

PREPARATION AND EVALUATION OF LOW-FAT BURGERS AND FORTIFIED BY CHICKPEA DURING FROZEN STORAGE

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Abstract

This study aimed to prepare beef burger formulae with low-fat content, substituted of beef fat by chickpea flour with different levels (٤, ٨, ١٢ and ١٥%). The obtained results showed that: Beef burgers are considered as a good source of carbohydrate, protein, crude fiber, ash content, minerals (K, Fe and Zn), antioxidant content (Polyphenols and flavonoids), essential amino acids, especially leucine and lysine and fatty acids (palmatic, stearic and oleic acid). Addition of chickpea flour to burgers decreased lipid oxidation (peroxide and thiobarbituric acid values) in either at zero time or during frozen storage. Addition of chickpea flour caused a decrease in total bacterial count, psychrophilic. Bacteria spore forming bacteria; fungi and yeasts in either at zero time or during frozen storage. During frozen storage, total bacteria, spore forming bacteria, fungi and yeasts were decreased. Sensory evaluation of burgers indicated that all samples were recorded highly acceptable score of color, texture, taste, order and overall acceptability by the panelists in either at zero time or at the end of storage.

ملخص البحث باللغة العربية :-

يهدف هذا البحث إلى إعداد البيف برجر المنخفض فى نسبة الدهن وذلك بإحلال مسحوق الحمص المجفف بنسب ٤، ٨، ١٢، ١٥% محل الدهن، وتم دراسة التركيب الكيميائي، العناصر المعدنية، مضادات الأكسدة، الأحماض الأمينية، الأحماض الدهنية وكذلك تم التقييم

المؤتمر السنوى الدولى الأول لكلية التربية النوعية – جامعة بنها فى الفترة من ٣٠ نوفمبر إلى ٣ ديسمبر ٢٠١٩م تحت عنوان الإبداعات التربوية النوعية من وجهة نظر مصرية إفريقية

الميكروبيولوجي والحسي خلال فترة التخزين بالتجميد على درجة حرارة -٢٠ درجة مئوية تحت الصفر لمدة ٣ شهور وكانت النتائج المتحصل عليها كما يلي:
يعتبر البرجر كمصدر جيد للكربوهيدرات، البروتين والألياف والمعادن والعناصر المعدنية (البوتاسيوم، الحديد، الزنك) وكذلك مضادات الأكسدة مثل الفينولات والفلافونويدات، الأحماض الأمينية الأساسية وخاصة اللبوسين واللايسين وكذلك الأحماض الدهنية وخاصة البلمتيك، الأستياريك، الأوليك .
كما أن إضافة مسحوق الحمض الجاف ادت إلى انخفاض معدل الأكسدة سواء عقب التصنيع أو خلال فترة التخزين بالتجميد حيث انخفض كل من رقم البيروكسيد، حامض الثيوباربتوريك. حدث انخفاض فى العدد الكلي للبكتيريا والمحببة للبرودة والمتجرثمة والفطريات والخمائر سواء فى بداية أو نهاية فترة التخزين بالتجميد وكانت جميع العينات مطابقة للمواصفات القياسية المصرية.
أظهر التقييم الحسي أن جميع العينات كانت مقبولة بدرجة حسية عالية لدى المحكمين من حيث اللون والطعم والرائحة والقوام والمظهر العام سواء عقب التصنيع أو فى نهاية مدة التخزين ودل التحليل الاحصائي عدم وجود فروق معنوية عند مستوى معنوية ٠,٥ فى كل من اللون، الرائحة، المظهر العام بينما لوحظ وجود فروق فى كل من القوام والطعم فى نهاية مدة التخزين.

INTRODUCTION

Meat is a noble food for man, as it contributes in the diet, with proteins of high biological value, and essential fatty acids (**Ferreria and Silva, ٢٠١٨**). The burger is defined as an industrialized meat product obtained from the ground beef of the animals, added or not of adipose tissue and ingredients, molded and subjected to suitable technological process, with characteristic texture, color, taste and smell.

Meat products such as burgers, sausages and meatballs have been developed using spices, food resources and meat from beef, pork, duck and quail to add value to them (**Ahmed and Nawab, ٢٠١٤; Adzitey, ٢٠١٥ and Kumari et al., ٢٠١٥**). Non-meat ingredients may be added to meat products with smaller quantities for binding, taste and flavor impartation (**FAO, ٢٠٠٧ and Mendiratta et al., ٢٠١٢**).

