

## Natural Antioxidant Changes in Fresh and Dried celery (*Apium graveolens*)

Manal A. Sorour<sup>1</sup>, Naglaa H. M. Hassanen<sup>2</sup>, Mona H. M. Ahmed<sup>2</sup>

<sup>1</sup>Food Engineering and Packaging Dept., Food Technology Research Institute, Agric. Research Center, Giza, Egypt

<sup>2</sup>Special Food and Nutrition Dept., Food Technology Research Institute, Agric. Research Center, Giza, Egypt

### Email address:

manal.sorour@yahoo.com (M. A. Sorour), naglaahassaneen@yahoo.com (N. H. M. Hassanen),

monahanafiahmed@yahoo.com (M. H. M. Ahmed)

### To cite this article:

Manal A. Sorour, Naglaa H. M. Hassanen, Mona H. M. Ahmed. Natural Antioxidant Changes in Fresh and Dried celery (*Apium graveolens*). *American Journal of Energy Engineering*. Special Issue: Energy Conservation in Food Industry. Vol. 3, No. 2-1, 2015, pp. 12-16.

doi: 10.11648/j.ajee.s.2015030201.13

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**Abstract:** The effect of temperature on natural antioxidant changes in fresh and dried celery was studied. Celery herbs were dried at 50 and 90°C using a laboratory scale hot air dryer. Fifteen phenolic components (gallic acid, protocatechuic acid, catechol, chlorogenic acid, syringic acid, caffeine, p-coumaric acid, ferulic acid, salicylic acid, cinnamic acid, chrysin, pyrogallol, ellagic acid, catechin and caffeic acid), five flavonoids components were identified in celery herbs (apigenin, hesperitin, luteolin, quercetin and rosmarinic) and three isoflavones components were identified in celery herbs (daidzein, genistein and isorhamnetin) were identified in celery herbs at 50 and 90°C. The chemical constituents of *apium graveolens* volatile oil were determined, the results observed that eleven components were isolated from *apium graveolens* essential oil and classified into five chemical categories namely, monocyclic terpenes (78.24%), bicyclic terpenes (14.88%), aliphatic hydrocarbons (1.79%), ketones (0.19) and sesquiterpene (2.89%). These identified compounds accounted for 97.99 % of the composition of *apium graveolens* essential oil. Organoleptic evaluation of *Apium graveolens* represented the mean scores and their statistical analysis indication for color, aroma, taste, texture and overall acceptability for biscuit treatments mixed with different concentrations of dried *Apium graveolens* at 50°C and 90°C.

**Keywords:** Drying of Celery, Phenolic Compounds, Flavor, Application of Dried Celery, Flavones

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## 1. Introduction

Air-drying is a traditional, low cost technique that is used to lower the water content of herbs at low temperatures. The drying at low temperatures protects against the degradation of the active constituents, but it is slow and metabolic processes may continue longer, which may lead to quality loss of the aromatic plants and subsequently of the produced added value products [1].

Temperature is one of the most important factors affecting antioxidant activity. Generally heating causes an acceleration of the initiation reactions and hence a decrease in the activity of the present or added antioxidants [2].

Phenolics, in particular, are thought to act as antioxidant, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic and anti-inflammatory, as well as reduce cardiovascular diseases [3].

Flavonoids show a strong antioxidant and radical

scavenging activity and appear to be associated with reduced risk for certain chronic diseases, the prevention of some cardiovascular disorders and certain kinds of cancerous processes. Flavonoids exhibit also antiviral, antimicrobial, and anti-inflammatory activities, beneficial effects on capillary fragility and an ability to inhibit human platelet aggregation, antiulcer and antiallergenic properties [4].

*Apium graveolens* Linn. (Apiaceae) has a long history of use in Ayurveda and Unani system of medicine. *Apium graveolens* L (Apiaceae) grows wild at the base of the north western himalyas and outlying hills in Punjab and in western India. *A. graveolens* has been used as a food, and at various times both the whole plant and the seeds have been consumed as a medicine. Celery seeds or celery seed extracts are used as flavoring agents and also in anti rheumatic formulations as the seeds have significance as arthritic pain relief, for treating rheumatic conditions and gout. Apart from the role in rheumatism, celery seeds proved its use in asthma,

bronchitis and inflammatory conditions [5].

