# CHEMICAL AND MICROBIAL STUDY OF MANGO AND APRIOT KERNELS SEEDS AND ITS EFFECT IN THE BEEF BURGER PRODUCTS

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# CHEMICAL AND MICROBIAL STUDY OF MANGO AND APRIOT KERNELS SEEDS AND ITS EFFECT IN THE BEEF BURGER PRODUCTS

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#### Abstract

Meat is a perishable food. both mango and apricot seed kernel extract can use as a natural food additive for extending the shelf-life of a variety of food products. The aim of the present work was to study the antioxidant and antimicrobial effect of ethanol apricot and mango seed kernels extract in beef burger stored at refrigerator temperature for different periods of time. The protein and fat content in apricot kernel extract (ASK) was five time higher than mango kernel extract (MSK). The ASK extract contain unsaturated fatty acids much higher than MSK. Vanillic was the highest amount of total polyphenols compounds found in ethanol mango kernel. While, pyrogallol was the highest compounds found in ethanol apricot kernel. Fresh samples that treated with sodium nitrite tended to have the lowest APC among all different samples investigated immediately after preparation. Both beef burger sample treated with mango and apricot kernels extract have APC lower than untreated samples. Sample treated with mango kernels extract had the lowest APC among all investigated samples after storage for 30 days. Accordingly, both extracts had a high nutritive value and broad antimicrobial spectrum against gram-positive and gramnegative bacteria. Moreover, the active antimicrobial component may be due to polyphenols found in both extracts.

Key words: Mango, Apricot, Kernel, Polyphenols, Antibacterial and Burger

# Introduction

Meat is a perishable food due to high moisture and nutrient contents, almost neutral in pH so meat is a good medium to be contaminated with microorganisms (**Post, 1996**). Nitrate and nitrite are additives commonly

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used in meat products curing for improvement of colour, flavour and as protection against the growth of microbial such as *Clostridium botulinum* (**Baji, 1984**). Nitrite has been implicated in a variety of long term health effects (**Walker and Krieble, 1990**).

However, increased consumer demand for more natural "preservative free" products during last years has led the food industry to consider the incorporation of natural antioxidants in food products. The use of natural antioxidants has the advantage of being more acceptable by the consumers as these are considered as "no chemical" (El-Bastawesy *et al.*, 2009).

Food antioxidants are compounds or substances that are present naturally in some ingredients or are intentionally added as food additive with the aim of inhibiting product oxidation and to inhibit the microbial growth (Halliwell, 1996).

Apricot kernels contain oil 53% in the seed and crude protein content 20-25% (**Demir and Cronin, 2005**). Apricot are high in health promoting phytonutrients, antioxidants, flavonoids (anthocyanins, flavonols and proanthocyanidins), and polyphenols (**Reed, 2002 and Sun et al., 2002**). Both water and methanol extracts inhibitory the growth of bacteria (**Ghazavi** *et al.,* **2008 and Yiğit** *and* **Mavi, 2009**).

Mango (*Mangifera indica L.*) is one of the most important tropical fruits. The mango seed kernels contain many macro and micronutrients (**Zein et al., 2005**). Soong and Barlow (2004) reported that mango seed has a strong antioxidant activity due to its high phenolic compounds content. These authors also observed that mango seed is a good source of phytosterols. Dried mango peel and kernel products can reduce total bacterial count, inhibited coliforms growth, and extended the shelf-life of pasteurized cow milk (Abdalla et al., 2007).

During the processing of mango and apricot, the seed is one of the main by-products. The seeds are not currently utilized for any commercial purpose and are discarded as a waste becoming a source of pollution. Both

mango and apricot seed kernel extract can use as a natural food additive for extending the shelf-life of a variety of foods products (**Ribero** *et al.*, 2007).

From the above mentioned data, it could be concluded that both mango and apricot seed kernel extract can use as a natural food additive for extending the shelf-life of a variety of foods products. Moreover, both of mango and apricot seed kernels have many macro and micro nutrient components. Accordingly, the aim of the present work was to study the antioxidant and antimicrobial effect of apricot and mango seed kernels extract in beef burger storage at refrigerator temperature for different periods of time.

