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Effect of Cooking Methods on Bioactive Compounds of Eggplant (*Solanum melongena* L.) and its Use in Preparing and Evaluating Frozen Baba Ghanoush Product During Storage

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Abstract

This research was undertaken to find out the effects of different cooking methods on bioactive compounds of eggplant. The effect of freezing and storage on microbial growth and sensory properties of the common Egyptian dish called Baba ghanoush were also studied. The results showed that eggplant contained a high percentage of crude fiber, crude protein, crude fat, and total ash. It can be noted that the mineral contents of fresh eggplant were Ca, Mg, Na and K as the major minerals. On the other hand, the results of total phenolics in the fresh, dry fresh, oven and steamed samples were 9.32, 75.78, 42.31 and 41.53 mg/100 g, respectively as gallic acid equivalent. The total flavonoids of the different eggplant treated samples varied between 0.46 to 0.7 mg equivalents of quercetin per 100 g. The results indicated that the antioxidant activity as well as IC_{50} of fresh, dry fresh, oven and steamed samples were 8.82, 87.20, 82.78, 90.23 and 10.86, 1.18, 1.21 and 1.12, respectively. Also, eighteen phenolic compounds were identified by HPLC. The color attributes L*, a*, and b* in case of Baba ghanoush prepared from oven eggplant slightly decreased with increasing the storage period. Significant reduction was noted in microbial growth with increasing the storage period up to 4 months in case of Baba ghanoush prepared either from oven and steamed eggplant. Sensory attributes of Baba ghanoush were well accepted by the panelists even after 4 months of storage.

Keywords: Eggplant**,** bioactive compounds, frozen baba ghanoush

1. Introduction

Vegetables are one of the richest sources of antioxidants (Vicente *et al*., 2014). Eggplant (*Solanum melongena* L.) is the most popular vegetable worldwide and one of an important market vegetables in Asian and Mediterranean countries. It is known for vegetables of diet food because of high moisture content and low caloric value. However, it is a good source of antioxidants as well as some phytonutrients. This plant is ranked amongst the top ten vegetables in terms of antioxidant capacity due to its phenolic and flavonoid constituents (Hung *et al*., 2004; Kandoliya *et al*., 2015). Preparation can preserve perishable foods, but may also cause changes in some important characteristics. (Muthukumarappan and Tiwari, 2010). Cooking may affect their compositions in both positive and negative way (Faller and Fialho, 2009; Mcdougall *et al*., 2010 and Chumyam *et al*., 2013). The processes of preparing vegetables increase the antioxidant content compared to raw vegetables (Ferracane *et al*., 2008; Pellegrini *et al*., 2009). However, quality can decrease after processing and cooking (Faller and Fialho, 2009). It is dependent on the nature of vegetables, time and temperature of the treatment followed (Rickman *et al*., 2007). Eggplants are versatile vegetables and could be processed by different techniques and cooking methods. Eggplants normally serve as side dishes during major meals and usually, boiled for a few sec. or deep-fried before being served.

However, these processings and heat-applied-cooking processes (frying, boiling, steaming and roasting), has a significant effect on the changes of chemical compositions such as bioactive components, antioxidant activities and physical characteristics including colour, texture, taste and flavour (Poelman *et al*., 2014; Fabbri and Crosby, 2015).

Recent studies of eggplant fruit have revealed that it is a good source of dietary fiber and vitamins and provides significant quantities of minerals such as P, K, Ca and Mg (Raigón *et al*., 2008; Okmen *et al*., 2009).

Extracts from eggplant are effective for curing cancer, high blood pressure, and hepatitis due to their content of anthocyanins and strychnine (Magioli and Mansur, 2005). Also, help in reducing many human diseases caused due to unbalanced diets or malnutrition. A balanced diet is known to be very important for human health. So, eggplant fruit contribute a major part of diet whether in fresh, dried, preserved by freezing, and cooked forms which still contain its essential nutrients such as amino acids, fatty acids, carbohydrates, minerals and vitamins (Okmen *et al*., 2009). Physical and chemical effects lead to killing microbes during food preservation by freezing (Archer, 2004). Therefore, this research was undertaken to find out the effects of different thermal processes (steam and oven) on antioxidants, flavonoids, phenolic substances found in eggplant, and to find out the effect of freezing on microbial growth and some sensory properties during the storage periods of one of the common Egyptian dish called Baba ghanoush.

