

## Effect of ultrafine grinding the dill seeds on nutritional and technological properties of pan bread

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### ABSTRACT

This study was conducted on two types of milling the dill seeds, namely, normal and ultrafine milling. Ultrafine milling converts insoluble fiber into soluble fiber and contained approximately the same essential amino acids and fatty acids contents compared to normal and ultrafine grinding dill seeds. An experiment was also conducted on the cells infected with colon cancer using different percentages of extracted milling types and found that the antitumor activity of ultrafine extract against HCT-116 cell line revealed growth inhibition at a concentration of 125 µg/ml was 40.19 % and IC<sub>50</sub> 189 ± 5.6 µg/ml. compared normal extract inhibition at a concentration of 2000 µg/ml was 29.11% and IC<sub>50</sub> >2000 µg/ml. Pan bread was prepared using different levels from dill seeds normal grinding (1,2 and 3%) and dill seeds ultrafine grinding (0.5, 0.8 and 1.1%) and the results cleared that all sensory attributes of pan bread made using 1 and 2% of dill seeds normal and 0.5 and 0.8% of dill seeds ultrafine grinding were significantly higher values than control. Moreover, ultrafine grinding dill seeds improved physical properties and retard staling of pan bread.

**Keywords:** dill seeds, ultrafine grinding, antitumor activity, pan bread staling.

### Introduction

Ultra-fine grinding is a useful tool for making superfine powder with good surface properties like dispersibility and solubility (Tkacova and Stevulova, 1998). It has also been used to decrease the particle size of wheat bran (Hemery *et al.*, 2011). Wang and Li (2011) recently found that the soluble dietary fiber content of wheat bran increased after ultra-fine grinding. Thus, ultra-fine grinding seems promising to improve the nutritional potential and sensory value of whole wheat flour products.

Superfine powders are easier to incorporate into food structure and for absorption by the body, which would consequently improve the quality and safety of food products and human health. (Zhu *et al.*, 2010).

Dietary fiber (DF), has attracted increasing interests in recent years as many studies have revealed that it might be involved in disease preventive and health promotive activities, including attenuation of blood cholesterol and glucose, laxative effect and reduction of risk of colon cancer, heart disease and obesity. Fiber is often classified as soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) (Huang *et al.*, 2007 and Gorinstein *et al.*, 2001).

Spices and herbs have been added to food since the ancient times, not only as flavoring agents, but also as food preservatives. They have also been used in folk medicine (Kabić *et al.*, 2008).

Functional foods are complex products and may contain many pharmacologically active and phytochemicals and these active ingredients may possess multiple biological activities rather than have only one effect on the human health (Nguyen *et al.*, 2014).

Anticancer potential of *Apiaceae* plants possesses the potential of being a promising anticancer agent in improving brain tumor therapy (Aydin *et al.*, 2013). Furthermore, dill showed non-cytotoxicity in normal cells, whereas it exhibited great anticancer activity on KB-Oral cavity and MCF7-Breast cancer cells (Peerakam *et al.*, 2014).

Dhiman *et al.* (2017) mentioned that the dill (*Anethum graveolens*) contains various chemical constituents like proteins, fatty oils, carbohydrates, furanocoumarin, polyphenols, minerals and many other biologically active compounds. Also besides *Anethum graveolens* has been use as a yurvedic medicine since the ancient times and it is popularly used as spices. It is an aromatic herb, which belongs to the family *Apiaceae*.

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The aim of the present study was to investigate the nutritional value and technological quality of pan bread using the ultrafine grinding of dill seeds and its effects on chemical composition, bioactive compounds, antioxidant and antitumor activities.

### **Materials and methods:**

#### **Materials:**

Wheat flour (72% extraction) was obtained from 6<sup>th</sup> October for Milling and Marketing Co., 6<sup>th</sup> October City, Egypt. Dried dill seeds (*Anethum graeolens* L.) from Medicinal and Aromatic Research Department Horticulture Research Institute Center, Giza, Egypt, instant active dry yeast, crystal white sugar, sodium chloride, corn oil, obtained from the local market, Cairo, Egypt

#### **Methods**

##### **Preparation of normal grinding dill seeds:**

Dill seeds were cleaned from all impurities including broken seeds, and then milled by perten laboratory mill to whole normal grinding dill seeds. The resultant powder was packed in poly ethylene bags until used.

##### **Preparation of ultrafine grinding dill seeds:**

The normal grinding dill seeds was micronized by a WZJ6 vibratory micro-mill (Jinan Beili Powder Machine, Jinan, China) to produce ultrafine grinding dill seeds.

##### **Transmission Electron Microscopy (TEM):**

Normal and ultrafine grinding dill seeds were examined using High Resolution Transmission Electron Microscope model EM-2100 made in Japan according to Casuccio *et al.* (2004).

#### **Analytical methods**

##### **Chemicals and reagents**

All chemicals, including Folin - Ciocaltea's reagent, DPPH (1, 1-Diphenyl-2-picryl-ydrazyl), standards of phenolic, flavonoids and all others reagents used in the study (analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA) .

##### **Chemical analysis:**

Protein, ash, crude fiber and fat content were determined according to the methods described in A.O.A.C. (2005). Nitrogen free extract (NFE) content was calculated by difference. Minerals content, Mg, P, K, Ca, Fe, Mn and Na of both normal and ultrafine grinding dill seeds were determined according to the method of A.O.A.C. (2005).

