Production of Drinking Milk Made with Anise Seed Extract

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ABSTRACT

Aqueous extract of anise seeds *(Pimpinella anisum L.*) was added to drinking milk at a rate of 10, 20, 30 % (v/v). The antimicrobial, antioxidation and sensory evaluation of drinking milk samples have been evaluated. Results revealed that addition of 10, 20, 30 ml aqueous extract of anise seeds caused 104.13, 112.77, 120.42% increase in total phenolics content in drinking milk, respectively. The microbial growth was obstructed. It could be concluded that aqueous extract of anise seeds can be used up till 30 % (v/v) in the preparation of drinking milk with increased health benefits and acceptable sensory attributes.

key words: Milk – Anise – Antioxidant activity – Antimicrobial activity.

INTRODUCTION

Anise or aniseed (*Pimpinella anisum* L., family *Apiaceae*) was used as whole, cracked or ground, fresh leaves, dry seeds and essential oil are full of aroma like the seeds (Charles, 2013). Anise is used widely as flavoring agent in food products such as bread, cookies, cakes, pretzels, fruit salads, juice drinks, jams, meat products, fish products and sweet rolls (Ağaoğlu *et al.*, 2007; Charles, 2013; Vasavada *et al.*, 2006).

Anise leaves are used to treat gastrointestinal problems and tooth pain. Its oil is used to treat lice and scabies. It is also used to treat colds and mouth fresheners. Anise seeds are used as food flavors in different regions of the world and are used by Europeans for the flavors of cakes, pastries and fruit salads. In France and Germany, they are added to cakes, apple sauce, sausages, fish and meat.

Anise is often used to aid digestion, improve appetite and decrease cramp and colic in infants. It is also a mild expectorant used to ease coughing and is used in lozenges and cough syrups. It is also used to promote lactation and decrease catarrh, often used in bronchitis. In India and Europe, it is chewed to freshen breath, but can also be used to induce sleep. If few seeds are taken with water, it will cure hiccups. Anise powder and aqueous extract are used as carminatives, antiseptics, diuretics, digestives, aphrodisiacs, and as a remedy for insomnia and constipation (Kreydiyyeh *et al.*, 2003). Besharati-Seidani *et al.* (2005) reported that anise has digestive, carminative, diuretic, and expectorant actions. Anise was shown to have antimicrobial properties (Robles-Zepeda *et al.*, 2011). The essential oil of anise showed strong nematocidal activity against *Meloidogyne incognita* (Ntalli *et al.*, 2011).

Mofleh *et al.* (2007) found that aqueous suspension of anise seed protects rats against chemically induced gastric ulcers. Anise significantly inhibited gastric mucosal damage induced by necrotizing agents and indomethacin. In pylorus-ligated Shay rats, anise suspension was used to significantly reduce the basal gastric acid secretion, acidity and completely inhibit the ulceration. They concluded that this antiulcer effect of anise was possibly prostaglandin mediated and/or through its antisecretory and antioxidative properties. The essential oils of anise were reported to have strong antioxidants activity (Topal *et al.*, 2008).

Therefore, the objective of this investigation was to produce drinking milk with added aqueous extract of anise seeds as a natural, health beneficial flavoring material. In addition, study the antimicrobial and antioxidative effect and the sensorial characteristics of anise-drinking milk.

MATERIALS AND METHODS

Materials

- Cow milk was obtained from herd of Damanhour Agricultural Secondary School.
- Anise seeds were obtained from the Taiba general supplies – herbs medicinal and plants Fayoum Governorate, Egypt.
- Butylated hydroxy toluene (BHT), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and solvents used for spectral and HPLC analyses were of HPLC grade and were obtained from Sigma Chemical Company, USA.
- Plate count agar, MaCconkey broth and potato dextrose agar (PDA) media were obtained from Oxoid Ltd., Basingstoke, Hampshire, England.

Methods

Preparation of anise aqueous extract

About 500g of anise seeds were washed carefully under running water, followed by distilled water, oven-dried at 55-60?C for 48 hr., and stored in air-tight plastic containers at room temperature in dark place till used. Approximately, 100 g of seeds were soaked with 500 mL distilled water for 24 hr. The extract was filtered, and the total volume was adjusted to 500 mL

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and then centrifuged at 4000 rpm for 15 min. the supernatant was collected and stored at -20°C.

