follows: in a 50 mL volumetric flask 1 mL of a standard solution of gallic acid, 6 mL of methanol, 2.5 mL of the Folin-Ciocalteau reagent, 5 mL of 7.5% Na_2CO_3 were added, reaching the final volume with purified water. The solutions were stored overnight and the spectrophotometric analysis was performed at 765 nm. The polyphenols were determined as follows: 2.5 g of extract was diluted with 50 mL methanol 80 % in falcon and was shaken at 240 rpm for 24 h. The sample was filtered twice with Whatman 0.2 µm. 500 µl of diluted extraction was added to 2.5 mL Folin-Ciocalteau reagent and 5 mL of Na_2CO_3 (7.5%). The samples were stored overnight, and the spectrophotometric analysis was performed at 765 nm (Capannesi *et al.*, 2000).

Statistical analysis

Results of the study were based on statistical package for the social sciences (SPSS) version 16.0 and differences among the means were determined for significance at $p<0.05$ by One-way ANOVA.

Results and discussion

The present work investigated the antimicrobial and antioxidant activity of Mocheh. The results of MIC and MBC/ MFC assays are presented in Table 1. The results showed that MIC of leaf extract of Mocheh on *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* was 128, 128, 128 and 128 mg/ mL respectively. The results showed that MBC/MFC of leaf extract of Mocheh on *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* was 256, 256, 256 and 256 mg/mL respectively. According to Kuete (2010), Kuete and Efferth (2010), the antibacterial activity of a plant extract is considered significant when MIC values are below 100 µg/mL, moderate when $100 \leq MIC \leq 625$ µg/mL and weak when $MIC > 625$ µg/mL (Victor Kuete, 2010; V. Kuete *et al.*, 2010; Tchinda *et al.*, 2017).

Table 2 shows the zone of inhibition exposed to different concentrations of aqueous extract of Mocheh by disk agar diffusion test. The results revealed that by increasing the concentration of aqueous extract of Mocheh, a greater zone of inhibition was observed. This implied that the gram-positive bacteria were more susceptible to this extract than the gram-negative bacteria. This observation is probably due to the presence of outer membrane in gram-negative bacteria that serves as a significant barrier in gram-negative species. Furthermore, the results introduced gram-positive bacteria as the most susceptible bacteria, an observation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by the active compounds of the examined extract (Al-Marzoqi *et al.*, 2015).

Table 1- Quantities of MICs and MBCs were obtained under the influence of various concentrations of aqueous extract of Mocheh leaves

Microorganisms	MIC (mg/mL)	MBC/MFC (mg/mL)
Aspergillus niger	128	256
Bacillus subtilis	128	256
Pseudomonas aeruginosa	128	256
Staphylococcus aureus	128	256

Results obtained from study of Al-Marzoqi et al. (2015) showed that active compounds of *Cardaria draba* (*Lepidium draba* L.) had wide spectrum antibacterial activity. The results revealed that both Gram-positive *Staphylococcus aureus* and *Staphylococcus saprophyticus* were susceptible to alkaloid and terpenoid compounds, while *Staphylococcus*

epidermidis was susceptible to alkaloid compounds only. In addition, all Gram-negative bacteria were resistant to active compounds except *Serratia* was susceptible to phenolic, alkaloid and terpenoid compounds, while *Proteus* and *Pseudomonas* were susceptible to terpenoid compounds only (Al-Marzoqi *et al.*, 2015).

About 20% of all investigated plants with antibacterial activity are rich in polyphenolic substances, tannins, catechins and polyphenolic acids. It is known that polyphenols can form heavy soluble complexes with proteins. Polyphenols may bind to bacterial adhesions and by doing so they disturb the availability of receptors on the cell surface (Brantner *et al.*, 1994).

In the study of phytochemicals compounds of Mocheh amounts of total phenolic content (TPC) and total flavonoid content (TFC), were 22.13 µg/mL and 18.88 mg/mL, respectively. The ability of aqueous extract of the Mocheh to quench DPPH free radical was measured. The extracts and BHT demonstrated a dose-dependent scavenging activity by reducing DPPH radical (Fig. 1). By plotting the graph of extract concentrations against the scavenging activity, a specific concentration of the sample that needed to provide 50% inhibition (IC_{50}) was calculated. IC_{50} for aqueous extract of the Mocheh was 168.21 µl/mL.

Table 2- Average inhibition zone (mm) of Mocheh leaves extract concentrations on some pathogenic microorganism's thorough well diffusion agar method

Microorganisms	Well diffusion agar			
	Concentrations (mg/mL)			
	100	200	300	400
Aspergillus niger	-[a]	-[a]	15.20± 0.42[b]	19.00± 0.58[c]
Pseudomonas aeruginosa	11.10± 0.33[a]	19.00± 0.25[b]	21.20± 0.40[c]	24.00± 0.50[d]
Bacillus subtilis	12.12± 0.36[a]	17.30± 0.44[b]	20.20± 0.59[c]	22.10± 0.57[d]
Staphylococcus aureus	13.30± 0.78[a]	18.00± 0.36[b]	20.50± 0.72[c]	22.30± 0.66[d]

Values are expressed as mean±standard deviations, $n = 3$; different letters (a, b, c and d) in each row show significant difference at $p \leq 0.05$.

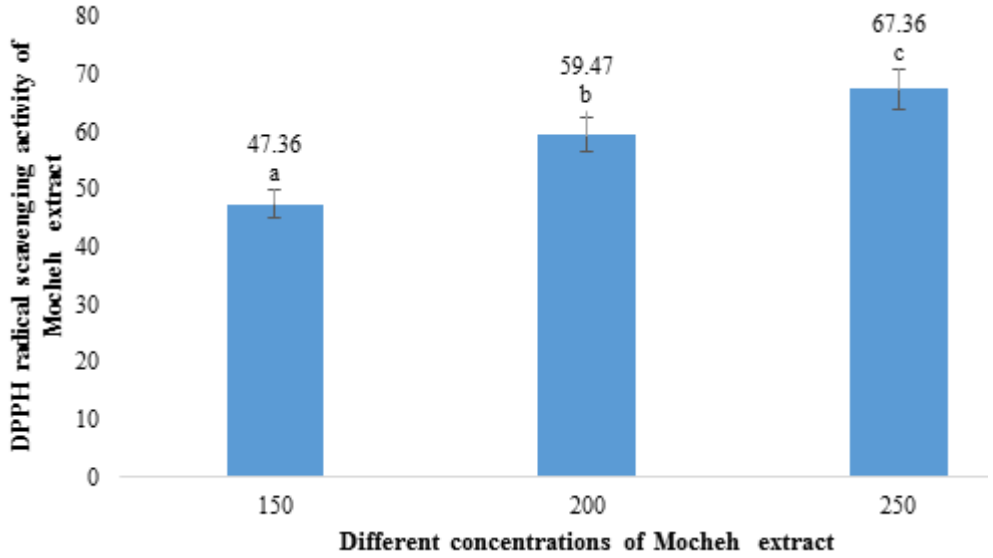

Fig. 1. DPPH radical scavenging activity of aqueous leaf extract of Mocheh.

Hussein (2016) identified the phytochemical compounds of the ethanolic extract of the leaves of *Lepidium draba* L. by gas chromatography–mass spectrometry, fourier-transform infrared spectroscopy and atomic absorption spectrophotometry techniques. The results showed that the leaves of this plant are a rich source of biologically active chemical

compounds, fibers and minerals. The author stated that the leaves of *Lepidium draba* L. can be used as a multifunctional drug source, although clinical trials are needed to prove the effectiveness (Hussein, 2016). Chyad et al. (2017) demonstrated that the extract of *Lepidium draba* had anticancer, analgesic and anti-inflammatory activities (Chyad, 2017).

Conclusion

According to the findings of this study, Mocheh extract showed more effective impact on the growth of gram-positive bacteria than the gram-negative ones. Since Mocheh is extensively distributed in Iran and its extract showed significant antimicrobial and antioxidant activities, this plant might be utilized as a raw material to produce natural antioxidants and/or preservatives for the food industry. Moreover, our findings are supporting the use of Mocheh as a traditional remedy in the treatment of gastrointestinal disorders.

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ارزیابی پتانسیل آنتی‌اکسیدانی و فعالیت ضدمیکروبی عصاره مچه (*Lepidium draba*) در شرایط برون‌تنی

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چکیده

استفاده از محصولات طبیعی مانند گیاهان دارویی، فرصت‌های نامحدودی برای معرفی مواد افزودنی جدید فراهم کرده است. از مچه برای تهیه سوپ، سالاد و غذاهای تازه استفاده می‌شود. این مطالعه با هدف سنجش فعالیت ضدمیکروبی و آنتی‌اکسیدانی گیاه مچه انجام شد. اثر ضدمیکروبی عصاره مچه در برابر *باسیلوس سوبتیلیس*، *سودوموناس آئروژینوزا*، *استافیلوکوکوس اورئوس* و *آسپرژیلوس نایجر* مورد آزمایش قرار گرفت. نتایج نشان داد که حداقل غلظت مهارکنندگی عصاره مچه برای *آسپرژیلوس نایجر*، *سودوموناس ائروژینوزا*، *باسیلوس سوبتلیس* و *استافیلوکوکوس اورئوس* به‌ترتیب ۱۲۸، ۱۲۸، ۱۲۸ و ۱۲۸ میلی‌گرم بر میلی‌لیتر بود. حداقل غلظت کشندگی عصاره نیز به‌ترتیب برای سویه‌های مذکور ۲۵۶، ۲۵۶، ۲۵۶ و ۲۵۶ میلی‌گرم بر میلی‌لیتر بود. مقادیر به‌دست آمده برای فلاونوئید کل و محتوای فنلی کل به‌ترتیب ۲۲/۱۳ (میکروگرم بر میلی‌لیتر)، ۱۸/۸۸ (میلی‌گرم در میلی‌لیتر) بود. فعالیت آنتی‌اکسیدانی برحسب IC_{50} ۱۶۸/۲۱ میکرولیتر در میلی‌لیتر بود. نتایج این مطالعه در مورد فعالیت‌های بیولوژیکی عصاره مچه احتمال استفاده از عصاره آبی برگ مچه را به‌عنوان منبع جدیدی از مواد ضدمیکروبی و آنتی‌اکسیدان طبیعی مواد غذایی و صنایع دارویی ارائه می‌دهد.

واژه‌های کلیدی: مچه، عصاره، باکتری‌های گرم مثبت و گرم منفی، فعالیت آنتی‌اکسیدانی.

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Research Full Papers

Development of low-fat chicken nuggets using fish protein concentrate in batter formulation

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Abstract

The effect of various levels (5, 10 and 15%) of fish protein concentrate (FPC) in batter formulation was investigated. The physicochemical properties of chicken nugget were evaluated in order to find the optimium level of FPC in batter formulation. Flow behavior showed that the control batter and a treatment contains of 7.5% FPC had higher viscosity. Moisture loss and fat uptake in control sample was higher than all treatments contain FPC in both deep fat and air fryer. Thicker crust resulted by higher level of FPC in batter leads less oil uptake during frying. Moreover, the samples contain FPC had the highest score in terms of texture and overall acceptability. In spite the fact that nuggets contain FPC had the high rate of our research priorities, however, the level around 15% considered as a limitation. Using desirability optimization, the range between 7.5- 8% of PFC in batter formulation was selected as the best level.

Keywords: Chicken nugget, Desirability, Fat absorption reduction, FPC, Frying

Introduction

Chicken nugget is the most popular restructured meat and a popular snack prepared using chicken meat which is consumed by adults and children as their favorite meal (Evanuarini and Purnomo 2011). However, healthy food is the most important priority for consumers and chicken nugget as a fried food with high calorie content is highly health concerns. Hence, nowadays many studies focus on the fat reduction in fried food (Adedeji, 2010; Mah, 2008).

Some factors like frying time and temperature, initial moisture content, post frying treatment and surface condition of food influence on fat absorption in frying (Rice and Gamble 1989; Gamble and Rice 1988; Adel-aal and Karara 1986; Fan and Arce 1986; Mittelman *et al.*, 1982; Pinthus and Saguy 1994; Lulai and Orr 1979; Ng *et al.*, 1957). In addition, surface properties act as a determining factor in the level of fat absorption and moisture loss. Batter described as a different level of flour to water mixture which food items are dipped inbefore frying (Loewe, 1990). Applying edible coating and batter system are considered as a convincing method to surface modification and fat uptake reduction (Adedeji and Ngadi, 2009; Pedreschi *et al.*, 2005; Mellema, 2003; Mallikarjunan *et al*., 1997).

Great emphasis on reduction in dietary fat intake to less than 30% of calories is suggested by health organizations (Saguy and Pinthus, 1995) and consumer demand for low fat fried food with good organoleptic attributes (Gennadios, 2002) encouraged researchers towards methods of oil absorption reduction with no adverse effect on other quality attributes such as taste, texture, and surface color during deep fat frying (Hansen, 1998). According to many researches, protein-based edible films and coatings proved to be a proper alternative for aforementioned issues. In other word, they can form structures and networks and contribute in improvement the food quality properties by interacting with other components (Kinsella *et al.*, 1994). The three-dimensional network formation enables proteins to

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physically entrap a large amount of water within the matrix (Hermansson, 1979). Furthermore, they possess hydrophilic properties and exhibit the ability of lipid barrier, a barrier between foods and frying medium (Krochta and De Mulder-Johnston, 1997), reduced moisture migration, improve breading adhesion, increased retention of natural juices and flavors and enhanced texture and appearance. So, in some cases protein can replace with the main part of the batter system, flour, to reach the aim.

On the other hand, in spite of the same nutritional value, some of the fish species are less favorable. In fact, they have no commercial value and the reasons ley behind it; can be an unusual size and unappealing taste. Hence, only a few fish species are usually consumed which cause fish consumption to be restricted to people of the specific level. But, nowadays the general view changes toward utilization of fish protein products in their meal. These are low cost and high quality, these can fill protein gap especially among poor people and diminish protein deficiency (Cordova-Murueta *et al.*, 2007). Furthermore, FAO (2006) reported that fish protein concentrates in human diet especially sustainable growing babies and pregnants is beneficial and improve the quality of life. It is also noted that fish by-products are able to reduce blood pressure, type-II diabetes (McCarty, 2003), improve glucose tolerance and insulin sensitivity (Lavigne *et al.,* 2000) when using as protein supplements in food products. Fish by-products can considered as an intermediate product and gain commercialization. In another word, they can be processed to a high quality and applicable fish products which can be utilized in human consumption as an ingredient in different food product formulations (Martin, 2012). Hence, using low-cost fishes is becoming increasingly important, as they can be converted to fish protein concentrate or fish protein hydrolyzate. It is possible to increase protein concentration by removing water, oil, bones and other materials (Finch, 1977). Based on this definition, Finch (1977) and Mackie (1983) stated that the FPC can be produced by the simple removal of water from fish flesh.

Besides all the sbove mentioned, functional properties are the considerable factor if the protein is utilized as an additive in food products. So the aim of this research is discovering the functional properties of FPC and its optimium level as an ingredient in batter formulations. Furthermore, to reach the low fat fried food with good organoleptic attributes, oil absorption reduction with no adverse effect on other quality attributes during deep fat frying, on the other hand the low cost and high quality of fish by- products were the motivation and sufficient reason of utilization fish protein concentrate in the batter system of chicken nugget and evaluation of product.

Materials and methods

Fish protein concentrate

Food-grade FPC from Saithe (Pollachius virens) was purchased from national marine research institute of Anzali prepared under sanitary conditions. According to Knobl et al. (1971) the procedure contains of two consecutive sets of step in which fish and isopropyl alcohol were mixed, then the solid and liquid were separated in a centrifuge. The final solid phase was de-solventized in a vacuum oven at 160˚C for about 18 to 22 h.