##### **Antioxidant activity:**

##### **Determination of total phenolic compounds**

The total phenolic compounds of dill seeds (normal and ultra-fine grinding) were determined calorimetrically using Folin-Ciocalteu reagent according to the method described by Lin and Tang (2007). The absorbance of the mixture was measured at 750 nm against blank of deionized water using spectrophotometer (Jenway 6705 uv /vis). Gallic acid was used as a standard. The data were expressed as mg gallic acid equivalent per gm sample (mg GAE/g).

##### **Determination of total flavonoids**

Total flavonoids of dill seeds (normal and ultra-fine grinding) in the different methanolic extracts (1gram sample: 100 ml methanol 80%) were measured spectrophotometrically as reported by Lin and Tang (2007). The absorbance was measured at 415 nm against blank of deionized water. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The flavonoid concentration is expressed as quercetin equivalents in mg per gram of extract.

### **Determination of antioxidant activity**

Antioxidant activity of dill seeds (normal and ultra-fine grinding) was determined through the evaluation of the free radical-scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The results were expressed as percentage of inhibition of the DPPH radical and was calculated according to the method of Allothman *et al.* (2009). The radical scavenging percentage was calculated by the following equation:

$$\text{Scavenging activity (\%)} = 1 - (\text{absorbance of extract} / \text{absorbance of control}) \times 100.$$

### ***In vitro* evaluation of antitumor activity of normal and ultrafine extract dill Seeds:**

Antitumor activity of normal and ultra fine grinding the dill seeds was determined according to the method of Mosmann (1983) as follows:

Dill seeds samples (normal and ultrafine grinding) were Aqueous extracted according to the method described by Guzmán-Gerónimo *et al.* (2017). Exactly 10g of normal or ultra fine grinding dill Seeds was added to 100 ml distilled water (w/v) at 70°C in flask then kept for 12 h. in water bath at 70°C, then samples were centrifuged at 4000 rpm for 15 min at temperature 5°C., and the supernatant was filtered. To evaluate antitumor activity of both normal and ultrafine grinding dill seeds against HCT-116 cells line the following concentrations were prepared from filtered supernatant (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/ml).

Mammalian cell lines: HCT-116 cells (colon carcinoma) were obtained from VACSERA Tissue Culture Unit. Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA.

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with distilled deionized water and filtered through a Whatmann No.1 filter paper.

Cell line Propagation: The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin.

All cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and were subcultured two times a week.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10<sup>4</sup> cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 500, 1000 and 2000 µg/ml). Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO<sub>2</sub> for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The incubation was continued for 24 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Micro plate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the micro plate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(OD<sub>t</sub>/OD<sub>c</sub>)]x100% where OD<sub>t</sub> is the mean optical density of cells treated with the tested sample and OD<sub>c</sub> is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC<sub>50</sub>), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA, USA).

#### **Determination of Amino-acids:**

Amino acids content were determined according to the method as described by Moore *et al.* (1958).

#### **Determination of fatty-acids:**

The standard procedure for analyzing the fatty acid contents of dill seeds (normal and ultrafine grinding) were used, the fatty acids were extracted and separated by the method described by Stroescu *et al.*, (2013).

#### **Determination of dietary fibre contents:**

Total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) contents of samples were determined with an enzymatic gravimetric procedure according to AOAC. (2005).

#### **Pan bread Preparation:**

The straight dough method for pan bread production was carried out according to the method described by AACC. (2010). A Pan bread recipe containing 100% wheat flour 72% ext. , instant active dry yeast (1.5 g), crystal white sugar (5 g), sodium chloride (1.5 g), corn oil (3 g) and kneading water 60 ml. A pan bread wheat flour 72% ext. was used as control and pan bread samples of normal and ultra fine grinding dill seeds were prepared by adding different levels (1 , 2 , 3%) and (0.5 , 0.8 , 1.1 %), respectively. These levels were chosen after experimental trails. Bread dough was prepared for each sample by mixing all ingredients in the mixer bowl for 2 min, fermented dough was left to rest for 20 min at 28- 30°C (first proofing) then the dough was divided into 150 g piece. The pieces were hand moulded and placed into pans for final proofing at 32-35°C and 80- 85 % relative humidity in fermentation cabinet for 60 min. Dough was baked in electrically oven at 210- 220°C for 15- 20 min. After baking, loaves were separated from the metal pan and allowed to cool at room temperature before sealed in polyethylene bags to prevent moisture loss then stored at room temperature (25± 2°C).

#### **Evaluation of pan bread qualities:**

##### **Physical properties:**

**Volume:** The volume of different types of the produced pan bread was determined by rape seeds displacement method (AACC., 2010).

**Weight:** Weight of pan bread was determined individually within one hour after baking the average of three pans was recorded.

##### **Specific volume:**

Pan bread was weighed individually within one hour after baking and specific volume was calculated according to the method of AACC. (2010) using the following equation:

$$\text{Loaf Specific volume (L.S.V)} = \text{Volume (cm}^3\text{)} / \text{Weight (gm)}.$$

##### **Organoleptic evaluation:**

Fresh samples of pan bread were organoleptically evaluated by taste panels from the staff in Food Tech. Res., Institute Agric. Res. Centre, Giza , Egypt. They were asked to score the internal characteristics of pan bread for general appearance(15), crust color(15), crumb color (15), distribution of crumb(15), taste (20) , flavor(20) and overall acceptability (100) according to the method described by AACC. (2010).