2. Materials and Methods

2.1 Materials

Eggplants (*Solanum melongena* L.) were purchased from the local market in Alexandria, (Fig 1). Egypt. Potassium sorbate and Citric acid (food grade) was obtained from the Department of Food Science and Technology, Faculty of Agriculture, Alexandria, Egypt. While the other ingredients such as salt, tahina and cumin were purchased from the local market in Alexandria, Egypt.

Fig 1. General appearance and cross section of eggplant fruit

2.2 Preparation of Samples

Eggplant (6 kg) was washed by tap water for 3 min, drained and divided into three parts. The first part was cut into medium slices (8 mm) and then treated with steam for 15 min, cooled and then peeled and chopped. The second part was treated in the oven for 30 min, peeled and chopped. The third part was peeled and cut into small slices and then divided into two parts. The first part was freeze-dried. The other part was used as a fresh sample for some analysis.

2.3 Methods

2.3.1 Technological Processes

2.3.1.1 Preparation of Baba ghanoush

The previous samples were used to prepare the Egyptian dish which is called Baba ghanoush. To (1500 g) each of steamed and oven sample, salt (15 g), tahina (150 g), cumin (3 g) and citric acid (3 g) were mixed and added to each sample together and then divided into polyethylene packages, and stored in the freezer (- 18 ºC) for 4 months.

2.4 Analytical Methods

2.4.1 Proximate Chemical Composition

Moisture content, crude fat, crude protein, crude fiber and total ash were determined according to AOAC (2006) Nitrogen free extract (NFE) was calculated by difference.

2.4.2 Mineral Content

Minerals including calcium, magnesium, manganese, iron, copper, and zinc were measured as described in AOAC method (2006) using Perkin Elmer 2380 Atomic Absorption Spectrophotometer**.** On the other hand, sodium and potassium were determined using flame photometer (model PFP7 PFP 7/C., England).

2.4.3 Ascorbic Acid

Ascorbic acid was determined in the fresh sample using 2.6, dichlorophenol indophenol dye according to the method of AOAC (2006).

2.4.4 pH Value

pH Value was measured using a digital meter Toledo MP 230 pH meter after mixing 10 g of sample with 10 mL distilled water.

2.4.5 Extraction of Samples

Sample preparation and extraction procedures were prepared by the modified method of Ahmed and Osama (2008). 1 g of the previously processed sample, as well as the raw sample was homogenized for 1 min in 50 mL of 80% methanol by using a mortar and pestle. The extract solution was centrifuged for 20 min at room temperature and the supernatant was collected and used to determine antioxidant capacities, total phenolic and total flavonoid contents.

2.4.6 Antioxidant Activity

2.4.6.1 DPPH Radical Scavenging Capacity Assay

The DPPH assay was performed as described by Bozin *et al.* (2006). The samples (from 0.5 to 15.5 μg / mL) were mixed with 1 mL of 90 μM DPPH solution and filled up with 95% MeOH, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. Ascorbic acid was used as a positive control.

The disappearance of DPPH was read spectrophotometrically at 515 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Inhibition of free radical by DPPH in (%) was calculated in the following way:

Inhibition % = 100 x (A_{blank} - A_{sample} / A_{blank}).

Where A blank is the absorbance of the control reaction mixture excluding the test compounds, and A sample is the absorbance of the test compounds. IC_{50} values, which represented the concentration of extraction of samples that caused 50% inhibition of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

2.4.6.2 Total Phenolic Content

The total phenolic content of methanol extract of eggplant was determined by the method of Ballard *et al.* (2010) using Folin-Ciocalteu reagent. The reaction mixture contained 0.2 mL of sample extract, 0.8 mL of distilled water, 0.1 mL of folin-ciocalteu's reagent and 0.3 mL of Na_2CO_3 (20 % w/v), the contents were mixed and kept for 30 min. The absorbance of the blue colored was read at 765 nm. The total phenolic content was calculated on a dry weight basis as gallic acid equivalents (GAE) from the calibration curve of gallic acid and the values were expressed as mg of gallic acid / 100 g sample.