Physicochemical analysis of FPC

Fat, moisture, crude protein and ash of fish protein concentrate were determined by standard procedures of Association of Official Analytical Chemists (1990). Crude protein content was determined using the Kjeldahl method (Kjeltec System, FOSS Tectator, Hoganas, Sweden). Crude lipid content was measured by the Soxhlet method (Soxtec System-Texator, Sweden). Ash content was determined by ashing samples about 5 hours at 550°C. Moisture content was determined by drying samples for 4 h at 105°C until constant weight was achieved. Carbohydrate content was calculated by difference. The pH of the FPC samples was measured according to Bragadottir et al. (2007).

Functional properties

Solubility

Solubility was determined based on the Eastman and Moore method (1984) with some modification. One g of powder was added to 100 ml of distilled water and mixed at high velocity in a mixer for 5 min. The solution was centrifuged for 5 min at 30.000× g. then 25 ml of the supernatant was oven-dried at 105ºC, for 5 h in a weighed Petri dishes. Weight difference of petri dishes before and after oven-drying described solubility (%).

Water holding capacity

The water holding capacity was measured using the method described by Taheri et al. (2013), FPC and distilled water in a ratio of 1 to 10 were mixed and centrifuged for 20 min at 1800×g. The difference between primarily volume of water and the supernatant determined as a milliliter of water absorbed per gram of protein.

Oil binding capacity

According to Taheri et al. (2013) a ratio of 1/10 FPC to sunflower oil was vortexed for 30 sec, let the mixture remains in a room temperature for 30 min. Then it was centrifuged for 10 min at 13600× g. The oil on the top was pulled and gram of the oil absorbed expressed as the oil binding capacity.

Batter and chicken nugget preparation:

The nugget core formulation was prepared by mixing %86 boneless and skinless chicken breast, %12.9 onion, %1 salt and %0.1 pepper in a mixer then shaped in 2.8*2.8 dimensions. Batter was prepared by mixing %90.8 wheat flour, %3.1 baking powder, %0.6 pepper, %5.5 salt w/w and a portion of 1.2 water to dry mix. In order to investigate the effect of FPC in batter formulation, wheat flour was replaced with 5, 10 and 15% FPC. Chicken samples were dipped in batter then fried in a deep fat (containing 1.5 litter sun flower oil at 170 ℃ for 8 min) and air fryer (170°C for 28 min). It should be noted, the time and temperature in each fryer determined based on pre-treatment test and according to device instruction. Fried nuggets were allowed to remove their excess oil on a tissue paper before consequent analysis.

Batter rheological test

Batters flow behavior was determined using a Bohlin rotational viscometer (Bohlin, Visco 88, Bohlin Instruments, UK). Proper spindles (C14, C25 & C30) were selected based on samples viscosity. Shear rate logarithmic elevation occurred over the range 14.2– 200 s^{-1} at 25°C. Batters flow behavior (shear stress-shear) was described based on the power law model:

$$\tau = k\dot{\gamma}^{n} \quad (1)$$

Where τ is the shear stress (pa), g: is the shear rate (s^{-1}), k is the consistency coefficient ($Pa.s^n$), and n is the flow behavior index (dimensionless).

Chicken nugget analysis:

Moisture content and Fat content

The whole samples were placed in a conventional oven (Memmert) for 24 h at 105˚C according to AACC (1986). Samples were weighted to determine dry basic moisture content followed by cooling in a desiccator. Fat content analysis was carried out by soxhlet extraction using the dried sample used for moisture content determination (AOAC, 1990).

Crust thickness

A computer vision system consists of illuminating lights placed 45 cm above the sample in a wooden box was used for crust thickness evaluation. A color digital camera (Canon EOS 1000D, Taiwan) was located vertically. Samples were cut vertically and images were captured with the above digital camera. The crust thickness was measured using Image J software (National Institutes of Health, USA) version 1.42e. The distance between two lines in the picture measured by straight line tool in the software considered as a crust thickness (Fig 1).

Sensory properties

Ten regular consumers of nugget were invited to evaluate color intensity, texture,

oiliness, internal moisture, flavor and overall acceptability using a 7-point hedonic scale. 1 represented extremely dislike and 7 extremely like. Evaluation was performed in a partitioned sensory laboratory, under the white fluorescent light, two sets of three digit coded samples were assessed and tap water was used between the samples consumption.

Fig. 1. The distance between two lines stands represent the crust thickness of nugget samples.

Statistical analysis and optimization

Data from randomized design were analyzed using analysis of variance. Treatments means were compared by Duncan's multiple range tests using SPSS 16.0 for Windows (SPSS Inc., Chicago, USA). Besides, multiple response method (desirability) was used in order to find optimized level of FPC based on physicochemical and sensorial characteristics.

Results and discussion

Physicochemical properties of FPC

The proximate composition of FPC used as a material for batter preparation is given in Table 1. Based on reports by Cordova-Murueta et al. (2007); Sathivel et al. (2009); Shaviklo et al. (2010a), fish species and added ingredients are the determinant factor of FPCs proximate composition. The protein content of FPC utilized in this research is mostly similar to freeze dried saithe protein isolate reported by Shaviklo et al. (2012); Barzana and Garcia-Garibay (1994). Also, Sen (2005) reported higher than 65% of protein content for FPC prototypes.

Table 1- Proximate composition

	Moisture	Fat	Protein	Ash	pH
FPC sample	4.15	0.5	94.25	1.1	6.78

Functionality

The functional properties of FPCs are depicted in Table (2). Drying process can affect physicochemical changes of protein and functional properties. So, based on different drying and processing conditions, no one can anticipate the same product from the same raw material (Shaviklo *et al*. 2010a, 2012).

FPC exhibited oil binding capacity of 1.97 g oil/g protein. According to He et al (2015), it can be concluded that the FPC with low oil-binding capacity can cause the fried food to absorb lower amount of oil. Water holding capacity of FPC is fairly similar to other products include freeze dried saithe protein isolate without additives, freeze dried Fish protein isolate with 5% sucrose and 0.2% phosphate, spray dried saithe with additives (2.5% sucrose and 0.2% phosphate), Freeze dried saithe without additives and Freeze dried saithe with additives (2.5% sucrose and 0.2% phosphate) which reported by Shaviklo et al. (2012) and Shaviklo et al. (2010c). Relatively high water holding capacity is attributed to high level of protein content of FPC. FPC protein solubility was higher than hake protein powder (HPP), soy protein concentrate (SPC) and pea protein isolate (Pires *et al*., 2012) however, it was lower than the egg white powder (Pires *et al.,* 2012) and Fish protein hydrolysates (Souissi *et al.,* 2007). The relatively low solubility of HPP and FPC may result from myofibrillar proteins solubility which is highly soluble at very acidic or alkaline pH and less soluble between 4.0 and 9.0 (Batista *et al*., 2006). Moreover, the distinction among several

results can be attributed to the difference in peptide length and the ratio of hydrophilic/hydrophobic peptides (Souissi *et al.*, 2007).

Table 2- Functional properties

	Solubility	WHC	OBC
FPC	24.13%	305%	1.97goil/gpr

Batter rheology

Apparent viscosity of different batter formulation was investigated as a function of shear rate (Table 3). Non-Newtonian shear thinning behavior was the obvious feature of all formulations. The power law ($R^2 \geq 0.91$) was the best model for describing the experimental data. Batter contains 15% of FPC and the standard sample had the highest and lowest viscosity respectively which represented that increasing the FPC in the batter formulation causing an enhancement in a batter viscosity. Viscosity significantly influenced by composition and proportion of ingredients, the solids–water relationship, temperature, solubility and water binding capacities of the dry ingredients, molecular weight, and structural association (Meyers 1989; Baixauli *et al*., 2003; Fiszman and Salvador 2003). Fiszman and Salvador (2003) and Sahin and Sumnu (2009) claimed that the proteins in batter provide a structure and increase the consistency of raw batters which is reflected by a rise in viscosity. Dogan et al. (2005) asserted that characteristics of dry ingredients have a key role in a batter viscosity development. They also explained that the highest viscosity of batter contains of soy flour is related to the maximum amount of bonded water among different flour. Therefore, it can be concluded that the higher viscosity of batter contains of FPC, compare with the control can be explained by higher amount of water bonded by FPC. Control batter providing low viscosity may be due to more free water available for movement like Grape Seed Powder- added batter in Kumcuoglu et al (2015). Batter viscosity influence on final product appearance, texture and oil uptake.

Table 3- Consistency index (K) and flow behavior index (n) of batter with different level of FPC

	K (Pa S^n)	n	R^2
Control	47.08	0.39	0.97
5%	117.12	0.29	0.96
10%	169.67	0.28	0.91
15%	219.56	0.19	0.98

Nugget moisture and fat content:

Batter formulation due to its level of FPC was significantly ($p<0.05$) influence on moisture loss in deep fat fried nugget. However, there have no significant ($p>0.05$) influence on air fried samples in case of moisture content. About deep fat fried samples, results showed that the addition of FPC up to the level of 10% leads to increase moisture, however the higher level (15%) decreased moisture content. It is worth to be noted that standard sample (with no FPC in batter) and nugget contain 5% FPC had the lowest and highest moisture content respectively in air fried samples (Fig 2).

Fat uptake was not significantly ($p>0.05$) influenced by FPC in the batter formulation in both deep fat and air frying. Analysis represented that the addition of FPC up to level of 10% in batter formulation causes a reduction in fat uptake. In fact, fat uptake had the opposite trend of moisture loss. Air fried samples contain 10% FPC also had significantly lower fat content than the others (Fig 3).

The higher moisture retention and consequently lower oil uptake may be related to the higher water holding capacity of FPC compared to the wheat flour and preventing water vaporization followed by lower porosity. Gamble et al. (1987); Krokida et al. (2000); Ufheil & Escher (1996); Ngadi et al. (2007) also reported the same results. Furthermore, they presented a negative linear relationship between moisture and oil content. Dogan et al. (2005) found that the soy flour in soy flour added batter can control water loss and oil uptake due to its high water binding capacity. Aminlari et al. (2005) used protein coating in order to reduce in water loss and oil absorption during frying.

Our finding about fat uptake reduction in protein based batter verified by He et al. (2015) using fish protein hydrolysates, Dehghan Nasiri

et al. (2010) using soy flour and Dogan et al. (2004), using soy and rice flour in batter formulation. As Salvador et al. (2005) reported, greater fat absorption attributed to the lower moisture retention during frying. FPC in batter formulation caused crust to be able to retain a higher amount of moisture and absorbed relatively lower oil than the control in the frying process. This important quality factor attributed to the higher water holding capacity of FPC and higher viscosity of batter containing FPC. High viscosity batter considered as a factor to control of moisture releasing and oil absorption during frying.

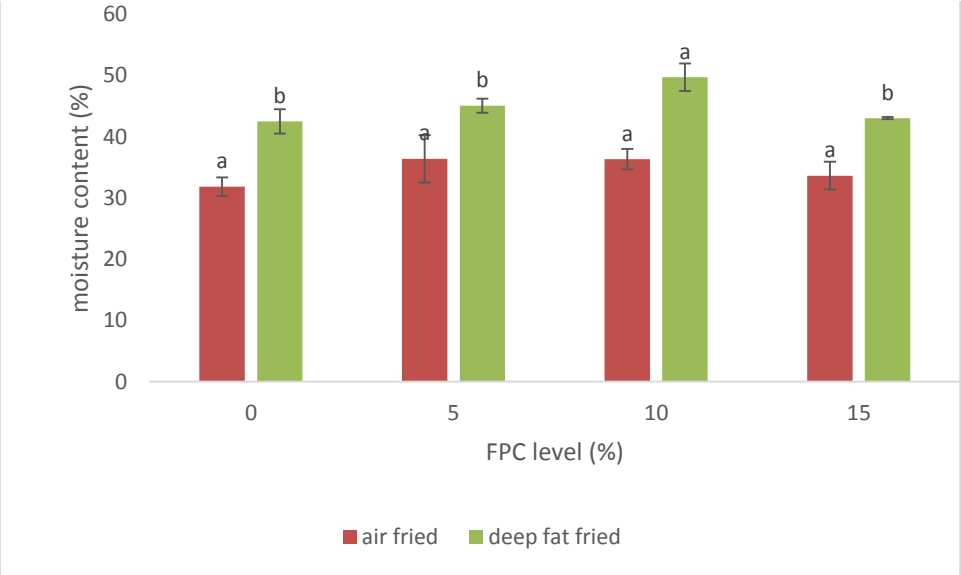

Fig. 2. Changes in moisture content by different level of FPC in batter formulation

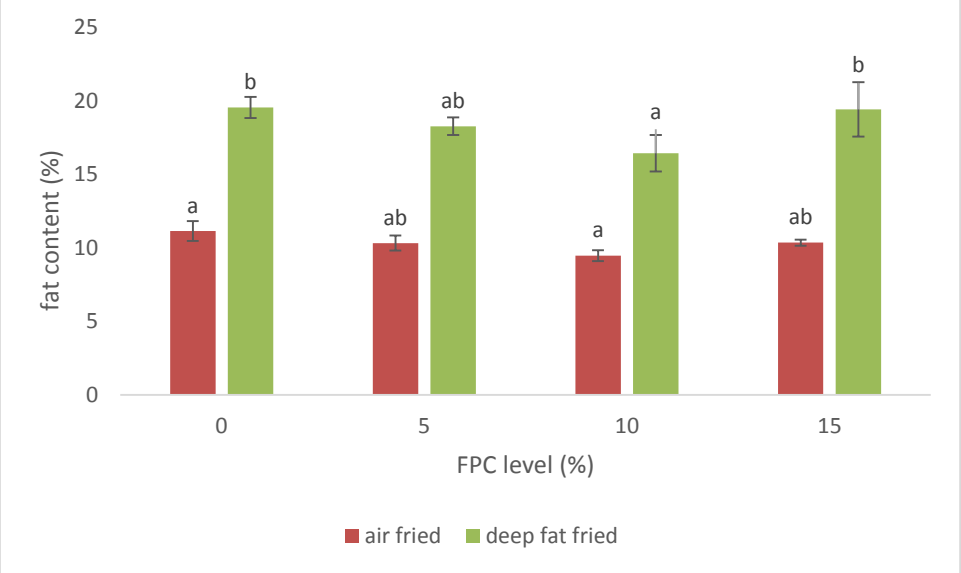

Fig. 3. Changes in fat content by different level of FPC in batter formulation

Crust thickness

Results of crust thickness analysis are presented in fig 4. The level of FPC significantly ($p<0.05$) affect the crust thickness in deep fat frying. Results showed that the thicker crust was obtained by the higher level of FPC in batter formulation and vice versa.

Air fat fried samples had the same trend of deep fat fried samples in terms of crust

thickness however, the effect of FPC level on crust thickness was not significant ($p>0.05$).

Oil absorption and distribution depends on several factors, however; it mostly occurs in the crust region (Gamble *et al*., 1987; Keller *et al*., 1986; Pinthus *et al*., 1995). Therefore, crust considered as a critical factor of oil distribution in fried food (Varela, 1988). Mariscal and bouchon, (2008) stated that the formation of crust during the pre-drying step causes the vacuum fried apples loss lower amount of water. Pinthus et al. (1995) has studied thickness as one of crust physical properties and oil uptake. They stated that developing the crust as a mass transfer resistance factor causes the frying process a mass transfer limiting process. McHugh et al. (1993) reported that mass transfer resistance of edible films attributed to the increasing of film thickness. Thickness as an important crust property can affect the oil absorption of fried food during the process. In the research performed by Moriera. (2004), chips with a thicker crust had lower total oil saturation. The analysis in the current research has shown that the oil uptake was affected by crust thickness, as increasing the crust thickness under the influence of FPC in batter formulation resulted to a decrease in oil uptake of final fried food.