##### **Determination of staling:**

Alkaline water retention capacity (AWRC) values of pan bread samples were measured after zero, 24, 48 and 72 hr. of storage at room temperature (25± 2°C) according to Kitterman and Rubanthalar (1971), as follow : Bread was cut into small pieces and dried in an electric oven at 50°C over night, then ground to pass through 60 mesh stainless steel sieve. Five grams of dried bread sample were placed into a 50 ml dry plastic centrifuge tube. Then, 25 ml of NaHCO<sub>3</sub> solution (8.4g

Sodium bicarbonate dissolved in one liter distilled water) were added. The tube was stoppered and shaken until all baked products became wet. Then, the mixture was left for 20 min with shaking every 5 min. The contents were then centrifuged at 1000 rpm for 15 min. After centrifugation, the supernatant was decanted and the precipitated was left for 10 min. at 45°C angel to get rid of free water. The percentage of the absorbed alkaline solution to 5 g of baked bread was calculated as follows: % AWRC= (W2 - W1 / Ws) × 100

W1 = Weight of empty tube (g).

W2=Weight of tube with sample after centrifugation (g).

Ws = Weight of sample. (g).

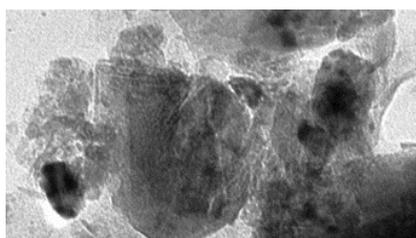
#### Statistical analysis:

Data were expressed as the means ± SD. Statistical analysis was carried out using one - way analyses of variance, ANOVA (Rao, and Blane, 1985).

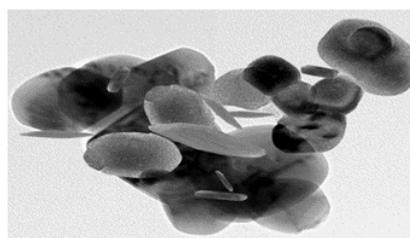
### Results and Discussions

#### Transmission Electron Microscopy (TEM) images of normal and ultra-fine grinding dill seeds:

TEM image in Fig (1) shows agglomeration of particles together formed irregular to sub rounded unclear edge particles with inner fiber structure so the fiber and particles act as one body in normal grinding dill seeds. Ultrafine grinding dill seeds shows no agglomeration where the particles separate from each other and from fiber with sharp edges. TEM images proof that dill seeds ultrafine composed of two particles shape (sub rounded and fiber) act as ultrafine-composite while normal grinding dill seeds show the fiber and particles appeared cemented and act as one body.



Normal grinding dill seeds



Ultrafine grinding dill seeds

**Fig. 1:** Transmission electron micrographs of, normal and ultrafine grinding dill seeds.

#### Nutritive and chemical composition of normal and ultrafine grinding dill seeds.

Data presented in Table 1. showed the proximate chemical composition of the dill seeds powders. First, the moisture content in dill seeds sample increased slightly under ultrafine grinding. Similarly, ultrafine grinding easily destroys cell structure and increases surface area given small particles. As a result, protein losses occur during the easy transfer from the interior of the cell to its exterior. Furthermore, ultrafine grinding significantly increased the soluble dietary fibre (SDF) value. However, it affected TDF value only marginally.

**Table 1:** Nutritive and proximate composition of normal and ultrafine grinding dill seeds (on dry bases).

Parameters	Dill seeds		
	Normal grinding	Ultrafine grinding	LSD
Moisture	7.64 <sup>a</sup> ±0.49	7.74 <sup>a</sup> ±0.10	0.80
Crude fibre	20.22 <sup>a</sup> ±0.08	19.81 <sup>b</sup> ±0.10	0.21
Protein	17.50 <sup>a</sup> ±0.50	16.83 <sup>a</sup> ±0.28	0.92
Fat	20.13 <sup>a</sup> ±0.60	20.25 <sup>a</sup> ±0.10	0.97
Ash	7.59 <sup>a</sup> ±0.10	5.78 <sup>b</sup> ±0.10	0.22
Nitrogen free extract (NFE)	34.55 <sup>b</sup> ±0.03	37.33 <sup>a</sup> ±0.37	0.60
Total Dietary Fiber (TDF)	22.34 <sup>a</sup> ±0.10	21.90 <sup>a</sup> ±0.26	0.45
Insoluble Dietary Fiber (IDF)	9.27 <sup>a</sup> ±0.10	4.35 <sup>b</sup> ±0.10	0.22
Soluble Dietary Fiber (SDF)	13.07 <sup>b</sup> ±0.07	17.41 <sup>a</sup> ±0.66	1.07

Each value represents the average from three replications.

\*Means in the same row with different letter are significantly different (P ≤ 0.05).

These results are consistent with those of previous studies and confirm that ultrafine grinding can effectively promote the redistribution of fibre components from insoluble to soluble fractions (Chau *et al.*, 2007).

#### Minerals of normal and ultrafine grinding dill seeds

The minerals content of dill seeds normal and ultrafine grinding are shown in Table (2) it could be noticed that the minerals were slightly change in element percent, which may be due to decomposition of some minerals by increasing energy of milling. The main elements percent still, as it is which meaning success of synthesis method without any huge chemical change or impurities.

**Table 2:** Minerals content of dill seeds normal and ultrafine grinding (%).

Minerals	Mg %	P %	K %	Ca %	Fe%	Mn %	Na %
Dill seeds normal grinding	7.49	10.33	11.95	25.29	0.68	0.01	0.40
Dill seeds ultrafine grinding	7.43	10.30	12.65	24.79	0.48	0.02	0.26

#### Amino acids content of normal and ultrafine grinding dill seeds

Data presented in Table (3) showed the essential amino acids and non-essential amino acids content of dill seeds normal and ultrafine grinding. It was observed that, dill seeds ultrafine grinding had a approximately the same amino acids contents (essential and non-essential) compared with dill seeds normal grinding.