# Materials and Methods

#### Materials:

• kernels:

Apricot and mango fruit kernels, were collected from Vitrac Company as by – products. The kernels were removed manually from the seeds, washed with tap water and then dried at (50 °C) in an oven for a week. The dried kernels were crushed using a commercial blender and stored at (- 18 °C ) in plastic package until analysis and extract the oil (Augustin and Ling, 1987).

# • Meat samples:

Six kilo grams of fresh meat were selected for this part of the study. The meat was collected from retail markets around Cairo City during summer 2014. Those samples collected in sterilized containers and transported to the laboratory under refrigeration and kept at under refrigerated condition until tested.

# **Preparation Of Beef Burger Formulated :**

Beef burgers were prepared as describe by (Abd-Elhak, et al., 2014)

- 1. Beef burger formulated was divided into six main leading brands as follow:-
- 2. The first group is control group (preserved without any preservatives).
- 3. The second group is preserved using sodium nitrite only (250 mg/kg).

- 4. The third group is preserved using1500 ppm of ethanol mango kernel extract.
- 5. The fourth group is preserved using 2500 ppm of ethanol apricot kernel extract.
- 6. The fifth group is preserved using 750 ppm of mango kernel extract and 125 ppm of apricot kernel extract.
- 7. 6 .The sixth group is preserved using (750 ppm of mango kernel extract + 125 ppm of apricot kernel extract ) + (125 mg of sodium nitrite).

# Methods:

# • Apricot Detoxification method:

Detoxification process has cotyledons of apricot seeds, after conducting a self enzyme hydrolysis of the amygdaline by emulsion enzyme located cotyledons seeds to produce hydrocyanic acid, which is causative to toxicity according to **Musa**, (2010).

# • Detoxification of the full split seeds before oil extraction:

The full apricots seeds (without grinding) are soaked and cracked in the amount of water by (1:12 w/v) at a temperature of 47 °C for a period of 30 hour with the change of water every two hours, and after detoxification was removed the water from cotyledons seeds and drying in the oven at a temperature of (50) °C for a week, and were then cotyledons apricot seeds grinding to remove toxicity by laboratory mill (**El-Adawy, 1992**).

# • Preparation of mango and apricot seed kernel extracts

Powdered kernels were extracted with Absolute ethanol was added to the apricot seed kernel powder at a ratio of 3:1 (v/w) and kept 24 hrs with gentle shaking at 40  $^{\circ}$ C. Filtered extracts were dried using a rotary evaporator at 45 $^{\circ}$ C and stored at 4 $^{\circ}$ C for further use (**Attia , 2000**).

#### Determination of chemical composition

Moisture content, crude protein, crude fiber, crude fat and ash of apricot and mango seed kernel powder were determined according to (AOAC 2007). Moreover, carbohydrate were calculated by difference.

# Determination of fatty acid

The fatty acid profile of ethanolic extract of mango and apricot kernels was determined according to ISO 5508 (1990) and ISO 5509 (2000) by gas chromatography (GC) as described by Nath, (1996)

# Determination of total phenolic compounds

The phenolic compounds of ethanolic extract of mango and apricot kernels were determined by HPLC according to the method of Goupy et al. (1999) using HPLC HewlletPackered (series 1050) equipped.

# • Preparation of beef burger for microbiological analysis:

About 50g of beef burger were aseptically weighed and grinded in sterilized hun. One gram of the grinded Beef Burger was transferred into another sterilized mortar for microbiological analysis where nine ml of sterilized saline solution was added and thoroughly mixed with the beef burgers and this represents 10 dilutions which were then used making further dilution according to **Karpinska et al. (2001)** 

# • Determination of total aerobic bacteria count:

The aerobic and anaerobic plate count were determined following the procedure proposed bv the International Commission on Microbiological Specifications for Foods (ICMSF, 1987). This medium was purchased from **El-Gomheria** Pharmaceutical Company America, Cairo, Egypt.

Microbiological examination was carried out every seven days interval from storage at refrigerator temperature. All count were done in triplicates. At each sampling time, the stored bags

were analyzed microbiologically according to the procedure recommended by the International Commission on Microbiological Specification for Foods (ICMSF). Serial dilutions were prepared as described by (ICMSF, 1987)

#### **Results and Discussion**

The moisture content of dried apricot seed kernels (ASK) was approximately 2.4%. Crude protein, total fat, crude fiber, carbohydrates and ash contents of ASK were found to be 26.5, 53.2, 13.5, 2.421 and 1.9% on a dry weight basis, respectively (table 1).