The aim of the work was to study the effect of drying air temperature (50 and 90°C) on the quality of celery herbs (phenolic, flavonoids, isoflavones and flavor of *apium graveolens*), and the evaluation of *Apium graveolens* when added to biscuits with different concentrations.

## 2. Materials and Methods

### 2.1. Materials

Fresh plant celery (*Apium graveolens*) was obtained from Agriculture Research Centre, Giza, Egypt during September, 2013.

### 2.2. Methods

#### 2.2.1. Drying of Celery Herbs

Drying of celery herbs were carried out using a laboratory scale hot-air dryer installed in Department of Food Engineering and Packaging, Agricultural Research Center at 50 and 90°C.

#### 2.2.2. Determination and Identification of Phenolic and Flavonoids Fraction of Celery Herb Using HPLC

Phenolic and flavonoids fraction compounds were identified determined by the method described by Schieber *et al.*, 2001 [6]. A high performance liquid chromatography system equipped with a variable wave length detector (Agilant, Germany) 1100, autosampler, quaternary pump degasser and column compartment. Analyses were performed with a C18 reverse phase packed stainless-steel column (Zorbax ODS 5  $\mu$  m 4.6 x 250 mm) . HPLC method started with linear gradient at a flow rate of 1.0 ml / min with mobile phase of water / acetic acid ( 98:2 v/v, solvent A) and methanol / acetonitril (50 : 50,v/v, solvent B) , starting with 5% B and increasing B to levels of 30% at 25 min,40 % at 35min,52 % at 40 min,70 %at 50 min,100 % at 55 min. The initial conditions were re-established by 5 min wash in both solvents. All chromatograms were plotted at 280 nm to estimated phenolic acids and at 330 nm for flavonoids. All components were identified and quantified by comparison of peak areas with external standards.

#### 2.2.3. Determination and Identification of Isoflavones Fraction of Celery Herb Using HPLC

Isoflavones fraction compounds were determined by the method described by Mantovani *et al.*, 2011 [7]. HPLC Samples of the purified extract had their bulk adjusted with methanol 80%, and filtered in polyethylene filters with PTFE membrane (Millipore Ltd. Bedford, E.U.A.) of 0.45  $\mu$ m pore, before the injection. Isoflavones compounds identification and quantification were carried out through high performance liquid chromatography (Gilson 321), with a secondary pump, deaerator, automatic injector, detector (UV-Visible), and the software program Boriwn version 1.5. The chromatographic conditions described by Song *et al.* (1998) were used C18 coated column Lichrospher, of Merck (250 x 4,6 mm, 5  $\mu$ m) was used, at 30°C; mobile phase was constituted of acetic

acid and methanol (19:1, v v-1) with 1 mL min.<sup>-1</sup> initial flow; detection at UV-Visible 254 nm; and injection volume of 20  $\mu$ L. The calibration curve was prepared using authentic standards in concentration of 0.25 to 0.1 mg mL<sup>-1</sup> diluted in mobile phase.

#### 2.2.4. Extraction of Essential Oils

The essential oil of *apium graveolens* was extracted by water distillation using a (Clevenger-type apparatus) for 4 hours. The obtained volatile oil was dried over anhydrous sodium sulphate and then holds in completely filled a glass bottle at -20°C until use [8].

#### 2.2.5. Separation and Identification of Essential Oils Constituents

The GC/MS technique (HP. 5890A) was used to identify the *apium graveolens* essential oil constituents, under the following conditions: packed capillary column (50m×0.2mm×0.3 thickness film of carbowax 20M), Helium was used as a carrier gas at flow rate 20 cm/sec, HP 7673A automatic injector was used to inject 2.0  $\mu$ L of diluted samples in ethyl alcohol (1:10, v/v) with split ratio 100:1, at 150°C, the oven temperature ( programmed) was set at 60°C for 10min and increasing gradually by the rate of 2.8°C/min to the final temperature (200°C) during 60 min. Mass spectrum was used to identify the constituents by comparing the samples spectrum with the data stored in Chemstation library which containing over 43,000 compounds.

#### 2.2.6. Biscuit Preparation

Biscuits were prepared according to the method reported by Naglaa and Gehad, 2011 [9].