Analysis of anise aqueous extract was 0.99% total solids, 0.2% protein, 0.27% ash and 581.1 total phenolics (mg/100 ml gallic acid equivalent).

Preparation of the mixtures of milk and anise extract

Milk was thermally treated at 90°C for 10 min. and then cooled to room temperature. The mixtures of milk and anise were prepared as follows: (1) control; milk without addition of anise extract, (2) 10 ml of anise extract + 90 ml milk, (3) 20 ml anise extract + 80 ml of milk, (4) 30 ml anise extract + 70 ml of milk, (5) only anise extract. Samples were kept for 15 days at refrigerator temperature (4±1?C) while they were analyzed every 5 days.

Chemical analysis

The samples were analyzed for total solids, protein, fat and lactose as described by Ling (1963). The pH values were measured using pH meter type 3320 Jenway LTD. (Felsted Danmow Essex CM63 IB, UK) and the titratable Acidity were measured according to A.O.A.C. (2000). Total Carbohydrate content was calculated by subtracting the total contents of moisture, crude protein, crude fat and crude ash from 100.

Determination of total phenolic content (TPC)

Total phenolic content (TPC) was determined according to Jayaprakasha *et al.* (2001) by using Folin-Ciocalteu reagent. 0.5 ml of sample was mixed with 0.5 ml of 10-fold-diluted Folin–Ciocalteu reagent. After 3 min, 4 ml of 7.5% sodium carbonate was added. The mixture was kept for 30 min in dark place at room temperature before the absorbance was measured at 765 nm using a spectrophotometer (model 2010, Cecil Instr. Ltd., Cambridge, UK). The results were expressed as milligrams of gallic acid equivalent per gram of dry weight (DW).

DPPH radical scavenging activity

Samples of anise-drinking milk were analyzed to determine its antioxidant activity. Free radical scavenging activity (RSA) of the samples were measured using the method of Brand-Williams *et al*., (1995). An aliquot 100µl of the sample solution was mixed with 2.9 ml of 1,1-diphenyl-2- pycrylhydrazyl (DPPH) in methanol. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a UV-visible spectrophotometer Specronic 20 D (Bauch and lamb). The antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = [1 - (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}})] \times 100.$$

All analyses were carried out in triplicates.

Microbiological Properties

Microbiological Analysis was carried out by standard plate count method. Samples were serially diluted by sterile peptone water, and 1mL aliquots of dilutions were used.

Total bacterial count was determined on plate count agar medium. From appropriate dilutions, 1 ml each was plated in duplicate using pour plate method. Plate count agar medium was used for the determination of total viable bacteria (Houghtby *et al.,* 1992). The plates for total viable bacteria count were incubated at 32?C for 48 hr. (Christen *et al.,* 1992)

Total coliforms and detection of *E. coli*, the most probable number (MPN) technique was used. One milliliter from each dilution was used to inoculate three series of three test tubes containing MaCconkey broth medium with Durham's tube (APHA, 1992). The tubes were incubated at 44?C for 24: 48 hr). The production of acid (yellow color) and gas (appear in Durham tube) from lactose indicate *E. coli* positive. A loop full of the positive tubes was cultured on eosin methylene blue (EMB) reference agar and incubated at 37?C for 24 hr (El-Hadedy and Abu El-Nour, 2012). Yeasts and molds were enumerated on Potato dextrose agar (PDA) followed by incubation at 25?C for 5 days (Frank *et al.,* 1992).

Sensory evaluation

The sensory evaluation of products was carried out 1, 5,10 and 15 days after treatment. Fifteen untrained consumers (made up of 6 females and 9 males, aged between 15 and 45 years old), were asked to describe the sensory attributes of anise milk drink. Color, odor, taste and overall acceptability of each milk drinks were evaluated using a hedonic scale from 9 to 1 (9 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely). All samples were presented to the assessors at room temperature under normal lighting conditions in transparent glass cups coded with random, three-digit numbers (Stone and Sidel, 1993). The sensorial test was conducted on eight sessions, in which the panelists evaluated 4 samples at a time, working in individual booths and drinking water for oral rinsing. The average value scores of all sensory evaluations were used in the analysis.