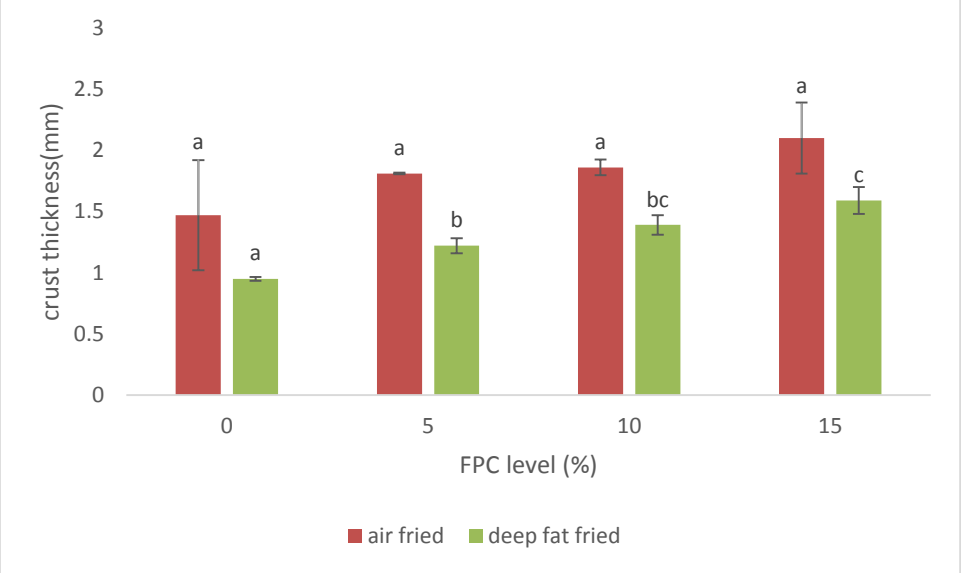

Fig. 4. Crust thickness influenced by different level of FPC in batter formulation

Sensory analysis

Texture was significantly ($p<0.05$) influenced by the level of FPC in batter formulation of air fried samples. However, the same factor was not significantly ($p>0.05$) influenced by the level of FPC in batter formulation of deep fat fried samples.

Addition of FPC in batter formulation had significant ($p<0.05$) influence on overall acceptability in both air and deep fat fried sample (Table 4).

Results of analysis showed that the addition of FPC up to level of 10% leads to higher score in texture evaluated by panelist and the sample contains of 15% had the least score among them.

Sample contains of 5% FPC in batter had the highest score in terms of overall acceptability in both types of frying.

Macaroni supplemented with 10% FPC was the most acceptable ones in terms of sensory properties (Costa *et al.*, 1990). Shaviklo et al. (2010) noted that 7% fish protein powder fortification of corn snack was more acceptable than the other in terms of texture and overall acceptability. Moreover, Shaviklo et al. (2010) reported that the ice cream fortified with or without fish protein powder had similar sensory quality.

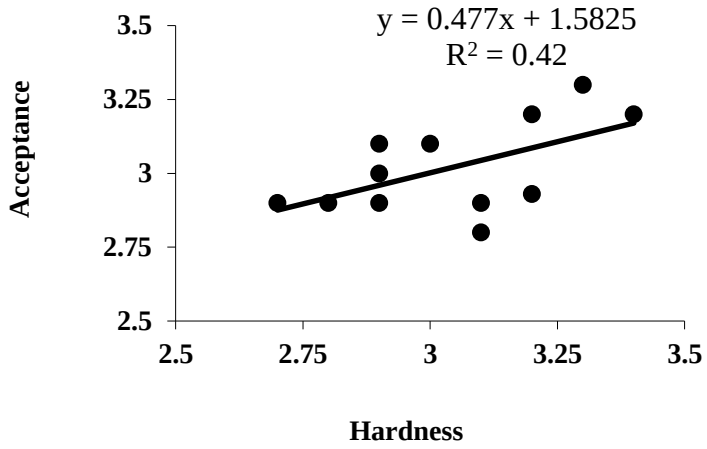

Fig. 5. Corelation between hardness and acceptance

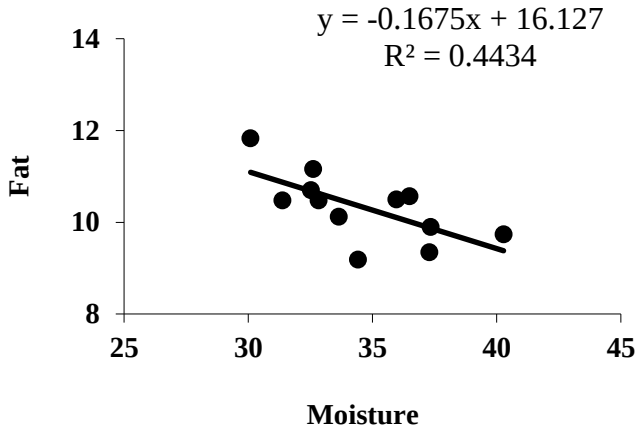

Fig. 6. Corelation between fat and moisture content

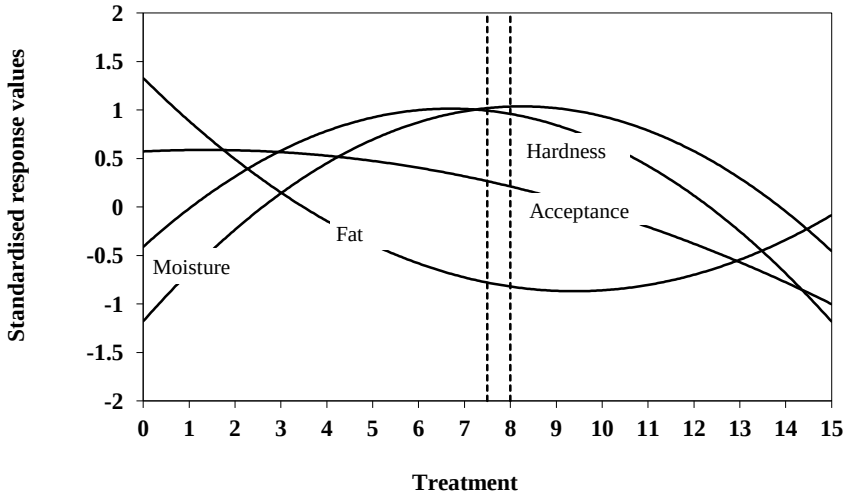

Fig. 7. Desirability diagram

Table 4- Effect of different level of FPC on texture and overall acceptability

Treatment (% FPC)	Texture (air fried)	Texture (deep fat fried)	Overall acceptability (air fried)	Overall acceptability (deep fat fried)
0	2.93^{a}±0.057	3^{a}± 0.1	3.06^{b}± 0.057	3.5^{b}± 0.0
5	3.3^{c}± 0.01	3.66^{b}± 0.3	3.23^{c}± 0.057	3.73^{c}± 0.057
10	3.13^{b}± 0.057	3.26^{ab}± 0.057	2.87^{a}± 0.068	3.4^{b}± 0.1
15	2.8^{a}± 0.1	3.03^{a}± 0.45	2.9^{a}± 0.0	3.1^{a}± 0.1

Mean value in each column with different color differ significantly ($p<0.05$)

Maneerote et al. (2009) reported that the overall acceptability of cracker with 10 g/100 g of fish powder content was slightly higher than the crackers with 5g/100 g of fish powder content.

The aim of this research was to reach a product with low fat content along with an ideal range of moisture content, high score in terms of texture and overall acceptability. Desirability is the best way of description of the aim in the current research.

Based on desired factors and according to desirability approach optimization, the relationship between texture and overall acceptability, as well as moisture and fat was statistically significant. Results, illustrated in fig.5 indicated that 42% of changes in product acceptability are characterized by texture. Hence, texture considered a substantial component to assess overall acceptability. Based on the regression model, each unit score increase in texture, results in 0.48 increase of overall acceptability. Moreover, results illustrated in fig.6 are shown that 44% of changes in fat content are characterized by moisture content. Each unit change in moisture content, causes 0.17 unit change in fat content however in a reverse trend.

In order to display variables with no similar unit of measurement in the same figure, they should be standardized. Then the standard data is drawn in a figure based on the quadratic model. Suggested model shows that 7.5-8% of FPC can keep the response in an optimized level (Fig 7).

Conclusions

Based on our priorities, the experimental results showed that FPC is preferred to wheat flour however up to level of 7.5-8% in batter formulation. Hence, FPC can be an appropriate alternative of wheat flour in battered products as a functional ingredient. Results showed that low value fish can be value added by using in processed food products. Although, further research is needed to apply them in other products.

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استفاده از کنسانتره پروتئین ماهی در فرمولاسیون خمیرآبه جهت تهیه ناگت مرغ کم چرب

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چکیده

تاثیر سطوح مختلف (۵،۱۰ و ۱۵٪) پودر پروتئین ماهی در فرمولاسیون خمیرآبه مورد بررسی قرار گرفت. به‌منظور دستیابی به مقدار بهینه پودر پروتئین ماهی، خصوصیات فیزیکوشیمیایی ناگت مرغ ارزیابی شدند. رفتار جریان خمیرآبه نشان داد که نمونه خمیرآبه شاهد و نیز نمونه حاوی ۷/۵ درصد ویسکوزیته بالاتری نسبت به دیگر نمونه‌ها داشتند. در هر دو روش سرخ کردن (سرخ کردن عمیق و هوا سرخ‌کن) افت رطوبت و جذب روغن در نمونه شاهد بالاتر از نمونه‌های حاوی پودر پروتئین ماهی بوده است. پوسته ضخیم‌تر که نتیجه مقدار بیشتر پودر پروتئین ماهی در خمیرآبه می‌باشد سبب جذب روغن کمتر طی سرخ کردن شده است. به‌علاوه، نمونه‌های حاوی پودر پروتئین ماهی امتیاز بالاتری از نظر بافت و پذیرش کلی کسب کرده است. علی‌رغم اینکه ناگت‌های حاوی پودر پروتئین ماهی اولویت‌های این تحقیق را برآورده کرده‌اند اما مقادیر حدود ۱۵ درصد سبب ایجاد محدودیت‌هایی شدند و بر اساس بهینه‌یابی مطلوبیت مقادیر ۸- ۷/۵ درصد پودر پروتئین ماهی در فرمولاسیون خمیرآبه به‌عنوان بهترین مقادیر تعیین شدند.

واژه‌های کلیدی: ناگت مرغ، مطلوبیت، پودر پروتئین ماهی، سرخ کردن، کاهش جذب روغن

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Research Full Papers
The antioxidant activity of lycopene and chlorophyll oleoresin and phenol stability of *Berberis vulgaris* extracts in cupcake formulation
Running title: The natural colered cakes

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Abstract

The application of natural ingredients in food formulations plays a key role in public health. The application of natural colorants is helpful in human health. Most of the natural colorants have additional roles such as antioxidant or antimicrobial activities. Natural colorants in foods especially in cake formulation, make an attractive view for Childs. In this study, the effects of three natural colorants of lycopene, chlorophyll, and *Berberis Vulgaris* extracts were investigated in cupcakes. The amounts of 0, 0.5, 1.5, and 2% of extracts were used. The moisture, volume increment, porosity, color parameters, total phenols, antioxidative effects of extracts, and sensory properties were evaluated. Results showed that all-natural colorants increase the final volume, porosity, oxidative stability, phenol content. Also, they reduced the moisture and lipid oxidation of samples. The red, green, and blue parameters of cakes decreased, especially in samples with *Berberis Vulgaris* extract. The lightness of all samples was significantly reduced after cooking but the lightness of samples with 2% lycopene oleoresin was not high. .Sensory evaluation showed that the color, flavor, and odor of cakes prepared with lycopene, and chlorophyll oleoresin had the highest scores. The cakes prepared with *Berberis Vulgaris* extract had the lowest scores in color, flavor, and odor, but the texture and porosity were the same as other samples. Finally, it can be concluded that lycopene oleoresin showed significant acceptance, antioxidant effect, and acceptable physical properties. Results can be helpful for researchers and food industries because the lycopene oleoresin showed a significant antioxidant activity after 3 weeks and the total phenols of Berberis Voulgaris extract showed significant stability after 3 weeks. Especially, the panelists have a significant reflection of the colored food. They propose them as an attractive materials for consumption.

Keywords: Natural colorant, Cupcake, Functional food, Natural antioxidant

Introduction

Bakery products are used by a wide range of consumers. Different types of cakes are a good choice for children and teenagers who are in an important period of their growth. Thus, by changing in food's formulation and making them functional or healthier foods, we can try to increase the health benefits of life.

Color is an important parameter in bakery products and has a great role on consumer purchasing decision. Color is the first factor that shows a wide range of quality factors like flavor, freshness, odor, healthy processing and handling, and make an attractive image for the consumer to choose. Since the color is the first factor for the attraction of consumers to choose a food product, and nowadays consumers are aware of the importance of application the natural ingredients in food formulations, application of natural colors in food formulation is a great step to make healthier foods. Also, some natural colors have other characteristics like antioxidant (Delgado-Vargas *et al.*, 2000, Bao *et al*., 2005), antimicrobial (Ranjbar and Ranjbar, 2016), and also health benefit properties.

Lycopene, as an important natural colorant, is investigated for nutritional, healthy, and nutraceutical properties. Lycopene has a reddish color, and also antioxidant and

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antimicrobial properties (Vitaglione *et al.*, 2005, Ozkan, Akyuz *et al*., 2012, Palmero, Panozzo *et al.*, 2014, Peker Akalin *et al*., 2016, Ranjbar and Ranjbar, 2016, Urbonaviciene and Viskelis, 2017). Studies on the health role of lycopene, emphasis on its antioxidant property as a key factor to preventing diseases in the digestive system, glands, different types of cancers, bones, and so on (Sies and Stahl, 1998; Shi and Maguer, 2000; Stacewicz-Sapuntzakis and Bowen, 2005; Sheriff and Devaki, 2013; Sroynak *et al*., 2013, Sahin *et al*., 2014, Sachdeva and Chopra, 2015; Sahin *et al.*, 2015).

Phenols are chemical constituents in plants that can play a different role in plants and also in human health. They can be found in many fruits and vegetables like grapes, berries, and tomatoes. They can promote health benefits through their antioxidant role. They can reduce the risk of metabolic syndromes and contribute to preventing some diseases (Laamech *et al.*, 2017). In recent years, medicinal plants are highly used in functional foods, and plants that contain antioxidants and other health beneficial components are sources of novel food formulations. The *Berberis vulgaris* extract has some effects like reducing blood pressure, cholesterol, and Triglycerides, makes the good effects on diabetics, preservation effects on neurons, and antibacterial and antioxidant effects like most antibiotics (Rahimi-Madiseh *et al*., 2017).

Oxidation is an important problem in processed foods that can affect the final physicochemical, shelf-life of product and consumer health. The addition of antioxidants can reduce the unwanted effects of oxidation. But today because of some doubts about health effects of synthetic antioxidants, the natural antioxidants can be a good candidate for processed foods. The addition of some antioxidants to cake batter is a good approach to make functional foods. Ranawana et al. (2018) studied the effect of beetroot on oxidative stability and functional characteristics of cakes. They found beetroot can significantly improve the antioxidant and phenolic profile, oxidative stability, and shelf-life of sponge cakes (Ranawana *et al*., 2018). Pasukamonset et al. (2018) also found the addition of *Clitoria ternatea* extract to cupcakes can increase the antioxidant activity and reduce the lipid peroxidation of sponge cakes (Pasukamonset *et al.*, 2018). Lu et al. (2010) studied the quality and antioxidant property of green tea powder in sponge cake as a flour replacer and found green tea cake is a good product with effective antioxidant properties (Lu *et al.*, 2010). Seo et al. (2010) optimized the amount suitable to add turmeric powder in sponge cake. They reported that by increasing the amount of turmeric powder in sponge cake batter, the hardness, gumminess, and chewiness, also increased but the sensory values decreased. They found the optimal ingredients were 1.6% turmeric powder, and 14.9% oil. Thus the turmeric powder can be easily used as a functional ingredient in sponge cakes (Seo *et al.*, 2010).

