EFFECT OF DRYING CONDITIONS AND EXTRACTION SOLVENTS ON TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT PROPERTIES OF MANGO SEED KERNEL EXTRACTS

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ABSTRACT

Mango seeds are a by-product of fruit factories that have low economic value. But, its high phenolic content makes it to be important source of natural antioxidants. The effect of drying methods (sun-drying and oven-drying at 50 °C) and extraction solvents (chloroform / methanol mixture (2:1, v/v), 80% ethanol and water) on total phenolics and antioxidant properties of mango seed kernel extracts (MSKE) were studied. Results showed that the combination of oven-drying and chloroform / methanol mixture (2:1, v/v) extraction was the most effective recovery method in relation to the yield of gained dried matter (15.17%), and quantity of extracted total phenolics per gram of extractable dry matter (102.5 mg). Antioxidant properties expressed as antioxidant index calculated from the ratio of induction period (time taken in hours to reach a peroxide value of 5 during storage at 80±2°C) of buffalo ghee sample treated with 5% (w/v) of MSKE concentrated liquid obtained from using oven-dried mango seed kernel and chloroform / methanol mixture extraction to induction period of control sample was 4.52, compared to 3.23 when 0.02% BHT was used. Using HPLC, nine phenolic compounds identified in oven-dried mange seed kernel extract resulted from chloroform / methanol mixture extraction method were qurecetin, catechin, vanillin, coumarin, tannin, rutin and cinammic, ferulic, and gallic, acids. It could be concluded that mange seed kernel powder can serve as potential source of natural antioxidants for application in food products due to their marked antioxidant properties.

Keywords: Mango seed kernel extract; phenolic compounds; antioxidant properties; buffalo ghee; synthetic antioxidant; butylated hydroxyl toluene (BHT).

INTRODUCTION

The importance of antioxidants for food applications has been underlined by numerous works. Synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and t- butyl hydroquinone (TBHQ) are widely used because they are effective and cheaper than natural types. However, the safety and toxicity of synthetic antioxidants are of important concerns (Imaida et al., 1983). Much attention has been focused on the use of natural antioxidants, to inhibit lipid peroxidation or to protect the human body from the oxidative damage by free radicals. Recently, a multitude of natural antioxidants have already been isolated from agricultural wastes such as solid wastes generated from the processing of vegetables and fruits, which cause serious environmental problems, such as water pollution, unpleasant odors and explosions and combustion (Zamorano et al., 2007). For instance, there are several million tons of mango seed wastes produced annually from the factories in Egypt.

These wastes must convert into value- added applications. One of these applications is to reuse of mango seed wastes as a low- cost source of natural antioxidants (Abdalla et al. 2007a).

Several studies have shown that mango seed kernels contain various phenolic compounds. Gallotannins and condensed tannin- related polyphenols were reported to be present in mango kernels (Arogba, 1997). In addition, polyphenols from dry mango kernel meal were found to contain tannic acid, gallic acid, and epicatechin in the ratio 17:10:1, respectively (Arogba, 2000). Abdalla et al. (2007a) have recently characterized the phenolic compounds in Egyptian mango seed kernels. The components included tannins, gallic acid, coumarin, ellagic acid, vanillin, mangiferin, ferulic acid, cinammic acid and unknown compounds.

The properties (e.g. antioxidant activity) of extracts from plants were found to be depent on the extraction solvents used (Durling et al., 2007; Lim and Murtijaya, 2007 and Spigno and De Faveri, 2007). Water, methanol, ethanol are commonly used to extract phyto-chemicals from plants due to the absence of toxicity. Since drying of mango seed kernels in the factory either by sun-drying or hot air oven-drying could reduce storage volume and extend their shelf life. Therefore, the objective of this study was to evaluate the effects of drying methods and extraction solvents on the total phenolics and antioxidant properties of mango seed kernel extracts to obtain a natural antioxidant that could be a synthetic antioxidant replacer in ghee preservation.

MATERIALS AND METHODS

Butter samples prepared from fresh raw cream obtained from fresh raw buffalo milk were heat clarified to ghee until the temperature reached 115°C. Five kilograms of mango (*Mangifera indica* L - Alphonso) seeds as waste were collected from private local fruit juice processing units at Giza, Egypt.

All chemicals used in this study were purchased from El-Gomhouria Co. for chemicals and medical requisites (Al-ameria, Cairo, Egypt), while butylated hydroxy toluene (BHT) was procured from Sigma (St. Louis, MO, USA).