Statistical analysis

Statistical analysis was performed according to SAS Institute (2017) using General Linear Model (GLM) with the main effect of addition ratios. Duncan's multiple range was used to separate among of three replicates at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of anise-drinking milk

Chemical composition of anise-drinking milk was shown in table 1.

Anise extract contained 0.99% total solids, 0.2% protein, 0.27% ash, and 581.1(mg/100 ml gallic acid equivalent) total phenolics.

Addition of 10, 20, and 30% aqueous extract of anise seeds to milk led to significant decrease in total solids (TS), protein fat, ash, and lactose contents of comparing with the control. Percentages of decrement were 9.10, 17.55, and 29.10 % in total solids; due to the addition of 10, 20, and 30% anise extract respectively. The protein content decrement was 9.52, 15.87, and 28.57%; fat content was 3.57, 19.64 and 28.57%; ash content was 8.45, 9.85 and 12.67%, total carbohydrates were 8.52, 21.30 and 34.08% respectively.

pH and titratable acidity

Data of pH and titratable acidity of anise-milk are shown in Table (2). Neither acidity nor pH values were changed significantly in all fresh samples, while some changes have been occurred during storage. Storage of anise-milk up to 15 days at 4°C led to slight insignificant increase in acidity after 5 days, while after 10 days of storage, the increment was significant in the samples contained 10 and 20 % anise extract. By the end of storage period, titratable acidity of all samples was increased significantly. On the other hand, pH values were moderately decreased. The lowest pH value after 15 days of storage was 6.12.

Total phenolic compounds and antioxidant activity

Data presented in Table 3 showed that drinking milk with anise seeds extract had the higher values of total phenolic compounds and antioxidant scavenging activity compared to control. In addition, total phenolic compounds in anise drinking milk were increased with the increasing ratio of anise extract. These variations could be attributed to the high antioxidative capacity of anise (581.1 mg/g DW). It can be noticed that addition of anise extract to milk was accompanied by high levels of total phenolic compounds. Therefore, anise-drinking milk could be considered as a useful source of antioxidative phenolic compounds. Antioxidative activity increased from 7.07% for control to 16.83, 22.3, 24.51% for T1, T2 and T3, respectively.

The antioxidative activity of herbal plants is mainly associated with the presence of phenolic compounds (Kim *et al.,* 1997).

In a study by Gulcin *et al.* (2003), the antioxidative properties of water and ethanolic extracts of anise seeds were evaluated using different antioxidant tests, and antioxidative activities were compared with synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol. Both extracts of aniseeds showed strong antioxidative activity, reducing power, DPPH radical and superoxide anion scavenging, hydrogen peroxide scavenging, and metal chelating activities compared to BHA, BHT, and α-tocopherol. Water extract exhibited greater antioxidative capacity than ethanolic extract.

Table1. Chemical composition (g/100 ml) of control and milk-anise drinks

treatment	Moisture	Total solids	Protein	Fat	Ash	Total Carbohydrate
Control	89.35 ^{b}	10.65^{a}	3.15 ^{a}	2.8 ^{a}	0.71 ^{a}	3.99 ^{a}
T1	89.95^{b}	9.68^{b}	2.85^{b}	2.7^{a}	0.65^{b}	3.7 ^{b}
T2	91.22^{a}	8.78^{c}	2.65^{c}	2.25^{b}	0.64^{b}	3.24 ^{c}
T3	92.45^{a}	7.55^{d}	2.25^{d}	2.0^{c}	0.62^{b}	2.78 ^{d}

T1 = 10 ml of anise extract + 90 ml milk,T2= 20 ml of anise extract + 80 ml of milk, T3=30 ml of anise extract + 70 ml milk. Values with different letters in the same column are significant differed at p< 0.05.