Celik et al. (2007) studied the effect of soapwort extract on the physical and sensory properties of sponge cake. They found egg white proteins can be replaced by soapwort extract and its application in cake formulation up to 75% did not change the physical properties of sponge cake (Çelik, Yılmaz et al. 2007). Toyosaki and Koketsu, (2007) investigated the antioxidant effects of silky fowl eggs and white leghorn eggs on the water-soluble part of baked sponge cake. They found that the silky eggs increased but the leghorn egg decreased the antioxidant effect and then the use of silky fowl eggs can improve the quality and oxidative properties of cakes.

In this study, cupcakes were prepared with natural colorants of lycopene and chlorophyll oleoresins, and *Berberis vulgaris* extract. The goal of this work was to investigate the stability of natural colors during baking and study the effect of natural colorant application in cake properties and consumer acceptance.

Materials and methods

Lycopene oleoresin (20% purity from fresh tomato (*Solanum lycopersicum*)) chlorophyll oleoresin (30% purity from spinach (*Chenopodiaceae* species)) and *Berberis*

vulgaris extract were purchased from a local company (San'at Pooya Torang CO. Iran). All other ingredients including flour, sugar, fresh eggs, vanilla, baking powder, and sunflower oil were purchased from a local market. Pure water was used.

Making the cupcakes

All samples had 100 g flour, 25 g fresh egg, 36 g sugar, 35 g water, 15 g oil, 1 g vanilla, and 1 g baking powder., the lycopene and chlorophyll oleoresins, and *Berberis vulgaris* extract was added to batters in 0, 0.5, 1.5, and 2% levels. These levels obtained from the pretreatment studies. At first, the oil and sugar were mixed to make a cream. Then eggs and vanilla were added and mixed for 5 min. after all, the flour (mixed with baking powder) and water were added gradually and mixed gently (Katomo, Japan). The equal weights of batter were poured into the same form of cups. Baking was done at 170°C for 25 min (Beikzadeh, Peighardoust et al. 2016). After reaching room temperature, the cakes were packaged into plastic zipper bags. Nine different formulations were prepared in this study.

Determination of total phenolic content and antioxidant property

Diluted samples of B*erberis vulgaris* extract cakes (0.50 ml) were added to 2.5 ml of diluted (1:10) Folin-ciocalteu reagent and kept for 4 min. Then 2 ml saturated sodium carbonate solution was added and after 2 h incubation at room temperature, the absorbance was measured by a spectrophotometer (LKB Novaspec II; Pharmacia, Sweden) at 760 nm against Gallic acid as a standard reference (mg GAE/g) (Li *et al.*, 2014). The total phenolic content was calculated as gallic acid equivalent (GAE) by the following equation:

TCVM= (T is the total phenolic content in $mg.g^{-1}$ of the extracts as GAE, C is the concentration of gallic acid established from the calibration curve in $mg.ml^{-1}$, V is the volume of the extract solution in ml and M is the weight of the extract in g (Abdelhady *et al.*, 2011).

The antioxidant property of cakes with lycopene and chlorophyll was determined according to the method of Lu et al. (2010). In order to measuring the peroxide value, about 1.0 g sample was treated with 25 ml of organic solvent mixture (chloroform/ acetic acid, 2/3: v/v). The mixture was shaken vigorously. Then 1 ml of saturated potassium iodide (Merck) solution was added and the mixture was kept in the dark for 5 min. After that, 75 ml of distilled water and 0.5 ml of starch solution (1%, w/v) was added as an indicator, and PV was determined by titrating the iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulfate solution. The PV was expressed as milli equivalents (meq) of peroxide per kg of lipid.

Physicochemical evaluations

Moisture content was determined by AACC (2000) method, about 2 h after the baking time. The volume index was determined as the sum of three height of the right side, left side, and the center of the midsection of the cakes. The porosity was measured by Image J processing software. About 2 h after the baking time, a central section cut was made and the color parameters (lightness (L*), Red, Green, and Blue) were determined. To determining the porosity, a 2× 2× 2 cm cut was made and scanned with a scanner (HP Scanjet G3010). With image J processing software and calculating the light and dark points, the porosity of cakes was measured (Khalilian Movahhed *et al.*, 2016).

Sensory evaluation

Twenty-five semi-trained panelists evaluated the sensory parameters of colored cake samples. A 5- Hedonic test (1= extremely dislike and 5= extremely like) was done. Panelists were from the food science students and the lab employees of the food science department of Semnan University. The samples were randomly numbered with three-digit numerical codes. The color, flavor, texture, porosity, and total acceptance of samples were studied (Khalilian Movahhed *et al.*, 2016).

Statistical analysis

All measurements were done in triplicate except for sensory evaluation. The significance

of difference among treatment means was determined by one-way analysis of variance (ANOVA) and Tukey test using Sigmaplot software (version 14). A *P* value of less than 0.05 was considered statistically significant. The sensory data were analyzed by chi-square (Khalilian Movahhed *et al.*, 2016).

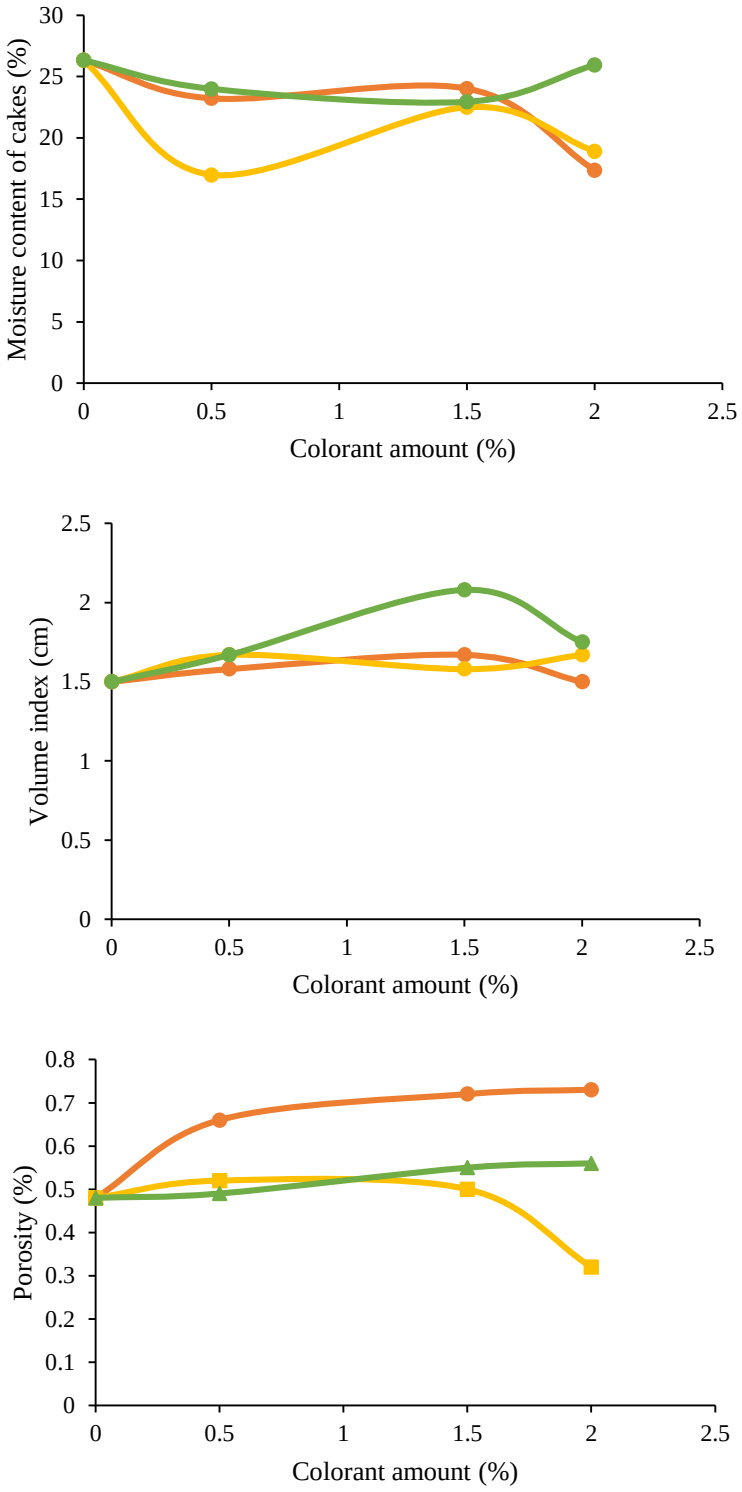


Fig. 1. The moisture content (a), Volume index (b), and porosity (c) of colored cakes. The (●), (■), and (▲) represents lycopene oleoresin, chlorophyll oleoresin, and *Berberis Voulgaris* extract, respectively.

Results and discussion

Physic-chemical properties of cupcakes

Figure 1 shows the changes in moisture content, volume index, and porosity of colored cake samples. According to the figure, the moisture content of all samples was reduced after adding natural colorant. Adding the chlorophyll oleoresin and 2% lycopene oleoresin to the cake batter, led to a significant reduction in moisture content. The *Berberis vulgaris* extract and lycopene oleoresin at 0.5, and 1.5% had a moderate effect on moisture reduction. A reduction in moisture might due to the oily nature of oleoresins. Because oil can lead to a decrease in water activity. The lycopene and chlorophyll oleoresins are a mixture of colorants, lipids, phospholipids, protein-phospholipid components, fatty acids (Shi and Maguer, 2000; Liu *et al*., 2010, Saini and Keum, 2018) that some of them can play as macromolecules and bind with free water molecules (Khalilian Movahhed *et al*., 2016). Also, all colorants showed a significant effect on the volume index of colored cakes. The oily nature of lycopene and chlorophyll oleoresins can have a positive effect on the volume increasing of batter during baking the cake. Brooker (1996) suggested the melting of lipid crystals develops the porosity of bread. He believed that melted crystals move to the interface of developing the air-crystals interface and helps the porosity and volume increasing of bread during the baking process.

As can be seen in figure 1-c, the porosity of samples increased after adding the natural colorants to the cake batter. This increase in samples with lycopene oleoresin is significant and can be explained by Brooker (1996) theory. Brooker (1996) also explained the crystal size has a significant reverse effect on the porosity of bread or cake. Oils contain small crystals can have a better effect on porosity than the oils with bigger crystals. The difference in the effect of lycopene oleoresin and chlorophyll oleoresin can be derived from this fact that perhaps the crystal sizes of the two oleoresins are different. This can be further studied in future. Also, because of the emulsifying effect of phospholipids, the observed increase in volume and porosity was possibly due to the oleoresins composition (Khalilian Movahhed *et al.*, 2016). It can be concluded that adding the lycopene and chlorophyll oleoresins to the cake batter, have a good effect on final product physical properties due to moisture reduction and increasing in volume. Also, lycopene oleoresin showed a good effect on the cake porosity that can be important if we consider the final interesting yellow-red color of the product.

Total phenols and antioxidant properties

Figure 2 shows the total phenol content of colored cake samples after baking. The total phenols of samples were monitored for 3 weeks and there were no significant changes during the 3-week storage of cake samples (Table 1). The antioxidants are sensitive colorants that their stability is important during the food formulations. Many factors such as pH, temperature, light, chelating agents, etc have a significant effect on the anthocyanin's stability (Taghvaei and Jafari, 2015; Zhao, Yu *et al.*, 2017; Chatham *et al.*, 2020). Thus different studies have done for increasing their stability and then their antioxidant activity (Martins *et al*., 2016, Ziabakhsh Deylami *et al.*, 2016; Bastos *et al.*, 2017; Rodriguez-Sanchez *et al*., 2017). For example, Pasukamonset et al. (2018) used *Clitoria ternatea* extract in sponge cake and found a significant increase in the phenol content of sponge cakes.

In this study, the reduction of phenol content in all samples was not so significant in 3 weeks (Table 2). Also, it was shown that the application of *Berberis Voulgaris* extract in the studied cake batter was effective on the stability of extract phenols (Table 1). This can be helpful to decide about selecting the food formulations that can be considered as a low-cost way for stabilizing the natural colorants without further processes.

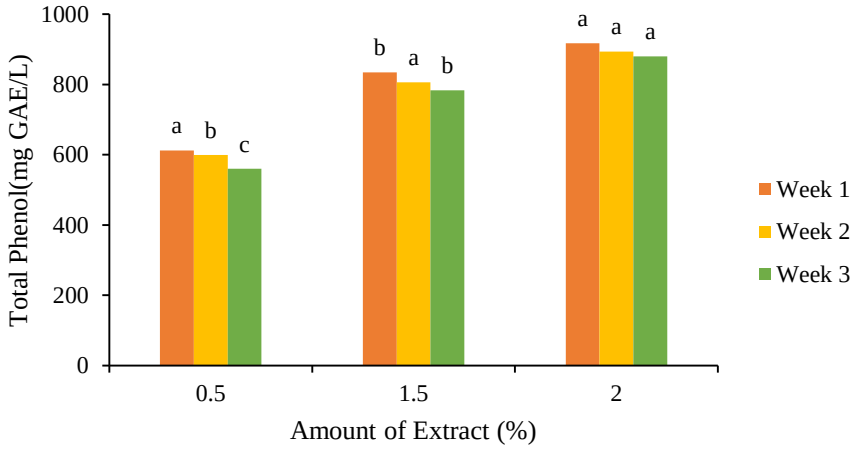


Fig. 2. Total phenol content of cake samples contains *Berberis Voulgaris* extract (the signs show the comparison between weeks)

Table 1- Total phenol content ANOVA during 3 Weeks

Week 1 and 2					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	682.667	682.667	0.0286	0.874
Residual	4	95605.333	23901.333		
Total	5	96288.000			
Week 1 and 3					
Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	1	3266.667	3266.667	0.126	0.740
Residual	4	103578.667	25894.667		
Total	5	106845.333			

Table 2- Comparison of Antioxidant activity of lycopene oleoresin during 3 Weeks

Week 1				
Comparison	**Diff of Means**	**t**	**P**	P<0.050
Control and treatments	1.883	29.255	<0.001	Yes
Week 2				
Comparison	**Diff of Means**	**t**	**P**	P<0.050
Control and treatments	2.520	63.000	<0.001	Yes
Week 3				
Comparison	**Diff of Means**	**t**	**P**	P<0.050
Control and treatments	1.013	3.041	0.038	Yes

As can be seen in Figure 3, the addition of lycopene oleoresin significantly increases the oxidative stability of cakes (Table 2). The peroxide value in all lycopene content samples is lower than a control for 3 weeks. In the first week, the lycopene oleoresin at 1.5% showed the lowest peroxide value. The peroxide value in week 2 is near week 1 and at week 3 increases. But the increase in samples with lycopene oleoresin at week 3 was almost lower than the peroxide value of control at week 1. It can be concluded that the addition of lycopene as a strong antioxidant can significantly help to maintain the lipid stability of cakes without the need to add synthetic antioxidants. Lu et al. (2010) showed the addition of green tea powder to the cake is effective in the antioxidant property of cakes (Lu *et al.*, 2010).