**Table 3:** Amino acids composition (mg/100g protein) of the normal and ultrafine grinding dill seeds.

Amino acids	Dill seeds	
	Normal grinding	Ultrafine grinding
Lysine	4.10	4.19
Leucine	6.20	6.88
Isoleucine	4.30	4.14
Threonine	4.80	4.41
Histidine	1.50	1.70
Valine	7.10	6.06
Methionine	0.80	1.00
Cistine	0.60	0.69
Phenylalanine	2.90	3.22
Tyrosine	2.50	2.77
Tryptophan	0.22	0.32
Total essential amino acid	35.02	35.38
Glutamic acid	8.40	10.21
Aspartic acid	9.60	10.54
Proline	5.11	5.53
Arginine	3.90	4.34
Glycine	23.17	20.02
Alanine	7.10	6.30
Serine	7.70	7.68
Total non- essential amino acid	64.98	64.62

#### Fatty acids content of the normal and ultrafine grinding dill seeds.

Fatty acids content of normal and ultrafine grinding dill seeds (% of total lipids) are presented in table (4). From the tabulated data, it could be stated that, normal and ultrafine grinding dill seeds are rich in unsaturated fatty acids particularly of oleic acid C18:1 and linoleic acid C18:2 content which were 48.15 , 42.11 and 49.40 , 40.06 % for normal and ultrafine grinding dill seeds respectively. Ultrafine grinding dill seeds contained approximately the same content of Myristic acid (1.60%) , palmitolic acid (0.28%) , Stearic acid (3.86%), Linolenic acid (0.52%) and Behenic acid (1.12%) compared with normal grinding dill seeds (0.26 , 0.22, 3.84, 0.36 and 0.55%, respectively) .

**Table 4:** Fatty acids content of normal and ultrafine grinding dill seeds. (% of total fatty acids).

No. of carbons	Common name	Dill seeds	
		Normal grinding	Ultrafine grinding
		Percent composition	
C14:0	Myristic acid	0.26	1.60
C16:0	Palmitic acid )	3.20	1.90
C16:1	Palmitoleic acid	0.22	0.28
C18:0	Stearic acid	3.84	3.86
C18:1	Oleic acid	48.15	49.40
C18:2	Linoleic acid	42.11	40.06
C18:3	Linolenic acid	0.36	0.52
C20:0	Arachidic acid	1.31	1.26
C22:0	Behenic acid	0.55	1.12
Total percentage		100	100

**Antioxidant activity of normal and ultrafine grinding dill seeds.**

In general antioxidants are the compounds which lead to inhibition or delay of the oxidation of other molecules such as the inhibition of the initiation or propagation of oxidizing chain reaction Rayner, (1998) and Hyun-Jung *et al.* (2008). Antioxidants contents including total phenol, total flavonoids of normal and ultrafine grinding dill seeds (% on dry basis) are presented in Table (5). It could be noticed that ultrafine grinding dill seeds had higher content of total phenol compounds (16.10 mg GAE/g) compared with normal grinding dill seeds(15.17 mg GAE/g), moreover total flavonoids was 10.60 and 7.76 mg QE/g. Concerning DPPH % value, ultrafine grinding dill seeds had higher content (85.54%) than normal grinding dill seeds (79.37%). These results are confirmed by those obtained by Zheng and Wang (2001); Guzmán-Uriarte *et al.* (2013).

**Table 5:** Total phenolic, total flavonoids and DPPH radical scavenging activity, of normal and ultrafine grinding dill seeds.

Parameters	dill seeds	
	Normal grinding	ultrafine grinding
Total phenolic contents(mg GAE/g)	15.17 <sup>a</sup> ±0.795	16.10 <sup>a</sup> ±0.389
Total flavonoids (mg QE /g)	7.76 <sup>b</sup> ±0.444	10.60 <sup>a</sup> ±0.096
DPPH %	79.37 <sup>b</sup> ±0.416	85.54 <sup>a</sup> ±2.563

GAE: Gallic acid equivalent. QE: quercetin equivalent.

\*Means in the same row with different letter are significantly different (P ≤ 0.05).

**Antitumor activity of normal and ultrafine extract dill seeds:**

*In vitro*, effect of normal and ultrafine extract dill seeds against human colon carcinoma cell line HCT-116 was evaluated. The current results in Table (6) indicating that increasing the concentration of extract affect cell lines in the range of from 3.90 to 2000 µg/ml leads to decrease cell population in the cell lines. Results illustrated in Table (6) was cleared that ultrafine extract dill seeds exhibited high inhibitory activity even at low concentrations. As the inhibitory activity of ultrafine dill seeds at 3.90 µg /ml reached 0% HCT-116 cell line compared normal extract which was inhibitory 0% at a concentration of 125 µg/ml. In the present study, the antitumor activity of ultrafine extract against HCT-116 cell line revealed growth inhibition at a concentration of 125µg/ml was 40.19 % and IC<sub>50</sub> 189 ± 5.6 µg/ml. compared normal extract inhibition at a concentration of 2000µg/ml was 29.11% and IC<sub>50</sub> >2000 µg/ml.