Table 2. pH and titratable acidity of control and milk-anise drinks during refrigerated storage at ±4 for 15 days

Treatment	pH				TA			
	Storage period (day)				Storage period			
	Fresh	5	10	15	Fresh	5	10	15
Control	6.37 ^{a}	6.07^{b}	5.98^{b}	5.59^{c}	0.18^{c}	0.19b^{c}	0.21^{ab}	0.23^{a}
T1	6.59^{a}	6.52^{b}	6.45^{c}	6.12^{d}	0.13^{b}	0.14^{b}	0.18^{b}	0.21^{a}
T2	6.62^{a}	6.57^{a}	6.48^{b}	6.15^{c}	0.13^{b}	0.14^{b}	0.17^{ab}	0.20^{a}
T3	6.62^{a}	6.68^{a}	6.53^{b}	6.19^{c}	0.13^{b}	0.14^{b}	0.13^{b}	0.19^{a}
Anise	6.15	6.16	6.16	6.17	0.075^{a}	0.074^{a}	0.073^{a}	0.073^{a}

T1 = 10 ml of anise extract + 90 ml milk,T2= 20 ml of anise extract + 80 ml of milk, T3=30 ml of anise extract + 70 ml milk. Values with different letters in the same row are significant differed at p< 0.05.

Table 3. antioxidant activity (%) and total phenolic compounds (TPC) in milk and anise-milk drinks

Treatment	Antioxidant activity (%)	TPC (mg/g DW)
Control	7.07^{e}	147.65^{e}
T1	12.97^{d}	191.05^{d}
T2	19.58^{c}	249.21^{c}
T3	26.19^{b}	307.37^{b}
Anise	66.12^{a}	581.10^{a}

T1 = 10 ml of anise extract + 90 ml milk,T2= 20 ml of anise extract + 80 ml of milk, T3=30 ml of anise extract + 70 ml milk. DW= Dry weight. Values with different letters in the same column are significant differed at $p< 0.05$.

Microbiological analysis

Table 4 showed that the total bacterial count and yeasts count of drinking milk was affected by the addition of anise extract as well as prolonging of the storage period at refrigerator temperature for 15 days. For fresh and stored products, samples with anise extract had significantly lowered total bacterial count and yeast counts than control sample, which may reflect the inhibitory effect of aqueous anise extract on the bacteria and yeast.

After 15 days of storage at the refrigerator temperature, there is an increase in total bacterial count in all samples, and there was an inverse relationship between bacterial count and the added level of anise extract. Yeasts were not detected in samples produced with 30 % of anise extract. In addition, molds and coliform group including *E. coli* were not detected in all samples until the end of the storage period. This may be due to the high hygienic conditions during processing, and the low storage temperature that inhibited their growth.

Gulcin *et al.* (2003), found that the ethanolic extract of aniseed showed significant inhibitory activity against all tested bacteria but not effective on *Candida albicans*, while, the antimicrobial effect of water extract was not detected against Gram-negative bacteria, *Pseudomonas aeruginosa*, and *Escherichia coli*, but it was effective against *Candida albicans*. Most micro-organisms were inhibited.

Sensory evaluation

Results of sensory properties of fresh and storage drinking milk revealed that addition of anise as an aqueous extract had significant effects on color, taste, odor and overall acceptability Table 5. As the added extract was increased, the score of color, taste, odor and overall acceptability was increased. In general, all drinking milk made with different levels of anise extract had acceptable color, taste, odor and overall acceptability. It could be concluded that the addition of anise extract did not negatively affect the natural color of milk, at the same time taste and odor were favorable, and consequently the product gained high score for overall acceptability.

CONCLUSION

Production of drinking milk with 10, 20, and 30% of anise seeds extract decreased slightly its total solids, protein, fat, ash, and lactose contents but significantly increased its total phenolic compounds and antioxidative activity. Addition of aqueous extract of anise seeds to milk up to 30% did not alter the color, while it imparted a favorable taste and odor and gained high score for the overall acceptability.

Table 4. Microbiological analysis of control and anise-milk drinks during refrigerated storage at ±4 for 15 days

treatnemt	Control		T1		T2		T3		Anise	
Test	Fresh	After 15 days	Fresh	After 15 days	fresh	After 15 days	Fresh	After 15 days	Fresh	At 15 days
Total Bacterial Count (c.f.u./ml)	$30*10^{4}$	$30*10^{6}$	$30 * 10^{2}$	70*10	$12*10^{2}$	$48*10^{2}$	$6*10^{2}$	$19*10^{2}$	8*10	$12*10^{2}$
Yeasts and Molds Count (c.f.u./ml)	32*10	32*10	50	40	30	40	40	30	n.d	n.d
Coliform group Count (c.f.u./ml)	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
E. coli	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

T1 = 10 ml of anise extract + 90 ml milk,T2= 20 ml of anise extract + 80 ml of milk, T3=30 ml of anise extract + 70 ml milk. CFU= Colony forming unit, n.d= not detected.