 Table (1): Chemical composition percentage of apricot and mango kernels powder.

Samples	Moisture	Fat	Protein fiber		Ash	Carbohydrate	
ASK	2.380	53.261	26.522	13.520	1.896	2.421	
MSK	5.632	11.356	5.911	11.140	2.225	63.736	

\* (ASK) Apricot seed kernels

<sup>\*</sup> (MSK) Mango seed kernels

The protein content reported in the current study was close to that reported by *Femenia et al.*, 1995 which reported that protein content of apricot kernel ranged from 14.1 to 45.3%. The chemical composition of apricot seeds kernels results obtained in this study were in general agree with the many previous studies (*Gupta et al.*, 2012). Moreover, cured protein is close to that obtained in *Lazos* study which reported that the cured protein in apricot kernel was 21.2% (*Lazos*, 1991).

The result of MSK obtained in the current study were in general agreement with the data obtained by (Zein et al., (2005), Abdalla et al., (2007) and Ashoush and Gadallah (2011). The MSK total lipid content obtained in this study was higher than that showed by (Youssef, 1999).

Total fat for apricot kernel detected in this study was in the range of total lipid obtained in other study which found the oil yields from apricot kernels ranged from 42.2% to 57.2% (*Musa et al., 2010*).

It should be noted that, the protein and fat content in ASK was five time higher than MSK. While, the carbohydrates content in MSK were much higher than ASK. This results is within with that obtained by *Abdalla* (2007) who confirmed that mango seeds kernel contained low amount of crude protein. Accordingly, the results indicated that the studied apricot kernel flours considered an important new protein sources. The previous opinion is agree with *El-Safy et al.*, 2012.

From the results illustrated in table (1), it was observed that the ASK was more nutritive than MSK because it's content more protein, fat and fiber. Accordingly, the investigated kernels flours can be used as an effective additive in foods such as meat and cereal products specially ASK flour.

From the previous chemical analyses, it could be concluded that, both of powder material contain fat with different concentration. Accordingly this study aimed to prepare alcohol extract from both apricot and mango seeds kernel. Then fatty acid composition was analyzed and the results were illustrated in table (2).

It should be noted that, the unsaturated fatty acid for apricot seed kernels extract was 91.5% and consisted mainly of oleic and linoleic acids. The saturated fatty acid content for apricot seed kernels extract was only 8.5%, with major fatty acid of palmitic and it consists 5.36%. Accordingly, the ability of some unsaturated fatty acids to reduce serum cholesterol level may focus attention on for spricot seed kernels extract (**Milosevic et al., 2010**). The results indicated that the apricot and kernel seeds extract contained several fatty acids such as Palmitic acid, Oleic acid and Linoleic acid. This result was in general closed to several studies (*Lazos, 1991 and* **Manzoor et al, 2012**).

	Fatty acids		Apricot seed kernels extract %	Mango seed kernels extract %	
saturated fatty acids	Palmitic acid	C16:0	5.36	4.68	
	Margaric acid	C17:0	0.03	ND	
	Stearic acid	C18:0	0.77	44.20	
	Arachidicacid	C20:0	1.50	2.81	
	Behenic acid	C22:0	0.01	0.17	
	Lignoceric acid	C24:0	0.06	ND	
	Total		8.5	51.86	
unsaturated fatty acids	Palmitoleic acid	C16:1	1.03	0.04	
	Ginkgolic acid	C17:1	012	ND	
	Oleic acid	C18:1	63.87	42.05	
	Linoleic acid	C18:2	26.10	4.96	
		C20:1	0.61	0.24	
sun	Total		91.5	47.86	

 

 Table (2): percentage of Fatty acids composition of Apricot and Mango seed kernels extract

In general, stearic acid was the main saturated fatty acid in, while oleic acid was the major unsaturated fatty acid in mango seed kernels. This result was in within with many studies which reported that the main fatty acids of total lipids were stearic acid (40–42%) and oleic (47–48%) of total fatty acids, and they together constituted 87–88% of total fatty acids (*Helmy* (1998) and El-Soukkary et al., (2000). Moreover, the current results were in agreement with earlier studies study which reported that the major fatty acids of Apricot extract were oleic, linoleic and palmitic (E1-Aal, Rahma and Khalil (1986) and (Manzoor et al.,(2012)).