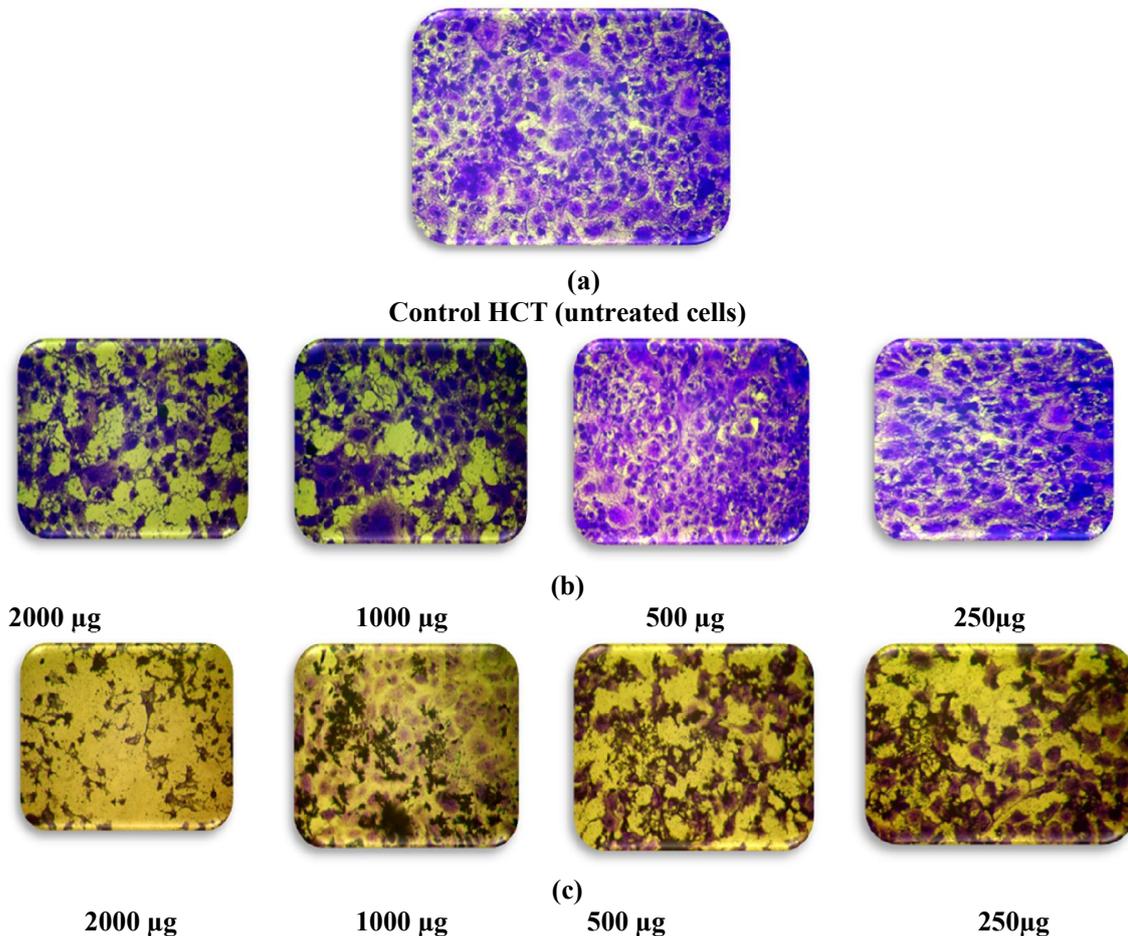
**Effect of normal and ultrafine extract dill seeds on morphological changes of HCT-116 cells.**

Fig. 2 (a, b and c) illustrated the morphological changes of HCT-116 cells untreated and treated tumor cell with dill seeds extracts clearly observed. The untreated tumor cells (control) showed adherent growth and completely homogenous layer a regular shape Fig.2 (a), meanwhile Fig.2 (b and c) the tumor cell was clearly decreased as a result of ultrafine extract dill seeds treatment with higher concentration.

**Table 6:** Evaluation of antitumor activity of dill seeds normal and ultrafine extract ( $\mu\text{g/ml}$ ) against HCT-116 cell line.

Sample conc. ( $\mu\text{g/ml}$ )	Inhibitory %	
	Dill seeds extract	
	Normal	Ultrafine
(Control) 0.0	0	0
3.9	0	0
7.8	0	1.3 $\pm$ 0.12
15.6	0	8.57 $\pm$ 0.55
31.25	0	16.05 $\pm$ 0.73
62.5	0	32.48 $\pm$ 2.64
125	0	40.19 $\pm$ 1.53
250	1.73 $\pm$ 0.09	59.24 $\pm$ 0.78
500	6.18 $\pm$ 0.21	67.51 $\pm$ 1.14
1000	14.87 $\pm$ 0.35	78.62 $\pm$ 0.91
2000	29.11 $\pm$ 0.72	90.46 $\pm$ 0.38
*IC <sub>50</sub>	>2000 $\mu\text{g/ml}$ .	$\mu\text{g/ml}$ .189 $\pm$ 5.6

\* IC<sub>50</sub>: value of the concentration an individual compound leading to 50% cell death.



**Fig. 2:** Effect of normal and ultrafine extracts dill seeds on morphological changes of HCT-116 cells. (a) Untreated cells (control). (b) Normal extract dill seeds. (c) Ultrafine extract dill seeds.