High animal fat diets are associated with several types of obesity, coronary heart disease, cardiovascular diseases and hypertension (**Serano et al., ٢٠٠٧ and Ozvural and Vural, ٢٠٠٨**). Recently, change in consumer's

preferences had led to expensive research on Low-fat foods (**Carrapiso, ٢٠١٧ and Yang et al., ٢٠١٧**).

The term "burgers" was taken originally from the word "hamburger" which presumably is a product that originated from Hamburg. Burger is one of the most important and popular meat products throughout the world. Most of European countries regulated that burgers should contain at least ٨٠% meat and ٢٠-٣٠% of fat content. The production process of burgers is composed of mixing the meat with the curing, flavoring and coloring agents to obtain acceptable sensory and technological properties (**Al-Marazeeq et al., ٢٠٠٨**).

Beef burgers is versatile, easy to prepare and relatively inexpensive (due to the use of low quality cuts of frozen meat), fat content is high (**Savell et al., ١٩٩١**). In recent years, the demand for low-fat meat products has been increased in order to avoid health risks associated with excessive fat intake (**Kirchner et al., ٢٠٠٠**).

Burgers are usually a feature of fast foods, most fast foods contain extremely high levels of trans fatty acids, which can lead to obesity, coronary disease and diabetes. Several studies have shown that the diets which rich in saturated fats and trans fats such as burger and fried meal caused an increase blood levels of low density lipoprotein (LDL) cholesterol that clogs the arteries (**Zoradia et al., ٢٠١١**).

Several studies highlighted the possibility of replacing fat with another ingredient or a combination of ingredients known as fat replacers (**Sayago Ayerdi et al., ٢٠٠٩**).

Addition of vegetable in meat products can improve can improve sensory functional properties and nutritional quality (**Turhan et al., ٢٠٠٧ and Ali et al., ٢٠١١**). Moreover, vegetables could serve as binders, fillers, fat replacers and also sources of dietary fibers and natural antioxidants (**Ali et al., ٢٠١١**).

Chickpea (*Ciceer arietum* L.) is a major legume it is considered as a good source of plant protein in many countries of Asia and Africa Moreover, it is a suitable source of dietary protein and their relatively

low levels of anti-nutritional factors (**Friedman, ١٩٩٦**). Also, chickpea is an important pulse crop with a wide range of potential nutritional benefited because of its chemical composition. Tocopherols, well – established natural antioxidants, were found in chickpea seeds in relatively similar amounts across all genotypes. In addition, it is a rich source in minerals especially calcium (**Marioli Nobile et al., ٢٠١٣**).

Chickpea (*Ciceer arietum*, L.) is an important and cheap source of vegetable protein which could be used as a substitute for animal protein and also contributing to the human diet in several developing countries. In Egypt, chickpea seeds found to be usually consumed at the raw green and tender storage (unripe stage) called Malana, or in the form of mature dry seeds. After parching the dry seeds could be consumed as a popular snake food, whole or decorticated after cooking and processing in different ways (**Alajaji and El-Adawy, ٢٠٠٦**). Chickpea is an important food legume and it is a major ingredient in many human diets (**Xu et al., ٢٠١٤**). They found that carbohydrate and protein were two major components in all type seeds.

Evidence suggests that pulse products, possibly due to their fiber content, play a role in the reduced risk of cardiovascular diseases (CVDs) (**Finley et al., ٢٠٠٧**), obesity (**Marinangeli and Jones, ٢٠١٢**), diabetes (**Sievenpiper et al., ٢٠٠٩**), and breast cancer (**Velie et al., ٢٠٠٥**). In addition to incorporating cooked whole pulses into the diet, pulses made into flours are another nutritionally beneficially dietary option (**USDA, ٢٠١٥**).

This study aimed to prepare beef burger formulae with low-fat content, substituted of beef fat by chickpea flour with different percentages (٤, ٨, ١٢ and ١٥%). Also, it could be obtained meat products with highly nutritional value, rich source of carbohydrate, fibers, and mineral contents, as well as it is the cheapest price and suitable for obesity and cardiovascular patient. In addition, chemical, physical, microbiological and sensory evaluations were studied during frozen storage period at -٢٠°C for ٣ months.