2.4.6.3 Identification of Phenolic Compounds by HPLC

The method of Shan *et al.* (2005) was used with some modifications in the HPLC device as follows: Phenolic compounds were extracted using 80 % methanol containing 2 ml 0.1 M sodium fluoride to prevent oxidation of phenolic compounds. Separation of the phenolic compounds was carried out using an HPLC system (Perkin Elmer Series 200) with a UV-visible detector (Perkin Elmer Series 200) at 290 nm. The mobile phase was 5 % formic acid in a gradient of methanol containing from 5 to 80 % final concentration. Compounds were identified by comparison with known standards obtained from Sigma – Aldrich Co.

2.4.6.4 Total Flavonoid Content

The total flavonoid content of eggplant extract was determined by the method of Nayanathara *et al*. (2016) according to a modified colorimetric method**.** Firstly, 5 mL of the sample solution was taken and 0.3 mL of 5% $NaNO_2$ solution was added. After 6 min, 0.6 mL of 10% $AlCl_3.6H_2O$ was added to the mixture, which was kept at room temperature for 6 min., followed by the addition of 2 mL of 1M NaOH and the total volume was made up to 10 mL with the addition of deionized water. The resulting solution was mixed well and immediately, the absorbance was measured at 510 nm on a UV-VIS spectrophotometer. For the blank, the extracts were replaced with an equal volume of deionized water. A standard calibration curve was prepared with various concentrations of quercetin (in deionized water). The total flavonoid content was expressed as the mg equivalents of quercetin per 100 g of sample.

2.5 Colour Measurement

Hunter Lab colourimeter (Ultra scan vis, USA) was used to measure the colour index. Five readings of the colour index of the Hunter scale (L^*, a^*, b^*) were recorded. The instrument was standardized during each sample measurement with a black and white tail (L^*= 99.1, a^* =-1.12, b^*= 1.26), where colour is represented by whiteness or brightness / darkness (L^*), redness / greenness (a^*) and yellowness / blueness (b^*) (Piggott, 1988).

2.6 Microbiological Analysis

Bacterial growth (total count), yeasts and fungi were determined by the method of Difco Manual (2003). 10 g of each sample were mixed with 90 mL of tryptone-salt broth and homogenized in a stomacher for 1 min. Dilution for subsequent microbiological analyses was prepared. Enumeration of total aerobic mesophilic flora and yeasts and fungi was done on plate count agar.

2.7 Sensory Evaluation

Colour, taste, odour, texture, (consistency) and overall acceptability of Baba ghanoush prepared from steamed and oven eggplant were assessed using 15 panelists of the Food Science and Technology Department, Faculty of Agriculture, Alexandria University. The panelists were asked to score the above attributes according to a standard hedonic rating score from 9 (like extremely) to 1(dislike extremely) as described by Wichchukita and O'Mahony (2014).

2.8 Statistical Analysis

The data were subjected to statistical analysis using analysis of variance (ANOVA). The average values (mean ± SD) were compared by using the least significant differences test (LSD-test) using a statistical package for social science software (SPSS) Version 21.

3. Results and Discussion

3.1 Chemical Composition

Table 1 shows the proximate chemical composition and mineral content of eggplant. It can be noted from Table 1 that the moisture content of fresh eggplant was 91.86%. On the other hand oven and steamed samples had 90.68% and 93.34% moisture, respectively (data not shown). In adulation, the pH value of the fresh eggplant was 5.12. The value slightly increased to 5.19 and 5.35 in the oven and steamed samples, respectively (data not shown). The data obtained in the present study are in accordance with the result reported by Osidacz and Ambrosio-Ugr (2013) and Djermoune *et al.* (2016) who found that the pH of cooked eggplant slightly increased compared with that of the fresh sample. This increase could be assigned to the good extraction of organic acid after softening the cooked sample to their degradation, during cooking, which produces a release of protons or could be ascribed to the reduction of available carboxylic groups of proteins.