**Sensory evaluation of pan bread:**

The sensory quality attributes of pan bread samples are presented in Table 7. The results indicated that taste, flavor and overall acceptability of pan bread made using different levels of dill seeds normal (1 and 2%) and ultrafine grinding (0.5 and 0.8%) were significantly higher values than

control. Pan bread quality of ultrafine grinding was superior to that of normal grinding. Meanwhile, pan bread produced from dill seeds normal grinding 3% and pan bread produced from dill seeds ultrafine grinding 1.1% were significantly lower in overall acceptability (81.94 and 82.87%), respectively.

**Table 7:** Sensory evaluation of pan bread made using different levels of dill seeds normal and ultrafine grinding.

Samples	Crust color (15)	Crumb distribution (15)	Crumb color (15)	Taste (20)	Flavor (20)	General appearance (15)	Overall acceptability (100)
Control	14.10 <sup>a</sup> ±0.08	14.03 <sup>a</sup> ±0.06	14.15 <sup>a</sup> ±0.18	18.08 <sup>b</sup> ±0.07	18.46 <sup>b</sup> ±0.14	14.00 <sup>a</sup> ±0.08	92.82 <sup>b</sup> ±0.26
WF + DNG 1%	14.15 <sup>a</sup> ±0.08	14.05 <sup>a</sup> ±0.06	14.46 <sup>a</sup> ±0.04	18.62 <sup>ab</sup> ±0.17	19.22 <sup>a</sup> ±0.15	14.30 <sup>a</sup> ±0.16	94.79 <sup>a</sup> ±0.16
WF + DNG 2%	14.06 <sup>a</sup> ±0.14	14.25 <sup>a</sup> ±0.08	14.53 <sup>a</sup> ±0.15	19.26 <sup>a</sup> ±0.10	19.51 <sup>a</sup> ±0.14	14.52 <sup>a</sup> ±0.14	96.12 <sup>a</sup> ±0.53
WF + DNG 3%	13.11 <sup>b</sup> ±0.17	13.15 <sup>b</sup> ±0.29	13.41 <sup>b</sup> ±0.05	14.46 <sup>c</sup> ±0.23	14.81 <sup>d</sup> ±0.02	13.02 <sup>b</sup> ±0.18	81.94 <sup>a</sup> ±0.13
WF + DUG 0.5%	14.47 <sup>a</sup> ±0.13	14.57 <sup>a</sup> ±0.16	14.58 <sup>a</sup> ±0.06	18.55 <sup>ab</sup> ±0.16	19.63 <sup>a</sup> ±0.14	14.03 <sup>a</sup> ±0.28	95.82 <sup>a</sup> ±0.25
WF + DUG 0.8%	14.57 <sup>a</sup> ±0.15	14.55 <sup>a</sup> ±0.1547	14.65 <sup>a</sup> ±0.1817	19.16 <sup>a</sup> ±0.15	19.50 <sup>a</sup> ±0.14	14.51 <sup>a</sup> ±0.14	96.94 <sup>a</sup> ±0.42
WF + DUG 1.1%	12.58 <sup>b</sup> ±0.21	13.45 <sup>b</sup> ±0.0476	13.45 <sup>b</sup> ±0.0476	14.11 <sup>c</sup> ±0.26	15.88 <sup>c</sup> ±0.05	13.12 <sup>b</sup> ±0.15	82.87 <sup>c</sup> ±0.32

**WF:** wheat flour, **DNG:** dill seeds normal grinding, **DUG:** dill seeds ultrafine grinding.  
 Means in the same column with different letter are significantly different ( $P \leq 0.05$ ).

**Specific volume of pan bread made using different levels of dill seeds normal and ultrafine grinding:**

Specific volume is one of the most important parameters which depends on flour properties mostly the protein network strength, under the targeted improvement of wheat flour, to measure the modification that could occur when ultrafine grinding used. The results which obtained in Table 8 indicated that there had a significant differences between normal grinding and ultrafine grinding for volume and specific volume of pan bread. This improvement due to increase the gluten strength by ultrafine grinding which lead to increase the retention rate of fermentation gas. The results are in agreement with that reported by Patricia and Caroline (2014) and El-Porai *et al.* (2013).

**Table 8:** Specific volume of pan bread made using different levels of dill seeds normal and ultrafine grinding.

Treatment	Weight (g)	Volume (cm <sup>3</sup> )	Specific volume (cm <sup>3</sup> /g)
Control	125.2 <sup>a</sup> ±5.01	445.07 <sup>bc</sup> ±5.00	3.55 <sup>b</sup> ±0.18
WF + DNG 1%	120.30 <sup>ab</sup> ±5.03	447.67 <sup>b</sup> ±7.50	3.72 <sup>b</sup> ±0.10
WF + DNG 2%	115.00 <sup>ab</sup> ±5.00	435.60 <sup>c</sup> ±5.50	3.79 <sup>b</sup> ±0.19
WF + DNG 3%	108.3 <sup>ab</sup> ±7.64	430.17 <sup>d</sup> ±5.00	3.97 <sup>ab</sup> ±0.34
WF + DUG 0.5%	117.67 <sup>ab</sup> ±4.50	450.00 <sup>ab</sup> ±5.00	3.82 <sup>ab</sup> ±0.19
WF + DUG 0.8%	125.00 <sup>a</sup> ±21.79	453.00 <sup>ab</sup> ±3.00	3.62 <sup>b</sup> ±0.61
WF + DUG 1.1%	105.00 <sup>b</sup> ±5.00	460.00 <sup>a</sup> ±10.00	4.38 <sup>a</sup> ±0.31

**WF:** wheat flour **DNG:** dill seeds normal grinding **DUG:** dill seeds ultrafine grinding  
 Means in the same column with different letter are significantly different ( $P \leq 0.05$ ).