The seeds were washed and peeled. The kernels were divided into two portions, the first was sun- dried in a green house for three days, and the second was dried in a hot air- oven at 50°C until a constant weight was attained (approximately six hrs in case of the last method). The dried kernels were chopped and ground in a stainless- steel grinder into fine powder, passed through a 60- mesh sieve and kept in closed dark glass bottles at 4°C until utilization.

Two solvents, 80% ethanol and chloroform / methanol mixture (2:1, v/v) were separately used to extract phenolics from mango seed kernels. 100 milliliters of each solvent were added to a flask containing 50 g of sun-dried or oven-dried material. Each flask was wrapped by aluminum foil to prevent light degradation during extraction. The flasks were shaken overnight at room temperature. Another 50 grams of sun-dried or oven-dried material were

stirred with deionized water (100 ml) at room temperature for 15 min as a third solvent. All mixtures were filtered through Whatman filter paper No 41 to obtain crude extracts according to the method of Puravankara *et al.*, (2000). Each solvent's crude extract filtrate was mixed with an equal volume of both distilled water and chloroform separately, and then the aqueous and organic phases were separated using a separation funnel.

Each aqueous layer containing all hydrophilic compounds obtained from all of the examined solvents was mixed with an equal volume of ethyl acetate. This step was repeated three times. After partitioning and phase separation, the top layers (ethyl acetate layers) were collected together and vacuum evaporated in a rotary flask evaporator to about 20 ml as described by Puravankara et al., (2000). All concentrated extracts were kept in a closed dark glass bottles at 4°C until utilization.

The mango seed kernel powder (MSKP) was analyzed for moisture, protein, fat, fiber and ash following the methods specified by AOAC (1990), and the carbohydrate content was calculated by difference. The total phenolic content of mango seed kernel extracts (MSKE) was determined using Folin-Ciocalteu's phenol reagent as shown by Slinkard and Singleton (1977). Briefly, 20 μ l of concentrated extract were mixed thoroughly with 1.16 ml distilled water and 100 μ l of Folin-Ciocalteu phenol reagent. After mixing for 3 min, 300 μ l of Na₂CO₃ solution (20% w/v) was added. The mixture was incubated in a shaking incubator at 40°C for 30 min, thereafter its optical density (O.D.) was measured at 765 nm. Gallic acid was used as a standard. The phenolic content was expressed as gallic acid equivalent using the linear equation based on the following standard curve as shown in Fig (1).

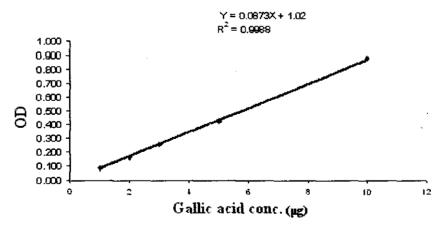


Fig. (1): Gallic acid standard curve

The phenolic compounds of the MSKE containing the highest total phenolic content were identified using high performance liquid chromatography (HPLC) according to the method described by Anderson and Pederson (1983). HPLC (Hewlett Packard Series HP 2100, UK) consisting of a model P 4600 pump with a Waters R401 detector, a U6K injector, and a Waters Bondapak C-18 column (30 cm × 4 mm) was used.

Sun- and oven- dried MSKEs were added separately to 200 g of freshly prepared buffalo ghee samples at levels of 0.5, 1, 3 and 5% (w/v). For comparison, the permitted synthetic antioxidant butylated hydroxy toluene (BHT) was added at 0.02% (w/v). The buffalo ghee samples with and without additives (control) were packed separately into screw capped 250 ml glass jars, covered externally with aluminum foil, and subjected to accelerated oxidation in an oven maintained at 80±2°C. Antioxidant properties of these samples were evaluated by monitoring their peroxide development at intervals of 12 hrs to record the time taken in hours to reach a peroxide value of 5, since, the ghee samples were presumed to be deteriorated after a peroxide value of 5 at 80°C (Parmar and Sharma, 1990).

Peroxide value (meq.O₂ / kg ghee) was determined according to AOCS (1989). To test the effectiveness of the additives, antioxidant indices were calculated according to Pruthi *et al.*, (1970). All tests were carried out in triplicate and average of results was presented.

The obtained data were statistically analyzed using 2×3 factorial design. Duncan's test was used to make the multiple comparisons (Steel and Torri, 1980). Significant differences were determined at P < 0.05.