Table 1. Chemical composition, pH values and mineral content of fresh eggplant (on dry weight basis)

Component	Value**
(%) Moisture content	91.86 ± 0.15
(%)Crude fiber	35.3 ± 0.03
(%)Crude protein	13.3 ± 0.05
(%) Crude fat	2.46 ± 0.04
(%)Total ash	7.86 ± 0.12
pH	5.12 ± 0.02
(%)* NFE	41.08 ± 0.06
	Minerals (mg/100 g)
Ca	78.00
Mg	56.20
Mn	0.99
Fe	7.61
Cu	5.80
Zn	4.50
Na	2032
K	2483

* Nitrogen Free Extract (calculated by difference)

** Mean value ± S.D. on dry weight basis

The result in Table 1 also declared that the content of crude fiber, crude protein, crude fat and total ash were 35.3, 13.3, 2.46 and 7.86 %, respectively. While the **NFE,** by difference was 41.08 %. In comparison with the results obtained in the present study, Neslihan (2006) and

Das *et al.* (2011) found that eggplant contained 1.5 % crude fiber, 1.9 % crude protein, 0.4% total fat and 5.2% carbohydrates based on wet weight.

According to the results obtained in the present study (Table 1), it can be noted that mineral contents of fresh eggplant were Ca, Mg, Na and K as the major minerals being 78.00, 56.20, 2032 and 2483 mg/100 g, respectively. In addition, microelements such as Mn, Fe, Cu and Zn were found in small concentrations being 0.99, 7.61, 5.80 and 4.50 mg/100 g, respectively. The data obtained in the present study are more or less in accordance with those reported by (Arivalagan *et al.* 2012; Arivalagan *et al*. 2013; Eze and Kanu 2014 and Ayaz *et al*. 2015). Generally, eggplant is a rich source of K and Na.

3.2 Bioactive Compounds and Antioxidant Activity of Eggplant

Table 2 shows the bioactive compounds as well as the antioxidant activity of eggplant

Table 2. Bioactive components and antioxidant activity of eggplant

Sample	Fresh sample	Dry fresh sample*	Oven sample**	Steamed sample**
Vitamin C (mg/100)	22.40 ± 0.05^{a}	20.15 ± 0.05^{a}	17.06 ± 0.05^{b}	19.52 ± 0.07^{a}
Total phenolics	9.32 ± 1.47^{a}	75.78 ± 2.2^{c}	42.31 ± 0.91^{b}	41.53 ± 0.91^{b}
Total flavonoids	0.46 ± 0.01^{a}	$10.7 \pm 0.01^{c.}$	$8.5 \pm 0.91 \pm^{bc}$	8.1 ± 0.02^{b}
DPPH inhibition (%)	8.82 ± 1.47^{a}	87.20 ± 1.83^{c}	82.78 ± 0.91^{b}	90.23 ± 0.91^{d}
IC_{50} (mg sample/mL)	10.86 ± 1.08^{b}	1.18 ± 0.07^{a}	1.21 ± 0.12^{a}	1.12 ± 0.08^{a}

* Dehydrated.(oven dried)

** Freeze dried.(lyophilized)

Data are presented as means ±SD.

Values with different superscripts within each column are significantly different at $P \leq 0.05$.

The results in Table 2 showed that the dry fresh sample of eggplant, oven and steamed ones contained 20.15, 17.06 and 19.52 mg/100 g ascorbic acid, respectively. Oven and steam processes slightly decreased the total amount of ascorbic acid compared with the fresh sample. On the other hand, the results in Table 2 showed that total phenolics in fresh, dry fresh, oven and steamed samples were 9.32, 75.78, 42.31 and 41.53 mg/100 g, respectively as gallic acid equivalent. The data in Table 2 showed that the total flavonoids of the different eggplant treated samples varied between 0.50 to 10.70 mg equivalents of quercetin per 100 g. The result in Table 2 also indicated that the values of inhibition increased with increasing the temperature. This indicated that the antioxidant activity (DPPH inhibition %) as well as IC_{50} (the concentration of extract in mg/mL needed to scavenge 50% of the DPPH radical) in fresh, dry fresh, oven and steamed samples were 8.82, 87.20, 82.78, 90.23 and 10.86, 1.18, 1.21 and

1.12, respectively. These values are mainly due to its high content of phenolics and flavonoids. In accordance with the results obtained in the present study, Chumyam *et al*. (2013) studied the antioxidant capacities of eggplants heated by boiling, steaming or microwaving. They found that the antioxidant capacities increased compared with the raw fruits, and the fruits heated by microwaving had the highest antioxidant capacities compared with boiling and steaming. Salerno *et al*. (2014) concluded that a highly positive correlation for each heat treatment was found between antioxidant capacity and total phenolic content of the four cultivars of purple skin eggplants. It has been reported that many antioxidant compounds in plants are mainly present as covalently bound forms with insoluble polymers. Heat liberates the antioxidant compounds, leading to an increase in antioxidant capacity (Choi *et al*., 2006).