**Staling of pan bread produced from different levels of dill seeds normal and ultrafine grinding:**

Alkaline water retention capacity (AWRC) is a simple and quick test to follow staling of bread. Higher values of AWRC mean higher freshness of bread (Yaseen *et al.*, 2010 and Giannone *et al.*, 2016).

The changes occurring in freshness characteristic of different pan bread samples stored at zero, 24, 48 and 72 h. at room temp 25 ±2°C are shown in Table 8. It could be observed that freshness of pan bread gradually decreased by increasing the period of storage. After 72 h. of storage, the lower reduction in stalling value (high freshness) was observed in pan bread prepared made using normal grinding (1, 2 and 3%) and ultrafine grinding (0.5, 0.8 and 1.1%) which reached to 165, 150, 140, 230, 237 and 242%, respectively compared to the higher reduction in staling value (low freshness) in pan bread prepared from (control) (181.67 %). Meanwhile, ultrafine grinding was the best compared to normal grinding and control. This may be due to its higher protein content which binding more moisture. The results are in agreement with those reported by Yaseen *et al.* (2010) and Salehifar *et al.*

(2010) who's mentioned that the reason for higher moisture in bread after storage is due to the content of protein. Therefore, the high level of protein regardless of its quality which provides the cysteine amino acid sufficiently to show a better improvement.

**Table 9:** Alkaline water retention capacity (AWRC %) of pan bread which made using different levels of dill seeds normal and ultrafine grinding.

Pan bread samples	Storage periods (h)			
	Zero	24	48	72
<b>Control</b>	224.33 <sup>a</sup> ±4.04	218.00±2.00	190.00 <sup>b</sup> ±5.00	181.67 <sup>b</sup> ±5.80
<b>WF + DNG 1%</b>	222.67 <sup>c</sup> ±2.51	214.00 <sup>c</sup> ±3.61	185.00 <sup>b</sup> ±5.00	165.00 <sup>c</sup> ±5.00
<b>WF + DNG 2%</b>	222.33 <sup>c</sup> ±2.51	211.00 <sup>c</sup> ±6.55	168.33 <sup>c</sup> ±7.63	150.00 <sup>d</sup> ±10.00
<b>WF + DNG 3%</b>	210.00 <sup>d</sup> ±5.00	193.33 <sup>d</sup> ±5.77	160.00 <sup>c</sup> ±10.00	140.00 <sup>d</sup> ±10.00
<b>WF + DUG 0.5%</b>	246.00 <sup>b</sup> ±5.29	242.00 <sup>b</sup> ±2.00	237.67 <sup>a</sup> ±4.50	230.00 <sup>a</sup> ±1.00
<b>WF + DUG 0.8%</b>	252.67 <sup>b</sup> ±2.51	248.67 <sup>b</sup> ±3.21	240.67 <sup>a</sup> ±10.50	237.00 <sup>a</sup> ±2.00
<b>WF + DUG 1.1%</b>	270.00 <sup>a</sup> ±10.0	260.00 <sup>a</sup> ±10.00	250.00 <sup>a</sup> ±10.00	242.00 <sup>a</sup> ±2.65

WF: wheat flour      DNG: dill seeds normal grinding      DUG: dill seeds ultrafine grinding

\*Means in the same row with different letter are significantly different ( $P \leq 0.05$ ).

### Conclusion

Therefore, it is concluded that dill seeds (ultrafine grinding) can be successfully incorporated in the development of functional pan bread which have good sensory acceptability. Dill seeds (ultrafine grinding) are rich source of dietary fibre, minerals and Antioxidant activity. This research will provide a new technology in the area of health science for the development of therapeutic approach for the management of life style disorder such as antitumor activity by simultaneous administration of nutritious bread formulation.

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### References

- AACC., 2010. Approved method of the American Association of Cereal Chemistry Published by American Association of Cereal Chemistry Inc. st. Paul. Minnesota. U.S.A.
- Alothman, M., R. Bhatand A.A. Karim, 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*, 115(3), 785-788.
- AOAC., 2005. Association of Official Analytical Chemists. Washington, D.C., USA. 18<sup>th</sup> Ed., Chapter 33 , pp. 10, 70-72 , Chapter 45, pp. 101.
- Aydin, E., H. Türkez and MS. Keles, 2013 . Potential anticancer activity of carvone in N2a neuroblastoma cell line. *Toxicology and Industrial Health* 31(8):764-772.
- Casuccio, G.S., S.F. Schlaegle, T.L. Lersch, G.B. Huffiman, Y. Chen and N. Shah, 2004. Measurement of fine particulate matter using electron microscopy technique. *Fuel Processing Technology*, 85: 763-779.
- Chau, CF., Y.T. Wang and Y.L. Wen, 2007. Different icronization methods significantly improve the functionality of carrot insoluble fiber. *Food Chem* 100:1402–1408
- Dhiman C., N. Kumar and P. Kothiya, 2017. Pharmacological actions of *Anethum graveolens* (Dill). *Journal of Pharmacy Research* 11 (5):511-516.
- El-Porai, E.S., A.E. Salama, A.M. Sharaf, A.I. Hegazy and M.G.E. Gadallah, 2013. Effect of different milling processes on Egyptian wheat flour properties and pan bread quality. *Annals of Agricultural Sciences*, 58(1): 51-59.