RESULTS AND DISCUSSION

The major composition of mango seed kernel powder(MSKP) presented in Table (1) indicated that the dry matter content of oven-dried mango seed kernel powder samples (ODMSK) (96.24%) was higher than that of sun-dried mango seed kernel powder samples (SDMSK) (93.89%). This was due to the use of steady and continuous heating in an oven during drying of mango seed kernels, which caused loss of accessible of their moisture. Consequently, ODMSK had higher solid compounds such as protein, fat, fiber, ash and carbohydrates (6.3, 11.1, 2.5, 2.1 and 78% on a dry weight basis, respectively). These results were in agreement with the data obtained by Zein et al. (2005), and Abdalla et al. (2007a).

Table (1): Effect of drying conditions of mango seed kernels on their chemical composition¹.

Samples	Chemical composition							
	Dry matter %	Protein %	Fat %	Fiber %	Ash %	Carbohydrates %		
ODMSK ²	96.24	6.30	11.1	2.5	2.1	78		
SDMSK ³	93.89	4.57	9.9	1.4	1.8	82.33		
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Dry weight basis

*Oven dried

Sun dried

The yield and total phenolics of MSKP varied significantly, according to the drying conditions and solvent type (Tables 2&3). The amount of extractable components expressed as percentage by weight of dry matter of MSKP ranged from 4.64% (sun drying + water extraction) to 15.17% (oven drying + Ch/MtOH extraction) (Table 2).

Table (2): Effects of drying conditions and extraction solvents on yield of extracted components of mango seed kernel powder

Danimar the and	Yield of	T		
Drying method	E	Mean		
<u> </u>	Chl/MtOH	80% Ethanol	Water	
Sun-drying	8.75° ± 0.16	12.84 ^b ± 0.08	$4.64^{1} \pm 0.20$	8.74 ± 1.19
Oven-drying	$15.17^{a} \pm 0.03$	$10.32^{\circ} \pm 0.13$	6.11 ^e ± 0.03	10.53 ± 1.31
Mean	11.96 ± 1.57	11.58 ± 1.04	5.38 ± 1.33	

^{*}Grams of extracted components per 100 g of dry matter in MSKP, ±SE.

Superscripts a,b,c,d,e,f: means that the same letter among the treatments are not significantly different.

Drying method = Significant.

LSD value at 0.05 for Extraction solvents = 0.28

LSD value at 0.05 for drying method × extraction solvents = 0.4

The content of phenolic compounds (expressed as gallic acid equivalents) of MSKEs as affected by drying method and type of solvent used was in the order: ODMSK (Chl/MtOH) > SDMSK (EtOH) > ODMSK (EtOH) > SDMSK Chl/MtOH) > ODMSK (H_2O) > SDMSK (H_2O) (Table 3).

Table (3): Effects of drying conditions and extraction solvents on total phenolic content of mango seed kernel powder.

Drying method	ng method Yield of extracted phenolices						
	Ex						
	Chi/MtOH	80% Ethanol	Water				
Sun-drying	69.3°± 0.39	80.8° ± 0.28	52.1 ± 0.17	67.4 ± 4.17			
Oven-drying	102.5° ± 0.18	$74.9^{\circ} \pm 0.14$	60.9° ± 0.05	79.43 ± 6.11			
Mean	85.9 ± 5.23	77.85± 3.45	56.5 ± 4.22	l			

^{*}mg of gallic acid per 100 gram of dry matter in MSKP. ±SE.

Superscripts a,b,c,d,e,f: means that the same letter among the treatments are not significantly different.

Drying method = Significant.

LSD value at 0.05 for extraction solvents = 0.52

LSD value at 0.05 for drying method × extraction solvents = 0.73

Oven-drying resulted in 47.9% higher total phenolics in chloroform/methanol extraction and 16.9% in water extraction than that of their corresponding results from sun-drying of MSK. This could be attributed to the degradation/ polymerization products of mango seed kernel polyphenols during oven-drying, which seem to be more soluble in chloroform/methanol mixture than water, and could react with Folin-Ciocalteu reagent to produce the blue color under alkaline conditions as stated by Yu et al., (2005). The obtaind results are also in partial agreement with the results of Nepote et al., (2002), who reported that during the drying of peanut skin in oven, products formed due to the Maillared reaction might contribute to the increase of total phenolics or phenolics- like complexes that contributed to higher absorbance readings.