#### 2.3. Organoleptic Evaluation of Biscuit Matricaria Chamomilla

The sensory evaluation of the obtained biscuit were carried out by a panel consisted of 10 panelists who asked to evaluate taste, aroma, color, texture and overall acceptability attributes according to the method reported by Amerine *et al.*, 1965 [10]

#### 2.4. Statistical Analysis

The obtained results were subjected to statistical analysis using the standard analysis of variance as outlined by Snedecor and Cochran, 1980 [11]

## 3. Results and Discussion

### 3.1. Chemical Characteristics of Dried Celery Herbs

The phenolic, flavonoids and isoflavones of *apium graveolens* fractionated, and identified by using HPLC.

### 3.2. Determination of Phenolic Components

The phenolic of celery herb are tabulated in Table (1). The results observed that fifteen phenolic components were identified in celery herbs (gallic acid, protocatechuic acid,

catechol, chlorogenic acid, syringic acid, caffeine, p-coumaric acid, ferulic acid, salicylic acid, cinnamic acid, chrysin, pyrogallol, ellagic acid, catechin and caffeic acid). Meanwhile, celery was dried at 50°C and 90°C caused detectable decrease in the total contents of the eight phenolic in celery. The increment of protocatechuic acid was (99.84, 64.2%), catechol was (150.33, 34.74%), chlorogenic acid was (31.16, 34.96%), salicylic acid was (3.10, 8.21%), chrysin was (313.04, 53.68 %), pyrogallol was (478.97, 40.11%) and ellagic acid was (97.76, 60.72 %) at 50°C and 90°C, respectively.

On the other hand, The increment percentages were 427.73 and 45.56% of gallic acid, 19.70 and 38.76% of syringic acid, 63.15 and 53.87 % of caffeine, 60.86 and 93.52% of p-coumaric acid, 48.80 and 95.93% of ferulic acid, 47.91 and 64% of cinnamic acid, 68.80 and 61.21% of catechin and 68 and 50.37% of caffeic acid for dried celery at 50°C and 90°C, respectively. The obtained data were in harmony with finding of [12-14].

**Table (1).** Phenolic acids of fresh and dried celery herbs using HPLC (ppm)

Phenolic acids	Fresh	Dried at 50°C	Dried at 90°C
Gallic	20.85	110.45	160.78
Protocatechuic	90.50	35.76	12.77
Catechol	7.53	18.85	25.40
Chlorogenic	65.30	85.65	115.60
Syringic	25.22	20.25	12.40
Caffeine	6.65	2.45	1.13
P-Coumaric	17.76	6.95	0.45
Ferulic	16.80	8.60	0.35
Salicylic	3.54	3.65	3.95
Cinnamic	0.48	0.25	0.09
Chrysin	0.23	0.95	1.46
Pyrogallol	75.26	435.74	610.54
Ellagic	235.56	465.85	748.75
Catechin	18.43	5.75	2.23
Caffeic	4.22	1.35	0.67

### 3.3. Determination of Flavonoids Components

Flavonoids constitute the largest group of plant phenols and account for over half of the 8000 naturally occurring phenolic compounds [15].

**Table (2).** Flavonoids of fresh and dried celery herbs using HPLC (ppm)

Components	Fresh	Dried at 50°C	Dried at 90°C
Apigenin	94.74	97.68	104.74
Hesperitin	92.76	136.54	194.65
Luteolin	43.45	76.48	110.26
Quercetrin	110.35	574.82	993.27
Rosmarinic	96.58	43.66	19.82

The flavonoids of dried celery herb are tabulated in Table (2). The results observed that five flavonoids components were identified in celery herbs (apigenin, hesperitin, luteolin, quercetrin and rosmarinic). Celery was dried at 50°C and 90°C caused detectable increase in the total contents of the four flavonoids. The increment percentages were (3.1, 7.22%) for apigenin, (47.19, 42.55%) for hesperitin, (76.01, 44.16%) for luteolin and (420.90, 72.79%) for quercetrin at 50°C and 90°C, respectively. Meanwhile, the decrement percentages

were (54.79, 54.60%) for rosmarinic at 50°C and 90°C, respectively. The obtained data were in harmony with finding of Wach *et al.*, [16].