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MATERIALS AND METHODS

Materials

Fresh Local beef meat from round cut was purchased from Cairo local market just after slaughtering, then transported covered with crushed ice to the laboratory of Specific Education, Benha University. Beef fat was also obtained from the same beef carcass. Grains of chickpea were cleaned from impurities, washed several time with clean tap water, soaked in water for about three days until swelling the grains and changed water to remove alkaloids. The grains of chickpea were blanched in amount of suitable water for ٢٠ min., then dried in an electric oven at ٥٠°C. The grains were blended in an electrical grinder to give a flour with suitable granules. The flour was packed in polyethylene bags and kept in refrigerator at $\pm 1^{\circ}\text{C}$ until used.

Other materials such as starch, fresh minced onion and garlic, sodium chloride white pepper and spices were obtained from Cairo local market.

Preparation of burgers

Beef meat and fat were cut into small slices, and minced with crushed ice by using an electric minter. The other materials, chickpea flour, minced onion and garlic, potato starch, white pepper, sodium chloride and spices mixture were added (Fernández-López, et al., ٢٠٠٦) and mixed together then homogenized well. The preparing burgers were carried out as the described by Aleson-Carbonell et al., (٢٠٠٥) as illustrated in table (١). Chickpea flour was replaced by beef fat with different ratio (٤.٠, ٨.٠, ١٢.٠ and ١٥.٠%). These ingredients were homogenized well and formulation was pressed into burgers (٨٠.٠ g weight, ٨.٠ cm diameter and ١.٠ cm thickness) by using Molinex-burger machine. Samples were packed in polyethylene bags and stored at -20°C for three

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months. Before using, samples were thawed over night at 4°C . Chemical analysis were done immediately just after defrosting and the rest of tests were made on samples after cooking burgers in sunflower oil for 2 min. for each side.

Table (١): The percentage of raw food ingredient used in prepared beef burger formulae.

Formulae	(١)	(٢)	(٣)	(٤)	(٥)
Food ingredients					
Minced beef meat	٦٠.٠	٦٠.٠	٦٠.٠	٦٠.٠	٦٠.٠
Minced beef fat	١٨.٠	١٤.٠	١٠.٠	٦.٠	٣.٠
Chickpea flour	٠.٠	٤.٠	٨.٠	١٢.٠	١٥.٠
Potato starch	١٠.٠	١٠.٠	١٠.٠	١٠.٠	١٠.٠
Sodium chloride	٠.٥	٠.٥	٠.٥	٠.٥	٠.٥
White pepper	٠.٥	٠.٥	٠.٥	٠.٥	٠.٥
Spices mixture	١.٠	١.٠	١.٠	١.٠	١.٠
Crushed ice	١٠.٠	١٠.٠	١٠.٠	١٠.٠	١٠.٠

Chemical analysis

Moisture content, protein, crude fiber and ash content of samples were determined according to the method described by AOAC, (٢٠٠٥). Results expressed as g/١٠٠ g on dry weight basis. Carbohydrates and total energy were calculated according to FAO/WHO/UNU, (١٩٨٥). Results of total energy were calculated as k.cal./١٠٠g on dry weight. Total lipids were determined as described by Bligh and Dyer, (١٩٩٥). Minerals of prepared beef burgers (Ca, K, Na, Fe and Zn) were determined according to the method described by American Association of Cereal

Chemists, (١٩٨٣) and Kirleis et al., (١٩٨٤) by using Atomic Absorption. Results expressed as mg/١٠٠ g on dry weight basis.

Total phenols of beef burgers was determined as described by **Velioglu et al., (١٩٩٨)**. Results were expressed as mg gallic acid equivalents /١٠٠g. Total flavonoids was carried out as the method of **Ordon et al., (٢٠٠٦)**. Results expressed as quercetin mg/g dry matter.

Amino acids were determined according to the methods described by **Winder and Egyum, (١٩٦٦) and AOAC, (٢٠١٢)** by using High Performance Amino Acids Analyzer Biochron ٣٠ Pharmacia Biotech. Food Technology Institute, Giza, Egypt. Results expressed as g/١٠٠ g on dry matter

Fatty acids of prepared beef burgers were determined according to the method described by **AOAC, (٢٠٠٠)** by using Gas chromatograph with FID detector in Agricultural Research Center, Food Technology Institute, Giza, Egypt.