It is worthy to note that solvents used for phenolics extraction also significantly affected the total phenolic concentration of MSKE, when equal volumes of solvents were used. Chloroform/methanol mixture was more effective in extracting phenolic compounds from ODMSK than both 80%

ethanol and water. The total phenolics resulting from extraction by chloroform/methanol mixture was 102.5 mg/g dry matter extracted, whereas 80% ethanol extract and water resulted in 74.9 and 60.9 mg/g dry matter extracted from ODMSK, respectively. These levels were compatible to the results reported by Puravankara *et al.*, (2000); Yu *et al.*, (2005); Abdalla *et al.*, (2007a) and Maisuthisakul and Gordon (2009).

Statistical analysis using ANOVA showed that there was a positive or negative effect for interaction between drying methods and extraction solvents, which indicated that drying method might enhance or reduce the extraction capability of solvent. For example, the extraction capability of chloroform / methanol mixture was enhanced by oven- drying method (102.5 mg galic acid/g dry matter extracted from MSK) while, sun- drying method was the superior with 80%ethanol for extracting phenolic compounds from MSK (80.8 mg galic acid/g dry matter extracted).

These findings were in line with Puravankara et al., (2000); Arabshahi-Delouee and Urooj (2007) and Maisuthisakul and Gordon, (2009) who found that solvents such as chloroform / methanol mixture, methanol or ethanol were the most effective in extracting phenolic compounds from MSK than water.

Antioxidant activity of mango seed kernel extracts (MSKE) is shown in Fig (2) and Table (4), It could be observed that 5% ODMSKE (Chl/MtOH) added to ghee samples, followed by SDMSKE (EtOH) added to ghee samples at the same level showed a marked increase in the antioxidant activity (induction period, time taken in hours to reach a peroxide value of 5 meg. of peroxide oxygen per kg of ghee) which was higher than that of 0.02% BHT containing ghee samples. Such induction periods were 398 h and 345 h for ODMSK (Chl/MtOH) and SDMSK (EtOH) containing ghee samples, respectively. Whereas, induction periods of both ODMSK (EtOH) and SDMSK (Chl/MtOH) containing ghee samples at level 5% were 279.2 and 267 h, respectively while that of 0.02% BHT containing ghee samples was 284 h. A noticeable decrease in induction periods of ghee samples mixed with ODMSK (H₂O) or SDMSK (H₂O) was observed as compared with all other treated samples, however, they showed higher induction periods than that of control sample at all levels (93.1, 102.6, 107.1, 140.2 h for ODMSK (H₂O) containing ghee samples and 92, 101.6, 105.2, 127 h for SDMSK (H₂O) containing ghee samples at 0.5, 1, 3 and 5% respectively). The correspondent figure for the control treatment was 88 h. The obtained results were in accordance with those of Arogba, (2000); Abdalla et al., (2007b) and Maisuthisakul and Gordon (2009) who, reported that the solvents used in extraction may affect the antioxidant activity of the extract, by their effect on the content and composition of the phenolic compounds extracted. Moreover, the obtained results revealed that the extraction with solvents such as chloroform / methanol mixture (2:1, v/v) or 80% ethanol was enough to dissolve most of the antioxidant components from mango seed kernel to have high antioxidant activity which was in line with the results of Maisuthisakul and Gordon, (2009).

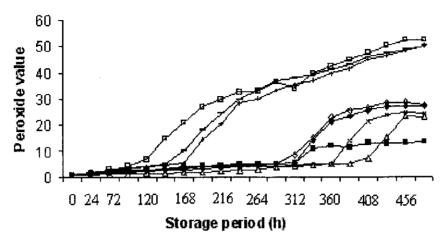


Fig. (2): Effect of addition 5% of mango seed kernel extracts (w/v) on the development of peroxide in ghee stored at 80°C ±2. □. Control; ■, 0.02% BHT; △, ODMSK(Chl/MtOH); ×,SDMSK(EtOH); ◆, ODMSK(EtOH); ◊, SDMSK(Chl/MtOH); +, CDMSK(H₂O); -, SDMSK(H₂O).

Table (4): Induction period (hrs)of buffalo ghee as affected by addition of mango seed kernel extracts (MSKE) at various level (w/v) and BHT at 0.02% (w/v).