From the above mentioned data, it could be concluded that, the Apricot seed kernels extract contain unsaturated fatty acids much higher than Mango seed kernels extract. On contrast, Mango seed kernels extract contain saturated fatty acids tended to have fat content approximately 6.5-fold higher than Apricot seed kernels extract.

Researchers have shown that foods rich in unsaturated fatty acids decrease risk of arrhythmias (abnormal heartbeats), which can lead to sudden death. Unsaturated fatty acids also decrease triglyceride levels, slow growth rate of atherosclerotic plaque, lower blood pressure and a possible lowering of cardiovascular disease (CVD) mortality (*AHA*, (2003) and *Hooper et al.*, (2004)). Accordingly, Apricot seed kernels extract has much power effect for reduction the risk of various forms of cardiac disease than Mango seed kernels extract.

Third: Flavonoids composition of ethanolic extract for apricot and mango seed kernels

Phenolic compounds has potential as cancer fighting molecules and many other health functions (**Ramos, 2007**). Though phenolic compounds are present in almost all foods of plant origin. fruits, vegetables, and beverages are the major sources of these compounds in the human diet (*Hertog, et al., 1995*).

phenolic compounds	Mango seed kernel extract( ppm)	Apricot seed kernel extract( ppm)	
Gallic	754.34	11.40	
Pyrogallol	3182.66	1278.78	
4-Amino-benzoic	18.30	11.27	
Protocatchchuic	144.88	57.82	
Catechein	321.05	190.37	
Chlorogenic	347.31	72.40	
Catechol	12228.52	127.30	
Epicatechein	1640.94	ND	
Caffeine	244.72	14.67	
p-oh-benzoic	947.56	319.22	
Caffeic	155.72	124.88	
Vanillic	13119.49	57.25	
ferulic	146.40	206.82	
Iso- ferulic	44.38	152.84	
e-vanillic	1459.53	269.39	
ellagic	539.51	221.55	
Alpha-coumaric	11.37	23.33	
benzoic	122.60	559.54	
salyciic	363.95	91.07	
3,4,5 Methoxy cinnamic	9.01	52.41	
coumarin	56.95	18.80	
p- coumaric	102.18	19.71	
cinnamic	1.77	10.01	

The phenolic compounds (mg/100g) of ethanolic extracts for mango and apricot kernel analyze by high- performance liquid chromatography (HPLC) against standard compounds. Data in table (3) showed that Vanillic was the highest amount of total polyphenols compounds found in ethanol mango kernel. While, Pyrogallol was the highest compounds found in

ethanol Apricot kernel. Epicatechein acid was found only in mango kernel extract. On the other hand, cinnamic acid was the lowest amount of total polyphenols found in both apricot and kernel. These results somewhat agree with those reported by Mohamed and Girgis (2005) and (Puravankara et al. 2000).

In general, all polyphenols component were higher in mango kernel extract as compared to apricot kernel extract. Based on the HPLC analysis, and by comparison with standards, 23 polyphenols could be identified or characterized in mango kernel extract, and 22 polyphenols in apricot extract. The polyphenols component isolated from mango kernel in this study were much higher than that obtained in **Puravankara et al.**, (2000) study , that identified six phenolic compounds in mango seed extracts, especially Gallic acid. This difference may be due to the different in mango category. Moreover, **Mohamed and Girgis (2005)** found that, separated six phenolic compounds, mainly coumaric, vanillin and ferulic acid.

From the above mentioned data it could be concluded that, mango kernels proved a source of phenolics. This result is applicable with other study (**Pura-vankara, et al., 2000**). From table 3 it be concluded that mango kernel is a rich sources of polypheols components. This finding is within with the finding reported by (**Abdalla et al., 2007**).