- Giannone, V., M.R. Lauro, A. Spina, A. Pasqualone, L. Auditore, I. Puglisi and G. Puglisi, 2016. A novel  $\alpha$ -amylase-lipase formulation as antistaling agent in durum wheat bread. *Food Science and Technology*, 65: 381-389.
- Gorinstein, S., Z. Zachwieja, M. Folta, H. Barton, J. Piotrowicz, M. Zember, M. Weisz, S. Trakhtenberg and O. Martin-Belloso, 2001. Comparative content of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry*, 49, 952–957.
- Guzmán-Gerónimo, R., E.A. Aparicio, O.G. Barradas, J. Chávez-Servia and T. Alarcón- Zavaleta, 2017. Chapter 4: Chemical, antioxidant, and cytotoxic properties of native blue corn extract. PP: 67-77. <http://dx.doi.org/10.5772/67574>.
- Guzmán-Urriarte, M.L., L.M. Sánchez-Magaña, G.Y. Angulo-Meza, E.O. Cuevas-Rodríguez, R. Gutiérrez-Dorado, S. Mora-Rochín, J. Milán-Carrillo, A. Valdez-Ortiz and C. Reyes-Moreno, 2013. Solid State Bioconversion for Producing Common Bean (*Phaseolus vulgaris* L.) Functional Flour with High Antioxidant Activity and Antihypertensive Potential. *Food and Nutrition Sciences*, (4): 480-490.
- Hemery, Y., M. Chaurand, U. Holopainen, A.M. Lampi, P. Lehtinen, V. Piironen, A. Sadoudi and X. Rouau, 2011. Potential of dry fractionation of wheat bran for the development of food ingredients, Part I: influence of ultra-fine grinding. *J. Cereal Sci.* 53: 1-8.
- Huang, Y.L., F. Sheu, M.H. Lee and C.F. Chau, 2007. Effects of particle size reduction of insoluble fibres by micron technology on various caecal and faecal indices. *Journal of the Science of Food and Agriculture*, 88: 435-441.
- Hyun-Jung, C.A., L.A. Qiang, P. K. Peter, Z. Ming, C. Fan and Y. Rickey, 2008. In vitro starch digestibility, expected glycemic index and some Physicochemical properties of starch and flour from common bean (*Phaseolus vulgaris* L.) varieties grown in Canada. *Food Res. Inter.* 41: 869–875.
- Kabić, D., A. Mišan, M. Sakač, Đ. Psodorov and I. Milovanović, 2008. Medical plant mixtures as additives in functional bakery food production. *Food Processing, Quality and Safety* 35(3):119-123.
- Kitterman, J.S. and G.L. Rubenthaler, 1971. Assessing the quality of early generation wheat selection with the micro AWRC test. *Cereal Sci., Today*, 16: 313-316, 328.
- Lin, J.Y. and C.Y. Tang, 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.*, 101(1): 140–147.
- Moore, S., D.H. Spackman and W.H. Stein, 1958. Chromatography of Amino Acid on Sulphonated Polystyrene Resins. An Improved System. *Analytical Chemistry*, 30: 1185-1190.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*; 65: 55-63.
- Nguyen, S., H. Huang, B.C. Foster, T.W. Tam, T. Xing, M.L. Smith, J.T. Arnason and H. Akhtar, 2014. Antimicrobial and P450 inhibitory properties of common functional foods. *Journal of Pharmacy and Pharmaceutical Sciences* 17(2):254-265.
- Patricia, M.G. and J. S. Caroline, 2014. Physicochemical and sensory characteristics of pan bread samples available in the Brazilian market. *J. Food Sci. Technol, Campinas*, 34(4): 746-754.
- Peerakam, N., J. Wattanathorn, S. Punjaisee, M.S. Buamongkol, P. Sirisa-Ard and S. Chansakaow, 2014. Chemical profiling of essential oil composition and biological evaluation of *Anethum graveolens* L. (seed) grown in Thailand. *Journal of Natural Sciences Research* 4(16):34-41.
- Rao, V.N. and K. Blane, 1985. PC-STAT, statistical programs for microcomputers. Version 1A. Department of Food Science and Technology, the University of Georgia, Athens, GA, USA.
- Rayner, M. 1998. Vegetables and fruit are good for us so why don't we eat more. *Brit. J. Nutr.*, 80: 119-120.
- Salehifar M., M. S. Ardebili and M.H. Azizi, 2010. Effect of wheat flour protein variations on sensory attributes, texture and staling of Taftoon bread. *Food Science and Technology*, 30(3), 833-837.
- Stroescu, M., A. Stoica-Guzun, S. Ghergu, N. Chira and I. Jipa, 2013. Optimization of fatty acids extraction from *Portulaca oleracea* seed using response surface methodology. *Industrial Crops and Products*, 43: 405-411.

- Tkacova, K. and N. Stevulova, 1998. Selected problems of the dispersity analysis of milled ultrafine powders. *Freiberger Forschungshefte A (Partikeltechnologie)* 841: 14-25.
- Wang, Y. and M.Q. Li, 2011. Effects of ultra-fine grinding on the properties of wheat bran. *Modern Food Sci. Tech.* 27: 271-274.
- Yaseen, A.A., A. Shouk and M.T. Ramadan, 2010. Corn-wheat pan bread quality as affected by hydrocolloids. *Journal of American Science*, 6(10): 684-690.
- Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.*, 49: 5165–5170.
- Zhu, K.X., S. Huang, W. Peng, H.F. Qian and H.M. Zhou, 2010. Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber. *Food Research International* 43(4): 943–948.