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Added	Sources of extracted phenolics							
antioxidant			MSK (EtOH)		MSK (H₂O)		BHT 0.02	Control
percentage								
(w/v)	OD	SD	OD	SD	OD	SD	%	
	101 ^b ±	93.6°	96.1 ^a	98°	93.1 ^{er}	92	284° ±	88 ^g
0.5	0.20	±	±	±	±	±	0.05	±
		0.12	0.15	0.12	0.12	0.21		0.06
	123.2 ^b	105°	108.8 ^d	112.4°	102.6	101.6 [†]	284ª	88 ^g
1	±	±	±	±	±	±	±	±
	0.23	0.15	0.15	0.12	0.29	0.06	0.05	0.06
	131.1 ^b	112 ^e	115.2 ^d	122.2 ^c	107.1 ^t	105.2 ^g	284ª	88 ^h
3	±	±	±	±	±	±	±	±
	0.55	0.24	0.10	0.40	0.24	0.16	0.05	0.06
	398ª	267 ^e	279.2 ^d	345°	140.2	127 ⁹	284 ^c	88 ⁿ
5	±	±	±	±	±	±	±	±
	0.25	0.17	0.23	0.06	0.10	0.06	0.05	0.06

OD = Oven dried. SD = Sun dried. ±SE.

Superscripts a,b,c,d,e,f,g,h: means that the same letter among the treatments are not significantly different.

LSD value at 0.05: for 0.5% = 1.43

LSD value at 0.05: for 1% = 1.59

LSD value at 0.05; for 3% = 0.87

LSD value at 0.05; for 5% = 1.48.

For more real comparison of oxidative stability between the ghee samples mixed with MSKE and other mixed with BHT as synthetic antioxidant, and control sample, the results were expressed in terms of antioxidant index values (ratio of induction period of treated sample to induction period of control sample (Table 5).

The data in Table (5) indicated that the antioxidant index values of ghee samples treated with 5% solvents-MSKE other than H_2O were comparable to those of 0.02% BHT. It is worthy to mention that, the antioxidant indices of ghee sample treated with 5% ODMSK (Chl/MtOH) followed by that treated with SDMSK (EtOH) were superior as compared with sample treated with 0.02% BHT. On the other hand, the antioxidant index values of the ghee samples treated with 5% ODMSK (EtOH) and SDMSK (Chl/MtOH) were slightly lower than that treated with BHT. Thus, the antioxidant indices for all treated samples with MSKE at 0.5, 1, 3 or 5% (w/v) were in order: ODMSK (Chl/MtOH) > SDMSK (EtOH) > ODMSK (EtOH) > SDMSK (Chl/MtOH) > ODMSK (H₂O) > SDMSK (H₂O).

Table (5): Antioxidant index¹ of buffalo ghee as affected by addition of mango seed kernel extracts (MSKE) at various levels (w/v) and BHT at 0.02% (w/v).

Added	Sources of extracted phenolics						
antioxidant percentage	MSK (Chi/MtOH)		MSK (EtOH)		MSK (H ₂ O)		BHT 0.02
(w/v)	OD	SD	QD	SD	OD	SD	%
	1.15 ⁵ ±	1.06 ^{bc} ±	1.09 ^{bc} ±	1.11 ^{bc} ±	1.06 ^{bc} ±	1.05° ±	3.23° ±
0.5	0.002	0.002	0.001	0.002	0.002	0.003	0.003
1	1.40 ^b ±	1.19 ^c ±	1.24 ^{bc} ±	1.28 ^{bc} ±	1.17° ±	1.15° ±	3.23° ±
	0.002	0.001	0.003	0.002	0.004	0.001	0.003
3	1.49° ±	1.27 ^{de} ±	1.31 [™] ±	1.39 ^{bc} ±	1.22 ^{de} ±	1.20°±	3.23 ^a ±
}	0.006	0.003	0.001	0.004	0.003	0.002	0.003
5	4.52° ±	3.03° ±	3.17° ±	3.92 ^b ±	1.59° ±	1.44 ^t ±	3.23° ±
	0.002	0.003	0.004	0.002	0.001	0.001	0.003

¹ Ratio of pre-deterioration period of treated sample to pre-deterioration period of control sample. ±SE.

OD = Oven dried. SD = Sun dried.

Superscripts a,b,c,d,e,f: means that the same letter among the treatments are not significantly different.