The average of total phenolic component obtained in this study was close to the value of total phenolic obtained in the recent studies which determine total phenolic (TPC) of leaves, peels, stem bark, and kernel of different mango varieties, and total flavonoid contents (TPC) in ranged from 630.89 to 11600.80 mg GAE/100g dry weight (**Choudhary and Swarnkar, 2011 and Badmus et al., 2012**).

Pyrogallol is predominating in both extracts. Pyrogallol is antioxidant and anti-inflammatoryand protected against chemically-induced liver toxicity in vivo. Most of polypheols component isolated from ethanol mango and apricot kernels extract constitute a significant part of a healthy diet due to their high antioxidant, anti-inflammatory and antimicrobial capacities (**Heim, et al., 2002**). Moreover, natural poltphenols are safe for

use as a food additive, functioning as an antioxidant in some countries, including Japan.

From the above mentioned data concluded that mango and apricot kernel extract can be used as a new natural antioxidant to improve the oxidative stability of some foods. This opinion is within with **Soong et al.**, (2004) results which reported that mango seed has a strong antioxidant activity due to its high phenolic compounds (Tanaka, 1999), rich in phytochemicals (Ashoush and Gadallah, 2011).

Thus utilization of mango and apricot kernels for extraction of oils seems to be a profitable proposition for the efficient utilization of stones/pits which otherwise is thrown as a waste. This opinion is agree with *Gupta and Sharma*.(2009), *Özcan*.(2010) and *Manzoor*, et al (2012) opinions.

Minced meat is usually a very perishable food so prolonging its shelf life is an important objective for producers. Accordingly, this study aimed to try extended the shelf life of one product of minced meat (beef burger) by adding natural extract.

Table (4): Total aerobic plate counts (APC) exhibited by beef burgers treated with mango and apricot kernels extract after storage at refrigerator temperature for different storage periods (cfu/g).

Storage periods	Zero time	7 days	14 days	21 days	30 days
Control	4×106 c	7.3×106 c	13.6×106 c	14.6×106 d	15×106 c
Sodium Nitrite	1×106 a	1.6×106ab	1.3×106 a	1.3×106 a	9×105ab
Mango seed kernels extract	2×106 b	1×106 a	3×105 b	8.5×105 c	6×105 a
Apricot seed kernels extract	2.3×106 b	1×106 a	1×106 a	2×106ab	9×105ab
Mango and apricot seed kernels extraction	2×106 b	1×106 a	1×106 a	2×106ab	1×106 b
(Mango and apricot seed kernels extraction) + (Sodium Nitrite)	3.6×106 c	2×106 b	4×106 b	3×106 b	1×106 b
Commercial samples	34×106 d	73×106 d	29×106 d	20.3×106 e	25×106 d

The total bacteria counts in beef burgers before and after addition of mango and apricot kernels extract storage for different periods of time at refrigerator temperature were shown in table (5). The results showed that, the total bacterial counts in fresh (untreated) beef burgers were  $4 \times 10^6$  cfu/g. Fresh samples that treated with sodium nitrite tended to have the lowest APC among all different samples investigated immediately after preparation. Even, there is significant differences have been observed between this samples and other investigated samples.

Both beef burger samples treated with mango and apricot kernels extract have APC lower than untreated samples, even statistical significant differences have been observed between them. Moreover, commercial samples tended to have the highest APC among all samples investigated immediately after preparation.

**Samples treated with half amount of sodium nitrite and half** amount of both kernel ethanol extract had APC higher than samples treated with pure extracts from mango and apricot kernels and sample treated with sodium nitrite. Even, statistical significant were observed between them.

Aerobic plate count (APC) for most of investigated samples after storage for different periods of time were increased with increasing the periods of time.

In weekly investigation, commercial samples tended to have the highest APC among all treated samples after storage at refrigerator temperature. It may be due to this sample had the highest APC before storage.

Aerobic plate count (APC) for control sample was higher than all another treated samples investigated after storage for different periods of time. It means that sodium nitrite, mango kernel extract and apricot kernel extract can play a positive role as antibacterial.

Sample treated with mango kernels extract had the lowest APC among all investigated samples after storage for 30 days, even lower than samples treated with sodium nitrite. After seven days of storage there no statistical significant difference were observed between samples treated with sodium nitrite or treated with ethanol mango or/and apricot kernels extract. This result agree with *Gadallah and abdel Fattah (2011)*.