Peroxide values of burgers were determined according to the method described by **AOAC, (٢٠٠٥)**. Results expressed as mill.equivalent/kg lipids. Meanwhile, thiobarbituric acid values (TBA) were carried out as the method described by **AOCS, (١٩٩٨)**. Results as expressed as mg malonaldehyde/kg sample. Total bacterial count; psychrophilic bacteria, spore forming bacteria; fungi and yeasts were determined according to **ICMSF, (١٩٧٨)**. Sensory evaluation of prepared beef burgers were carried out as described by **Watts et al., (١٩٨٩)**. Statistical analysis of the obtained data for three replicates was carried out by **SPSS, (١٩٩٨)**. Values of $p \leq 0.05$ were considered as significantly difference.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition of both fresh local beef meat and chickpeas flour was shown in table (٢). The obtained results showed that beef meat was consisted of ٧٣.٥٢% moisture content, ١٧.٩١% crude protein, ٧.٠٥% fat, ٠.٥٩% crude fiber, ٠.٥١% ash content and ٠.٤٢% carbohydrate (g/١٠٠ g on fresh weight basis).

On the other hand, chickpea flour was composed of ٢٤.٣٢% plant protein, ٤.٧٦% fat, ٢.٤٧% crude fiber, ٢.٦٢ ash content and ٦٥.٨٣% carbohydrate (g/١٠٠ g on dry weight basis. These results are confirmed by **Atul et al., (٢٠١١)** and **Al-Shemary, Fatma, (٢٠١٩)**. Generally, chickpea is considered as a good source of protein, carbohydrate, crude fiber and ash content. Total energy of local fresh meat was ١٣٦.٨ k.cal/١٠٠g on fresh weight basis. Meanwhile, total energy of chickpea flour was ٤٠٣.٤ k.cal/١٠٠g on dry weight basis.

Table (٢): Chemical composition of both beef meat and chickpea flour.

Constituents	Ingredients	Local fresh beef meat (g/١٠٠ fresh weight)	Chickpea flour (g/١٠٠ dry weight)
Moisture %		٧٣.٥٢	٧.٩٢
Crude protein %		١٧.٩١	٢٤.٣٢
Fat%		٧.٠٥	٤.٧٦
Crude fiber %		٠.٥٩	٢.٤٧
Ash%		٠.٥١	٢.٦٢
Carbohydrates%		٠.٤٢	٦٥.٨٣

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Total energy (k.cal/١٠٠g) dry weight	١٣٦.٨	٤٠٣.٤
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Table (٢) illustrates chemical composition of prepared beef burgers replaced of beef fat by chickpea flour during frozen storage period moisture percentage of prepared beef burger formulae replaced of beef fat by chickpea flour with different ratio (٤.٠ , ٨.٠ , ١٢.٠ and ١٥.٠%) during frozen storage at -20°C for ٣ months. The results showed that the moisture content was ranged from ٤٧.١% to ٥٦.٣%, the highest percentage of moisture was found in control samples and the lowest was in formula (٥) which contained ١٥.٠% chickpea flour. The higher addition of chickpea flour, the lower percentage of moisture content was observed. During frozen storage period, the moisture content was gradually decreased as the prolonged storage time proceeded until reached between ٤٣.٧% and ٥٠.١%. Also, moisture content was decreased as the level of chickpea flour increased. The same conclusion was found by **Mehram, Eman**, (٢٠١٧) and **Morcy, Hayam**, (٢٠١٧). The decrease of moisture content during frozen storage may be due to the escape of a moisture in the drip loss during thawing (**Emam**, ١٩٨٧).

The obtained results indicated that crude protein was ranged from ٤٦.٠ to ٥٠.٦% g/١٠٠ g on dry weight basis, control sample was recorded the lowest percentage of protein, meanwhile, formula (٥) was the highest percentage. The protein content was increased as the level of chickpea increased, this increase of crude protein could be due to that chickpea is rich source of plant protein as shown in the previously results in table (٣). Chickpea is considered as a good source of plant protein (**Atul et al.**, ٢٠١١).

It is evident from the results that fat percentage of prepared beef burgers was ranged from ١٤.٩٥ to ٢٨.٠٢%, the highest percentage of fat was found in control sample (without chickpea flour) and the lowest percentage was in formula (٥) i.e fat content was decreased as the level of chickpea flour increased. This decrease of fat could be due to that chickpea flour contained low fat percentage as shown in the previously results in table (٢).

The results also indicated that carbohydrate of prepared beef burgers was ranged from ١٧.٦٩ to ٢٤.٠٤% g/١٠٠ g on dry weight basis. Formula (٥) was the highest percentage of carbohydrate. Also, chickpea is a rich source of carbohydrate (**Al-Shemary, Fatma, ٢٠١٩**).