3.3 Identification of Phenolic Compounds by HPLC

Table 3 shows the phenolic compounds of the methanolic extract of freeze-dried eggplant powder.

Table 3. Identification of phenolic compounds by HPLC

phenolic compound	Concentration (ppm)*
Protocatchoic acid	77.51
Catechein	221.81
Chorogenic acid	71.80
Caffeine	68.85
Caffeic acid	25.99
Vanillic acid	25.04
P-Coumaric acid	6.10
Ferulic acid	0.73
Iso- Ferulic acid	13.49
Ellagic	25.09
Oleuropen	28.76
Alpha- Coumaric acid	0.68
Quercetin	52.44
Salycillic acid	31.48
3,4,5-Methoxy-Cinnamic acid	10.07
Coumarin	3.90
Cinnamic acid	0.58
Rutin	21.23

* on dry weight basis.

Eighteen compounds could be identified. The most predominant compounds were catechein, protocatchoic acid, chorogenic acid, caffeine and quercetin. The other compounds were found in smaller amounts. Comparing with the results obtained in the present study, Scorsatto *et al*. (2017) determined phenolic acids of eggplant flour prepared from the whole fruit dehydrated in an oven. They found the presence of chorogenic acid, caffeic acid and ferulic acid. On the other hand, Mansoura (2019) identified and quantified the different flavonoids in eggplant peel. She found that the highest levels of flavonoid compounds were quercetin 3-diglucoside, myricetin-3- galactoside and quercetin-3- rhammoside.

3.4 pH Values, Colour Parameters and Microbiological Tests for Baba Ghanoush Product

Table 4 Shows the pH value, colour parameters: L*, a*, b* and microbiological tests during different storage periods at (-18 °C) of the product (Baba ghanoush).

Table 4. pH values, colour parameters and microbiological tests for Baba ghanoush product

Storage periods		Oven samples			Steam samples		
Parameter		Zero time	2 month	4 month	Zero time	2 month	4 month
pH		4.67±0.08[a]	4.58±0.1[a]	4.53±0.2 [a]	4. 8±0.07 [a]	4.78±0.1 [a]	4.77±0.2 [a]
Hunter Lab	L*	60.50±0.3 [a]	59.40±0.2[a]	60.75±0.2 [a]	58.79±0.18 [a]	61.72±0.2[a]	62.81±0.2 [a]
	a*	2.44±0.06 [a]	2.21±0.07[a]	2.94±0.07[a]	1.42±0.08 [a]	1.31±0.07[a]	1.98±0.1 [a]
	b*	19.34±0.1 [a]	19.50±0.1[a]	20.97±0.1 [a]	18.99±0.1 [a]	20.29±0.1 [a]	22.50±0.1 [b]
Microbiological tests CFU/g	Total count	29 x10^2±0.1 [a]	9 x10±0.1 [b]	2 x10±0.1 [c]	35 x10^2±0.1 [a]	8 x10±0.1 [b]	2 x10±0.1 [c]
	Yeast and fungi	3.5 x10±0.1 [a]	1.5 x10±0.1 [a]	ND	2.5 x10±0.1 [a]	2 x10±0.1 [a]	ND

*ND = Not Detected

Data are presented as means ±SD.

Values with different superscripts within each row column significantly different at P≤ 0.05.

It can be noted that pH values of the oven and steamed samples before storage were 4.67 and 4.8, respectively. On the other hand, the results declared that after storage for 2 and 4 months, the values of pH were more or less the same for both samples. Concellon *et al*. (2007) and Djermoune *et al*. (2016) found that the pH of eggplant was 5.54. A slight increment in the pH value was noted after 2 days of frozen storage. The increase in the pH value continued until day 13 of frozen storage and afterward remained constant around pH 6.48.

Table 4 shows the colour attributes of Baba ghanoush prepared from oven and steamed eggplant and stored at -18 ^{0}C for 4 months. It can be noted that all the colour attributes L*, a*, and b* in case of oven eggplant slightly decreased with increasing the storage period with slight significant differences. On the other hand, the above attributes in case of steamed eggplant slightly increased with increasing the storage period with slight significant differences. Lee and Coates (2002) found that the overall colour values L*, a*, and b* changed in flame grapefruit juice concentrates during storage at -23 °C.