From the above mentioned data it could be concluded that mango and apricot kernels extract reduced aerobic plate of beef burgers, even after storage for 30 days as refrigerator temperature. These results might be due to a variety of phenolic compounds such as falvonds and gallotannins present in the mango and apricot kernels extract. This observation is agree with those report by *Pura-vankara et al* (2000), *Kabuki et al.* (2000), *Engels et al* (2009) and (*Tian et al.*, 2009).

It is striking that the mean total APC present in investigated beef burger is lower than the recommendation safety limit proposed by Egyptian Standards which proposed that total APC in beef burger should not excess than  $10^6$  cell/g (*E.O.S*, 2005).

From the above mentioned data it be concluded that the above results apricot, and mango kernels are rich in nutrients and could be utilized for cooking and some food products. These results reported that the both extracts had a broad antimicrobial spectrum against gram-positive and gram-negative bacteria. Moreover, the active antimicrobial component may be due to polyphenols found in both extracts.

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دراسة كيميائية و ميكروبية لبذور المشمش و المانجو و تأثيره علي البيف برجر

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#### الملخص العربى

ان اللحوم من الأطعمة القابلة للتلف لـذلك فاننا نضيف بعض المضيفات الطبيعية الغذائية لأطالة فترة الصالحية وهذة المضيفات مستخرجة من مصادر طبيعية و هي بذور المانجو و بدور المشمش و كان الهدف من تلك الدراسة هو استخراج مضادات الأكسدة و مضادات الميكروبات من بذور المشمش و بذور المانجو و اضافتها لبر جر اللحم البقري و تخزينها في الثلاجة لفترات زمنية مختلفة و توصلت النتائج الي ان محتوي البروتين و الدهون المستخرج من نوي المشمش اعلي خمسة محرات من المستخرج من نوي المانجو و ايضا مستوي الاحماض الدهنية الغير مشبعة اعلي من مرات من المستخرج من نوي المانجو و ايضا مستوي الاحماض الدهنية الغير مشبعة اعلي من المستخرجة من نوي المانجو و كانت مجموعة مركبات البولي فينول المستخلصة في الايثانول لنواة المانجو اكبر من مثلتها في بذور المشمش. تم تحضير العينات من البر جر البقري مع اضافة الحد الادني لنترات الصوديوم لعينة من العينات و عولجت باقي العينات بزيوت بذر المشمش و المانجو فوجد ان العد الكلي لجميع العينات اللتي عولجت بمستخلصات بنور المشمش و المانجو فوجد ان العد الكلي لجميع العينات اللتي عولجت باقي العينات بزيوت بذر المشمش و المانجو فوجد المي الادني لنترات الصوديوم لعينة من العينات و عولجت باقي العينات الويا أو المانجو و حفظت لمة من المانجو الحميع العينات اللتي عولجت بمستخلصات بذور المشمش و المانجو و حفظت لمة مت يوم الادني لندرات الصوديوم لعينة من العينات و عولجت باقي العينات بزيوت بذر المشمش و المانجو فوجد الدي لي الهذ اللتي لم الكنترول التي لم يضف اليها أي اضافة حيث قل العدد الاجمالي ان العد الكلي لجميع العينات اللتي لم الكنترين لمدة ٣٠ يوم المي أن العدد الاجمالي الميكروبات العنقودية من لحم البر جر حتي بعد التخزين لمدة ٣٠ يوم إن النلاجة الإضافة الي ارتفاع الميكروبات العنقودية من لحم البر جر حتي بعد التخزين لمدة ٣٠ يوم إن النزاجة المانو وقد يكون الميكروبات العنقودية من لحم البر جر حتي بعد التخزين لمدة ٣٠ يوم إلى النويا و المانية الي ارتفاع الميكروبات العنقودية من لحم البر جر حتي بعد التخزين لميت م يوم إلى النزم و السائبة و قد يكون المي علومود البولي فينول الموجود في مستخلصات بذور الممش و المانجو و .

قسم الاقتصاد المنزلي – تغذية و علوم الاطعمة كلية التربية النوعية – جامعة عين شمس

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