It is obvious from the results that crude fiber of prepared beef burgers was ranged between ٣.٠ and ٤.٧١% (g/١٠٠g on dry weight basis). The highest value of crude fiber was found in formula (٥) and control sample recorded the lowest value. This could be that chickpea is considered as a good source of crude and dietary fibers (**Al-Shemary, Fatma, ٢٠١٩**).

The results also showed that ash content of prepared burgers was ranged from ٣.١٠ to ٥.٧% g/١٠٠ g on dry weight basis. Ash content was increased as the level of chickpea flour increased. This increase could be due to that chickpea is a rich source of ash content as illustrated in the previously results in table (٢). In addition, chickpea is a good source of minerals (**Marioli Nobile et al., ٢٠١٣**).

It was found that both protein and fat percentage of all prepared burgers were decreased during frozen storage until reached from ٤٢.٢ to ٤٧.٣% in crude protein and from ١١.٧ to ٢٦.٣% in fat content. This decrease of protein may be due to the part of total nitrogen which escape in

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the drip loss through thawing process. The same trend of results was noticed by (Mehram, Eman ٢٠١٧).

Both fat and protein content were decreased in contrast, carbohydrate, fibers and ash content were increased during frozen storage period. These results are in agreement with those obtained by Morcy, Hayam, (٢٠١٧), and Ayed, Najlh, (٢٠١٨). Finally, it could be concluded that prepared burgers by replaced chickpea flour were considered as a good source of carbohydrate, protein, crude fibers and ash content. Meanwhile, it contained low-fat content and formula (٥) showed that best results.

Table (٣): Chemical composition of prepared beef burgers replaced of beef fat by chickpea flour (CF) during frozen storage.

Formulae Constituents	(١) Control	(٢) ٤% (CF)	(٣) ٨% (CF)	(٤) ١٢% (CF)	(٥) ١٥%(CF)
Moisture content					
Zero time	٥٦.٣	٥٣.٤	٥١.٧	٤٨.٥	٤٧.١
٣ months	٥٠.١	٤٨.٣	٤٦.٢	٤٤.٥	٤٣.٧
Crud Protein (%)					
Fat (%)					
Zero time	٢٨.٠٢	٢٧.٣٧	٢٥.٥٦	١٨.٤٦	١٤.٩٥
٣ months	٢٦.٣	٢٥.٨	٢٤.١	١٦.٣	١١.٧
Ash (%)					
Zero time	٣.١٠	٤.٩٠	٥.٣٠	٥.٥٠	٥.٧٠
٣ months	٣.٠	٥.٦	٦.١	٦.٤	٧.٠
Crude fiber (%)					
Zero time	٣.٠٥	٣.٣٠	٣.٥٥	٣.٩٣	٤.٧١
٣ months	٣.٠	٤.٨	٦.٥	٦.١	٦.٤
Carbohydrates (%)					
Zero time	١٩.٨٣	١٧.٧٣	١٧.٦٩	٢٣.٨١	٢٤.٠٤
٣ months	٢٠.٠	٢٠.٠	٢٠.٧	٢٥.٥	٢٨.٦

Results expressed as g/100 g on dry weight

Antioxidant content

Table (٤) shows antioxidant contents of prepared beef burgers substituted of beef fat by chickpea flour with different ratio (٤, ٨, ١٢ and ١٥%). The results revealed that phenols of beef burgers was ranged between ١٠٨.٩١ to ١٥٩.٤٤ mg/100 g gallic acid equivalent. The highest value of total phenolics was found in formula (٢) contained ٤% chickpea flour. On the other hand total flavonoids was ranged from ١٥.٢٥ to ٤٣.٨ mg/100 g quercetin equivalent, the highest value was found in formula (٥) and the lowest one was in control samples (without addition of chickpea flour). It is evident from the results that the higher addition of chickpea flour, the higher value of total flavonoids was noticed. This results could be due to the chickpeas is a good source of antioxidant contents (Al-Shemary, Fatma, ٢٠١٩).

Chickpeas is a rich source of tochoferols (Boschin and Arnolds, ٢٠١١). In addition, El-Nahas, (٢٠٠٢) found that both green and dry chickpeas is a good source of antioxidant contents, especially chlorogenic acid which is considered the most important of the cinamics group. Generally, beef burgers by the addition of chickpea flour is considered as a good source of antioxidant contents and formula (٥) was the best results in both polyphenols and flavonoids.