### 3.4. Determination of Isoflavones Components

The isoflavones of dried celery herb are tabulated in Table (3). The results observed that three isoflavones components were identified (daidzein, genistein and isorhamnetin). Celery was dried at 50°C and 90°C caused detectable increase in the total contents of the two isoflavones. The increment percentages were (136.47, 96.42%) for genistein and (96.94, 82.45%) for isorhamnetin at 50°C and 90°C, respectively. Moreover, the decrement percentages were (37.61, 39.95%) of daidzein at 50°C or 90°C, respectively. The obtained data were in harmony with finding of Manal and Sahar [13]

**Table (3).** Isoflavones of fresh and dried celery herbs using HPLC (ppm)

Components	Fresh	Dried at 50°C	Dried at 90°C
Daidzein	1820.45	1135.72	681.92
Genistein	30.84	72.93	143.25
Isohamnetin	484.32	953.85	1671.36

### 3.5. Chemical Composition of Essential Oil

Essential oils of food products affect their nutritional availability due to their direct responsibility for consumer acceptance or rejection. Thus, the changes occurred in the chemical composition of the essential oil of two collective samples of celery dried at 50°C and 90°C.

The percent of essential oil for fresh celery was 0.72%, the obtained data are in harmony with the findings of Fazal and Singla [5]. The chemical constituents of *apium graveolens* volatile oil are tabulated in Table (4). From these results, it could be indicated that eleven components were isolated from *apium graveolens* essential oil. These components were identified and classified into five chemical categories namely monocyclic terpenes (78.24%), bicyclic terpenes (14.88%), aliphatic hydrocarbons (1.79%), ketones (0.19) and sesquiterpene (2.89%). The percent of these identified compounds were 97.99% of *apium graveolens* essential oil and the remainder portion was 2.01% representing seven unknown constituents. The first chemical group as shown in Table (4) was monocyclic terpenes which consists of two compounds (d-Limonene, 77.65% and  $\gamma$ -terpinene, 0.59%). Limonene was reported as the major constituent of *apium graveolens* essential oil by Misic *et al.*, and Fazal & Singla [5 and 17]. The second recorded chemical group was bicyclic terpenes which consists of four components ( $\alpha$ -pinene, 1.36%, camphene, 0.29%, Sabinene, 1.72% and  $\beta$ -pinene, 11.55%). The third identified chemical group was aliphatic hydrocarbons which consists of one compound ( $\beta$ -myrcene, 1.79%). This compound was reported as constituent of *apium graveolens* essential oil by Misic *et al.*, 2008 [17]. The fourth identified chemical group was ketones which consist of one compound (L-carvone, 0.19%). The five chemical group was sesquiterpene which consists of three compounds ( $\beta$ -caryophyllene, 1.11%,  $\beta$ -selinene, 0.93% and  $\alpha$ -selinene

0.85%).

The results observed that dried celery at 50°C and 90°C caused detectable decrease in the total contents of five chemical groups. The percent of monocyclic terpenes was decreased to (5.77, 11.37%), (27.75, 41.66%) for bi-cyclic terpenes, (20.67, 50.27%) for aliphatic hydrocarbons, (42.10, 63.15%) for ketones and (19.03, 41.17%) for sesquiterpene at 50°C and 90°C, respectively. The obtained data were in harmony with finding of Okoh *et al.*, 2008 [18]

**Table (4).** Chemical components of celery (*apium graveolens*) essential oils fractionated and identified by GC/Mass technique.

Chemical compounds	Area%		
	fresh	50°C	90°C
1-Monocyclic terpenes:			
d-Limonene	77.65	73.34	69.13
γ-terpinene	0.59	0.38	0.21
Total :	78.24	73.72	69.34
2-Bi cyclic terpenes:			
α-pinene	1.36	0.97	0.75
Camphene	0.29	0.15	0.08
Sabinen	1.72	1.45	1.22
β-pinene	11.51	8.18	6.63
Total :	14.88	10.75	8.68
3-Aliphatic hydrocarbons:			
β-Myrcene	1.79	1.42	0.89
Total :	1.79	1.42	0.89
4- Ketones:			
L-Carvone	0.19	0.11	0.07
Total:	0.19	0.11	0.07
5-Sesquiterpene:			
β-Caryophyllen	1.11	0.90	0.77
β-selinene	0.93	0.78	0.54
α- selinene	0.85	0.66	0.39
Total:	2.89	2.34	1.70
6-Unknown:	2.01	11.66	19.32