HPLCSeparation of mango seed kernel extract, chromatogram indicating separation and identification of individual phenolic compounds in mango seed kernel extract (chloroform / methanol mixture -2:1, v/v) was shown in Fig (3). The results indicated that mango seed kernel (MSK) contained different phenolic compounds such as qurecetin, which represented 29% of total polyphenols, followed by 22% of catechin then 20% vanillin. MSKE also contained high amounts of coumarin and cinammic and ferulic acids, while gallic acid, tannin and rutin were found in lower amounts than other phenolic compounds. These results are in agreement with those reported by Abdalla et al. (2007a) and Mohamed and Girgis (2005) since Mohamed and Girgis (2005) separated six phenolic compounds, mainly coumaric, vanillin and ferulic acid, while Abdalla et al. (2007a) separated eight phenolic compounds.

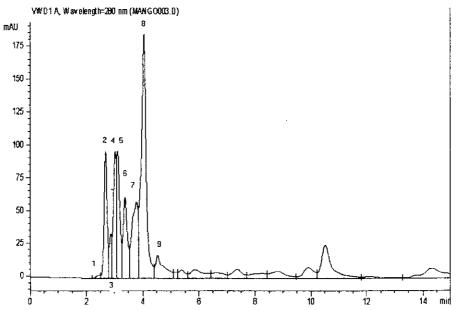


Fig. (3): HPLC chromatograms of mango seed kernel extract (Ch/MtOH).

Peaks identified: 1.rutin, 2.catechin, 3.tannin, 4.coumarin,
5.cinnamic acid, 6.ferulic acid, 7.vanillin, 8.qurecetin, 9.gallic acid.

In conclusion, the results of this study indicated that mango seed kernel (MSK) was very rich in phenolics and its total phenolics content was as high as 52.1-102.5 mg/gram dry matter extracted from mango seed kernel, depending on drying conditions and extraction solvents. It could be concluded that using hot air oven-drying, and chloroform / methanol mixture (2:1, v/v) extraction, is the most suitable procedure for the preparation of a mango seed kernel extract as natural antioxidant.

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تأثير ظروف التجفيف ومذيبات الاستخلاص على محتوى مستخلصات نواة بذرة المانجو من المركبات الفينولية وخواصها المضادة للكسدة

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تعتبر بذور المانجو ناتجا من النواتج الثانوية لمصانع عصائر الفاكهة أو من مخلفاتها قليلـــة القيمـــة الإقتصادية. إلا أن محتواها العالى من الفينو لآت جعلها مصدرًا هاما لمضادات الأكسدة الطبيعية . لذلك ذرس تأتير كلا من طرق التجفيف لنواة بذرة المانجو كـــالتجفيف الشمــسي أو بـــالفرن علـــي ٥٠ ٥م ومـــذيبات الإستخلاص مثل خليط الكلوروفورم/ميثانول والإيثانول ٨٠٪ والماء على محتوى مستخلص نــواة بــنرة المانجو من الفينو لات و على خواص ذلك المستخلص المضادة للأكسدة.

وقد أظهرت النتائج ما يلي:

- كان الجمع بين تجفيف نواة بذرة المانجو بالفرن واستخلاص ما بمسحوقها من مركبات فينولية بواسطة خليط الكلوروفورم/ميثانول أكثر فاعلية عن طريقة التجفيف ومذيبات الإستخلاص الأخرى بالنسبة لكميـــة المادة الجافة القابلة للإستخلاص (١٥,١٧٪) وكمية ما تحتويه تلك المادة الجافة من فينولات (۲۰۵ ملجم/جم).
- كان مؤشر مضاد الأكسدة النسبي (نسبة أقصى فترة زمنية لمقاومة السمن المعامل بمضادات الأكسدة إلى أقصىي فترة زمنية لمفاومة السمن الغير معامل بمضادات الأكسدة) للسمن الجاموسي المخزن على عالم ٥٨٠م والمعامل بـــ ٥٪ من المستخلص بواسطة خليط الكلوروفورم/ميثانول من نواة بـــذرة المـــانجو المجففـــةُ بالفرن (٤٠٥٢) أكبر من متيله للسمن الجاموسي المخزن على نفس درجة الحرارة والمعامل بـ ٢٠٠٠٪ .(٣, ٢٣) BHT
- عُرفت تسعة مركبات فينولية بالمستخلص السابق وهي كورستين وكاتشين و فانيلين وكومارين وتسانين وروتين و الأحماض سينامبك وفير وليك و چاليك.

مما سبق يتضح انه يمكن استخدام أنوية بذور المانجو كمصدر لمضادات الأكسدة الطبيعية القادرة على أن تحل محل مضادات الأكسدة الصناعية.

قام بتحكيم البحث

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