Table (٤): Antioxidant content of prepared beef burgers, replaced of beef fat by chickpea flour.

Treatments	(١) Control	(٢)	(٣)	(٤)	(٥)
Antioxidants		٤% (CF)	٨% (CF)	١٢% (CF)	١٥% (CF)
Total phenols	١٢٤.٩٣	١٥٩.٤٤	١١٩.٨٢	١٠٨.٩١	١٣٨.٦١

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Total flavonoids	١٥.٢٥	٣٠.٥٩	٣٩.٩٨	٣٤.١٨	٤٣.٨٠
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* Results expressed as mg/١٠٠ g (galic acid equivalent) on dry weight basis.

* Results expressed as mg/١٠٠ g (Querectin equivalent) on dry weight basis

Minerals

Table (٥) illustrate mineral contents of prepared beef burger formulae substituted of beef fat by chickpea flour with different levels (٤.٠, ٨.٠, ١٢.٠ and ١٥.٠%). Macroelements of Ca of burger samples was ranged from ١٩.١٢ to ٢٨.١٥ mg/١٠٠g on dry weight basis, while P was ranged between ٣٩.٠ and ٤٧.٠ mg/١٠٠g dry matter. It is obvious from the results that Mg was ranged from ٦٠.١٨ to ٧٣.٧٩ mg/١٠٠g dry weight, the highest value was in formula (٥) which contained ١٥.٠% and the lowest value was found in control sample (without addition of chickpea flour). Also, K of burger samples was ranged between ١٩.٤٦ and ١١٦.٣ mg/١٠٠g on dry weight, the highest value was also found in formula (٥) and the lowest one was in control samples. It is worthy to mention that both Mg and K were increased as the level addition of chickpea increased. This results could be due to that chickpea is considered as a good source of studied minerals (**Marioli Nobile et al., ٢٠١٣**).

On the other hand, microelements of Fe of prepared burger samples was ranged from ٧.١ to ١١.٢٧, while Zn was ranged between ٧.٥٨ and ١١.٩٢ mg/١٠٠g dry matter. Formula (٥) was the highest value of Fe. This results could be due to that chickpea is a good source of microelements (**Mariol Nobile et al., ٢٠١٣**). It is worthy to mention that consuming ١٠٠g from all prepared beef burgers could cover the daily requirements of Fe and Zn human consumption. Prepared beef burgers by using

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chickpea flour is considered as a good source of Fe, K and Mg and formulae (٥) was the best formula of these minerals.

Table (٥): Mineral contents of prepared beef burgers, replaced of beef fat by chickpea flour (CF).

Formulae Minerals	(١) Control	(٢) % (CF)	(٣) % (CF)	(٤) % (CF)	(٥) % (CF)
Macroelements (mg/١٠٠ g dry weight)					
Ca	٢٥.٢٢	١٩.١٢	٢٠.٢٨	٢٦.٩١	٢٨.١٥
P	٣٩.٠	٤١.٠	٤٢.٠	٤٧.٠	٣٩.٠
Mg	٦٠.١٨	٦٥.٩١	٦٨.٣٧	٦٩.١٣	٧٣.٧٩
K	١٩.٤٦	٨٣.٠٦	١١٦.٢	١٠٦.٣	١١٦.٣
Microelements (ppm /١٠٠ g dry weight)					
Fe	٨.٨٧	٧.١	١٠.٣٧	١١.٠٩	١١.٢٧
Zn	١١.٩٢	٧.٥٨	٨.١٤	٩.٣٢	٨.٠١

Results calculated as mg/١٠٠ g on dry weight basis.

Amino acids

Table (٦) illustrates amino acid composition of prepared beef burger formulae substituted of beef meat by chickpea flour during frozen storage period at -٢٠°C for ٣ months. The results showed that there are ١٧ amino acids could be separated and identified by Amino Acid Analyzer. These amino acids are ١٠ essential and ٧ non-essential amino acids. The major essential amino acids are leucine which ranged from ٣.٤١ to ٤.٠٧ mg/١٠٠ gram on dry weight basis, followed by lysine (٣.٢٨ – ٤.١٧) mg/١٠٠ g on dry weight basis. On the other hand, total essential amino acids was ranged between ١٩.٩٥ and ٢٢.٨٢ mg/١٠٠ g on dry weight gain, the highest percentage was found in control sample and the lowest one was in formulae (٤). This results could be due to that beef meat is a rich source in essential amino acids. It is evident from the abovementioned results that total non-essential amino acids was ranged from ٢٣.١٩ to ٢٦.٤١ mg/١٠٠g on dry weight basis. The predominant

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non-essential amino acids of prepared beef burgers was glutamic acid (٧.١٩-٨.٥٩), followed by aspartic acid (٤.١٥-٤.٧٩) mg/١٠٠ g on dry weight basis.