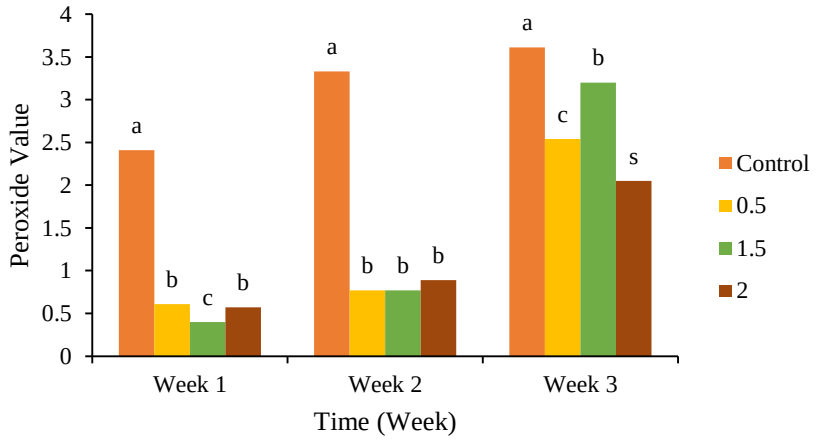


Fig. 3. Total peroxide value of colored cake samples with lycopene oleoresin during 3 weeks
(The signs show the comparison between each week)

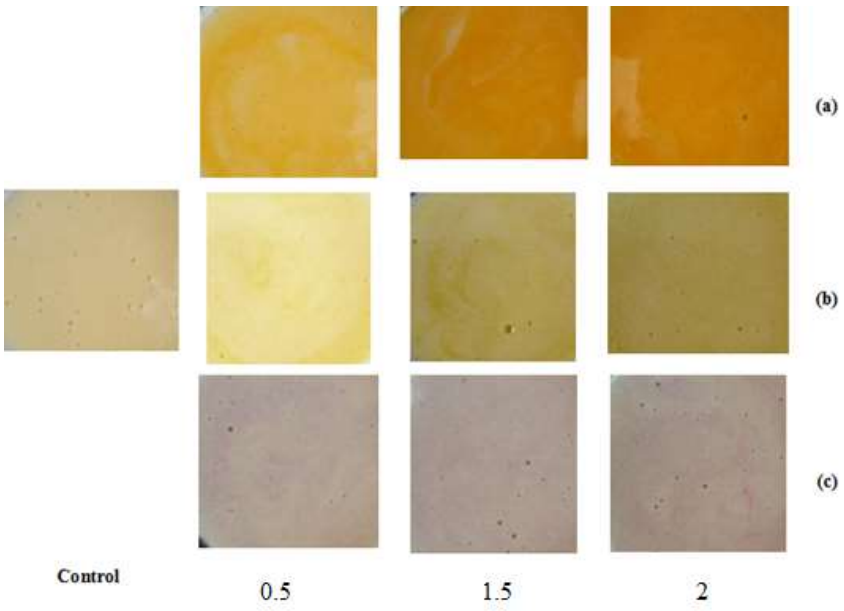


Fig. 4. The color of batters colored with lycopene oleoresin (a), chlorophyll oleoresin (b), and *Berberis Vulgaris* extract (c), respectively.

Color of samples

Color is the most important factors in consumer acceptance of processed food. Color is effective in attracting consumers in purchasing a food product. But the most important challenge of food colors is the maintenance of color during processing. The changes in pH, acidity, the interaction between food components, and the temperature of the food processing can affect the final color of processed foods. Natural colorants have a sensitive structure out of their source (Tonnesen *et al.*, 2002; Shahid *et al*., 2013; Taghvaei and Jafari, 2015; Torres *et al*., 2016). Therefore, their application and formulation must be carefully chosen. This needs a wide study about the natural colorants in different food formulations and processing conditions. Calvo and Salvador. (2000) have examined the stability of four natural colorants of annatto (orange), chlorophyllins (green), cochineal (red), and curcumin (yellow) during gel making. They made their samples with gelatin, a mixture of xanthan and locust bean gum,

sugar, and natural colorant. Then they measure the color (with a Hunter Labscan II colorimeter) and sensory parameters (a team of 10 judges). They found that the cochineal and curcumin natural colorants can replace the synthetic ones in jellies (Calvo and Salvador 2000). Pasukamonset et al. (2018) found the application of *Clitoria ternatea* extract in sponge cake can decrease the lightness, redness, and yellowness (Pasukamonset, Pumalee et al. 2018). Lu et al. (2010) showed that the addition of green tea powder to cake formulation decrease crust *L*, *a*, *b*, and crumb *L*, *b* values of samples.

Figures 4 and 5 show the color of batter (before baking) and cakes (after baking) containing the lycopene and chlorophyll oleoresin, and *Berberis vulgaris* extract. Seo et al. (2010) reported that turmeric powder decreased the brightness, and increased redness and yellowness in sponge cakes. In this study, the color parameters were determined for batters and final cakes, and the data are shown in Table 3.

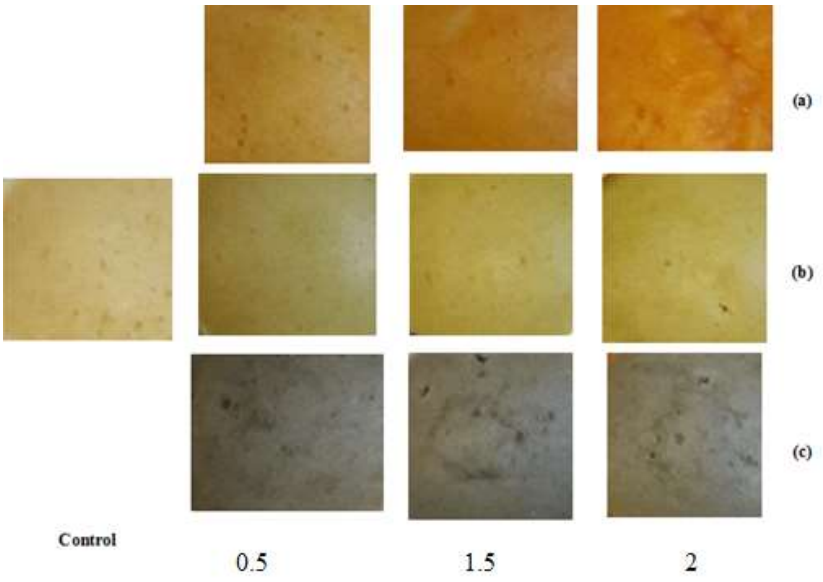


Fig. 5. The color of cakes colored with lycopene oleoresin (a), chlorophyll oleoresin (b), and *Berberis Vulgaris* extract (c), respectively.

Table 3 shows the lightness, redness, greenness, and blueness parameters of the cake colors. The lightness of all samples was reduced after baking but the lightness of samples with 7% lycopene oleoresin was not high. After baking, the redness, greenness, and blueness parameters of cakes also decreased. It is demonstrated that the addition of lycopene, chlorophyll, and *Berberis vulgaris* extracts to the batter, changes the color to the red, green, and mixture of them in each sample, respectively. Lycopene makes a red-yellow color in cakes and chlorophyll changes the final color of cakes to green-blue. The samples with *Berberis vulgaris* extract have a mixture of colors of red, green, and blue. But after baking, the decrease in color in samples with *Berberis vulgaris* extract was more than other oleoresins. This may be due to the oily nature of lycopene and chlorophylls and also due to their color intensity in pH about 6-7 (Dabas and Kean, 2015). The *Berberis vulgaris* extract is pH sensitive because of the chemical structure of colorants (Li *et al*., 2014) and perhaps this significant reduction in color parameters in cakes with *Berberis vulgaris* extract was due to its sensitivity to pH of baking powder which was added to the cake batter.

Sensory Evaluation

Panelists scored overall acceptance of colored cakes as good. They believed that colored cakes are interesting for consumption in comparison with a non-colored cake. As can be

seen in Figure 6 and Table 4, the panelists recorded the high scores for cakes containing lycopene oleoresin. The cake with 0.5% of lycopene oleoresin had the highest overall acceptance. The cakes with 0.5 and 1.5% chlorophyll oleoresin had the second scores in overall acceptance. The color, flavor, and odor of cakes with lycopene and chlorophyll had the highest scores. The texture was approximately the same in all samples. Pasukamonset et al. (2018) reported that the *Clitoria ternatea* extract did not affect overall acceptability between the control and the cake containing *Clitoria ternatea* extract.

The panelists did not give high scores for cakes with *Berberis vulgaris* extract. The cakes with *Berberis vulgaris* extract had the lowest scores in color, flavor, and odor, but the texture and porosity were the same as other samples. Lu et al. (2010) reported that there was no difference between the control and cakes with different levels of green tea powder except the 30% level of green tea powder samples that obtained the lower rate in sensory evaluation.

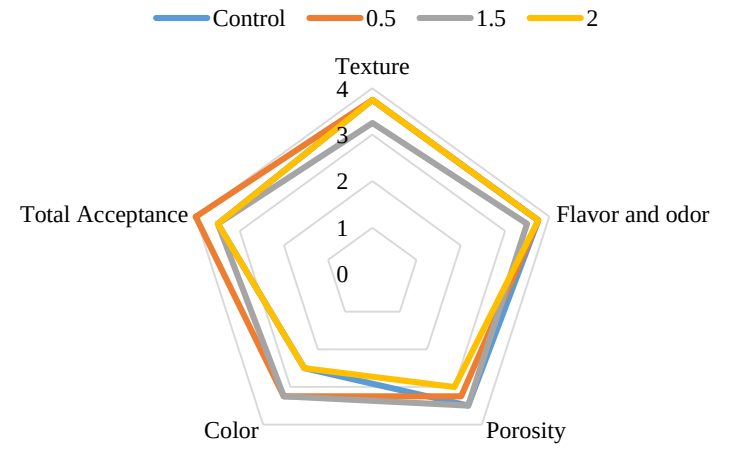
Table 3- Color Parameters of batters and cakes

	Amount of extract	L*		Red		Green		Blue	
		Batter	Cake	Batter	Cake	Batter	Cake	Batter	Cake
Control	0%	188±31.5^{b}	176±56.9^{a}	194±18.87^{a}	187.51±40.22^{a}	177.54±30.28^{a}	167.23±44.45^{a}	133.83±44.21^{ab}	109.10±45.08^{a}
Lycopene oleoresin	0.5%	179±22.5^{b}	139±67.91^{b}	205.01±29.88^{a}	162.46±15.176^{b}	163.48±16.23^{ab}	124.88±2.1^{b}	63.06±26.56^{c}	51.93±12.09^{d}
	1.5%	140±16.5^{b}	126±44.52^{b}	181.62±6.49^{b}	160.76±13.47^{b}	120.46±26.79^{d}	106.53±16.25^{b}	29.51±60.11^{d}	12.095±51.93^{e}
	2%	141±15.5^{b}	138±51.52^{b}	188.62±13.49^{a}	184.55±37.26^{a}	120.14±27.11^{d}	112.74±10.04^{b}	19.49±70.13^{d}	9.33±54.69^{e}
Chlorophyll oleoresin	0.5%	216±59.5^{a}	140±8.67^{b}	145.77±29.36^{d}	128.99±18.29^{c}	132.56±14.69^{c}	115.40±7.38^{b}	76.57±13.05^{c}	44.92±19.1^{d}
	1.5%	142±14.5	161±32.44^{a}	169.54±5.59^{c}	154.56±7.27^{bc}	152.62±5.37^{b}	135.13±12.35^{bc}	74.11±15.51^{c}	70.33±6.31^{c}
	2%	153±3.5^{b}	164±36.76^{a}	173.86±1.27^{c}	164.34±17.06^{b}	155.77±8.52^{b}	147.37±24.59^{b}	82.92±6.7^{c}	73.47±9.45^{c}
Berberis vulgaris extract	0.5%	123±33^{c}	87±33.1^{c}	170.20±4.93^{c}	98.50±48.78^{c}	156.28±9.02^{b}	94.93±27.85^{c}	144.38±54.76^{a}	80.40±16.38^{bc}
	1.5%	139±17.5^{b}	114±21.01^{bc}	158.11±17.02^{c}	109.06±38.22^{c}	142.94±4.31^{bc}	106.05±16.73^{b}	130.48±40.86^{ab}	91.40±27.38^{bc}
	2%	144±12.5^{b}	126±27.5^{b}	164.60±10.53^{c}	122.11±25.17^{bc}	150.74±3.48^{b}	117.56±5.22^{b}	141.86±52.24^{a}	97.23±33.21^{b}

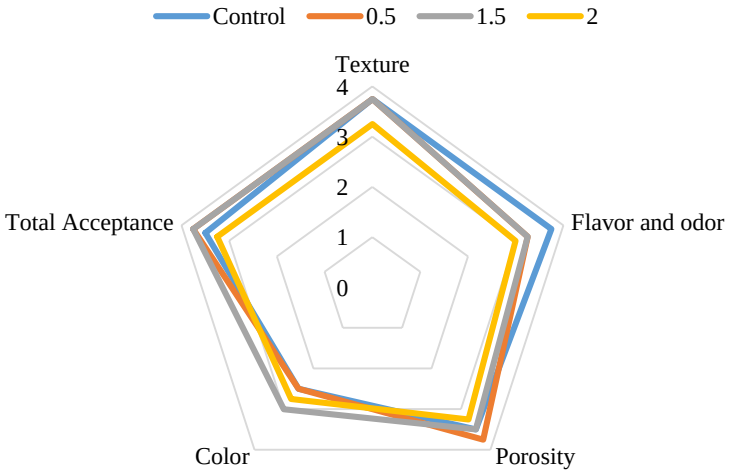
The signs show the comparison between each group

Conclusion

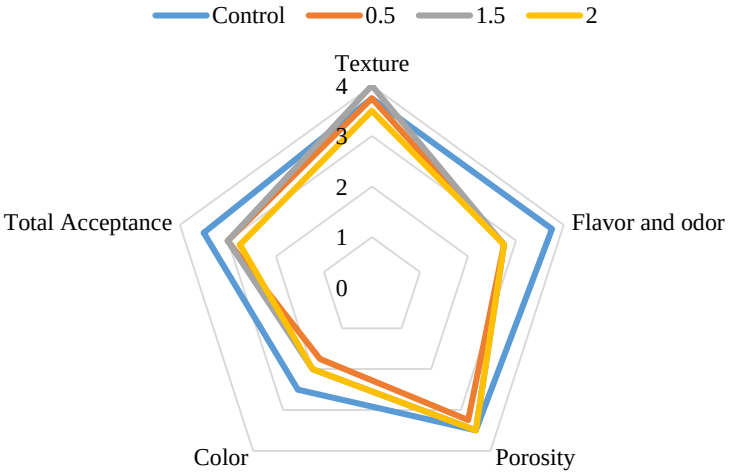
Natural colorants are suitable ingredients for coloring the processed foods. Some of the natural colors have also shown other characteristics like antioxidant, emulsifying, antibacterial property, and also health benefits. It is worthy to study the different natural colorants in different foods according to their physicochemical conditions like the method of extraction, stabilization, and application of colors and the food parameters like pH, acidity, composition, and type of processing on the final effects of colorant in foods. This study may be helpful for industries that apply natural colorants. In this study, it was shown that lycopene, chlorophyll, and *Berberis vulgaris* extracts could make an interesting color to attract consumers. Also, data have shown that the application of these natural colors, helps to improve some physical properties of cakes, and increases the phenol contents and oxidative property. It is a good step for human health, especially when we know that the major consumers of cakes are children.



(a)



(b)



(c)

Fig. 6. The sensory evaluation of colored cake: (a) lycopene oleoresin, (b) chlorophyll oleoresin, (c) *Berberis Voulgaris* extract, respectively.

Table 4-ANOVA for sensory evaluation

Source of Variation	DF	SS	MS	F	P
Between Groups	4	6.950	1.737	12.169	<0.001
Residual	45	6.425	0.143		
Total	49	13.375			

By increasing the natural additives in food formulations, and the tendency towards the natural sources used in the food industry, it has been a good area of study about the effect of different food formulations and processing on natural additives which can be linked by their health effects in human. Finally, it can be concluded that lycopene oleoresin is a good natural colorant to make colored cakes. It showed significant acceptance, antioxidant effect, and cake physical properties. The lycopene oleoresin at 0.5% level is proposed according to the overall acceptance of panelists and physico-chemical analysis of the final cake.