### 3.6. Organoleptic Evaluation of Celery (*apium graveolens*)

Table (5) represents the mean scores and their statistical analysis indication for color, aroma, taste, texture and overall acceptability for biscuit treatments mixed with different concentrations of dried celery (*apium graveolens*) herbs at 50°C and 90°C. The data in Table (5) indicated that there are no significant differences in the quality attributes i.e., texture of biscuit which manufactured with or without (control) addition of different levels of *apium graveolens*. According to the results, the mean score of aroma, taste and overall acceptability of biscuit samples increased as the concentration of *apium graveolens* increased from 0.1 to 0.3%. Therefore, high significant differences were found among these samples and their control sample. However, no significant differences were found in the acceptability among all biscuit samples incorporated different concentrations of *apium graveolens* dried at 90°C (0.1, 0.2 and 0.3%). On the other hand, the biscuit samples formulated with 0.1% , 0.2% and 0.3% of *apium graveolens* dried at 50°C had higher aroma and taste scores in comparison with biscuit samples of the control and celery dried at 90°C. The obtained data are in harmony with the findings of Lawiess and Naglaa & Gehad, [13 and 19]

**Table (5).** The mean scores of organoleptic evaluation of biscuits manufactured by addition of dried celery *apium graveolens* herbs at 50°C and 90°C.

Treatments	Color	Aroma	Taste	Texture	Overall quality	Accept ability
Control	10.0 <sup>a</sup>	7.0 <sup>d</sup>	7.0 <sup>d</sup>	9.0 <sup>a</sup>	8.0 <sup>e</sup>	+
Dried celery at 50°C						
<i>Apium graveolens</i> (0.1%)	9.0 <sup>b</sup>	9.0 <sup>b</sup>	9.0 <sup>b</sup>	8.0 <sup>b</sup>	9.00 <sup>b</sup>	+++
<i>Apium graveolens</i> (0.2%)	9.0 <sup>b</sup>	10.0 <sup>a</sup>	10.0 <sup>a</sup>	8.0 <sup>b</sup>	9.50 <sup>a</sup>	++++
<i>Apium graveolens</i> (0.3%)	8.0 <sup>c</sup>	10.0 <sup>a</sup>	10.0 <sup>a</sup>	8.0 <sup>b</sup>	9.50 <sup>a</sup>	++++
Dried celery at 90°C						
<i>Apium graveolens</i> (0.1%)	8.0 <sup>c</sup>	8.0 <sup>c</sup>	8.0 <sup>c</sup>	8.0 <sup>b</sup>	8.0 <sup>c</sup>	++
<i>Apium graveolens</i> (0.2%)	7.0 <sup>d</sup>	9.0 <sup>b</sup>	9.0 <sup>b</sup>	8.0 <sup>b</sup>	8.5 <sup>cd</sup>	++
<i>Apium graveolens</i> (0.3%)	7.0 <sup>d</sup>	9.0 <sup>b</sup>	9.0 <sup>b</sup>	8.0 <sup>b</sup>	8.5 <sup>cd</sup>	++
LSD	0.01779	0.02516	0.0251	0.00562	0.03081	

(+) The sample is acceptable.

(+++ ) The sample is very good acceptable.

(++) The sample is good acceptable.

(++++ ) The sample is excellent acceptable.

\*Any two means, at the same column , have the same letter did not significantly different at 5% or 1% level of probability.

## 5. Conclusion

Celery herbs were dried at 50 and 90°C using a laboratory scale hot air dryer and the phenolic, flavonoids and isoflavones of *apium graveolens* fractionated, and identified by using HPLC. Drying of celery herbs at 50°C and 90°C caused detectable decrease in the total contents of the eight phenolic acids (protocatechuic, catechol, chlorogenic, salicylic, chrysin, pyrogallol and ellagic acid), while it caused an increment in the total content of gallic, syringic, caffeine, p-coumaric, ferulic, cinnamic, catechin and caffeic. Five flavonoids components were identified in celery herbs (apigenin, hesperitin, luteolin, quercetrin and rosmarinic) and three isoflavones components were identified (daidzein, genistein and isorhamnetin) in dried celery herbs. The chemical constituents of *apium graveolens* volatile oil were determined, the results observed that eleven components were isolated from *apium graveolens* essential oil and classified into five chemical categories namely, monocyclic terpenes, bicyclic terpenes, aliphatic hydrocarbons, ketones and sesquiterpene. Organoleptic evaluation of *Apium graveolens* represented the mean scores and their statistical analysis indication for color, aroma, taste, texture and overall acceptability for biscuit treatments mixed with different concentrations of dried *Apium graveolens* at 50°C and 90°C.

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