In addition, total amino acids of prepared beef burgers ranged between ٤٣.١٤ and ٤٩.٢٣ mg/١٠٠g on dry weight. The highest value was found in control samples and the lowest one was in formula (٤) which contained ١٢% chickpea powder. This results may be due to that beef meat is considered as a good source of animal protein and essential amino acids (Emam, ١٩٩٠, and Ferreria and Silva, ٢٠١٨). It is worthy to mention that, total essential amino acids and total amino acids were slightly decreased as the percentage addition of chickpea flour increased. All prepared beef burger formulae are considered as a good source of essential amino acids especially, leucine and lysine, as well as control sample was recorded the best results of EAA.

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Table (١): Amino acids of prepared burgers substituted of beef fat by chickpea flour.

Formulae	(١) Control	(٢) ٤% (CF)	(٣) ٨% (CF)	(٤) ١٢% (CF)	(٥) ١٥% (CF)
Essential amino acids (EAA)					
Therionine (THR)	٢.٣٤	٢.٠٦	٢.٢٢	١.٤٨	١.٨٦
Valine (VAL)	٢.٥٥	٠.٢٦	٢.٣٠	٢.١٢	٢.٣٢
Isoleucine (ILE)	٢.٢٦	٢.١٠	٢.١٦	١.٩٥	٢.٠١
Leucine (LEU)	٤.٠٧	٣.٦١	٣.٨٥	٣.٤١	٣.٥٢
Tyrosine (TYR)	١.٤٧	١.٦٩	١.٧٦	٠.٨٩	١.٥٤
Phenyl alanine (PHE)	١.٨٨	٢.٠٠	٢.٠٤	١.٨٥	١.٨٧
Histidine (HIS)	١.٨٤	١.٧٣	١.٧٨	١.٦٥	١.٦٦
Lysine (LYS)	٤.١٧	٣.٥٣	٣.٧١	٣.٢٨	٣.٣٣
Cystine (CYS)	٠.٧٨	٠.٥٦	٠.٥٥	٢.١٩	١.٠١
Methionine (MET)	١.٤٦	١.٢٦	١.٤١	١.١٣	١.٠٩
Total EAA	٢٢.٨٢	٢٠.٠٠	٢١.٧٨	١٩.٩٥	٢٠.٢١
Non-essential amino acids (NEAA)					
Aspartic (ASP)	٤.٧٩	٤.٣٠	٤.٦١	٤.٣٩	٤.١٥
Serine (SER)	١.٩٩	١.٧٥	٢.٠٢	١.٠٦	١.٥٩
Glutamic acid (GLU)	٨.٥٩	٧.٧٢	٨.٢٢	٧.١٩	٧.٨٥
Glycine (GLY)	٢.٨٢	٢.٤٦	٣.٢٧	٢.٥١	٢.٥٥
Alanine (ALA)	٣.٢١	٣.٤٤	٣.٨٣	٣.٣٦	٣.٣٢
Arginine (ARG)	٣.٢١	٣.١١	٣.٤٢	٣.١٤	٢.٩٥

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Proline (PRO)	١.٨	٢.١٠	٢.١٥	١.٥٤	٢.٠٣
Total NEAA	٢٦.٤١	٢٤.٠	٢٧.٥	٢٣.١٩	٢٤.٤٤
Total AA	٤٩.٢٣	٤٤.٠	٤٩.٢٨	٤٣.١٤	٤٤.٦٥

Results expressed as g/١٠٠ g on dry weight

Fatty acids

Data in table (٧) illustrates the fatty acid composition of lipids extracted from prepared beef burger formulae substituted of beef fat by chickpea flour with different ratio (٤, ٨, ١٢ and ١٥%) during frozen storage at -٢٠°C for ٣ months. The obtained results showed that there are nineteen fatty acids could be separated and identified by Gas liquid Chromatography (GLC). These fatty acids are, five saturated fatty acids (SFA), twelve unsaturated fatty acids (USFA) and two odd carbon number fatty acids.