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نشریه پژوهشهای علوم و صنایع غذایی ایران
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فعالیت آنتی‌اکسیدانی اولئورزین لیکوپن و کلروفیل و پایداری فنولی عصاره زرشک در فرمولاسیون کیک فنجانی

آزاده رنجبر ندامانی*

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تاریخ پذیرش: ۱۳۹۹/۱۰/۰۸

چکیده

کاربرد عناصر رنگی در فرمولاسیون مواد غذایی نقش کلیدی در سلامت عمومی بازی می‌کند. کاربرد رنگدانه‌های طبیعی در سلامت بشر مفید است. بیشتر رنگدانه‌های طبیعی نقش‌های اضافی مانند آنتی‌اکسیدانی و ضدمیکروبی نیز دارند. رنگدانه‌های طبیعی در مواد غذایی و به‌ویژه در فرمولاسیون کیک، یک ظاهر جذاب برای جلب کودکان ایجاد می‌کند. در این مطالعه، اثر سه رنگدانه لیکوپن، کلروفیل و عصاره زرشک کوهی در کیک فنجانی بررسی شد. مقادیر صفر، ۰/۵، ۱، ۱/۵، و ۲٪ استفاده شدند. رطوبت، افزایش حجم، تخلخل، پارامترهای رنگی، فنول کل، اثرات آنتی‌اکسیدانی رنگدانه‌ها، و ویژگی‌های حسی ارزیابی شدند. نتایج نشان دادند تمام رنگ‌های طبیعی حجم کیک، تخلخل و پایداری اکسیداتیو را افزایش دادند. همچنین باعث کاهش رطوبت و اکسیداسون لیپیدی نمونه‌ها شدند. پارامترهای قرمز، سبز و آبی کیک‌ها به‌ویژه در نمونه حاوی عصاره زرشک بعد از پخت کاهش یافتند. ارزیابی حسی نشان داد رنگ، طعم و بوی کیک‌های حاوی لیکوپن و کلروفیل بیشترین امتیاز را به‌دست آوردند. کیک‌های حاوی عصاره زرشک کمترین امتیاز را در رنگ، مزه و بو داشتند اما بافت و تخلخل با سایر نمونه‌ها برابر بودند. در نهایت می‌توان نتیجه گرفت که لیکوپن اولئورزین پذیرش بارزی در ارزیابی حسی، اثرات آنتی‌اکسیدانی، و ویژگی‌های فیزیکی کیک از خود نشان داد. نتایج می‌توانند برای محققین و صنایع غذایی مفید باشند. زیرا اولئورزین لیکوپن یک فعالیت آنتی‌اکسیدانی بارز بعد از ۳ هفته نگهداری کیک‌ها از خود نشان داد. همچنین فنل کل عصاره زرشک بعد از سه هفته پایداری مطلوبی را دارا بود. به‌ویژه اینکه آزمونگرهای حسی عکس العمل بارزی نسبت به کیک رنگی از خود نشان داده و آن‌ها را برای مصرف جذاب می‌دانستند.

واژه‌های کلیدی: رنگدانه طبیعی، کیک فنجانی، غذای عملگر، آنتی‌اکسیدان طبیعی.

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Research Full Papers
Effect of food processing on aflatoxin reduction in cereals and nuts: A meta-analysis approach

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Abstract

Fungal toxins, mycotoxins such as aflatoxins, are compounds produced by different fungi during the growth and reproduction period. The most important fungal toxins that jeopardize human health are aflatoxins, which are produced by *Aspergillus* fungi and can grow in all crops. With toxic and carcinogenic effects of aflatoxins, many studies were performed using different methods to eliminate or reduce the amount of aflatoxin in cereals and nuts. On the contrary, using different methods for reducing aflatoxins in cereals and nuts make it impossible or difficult for researchers who study one or few related articles. This paper was conducted to review, investigate and do a meta-analysis on the results of the studies conducted and aimed to answer this general question as by which method can further reduce the amount of aflatoxin in cereals and nuts. Results showed that the methods of UV-irradiation, Ozone & UV irradiation and citric acid were the most important methods by 0.469, 0.441, and 0.427 of effect size respectively.

Keywords: Cereals, Food processing, Meta-analysis, Nuts.

Introduction

Fungal toxins, mycotoxins, are compounds produced by different fungi during the growth and reproduction period. Natural fungi found in human food sources, which mainly include three genera *Aspergillus*, *Fusariums*, and *Penicilliums* (Mannaa and Kim, 2017; Nešić *et al*., 2021). The most important fungal toxins that jeopardize human health are aflatoxins, which are produced by *Aspergillus* fungi and can grow in all food crops (Kumar *et al.*, 2017; SINGH *et al*., 2021). The fungi multiply rapidly in hot and humid environments, generate toxins, and very keen on nuts and grains. Aflatoxins production mostly in crops and under dehydration stress conditions, high temperature, pest and mechanical damage, rainfall and improper storage(Kumar *et al.*, 2017). With toxic and carcinogenic effects of aflatoxins , a variety of methods-- physical methods (e.g., heating, microwave, gamma, and UV, etc.,), chemical methods (e.g., the use of chlorine, ozone, sodium bisulfate, hydrogen peroxide), and mechanical methods have been studird for eliminating or reducing the amount of aflatoxin in food crops (Javanmardi *et al*., 2020; Khoori *et al*., 2020; Marshall *et al.*, 2020; Martins *et al*., 2017; Pankaj *et al.*, 2018; Patras *et al.*, 2017; Roohi *et al*., 2020; Vijayalakshmi *et al*., 2018).

Meta-analysis is a statistical method by which independent and separate research results are obtained to achieve general results about treatments. In other words, it is a concise form of previous studies, which encourages a precise estimation of indicators and explanation of incongruences in research findings (Khaneghah *et al*., 2020). Using meta-analysis, researchers can justify contradictions and discrepancies in research and arrive at conceivable, stricter and more valid conclusions. When the effect of a treatment is consistent from one study to another, a meta-analysis is useful in identifying this common effect, and when the effect changes from a study to another, meta-analysis may help

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identify the cause(s) of this change (Keikotlhaile *et al*., 2010). Meta-analysis can be considered as a review of research background which is based on a knowledge accumulation paradigm because the most important assumption of this paradigm is that knowledge is accumulated as researchers proceed with their studies following the results and findings of former researchers (Pagliai *et al., 2021)*. The statistical unit in meta-analysis is research conducted before. The meta-analysis approach is to arrive at more reliable conclusions, and useful and effective in giving prominence and adjusting existing gaps and bottlenecks in the research background of the subject under study, providing the researcher with essential insight into new approaches to study (Hoye and Elvik, 2010; Keikotlhaile *et al*., 2010).

Table 1- A summary of research on the effects of different processes on the reduction of aflatoxins in cereals and nuts

Reference	Processing	Commodity
Pukkasorn *et al*., 2018	Ultra superheated steam	**Peanuts**
Martins *et al*., 2017	Roasting	**Peanuts**
Rastegar *et al*., 2016	Roasting	**Pistachio**
Lee *et al*., 2015	Heating process	**Soybean**
Mohamadi *et al*., 2012	Cooking	**Rice**
Arzandeh & Jinap, 2010	Heating	**Peanuts**
Tatrishrili *et al*., 2019	Microwave heating	**Peanuts**
Schmidt *et al*., 2019	Microwave heating	**Wheat**
Meenatchi *et al*., 20115	Microwave heating	**Maize**
Basaran & Akhan, 2010	Microwave heating	**Hazelnuts**
Zhang *et al*., 2020	Microwave heating	**Corn**
Patill *et al*., 2019	Microwave heating & Gamma irradiation	**Peanuts**
Li *et al*., 2019	Ozone & vu irradation	**Peanuts**
Porto *et al*., 2019	Ozone	**Corn**
El- Desouky *et al*., 2012	Ozone	**Wheat**
Bashiri *et al*., 2013	Ozone	**Pistachio**
Ferreira *et al*., 2020	Ozone	**Brazil nuts**
Chen *et al*., 2014	Ozone	**Peanuts**
Luo *et al*., 2014	Ozone	**Corn**
Abuagela *et al*., 2019	Citric acid	**Peanuts**
Jubeen *et al*., 2020	Citric acid, lactic and propionic acid	**Almond, peanut, pistachio, Walnut**
Rastegar *et al*., 2017	Citric acid	**Pistachio**
Safara *et al*., 2010	Citric acid	**Rice**
Ghanghro *et al*., 2016	UV radiation	**Wheat**
Basaran, 2009	UV radiation	**Hazelnuts**
Mazaheri, 2012	UV radiation	**Pistachio**
Jubeen *et al*., 2012	UV radiation	**Ground Nut**
Patil *et al*., 2019	Gamma irradiation	**Peanuts**
Serra *et al*., 2018	Gamma irradiation	**Corn**
Assunca *et al*., 2015	Gamma irradiation	**Nuts**

Despite many studies and the use of different methods to eliminate or reduce the amount of aflatoxin in cereals and nuts, no precise conclusion has ever been made so far about the

studies. On the contrary, using different methods for reducing aflatoxins in cereals and nuts make it impossible or difficult for researchers who study one or few related articles. Therefore, the research was conducted to review, investigate and do a meta-analysis on the results of the studies conducted and aimed to answer this general question as by which method can further reduce the amount of aflatoxin in cereals and nuts.

Materials and methods

The terms "aflatoxin reduction", "aflatoxin elimination" "processing", "cereals", "nuts", and "effects" were used as keywords in Google Scholar, Scopus, ISI WEB of knowledge, which allows for further investigations into different processes of aflatoxin reduction or elimination in cereals and nuts. Moreover, the article reference section was used for a better search. Afterward, such data as a type of process, crop, and its influence on the reduction or elimination of aflatoxin level were collected in Microsoft Excel spreadsheet (Table 1).

Meta-analysis calculations

Meta-analysis is widely used to combine the results of previous research (Hoye and Elvik, 2010). To make meta-analysis dispose of errors, we need to collect all studies conducted in a particular field, because failure to consider all studies would result in dissemination error, making results less reliable (Caird *et al.*, 2008; Thornton and Lee, 2000). Meta-analysis is divided into six consecutive stages; clear expression of a problem and hypothesis, determining criteria for including independent studies in meta-analysis, search and retrieval of resources and related studies, data coding, and statistical analysis of select studies, synopsis and report of results, and explanation of result application (Wang and Bushman, 1999). The meta-analysis is built on the size of an effect (r) (Caird *et al*., 2008).

Results and discussion

Calculating effect size

The size of an effect indicates the ratio of significance test to study the size. The concept of effect size was introduced by Cohen's studies, emphasizing its importance. Cohen contends that it is not enough to focus only on a significant level to approve or disapprove a hypothesis, but effect size has to be seriously taken into account (Kouba and Lysek, 2019). Meta-analysis invariable can calculate the size of an effect by working out mean, variance, and standard deviation values of groups or methods. But the most common statistics in this field are (r) and (d). The most important formula for calculating the effect size (ES) are those proposed by (Lipsey and Wilson, 2001) as follows;

$$d=\frac{2t}{\sqrt{df}}\quad d=\frac{2\sqrt{f}}{df}\quad d=\frac{2\,r}{\sqrt{1-r^2}} \tag{1}$$

$$r=\sqrt{\frac{x^2}{n}}\quad r=\sqrt{\frac{t^2}{t^2+df}}\quad r=\sqrt{\frac{F}{F+df}} \tag{2}$$

In addition to the above statistics, Fisher's Z index was used to make sure the research results are reliable. Therefore, both r and z indices were used in this research, which will be addressed later. For the significance test of a hypothesis in meta-studies, there is a need for at least five studies (Thompson *et al.*, 1997). Therefore, certain methods were introduced in the meta-analysis in this research, which the number of available studies was more than 5.

Dissemination bias

Before we determine significance test of hypotheses in this research, it is first necessary to examine the homogeneity and heterogeneity of effect size of each hypothesis. One of the methods used for dissemination bias is the use of a funnel plot. Examining the plot in this study showed that all graphs are symmetrical in this study, and no black dot indicating asymmetry was seen, which represents the lack of any publication bias (Fig 1).

However, meta-analysis argue that the results of funnel plots are not reliable enough, and it is better to use inferential tests to complete research results. To this end, two common inferential tests in meta-analysis

studies- correlation and regression, which are based on diagram results- were utilized.

The first method for checking publication bias was the use of Begg and Mazumdar's rating correlation, as its formula is as follows (Sahebi *et al.*, 2021).

$$t^*_i = \frac{t_i - t'}{\sqrt{V_i}} \quad (3)$$

The second test was regression, which was calculated by the following formula if it was above (Lin and Chu, 2018):

$$Z_i = \beta_0 + \beta_1 \frac{1}{\sqrt{V_i}} + \varepsilon_i \quad (4)$$

The statistical results of regression and correlation indicate that the null hypotheses in $\alpha = 0.05$ cannot be excluded in either of the two methods, so the funnel plot is symmetric, and the evaluation is free from dissemination error (table 2).

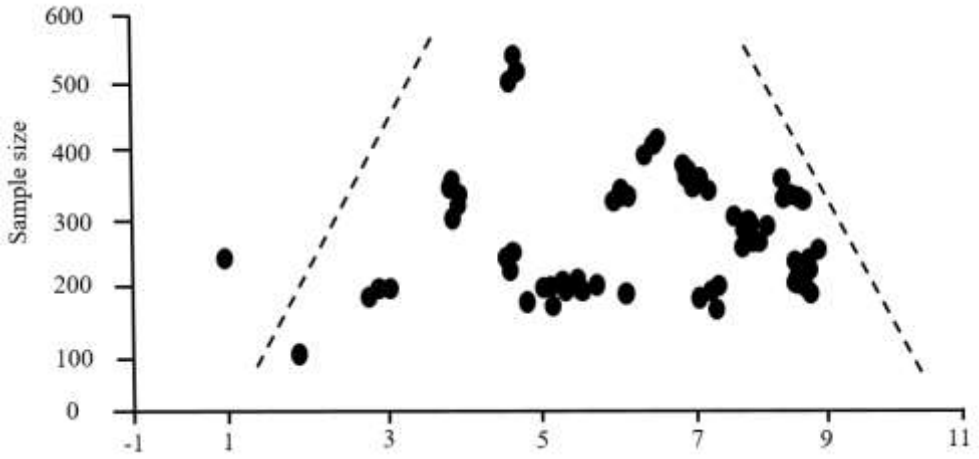

Fig. 1. The logarithm of the odds ratio in the studies used

Table 2- Results of examining dissemination error

Method	Regression		Correlation test	
UV-irradiation	**z**	**P-value**	**t**	**P-value**
ozone	-0.692	0.152	0.124	0.15
Gamma irradiation	-0.862	0.325	-0.251	0.152
microwave heating	-0.782	0.142	-0.214	0.174
citric acid	-1.062	0.257	-0.551	0.324
Ozone & uv irradiation	-0.699	0.324	-0.141	0. 264
conventional thermal	-0.771	0.152	-0.201	0.324
Microwave heating & Water	-0.855	0.103	-0.238	0.157

Q and τ^2 heterogeneity tests

Following the examination of dissemination bias in studies, there is a need for examining homogeneity tests or the heterogeneity of effect size for each method. In doing so, Cochrane (Q) and τ^2 are used. Q test follows χ^2 dissemination with g^{-1} degree of (Shadish and Haddock, 2009):

$$Q = \sum_{i=1}^{g} w_i y_i^2 - \left[\left(\sum_{i=1}^{g} w_i y_i \right) \Big/ \sum_{i=1}^{g} w_i \right] \quad (5)$$

To derive τ^2, we can use the following equation (Pigott, 2012):

$$\tau^2 = \frac{Q - (g-1)}{C} \quad (6)$$

C is an estimator, which is derived from the following equation;

$$C = \sum_{i=1}^{g} w_i - \left[\sum_{i=1}^{g} w_i^2 \Big/ \sum_{i=1}^{g} w_i \right] \quad (7)$$

If the effect sizes are homogenous, a fixed effect model is used, if they are heterogeneous, random effects are used for testing each method used in the research. The results of Q and τ2 were significant for all methods and processes, indicating heterogeneity of effect size; in which case, we should study the research hypotheses by using the random effect model (Table 3).