Table 5. Results of sensory evaluation of anise-milk drink

Storage Period (days)	Attributes	control	T1	T2	T3
Fresh	Color	8.3±0.16^{d}	8.4±0.11^{c}	8.5±0.1^{b}	8.6±0.15^{a}
	Taste	7.9±0.14^{c}	8.2±0.19^{b}	8.4±0.13^{a}	7.8±0.11^{c}
	Odor	7.3±0.13^{b}	7.9±0.22^{a}	8.0±0.13^{a}	8.0±0.17^{a}
	overall acceptability	7.9±0.09^{d}	8.2±0.12^{b}	8.3±0.24^{a}	8.1±0.14^{c}
5	Color	8.1±0.27^{b}	8.1±0.21^{b}	8.2±0.19^{a}	8.2±0.15^{a}
	Taste	7.5±0.13^{b}	8.0±0.24^{a}	8.1±0.15^{a}	7.6±0.14^{b}
	Odor	7.1±0.22^{b}	7.7±0.17^{a}	7.8±0.11^{a}	7.8±0.09^{a}
	Overall acceptability	7.6±0.19^{c}	7.9±0.14^{ab}	8.1±0.19^{a}	7.8±0.11^{b}
10	Color	8.0±0.17^{b}	8.0±0.32^{b}	8.2±0.09^{a}	7.9±0.14^{c}
	Taste	7.1±0.12^{b}	7.8±0.38^{a}	7.8±0.09^{a}	7.2±0.21^{b}
	Odor	6.9±0.27^{b}	7.4±0.24^{a}	7.5±0.05^{a}	7.5±0.19^{a}
	Overall acceptability	7.3±0.36^{c}	7.7±0.23^{b}	8.0±0.28^{a}	7.6±0.22^{b}
15	Color	7.7±0.14^{b}	7.8±0.23^{b}	8.0±0.29^{a}	7.7±0.35^{b}
	Taste	6.8±0.23^{c}	7.5±0.11^{a}	7.5±0.13^{a}	7.0±0.23^{b}
	Odor	6.6±0.11^{b}	7.1±0.17^{a}	7.2±0.09^{a}	7.1±0.11^{a}
	Overall acceptability	7.0±0.20^{d}	7.5±0.12^{b}	7.8±0.31^{a}	7.3±0.23^{c}

T1 = 10 ml of anise extract + 90 ml milk,T2= 20 ml of anise extract + 80 ml of milk, T3=30 ml of anise extract + 70 ml milk. Values (Mean ± standard deviation). Values with different letters in the same row are significant differed at $p< 0.05$.

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الملخص العربي

إنتاج مشروب اللبن المضاف إليه مستخلص اليانسون

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يستخدم اليانسون على نطاق واسع كمادة منكهة في كثير من الأغذية. في هذه الدراسة تم تقييم التركيب الكيماوي والنشاط المضاد للميكروبات والفينولات الكلية ونشاط تضاد الأكسدة والتقييم الحسي للبن المصنع بإضافة المستخلص المائي لليانسون بنسب ١٠، ٢٠، ٣٠ %. وقد بلغت الفينولات الكلية في اليانسون ٥٨١,١ مجم/جم (وزن جاف). وقد أظهرت النتائج أنَّ إضافة مستخلص اليانسون قد نتج عنه زيادة في المحتوى من المركبات الفينولية المضادة للأكسدة. كما بيّنت النتائج أن اليانسون جيّد في نشاط تضاد الأكسدة، وفي إعاقة نمو الميكروبات في المشروب الناتج. كما بيّنت نتائج التقييم الحسي للمنتجات أنه يمكن استخدام اليانسون حتى ٣٠مل / ١٠٠مل مشروب لإعطاء ناتج ذي تقبُّل حسي مميز.

الكلمات المفتاحية: اللبن، اليانسون، تضاد الأكسدة، تضاد الميكروبات.