Effect of frozen storage of Baba ghanoush prepared from oven and steamed eggplant in their content of total count and yeast and fungi is shown in Table (4). It can be noted that the total aerobic mesophilic counts of Baba ghanoush prepared from oven eggplant at zero time was 29 x10^2 CFU/ g while the yeast and fungi count was 3.5 x10 CFU/ g. A significant reduction was noted in total count, yeast, and fungi with increasing the storage period up to 4 months. The same trend was found in case of Baba ghanoush prepared from steamed eggplant.

Archer, (2004) found that freezing is an ancient technology for preserving foods. In addition, Wójcik and Jadczak (2007) studied microbiological analysis of fresh plant material - shoots with leaves (whole and cut into 2-3 cm pieces) of plants: basil, marjoram and frozen pieces (after freezing and storage for 12 months at -25°C). The results showed that in the fresh whole plants, a total number of mesophilic aerobic bacteria ranged from 3.9 in marjoram to 6.7 log CFU/g in basil to 3.5 log CFU/g. Frozen storage led to induced differentiated decreasing of microbiological contamination of tested material. After 12 months of frozen storage, an average survival percentage of bacteria was 10%, of moulds and yeasts, respectively 44% and 48%, in relation to number of these microorganisms in fresh cut spice plants before freezing.

3.5 Sensory Evaluation of Baba Ghanoush Products

Table 5 shows the sensory attributes of Baba ghanoush prepared from oven and steamed eggplant (as shown in Fig 2) and frozen storage at -18 ^{0}C for up to 4 months. It can be noted that all the products were well accepted by the panelists even after 4 months of storage without any significant difference between the different sensory attributes. In accordance with the results obtained in the present study, Uthumporn *et al*. (2016) studied the organoleptic properties when eggplant was cooked at three different cooking methods including frying, grilling and steaming. The results showed that cooking by steam gives better results of colour, texture, taste, aroma and the overall acceptability of the final food products.

Table 5. Sensory evaluation of Baba ghanoush stored at -18 ^{0}C for different periods

Sample Attributes	Oven sample			Steamed sample		
	Zero	2 month	4 moth	Zero	2 month	4 moth
Colour	7.56±0.73^{a}	7.44±0.81^{a}	7.44±0.51^{a}	8.56±0.63^{a}	8.19±0.66^{a}	8.56±0.51^{a}
Taste	7.69±0.87^{a}	7.44±0.81^{a}	7.38±0.89^{a}	8.13±0.5^{a}	8.00±0.63^{a}	8.25±0.58^{a}
Odour	7.88±1.09^{a}	7.67±0.87^{a}	7.75±0.77^{a}	7.94±0.57^{a}	7.69±0.60^{a}	8.19±0.54^{a}
Texture	7.69±1.01^{a}	7.69±1.01^{a}	7.69±0.95^{a}	8.31±0.70^{a}	8.00±0.73^{a}	8.38±0.62^{a}
Overall acceptability	7.69±0.95^{a}	7.69±0.95^{a}	7.44±0.63^{a}	8.19±0.63^{a}	8.07±0.44^{a}	8.31±0.60^{a}

Data are presented as means ±SD.

Values with different superscripts within each column are significantly different at P≤0.05.

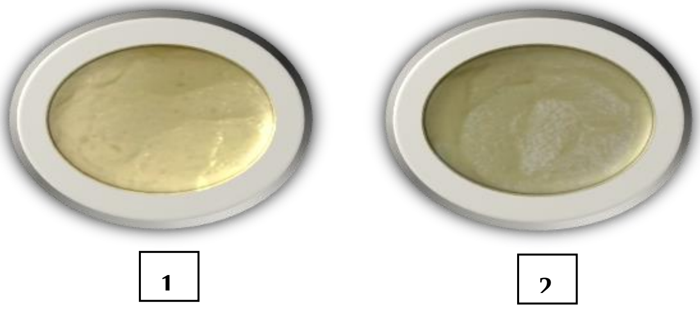

Fig 2. General appearance of Baba ghanoush Product Prepared by two different cooking methods:1- Steam, 2- Oven

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