Table 3- Results of Q and I^2 heterogeneity

Methods	Q	Sig	Result	τ^2	Result
UV-irradiation	329.841	0.000	Heterogeneous	98.74	Heterogeneous
ozone	238.61	0.000	Heterogeneous	95.63	Heterogeneous
Gamma irradiation	52.63	0.000	Heterogeneous	91.63	Heterogeneous
microwave heating	263.84	0.000	Heterogeneous	97.34	Heterogeneous
citric acid	85.652	0.000	Heterogeneous	92.33	Heterogeneous
Ozone & uv irradiation	297.63	0.000	Heterogeneous	99.52	Heterogeneous
conventional thermal	152.36	0.000	Heterogeneous	94.68	Heterogeneous
Microwave heating & Water	138.76	0.000	Heterogeneous	93.55	Heterogeneous

In the random effect model, it is postulated that the effect size of a random variable has a normal distribution with θ mean and τ^2 variance (Pigott, 2012). Therefore, the random effect model is composed of two parts sampling variance (V_i) and between-studies variance (τ^2), which is obtained by the following equation:

$$v_i^* = \tau^2 + v_i \quad (8)$$

Finally, the statistical weight of the effect sizes is obtained in the random effect model by the following equation;

$$w_i^* = \frac{1}{v_i^*} \quad (9)$$

Calculating the statistical weight obtained either by fixed or random effect model, the mean weight of the effect is obtained based on the sum of effect sizes from the following equation;

$$\bar{y}_{\bullet} = \exp\left(\sum_{i=1}^{g} w_i y_i \Big/ \sum_{i=1}^{g} w_i \right) \quad (10)$$

To estimate the mean weight of an effect within 95% reliability range, the following equation is used;

$$95\%CI = \exp\left[\left(\sum_{i=1}^{g} w_i y_i \Big/ \sum_{i=1}^{g} w_i \right) \pm 1.96 \Big/ \sqrt{\sum_{i=1}^{g} w_i} \right] \quad (11)$$

Moreover, for integrating effect sizes according to the weighting method (Stouffer), the following formula are used (Wj is the same sample size in each method).

$$Z_r = 0.5 \log_e \left[\frac{1+r}{1-r} \right] \quad (12)$$

$$Fisher\ Z = \frac{\sum w_j Z_r}{\sum w_j} \quad (13)$$

The results of Table 4 indicate that the methods of UV-irradiation, Ozone & UV irradiation and citric acid were the most important methods by 0.469, 0.441, and 0.427 respectively.

Conclusion

The meta-analysis method was used to summarize the effect of different food processes on the reduction and elimination of aflatoxin in cereals and nuts. This method determines which food process is more capable

of reducing aflatoxin levels in cereals and nuts. The results of this study showed that among the methods used to reduce or eliminate aflatoxin in cereals and nuts, UV-irradiation, Ozone & UV irradiation and citric acid were the most effective.

Table 4- The results of the significance test of effect size for each method

Methods	Model	Number of studies	R^2	95% CI	Z	Sig	Results
UV-irradiation	Random	10	0.462	0.234-0.722	4.869	0.000	Confirm
Ozone & uv irradiation	Random	8	0.441	0.266-0.634	3.527	0.000	Confirm
Citric acid	Random	8	0.427	0.317-0.544	2.658	0.015	Confirm
Conventional thermal	Random	6	0.367	0.248-0.588	4.025	0.004	Confirm
Microwave heating	Random	8	0.328	0.185-0.511	3.698	0.002	Confirm
Microwave heating & Water	Random	7	0.246	0.098-0.421	4.297	0.001	Confirm
Ozone	Random	7	0.204	0.145-0.285	2.367	0.004	Confirm
Gamma irradiation	Random	7	0.178	0.105-0.261	3.597	0.005	Confirm

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چکیده

مایکوتوکسین‌ها (سموم قارچی) مانند آفلاتوکسین ترکیباتی هستند که توسط قارچ‌های مختلف در طول دوره رشد و تولیدمثل تولید می شود. با توجه به اثرات سمی و سرطان‌زا بودن آفلاتوکسین، از روش‌های مختلف برای کاهش یا از بین بردن مقدار آفلاتوکسین در غلات‌ها و مغزها استفاده شده است. برای مقایسه روش‌های مختلف که برای کاهش یا حذف میزان آفلاتوکسین استفاده می‌شود چون در هر مقاله از یک یا چند روش استفاده شده است بنابراین مقایسه کارایی روش‌های مختلف امکان‌پذیر نیست بنابراین در این پژوهش با استفاده از روش فراتحلیل، روش‌های مختلف که برای کاهش یا حذف میزان آفلاتوکسین در غلات و مغزها استفاده شده است با هم مقایسه شد. نتایج نشان داد که روش‌های استفاده از اشعه فرابنفش، ترکیب روش ازن- فرابنفش و استفاده از اسید سیتریک با اثر اندازه ۰/۴۶۹، ۰/۴۴۱ و ۰/۴۲۷ دارای بیشترین کارایی در کاهش میزان آفلاتوکسین در غلات و مغزها داشتند.

واژه‌های کلیدی: غلات، فرایندها، فراتحلیل، مغزها

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Evaluation of chlorophyll content, antioxidant activity and antimicrobial effect of Dandelion leaves extract

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Abstract

In this research, the chlorophyll content, total flavonoid content (TFC), total phenolic content (TPC) and antioxidant activity of aqueous extract of Dandelion was determined. In addition, the effect of aqueous extract of Dandelion, obtained by maceration, was tested on various food borne and food spoilage microorganisms. Antimicrobial activity of leaf extract of Dandelion was investigated using disk agar diffusion, well agar diffusion, minimum inhibitory concentration and minimum bactericidal/fungicidal concentration methods. The values obtained for TFC, TPC, chlorophyll a and b were 22.82 (µg/mL), 116.89 (mg/mL), 0.081 (mg/l) and, 0.063 (mg/l), respectively. The result of the radical scavenging activity was IC_{50}= 68.81µl/mL. The results showed that MIC of leaf extract of Dandelion on *Aspergillus niger*, *Salmonella typhimurium*, *Bacillus subtilis* and *Staphylococcus epidermidis* was 512, 256, 256 and 64 mg/ml respectively. MBC for *Aspergillus niger* was more than 512, for *Salmonella typhimurium*, and *Bacillus subtilis* was 512, and for *Staphylococcus epidermidis* was 128 mg/ml. In addition, the diameter of inhibitory growth zone in well diffusion method was more than disk diffusion. Generally, it can be stated that the aqueous leaf extract of Dandelion on Gram-positive bacteria showed more antimicrobial activity than Gram-negative bacteria, while there is no significant inhibition on mold. Based on the results of this study, Dandelion aqueous extract might be used as a natural agent to prevent the growth of food borne microorganisms, particularly, that cause food poisoning that leading to the reduction of gastroenteritis risk.

Keywords: Antimicrobial activity, Antioxidant activity, Dandelion, Pathogenic microorganisms.

Introduction

Dandelion is originally from Western Europe and Northern Asia; it is widely distributed through Europe, Asia, and America. It blossoms almost the whole year and grows in the autumn and is found in fields, gardens, wild lands and by the roadsides, at altitudes ranging from sea level to two thousand meters (Escudero *et al*., 2003). Dandelion, is a member of the Asteraceae/ Compositae family. It is a perennial herb, native throughout the Northern hemisphere. Traditionally, Dandelion has been used for centuries as a remedy for various ailments due to its antidiabetic, choleretic and diuretic properties (Sigstedt *et al*., 2008; You *et al*., 2010). The variety of health benefits associated with the use of Dandelions has been attributed to specific *Taraxacum* species as extracts of the whole plants or specific plant parts (Cragg., 2007). Dandelions play a pivotal role in traditional medicine for treatment of breast, uterine and lung tumors as well as hepatitis and digestive diseases, kidney disease, liver and spleen disorders, eye problems and diarrhea (Schütz., *et al*. 2006; Sigstedt., *et al*. 2008). The young leaves and flowers are very appreciated in salads, while roasted roots are used as coffee substitutes (Dias *et al*., 2014).

Identification of plant products or alternative medicines which could limit reactive oxygen species (ROSs) is necessary to help protect the liver from possible damage. ROSs, such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive,

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toxic molecules, which are generated normally in cells during metabolism. (Baba and Malik., 2015). Antioxidants are substances that delay or prevent the oxidation of inter- or intra-cellular oxidizable substrates from oxidative stress. Some bioactive compounds, which are generally found in plants, have been determined to protect cells from oxidative stress by preventing the formation or detoxifying free radicals, resulting in prohibition variety of pathophysiological problems (You., *et al.* 2010).

Free radicals have been implicated in the development of a number of disorders, including cancer, neurodegeneration and inflammation, giving rise to studies of antioxidants for the prevention of diseases. The presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Baba and Malik., 2015; Mohsenipour., 2015).

In the other hand, the growing bacterial resistance to antibiotics has become a major concern worldwide which prompting a resurgence in research of the antimicrobial role of herbs against resistant strains. A lot of plants have been recognized as valuable resources of natural antimicrobial compounds. Plant extracts offer considerable potential for the development of the new agents against infections currently difficult to treat (Wendakoon., *et al*. 2012).

Therefore a renewed interest in natural substances has focused attention on plants rich in bioactive compounds well known for their antimicrobial and antioxidant properties being investigated for their antioxidant properties, and the demand for natural antioxidants and food preservatives is increasing (Baba and Malik, 2015; Mohsenipour, 2015).

The objectives of this research were to measure the chlorophyll content (CC), total flavonoid content (FC), total phenolic content (TPC) and the antioxidant activity (AA) of Dandelion leaves extract in addition to its free radical scavenging activity. The other aim of this study was to investigate the antibacterial and antifungal effects of Dandelion leaves on various food borne microorganisms "*in vitro"*.

Materials and methods

Extract preparation

Fresh plant Dandelion was collected in spring locally in April, 2017 from the green area of Ferdowsi University of Mashhad (Mashhad, Khorasan Razavi), Iran. The taxonomical identification of the plant was confirmed by Ferdowsi University of Mashhad, Institute of Plant Sciences. Plants were extracted as described by Sigstedt *el al.*, (2008) with some modifications. Freshly collected leaf of Dandelion were rinsed with running water, shade- dried and then powdered using an electrical blender (Bosch Limited, Germany). For successive extraction, 50 gram of plant powder was macerated in 250 ml of water. Extracts was prepared using the maceration process for 72 h under constant shaking and filtered with Whatman No. 1 filter paper. The extract was evaporated to dryness under reduced pressure using a rotary evaporator and then was incubated at 37°C to complete evaporation of solvent. Percentage yield of the extract (w/w) is calculated as follows: Percentage yield of the extract (w/w) $= \frac{W1}{W2} * 100$, W_1= Weight of plant powder was macerated (g) and W_2= Weight of extract obtained (g)

Chlorophyll content

In a 15 ml volumetric flask, 100 mg of aqueous extract was dissolved in 10 ml of 80% acetone. The volumetric flask was kept in ice and dark environment for 30 minutes. The sample was centrifuged at 3000 rpm (Sigma, Germany) for 10 min at 4°C. Immediately after centrifugation, the falcon was transferred to ice before rapid measurement with spectrophotometer. The absorbance of the solution was read at three wavelengths including 663.2, 646.8 and 470 nm (Roshanak *et al.*, 2015). Chlorophyll concentrations were

calculated in mg/l of acetone according to the following formula:

Ca (mg/l)= (12.25*D66302)– (2.79*D646.8)

Cb (mg/l)= (21.50*D646.8)– (5.10*D663.2)

Ca: Chlorophyll a
Cb: Chlorophyll b

Total flavonoid content

Total flavonoid content was determined spectrophotometrically using a method based on the formation of a complex flavonoid–aluminum, according to Chang *et al.*, (2002) with some modifications. Quercetin was used to make the calibration curve. Ten mg of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 510 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water to give blank. Similarly, 0.5 mL of extract was reacted with aluminum chloride for determination of flavonoid content as described above (Chang *et al.*, 2002).

The calibration equation for quercetin was obtained as y=0.0107x+ 0.135 (R^2= 0.994), where x is the absorbance and y is the concentration of quercetin in mg/l.

Total phenolic content

Briefly, 2.5 g of the finely dried extract powder was mixed with 50 ml methanol 80% in falcon and was shacked in 240 rpm for 24 h. The sample was filtered twice with Whatman 0.2µm. TPC was determined using Folin-Ciocalteu method. Then, 500 µl of diluted extract, 2.5 ml Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate were also mixed. After heating at 45°C for 15 min, the absorbance was measured at 765 nm against methanol 80% as blank. TPC was expressed as Gallic acid equivalent/g dry weight of sample. The calibration equation for Gallic acid was obtained as y=17.422x+0.0872 (R^2=0.998), where x is the absorbance and y is the concentration of Gallic acid in mg/l (Capannesi *et al.*, 2000).

Antioxidant properties

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Briefly, 200 µL of extract (150- 200- 250 µg/mL) was mixed with 3.8 mL DPPH solution and incubated in the dark at room temperature for 1 h. The absorbance of the mixture was then measured at 517 nm. The control sample contained all the reagents except the extract. The percentage inhibition was calculated using the following equation:

% inhibition=100× (A of control−A of sample)/A of control (Baba and Malik., 2015; Roshanak *et al.*, 2015).

Reducing power

2.5 mL of extract and Butylated hydroxytoluene (BHT) were mixed with 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and were incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 200 g for 10 min. 2.5 mL of the supernatant was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The absorbance at 700 nm was measured against distillated water as a blank. The increased absorbance of the reaction mixture correlates with greater reducing power (Ardestani and Yazdanparast, 2007)

Microorganisms and culture conditions

Aspergillus niger (PTCC 5010), *Salmonella typhimurium* (PTCC 13311), *Bacillus subtilis* (PTCC 1023), and *Staphylococcus epidermidis* (PTCC 1435), were procured from microbial collection, Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad. Microbial strains were incubated 24 hours for bacteria and 72 hours for fungal strains before the antimicrobial tests were performed. 0.5 McFarland standard was

used for preparation of microbial suspension, which was equivalent to 1.5×10^8 CFU/ml of microorganism (Amin Mir *et al.*, 2016).

Minimum inhibitory concentration (MIC) and Minimum bactericidal/ fungicidal concentration (MBC/ MFC)

MICs were determined by the broth microdilution method. Serial dilutions of the aqueous extract of Dandelion (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 mg/mL) in broth medium were prepared on a microtiter plate, and microbial suspensions were added to the micro wells at 1.5×10^8 CFU/ ml. It is worth to mention that Mueller Hinton Broth (MHB) (Sigma-Aldrich) was used for bacterial strains and Potato Dextrose Broth (PDB) (Sigma-Aldrich) was used for fungal strain. The microtiter plates were then incubated at 37°C for 24 h for bacterial strains and 25°C for 72 h for fungal strain. Activity was recorded as red coloration in the wells after addition of Triphenyltetrazolium Chloride (concentration of 5 mg/ mL) and incubation for 30 minutes. MICs were determined as the lowest concentration that prevented visible growth (Baba and Malik, 2015). 100 µL of the culture from each well in which the red color was not observed, was streaked on Mueller Hinton Agar (MHA) (Sigma-Aldrich) for bacterial strains and Potato Dextrose Agar (PDA) (Sigma-Aldrich) for fungal strains. The plates were incubated at 37°C for 18-24 h for bacterial strains or 25°C for 72 h for fungal strains and the lowest dilution that yielded complete inhibition of growth was taken as MBC or MFC (Wendakoon *et al*., 2012).

Well diffusion agar (WDA) method

20 ml of sterile MHA for bacterial strains and PDA for fungal strain was poured into plates and allowed to set. The plates were then seeded with 100 µL of a 24-hour old culture using a sterile glass rod to spread the culture, and then the plates were kept for drying. Wells were made on the plates with sterile whole puncture (6 mm diameter). 60 µL of the plant extract (100, 200, 300 and 400 mg/ml) was poured in each respective well. The plates were then incubated at 37°C for 18-24 h for bacterial strains or 25°C for 72 h for fungal strain. The antimicrobial activity of the plant extract was assessed by an inhibition zone surrounding the well and inhibition zone diameter (IZD) was measured and expressed in millimeter (Sohail *et al.*, 2014).

Disc diffusion agar (DDA) method

10 µL of MHA for bacterial strains and PDA for fungal strain were prepared and fresh inoculum was spread over the surface of the media. The sterile filter paper discs of size 6 mm were dipped into the extract solution of different concentrations (100, 200, 300 and 400 mg/ml). Then the disc was placed over the center of medium surface and the plates were incubated at 37°C for 18-24 h for bacterial strains or 25°C for 72 h for fungal strain. Inhibition zone diameter was reported in millimeters (Espinel-Ingroff *et al*., 2002; Awoyinka *et al.*, 2007).

Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Fisher Test with $\alpha = 0.05$. This analysis was carried out using Minitab v. 18.0.

Results and discussion

The type and level of biological activity exhibited by any plant material depends on many factors, including the plant part, geographical location, soil conditions, harvest time, moisture content, drying method, storage conditions, and post- harvest processing. For example, the relatively high temperatures that can be generated during tissue grinding can denature chemical constituents. Also, the extraction solvent, time period, and temperature can affect the level and composition of secondary metabolites extracted from plant tissues. Many solvents are used for extraction including water, methanol, ethanol and acetone separately or mixed with water. In this study water was chosen as extraction solvent because it is quite safe for human consumption as

compared with other organic solvents (Wendakoon *et al.*, 2012).

The results of MIC and MBC/MFC tests showed that *Staphylococcus epidermidis* was the most susceptible and *Aspergillus niger* was more resistant to the aqueous extract of Dandelion leaves respectively. According to Kuete (2010), Kuete and Efferth (2010), the antibacterial activity of a plant extract is considered significant when MIC values are below 100 µg/ mL, moderate when 100≤ MIC ≤ 625 µg/mL and weak when MIC> 625 µg/mL (Voukeng *et al.* 2017). Consequently, the antimicrobial activity observed with Dandelion (64≤ MIC≤ 512 mg/mL) can classified as weak for bacterial and fungal strain. In our previous research we also found that MIC of leaf extract of Dandelion (*Taraxacum pseudocalocephalum*) on *Candida albicans*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria innocua*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* was 128, 128, 256, 256, 256, 256 and 512 respectively (Shahidi *et al*., 2019).

The results of the antimicrobial activity of the aqueous extract of Dandelion leaves according to well diffusion agar and disk diffusion agar methods are depicted in Table 2.

Table 1- MIC and MBC/MFC of Dandelion leaves extract on some pathogenic bacteria and fungi

Microorganisms	MIC (mg/mL)	MBC or MFC (mg/mL)
Aspergillus niger	512	512<
Salmonella typhimurium	256	512
Bacillus subtilis	256	512
Staphylococcus epidermidis	64	128

Table 2- Average inhibition zone (mm) of Dandelion leaves extract concentrations on some pathogenic microorganisms thorough DDA and WDA antimicrobial methods

Microorganisms	Zone of inhibition (mm)							
	Well diffusion agar				Disk diffusion agar			
	Concentrations (mg/mL)				Concentrations (mg/mL)			
	100	200	300	400	100	200	300	400
Aspergillus niger	8.20±0.22	10.20±0.36	12.10±0.22	14.00±0.42	7.10±0.55	8.00±0.37	10.70±0.22	11.00±0.55
Salmonella typhimurium	9.60±0.43	11.40±0.15	14.00±0.44	17.40±0.36	9.40±0.37	10.10±0.42	12.60±0.36	15.20±0.42
Bacillus subtilis	8.12±0.27	12.50±0.34	17.00±0.30	20.60±0.22	8.20±0.29	10.10±0.57	12.50±0.48	16.00±0.35
Staphylococcus epidermidis	10.00±0.41	14.30±0.29	16.20±0.21	18.30±0.35	8.00±0.16	10.30±0.55	12.50±0.22	17.10±0.52

The results showed that by increasing the concentration of extract from 100 mg/mL to 400 mg/mL, the IZD increased significantly. The maximum and minimum effects of the aqueous extract of plant leaves were observed on *Staphylococcus epidermidis* and *Aspergillus niger*respectively. As a result, Gram-positive bacteria were generally more susceptible than Gram-negative bacteria to aqueous extract of Dandelion. Possibly because of the presence of outer membrane that serves as an effective barrier in Gram- negative species (Al-Marzoqi *et al.*, 2015). Mean IZD by well agar method was higher than disk agar method. Also the strains showed a smaller IZD at lower concentrations. Perhaps higher IZD in well agar diffusion is related to the direct contact of the aqueous extract of Dandelion with the microbial strains. In addition, in the disk agar diffusion method, the antimicrobial agent from the disk should be transferred to the surface of the medium.In this case factors such as

Temperature and time can be effective in releasing antimicrobial agent (Klančnik *et al.*, 2010).

Polyphenolic substances, tannins, catechins and polyphenolic acids are abundantly found in about 20% of the plants with high antimicrobial properties. It is known that polyphenols can form heavy soluble complexes with proteins. Polyphenols may adhesive to bacterial and causing disrupt for accessing to cell surface receptors (Brantner and Grein, 1994). For example, flavonoids are polyphenolic heterocyclic compounds and an integral constituent of food, fodder and a prominent antioxidative compounds. The antioxidant ability of flavonoids resides mainly in their tendency to donate hydrogen atoms and thereby scavenge the free radicals generated during lipid peroxidation (Agarwal and Verma., 2011).

Although a pro-oxidant activity of chlorophylls under light, which could be understood as a transfer of the energy of singlet-excited chlorophyll to oxygen that would form reactive oxygen species, has been studied. However, many studies also reported that chlorophylls and pheophytins provide protection by preventing autoxidation of vegetable edible oils stored in the dark and suggested a hydrogen donating mechanism breaking the radical chain reactions (Lanfer-Marquez *et al.*, 2005).

Table 3 showed phytochemicalcompounds of Dandelion. The ability of aqueous extract of the Dandelion leaf to quench DPPH free radical was measured. The extracts and BHT demonstrated a dose-dependent scavenging activity by reducing DPPH radical (Fig. 1). By plotting the graph of extract concentrations against the scavenging activity, a specific concentration of the sample that needed to provide 50 % inhibition (IC_{50}) was calculated. IC_{50} for aqueous extract of the Dandelion was 68.81 µl/ml.

Table 3- Average mean of total phenolic content, total flavonoid content and chlorophyll content of aqueous leaf extract of Dandelion

Chemical test of aqueous extract of Dandelion	result
Total phenolic content (µg/mL)	22.82± 97
Total flavonoid content (mg/mL)	116.89± 2
Chlorophyll content (mg/L)	Ca:0.081±0.0 Cb:0.063±0.002

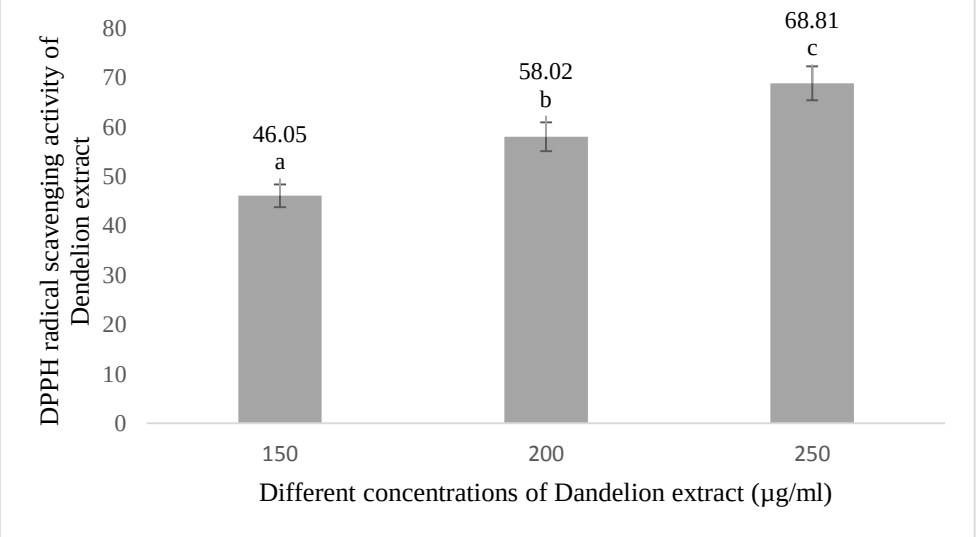

Fig. 1. DPPH radical scavenging activity of aqueous leaf extract of *Taraxacum pseudocalocephalum*. Bars with different letters are significantly different.

In a study by Ali *et al.*, (2016) antimicrobial Activity of dichloromethane, ethylacetate, methanol and water extracts of stem, root and flower of *Taraxacum officinale*, against *Streptococcus mutans, Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus aureus* and *Pseudomonas aeruginosa* was evaluated. They addressed that among all types of plant extracts, the methanolic extracts were found to bear the highest antimicrobial potential against all examined bacterial strains, followed by the ethylacetate, the dichloromethane and the water extracts of the plant respectively. Among the plant parts observed, roots were observed to be more effective in inhibiting the growth of microorganisms followed by flower extracts. The stem extracts have a little effect on the growth of microorganisms (Amin Mir *et al.*, 2016).

Baba and Malik (2015) evaluated the antioxidant and antimicrobial activity of a methanolic extract of the roots of *Arisaema jacquemontii* and showed this extract prevented the growth of both Gram-positive and Gram-negative bacteria, at an MIC of 0.24– 0.41 mg/mL. They reported that antimicrobial and antioxidant activities of the extracts were positively associated with the total phenolic and flavonoid contents of the extract.

Antibacterial activity of the plant *Taraxacum officinale* leaves extracts including methanol, chloroform, and distilled water was investigated by Sohail et al. (2014). The results of this study showed that methanol and chloroform extracts of *Taraxacum officinale* were found to be effective against all tested pathogenic bacteria (*P. aeruginosa, E. coli, S. aureus, Bacillus Subtilis* and *Micrococcus luteus*), while water extracts showed no activity. MIC of the extracts against these bacterial strains was in the range of 0.30 mg/ml. They reported that phytochemical analysis result indicates the presence of secondary metabolites like Alkaloids, Tannins, and Flavonoids which may be responsible for antibacterial activity and the extracts of *Taraxacum officinale* have potential against growth of all tested pathogenic strains.

Xue et al. (2017) evaluated compounds extracted from different parts of Dandelion. Their study showed Dandelion leaf had the highest, while root had the lowest level of total phenolic and flavonoid contents. Among eleven phenolic acids and flavonoids which were identified, chicoric acid was the main component in all parts of Dandelion. Consistent with the total content of phenolics and flavonoids, leaf extract had the highest total antioxidant and DPPH scavenging activity.

Antibacterial activity of water-soluble polysaccharides from the *Taraxacum officinale* was investigated by Wang (2014). The result of this study showed that polysaccharides extracted from Dandelion (PD) displayed high antibacterial activity at a concentration of 100 mg/mL against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and PD may be a viable option for use as a food preservative (Wang, 2014).

Sangeetha and Ezhilarasan. (2016) evaluated the antimicrobial effect of Dandelion against oral pathogens by the minimum inhibitory concentration and minimum bactericidal concentration. They reported that Dandelion shows high sensitivity against cariogenic microbes such as *Enterococcus faecalis* and *Streptococcus salivarius* and *Taraxacum officinale* suggested as a useful herb in order to control dental caries and endodontic infections.

Qian et al. (2014) prepared oligosaccharides from Dandelion (*Taraxacum officinale*) by hydrolysis with hydrogen peroxide and investigated their antibacterial activity. They reported that the oligosaccharides showed high antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*, indicating that Dandelion-derived oligosaccharides have the potential to be used as antibacterial agents.

Conclusion

Although, many solvents are used for extraction, in this study water was chosen as extraction solvent because it is quite safe for human consumption as compared with other organic solvents. Generally, it can be stated that the aqueous leaf extract of Dandelion on Gram-

positive bacteria showed more antimicrobial activity than Gram-negative bacteria, while there is no significant inhibition on mold. The results of MIC and MBC/ MFC tests showed that *Staphylococcus epidermidis* was the most susceptible and *Aspergillus niger* was the most resistant strains to the aqueous extract of Dandelion leaves respectivel. As previously mentioned, polyphenolic substances are abundantly found in plants with high antimicrobial properties. The results of this study also showed that the content of phenolic compounds and especially the flavonoids of dandelion is in considerable quantities. According to our knowledge, Dandelion aqueous extract might be used as a natural agent to prevent the growth of food borne microorganisms, particularly, the cause of food poisoning that leading to the reduction of gastroenteritis risk.

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ارزیابی میزان کلروفیل، فعالیت ضداکسایشی و اثر ضدمیکروبی عصاره برگ قاصدک

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چکیده

در این پژوهش محتوای کلروفیل، محتوای فلاونوئیدی کل، محتوای فنل کل و فعالیت ضداکسایشی عصاره آبی قاصدک مورد بررسی قرار گرفت. همچنین، تأثیر عصاره آبی خبررسانک به‌دست آمده به روش خیساندن، بر برخی میکروارگانیسم‌های عامل فساد و مسمومیت ناشی از مواد غذایی آزمایش شد. فعالیت ضدمیکروبی عصاره برگ قاصدک با استفاده از روش‌های انتشار دیسک و چاهک، حداقل غلظت بازدارندگی و حداقل غلظت کشندگی مورد بررسی قرار گرفت. مقادیر به‌دست آمده برای TFC، TPC ، کلروفیل a و b به‌ترتیب ۲۲/۸۲ (میکروگرم بر میلی‌لیتر)، ۱۱۶/۸۹ (میلی‌گرم بر میلی‌لیتر)، ۰/۸۱ (میلی‌گرم بر لیتر) و ۰/۰۶۳ (میلی‌گرم در لیتر) بود. فعالیت به دام‌اندازی رادیکال‌های آزاد، بر حسب IC_{50} برابر با ۶۸/۸۱ میکروگرم بر میلی‌لیتر بود. نتایج نشان داد که MIC عصاره برگ قاصدک بر *Aspergillus niger*، *Salmonella typhimurium*، *Bacillus subtilis* و *Staphylococcus epidermidis* به‌ترتیب ۵۱۲، ۲۵۶، ۲۵۶ و ۶۴ میلی‌گرم بر میلی‌لیتر بود. MBC عصاره برگ قاصدک بر *Aspergillus niger*، *Salmonella typhimurium*، *Bacillus subtilis* و *Staphylococcus epidermidis* به‌ترتیب بیش از ۵۱۲، ۲۵۶، ۲۵۶ و ۱۲۸ میلی‌گرم بر میلی‌لیتر به‌دست آمد. نتایج نشان داد که قطر هاله بازدارندگی در روش چاهک آگار بیشتر از روش دیسک دیفیوژن بوده و سویه‌ها در غلظت بالاتر قطر هاله بازدارندگی بیشتری نشان دادند. به‌طور کلی می‌توان اظهار داشت که عصاره آبی گیاه قاصدک روی باکتری‌های گرم مثبت فعالیت ضدمیکروبی بیشتری نسبت به باکتری‌های گرم منفی داشت، در حالیکه اثر بازدارندگی چشمگیری بر کپک‌ها نداشت. بر اساس یافته‌های این پژوهش، عصاره آبی قاصدک می‌تواند به‌عنوان یک ماده طبیعی برای جلوگیری از رشد میکروارگانیسم‌های عامل فساد مواد غذایی و به‌ویژه عامل مسمومیت غذایی و با هدف کاهش خطر ابتلا به بیماری‌های گوارشی، مورد استفاده قرار گیرد.

واژه‌های کلیدی: قاصدک، فعالیت ضدمیکروبی، فعالیت ضداکسایشی، میکروارگانیسم‌های بیماری‌زا.

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