It is obvious from the results that three fatty acids are ($\text{C}_{16:0}$, $\text{C}_{18:0}$ and $\text{C}_{18:1}$) only are predominant and constituents ٨٢.٨٨% of the total fatty acids, while others are in minor quantities i.e the remaining sixteen fatty acids constituents only ١٧.١٢% of the total fatty acids. The three major fatty acids are palmitic $\text{C}_{16:0}$, (٢٨.٨٦%), stearic, $\text{C}_{18:0}$, (١٩.٣٥%) and oleic, $\text{C}_{18:1}$ (٣٤.٦٧%). There are two odd carbon number fatty acids, pentaenoic, $\text{C}_{15:0}$, (١.٣٦%) and heptadecanoic, $\text{C}_{17:0}$, (٢.٥٥%) of the total fatty acids. Total saturated fatty acids was ranged from ٣٨.٢ to ٥٤.٥٣% of the total fatty acids, while unsaturated fatty acids was ranged between ٤١.١ and ٥٨.٥٢% of the total fatty acids.

It is worthy to mentioned that total saturated fatty acids of beef burger was decreased as the level addition of chickpea flour increased. In contrast, unsaturated fatty acids increased with the increasing of chickpea flour. However, linoleic acid $\text{C}_{18:2}$ was ranged between ٢.٨٢ and ١١.٥٥% of the total fatty acids. The highest percentage was found in formula (٥) and the lowest was found in control samples.

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KS ratio of lipid extracted from prepared beef burgers was increased as the level chickpea flour increased. This increase could be due to that chickpea contained unsaturated fatty acids (Gul et al., ٢٠٠٨ and Marioli Nobil, ٢٠١٣). Prepared beef burgers replaced of beef fat by chickpea flour is considered as a good source of fatty acids, especially palmitic, stearic and oleic acid.

Table (٧): Fatty acid composition of prepared beef burgers substituted of beef fat by chickpea flour.

Fatty acids	Formulae	(١) Control	(٢) % (CF)	(٣) % (CF)	(٤) % (CF)	(٥) % (CF)
	Essential amino acids (EAA)					
Caprylic	(C _{١٢:٠})	٠.٠	٠.٠	٠.٠	٠.١٦	٠.١٩
Myristic	(C _{١٤:٠})	٣.٩٦	٤.١٠	٣.٨٨	٣.٣٨	٢.٧٤
Palmitic	(C _{١٦:٠})	٢٨.٨٦	٢٩.٣٤	٢٧.٦٢	٢٥.٨٩	٢٤.٣٨
Stearic	(C _{١٨:٠})	١٩.٣٥	٢٠.٨٧	١٨.٣٥	١٦.٦٤	١٠.٧٣
Arachidonic	(C _{٢٠:٠})	٠.٢١	٠.٢٢	٠.٢١	٠.٢٣	٠.١٧
Total SFA		٥٢.٣٨	٥٤.٥٣	٥٠.٠٦	٤٦.٣٠	٣٨.٢١
Tetradecenoic	(C _{١٤:١})	٠.٥٢	٠.٥٥	٠.٦٢	٠.٥٩	٠.٨٥
Palmitoleic	(C _{١٦:١})	٢.٥٥	٢.٥١	٢.٨٣	٢.٨٩	٣.٤١
Decatrienoic	(C _{١٦:٣})	٠.٥٣	٠.٥٠	٠.٥٥	٠.٥٤	٠.٥٧
Oleic	(C _{١٨:١})	٣٤.٦٧	٣٣.٣٠	٣٦.٣٤	٣٧.٥٠	٣٩.٣٠
Vaccinic	(C _{١٨:١})	٠.٤٣	٠.٤١	٠.٤٦	٠.٤١	٠.٣٤
Linoleic	(C _{١٨:٢})	٢.٨٢	٢.٥٩	٤.١٥	٥.٢٠	١١.٥٥
Gamma linolenic	(C _{١٨:٣})	٠.١٦	٠.١٦	٠.١٧	٠.١٦	٠.١١
Linolenic	(C _{١٨:٣})	٠.٣٢	٠.٢٦	٠.٣٦	٠.٣٤	٠.٨٧

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Octadecatetraenoic (C _{18:4})	٠.٢٢	٠.٢٣	٠.٢٦	٠.٣١	٠.٣٥
Eiosaenoic (C _{٢٠:٤})	٠.٤٠	٠.٣١	٠.٣٢	٠.٥٩	٠.٥٤
Gadoleic (C _{٢٠:١٥})	٠.١٨	٠.١٥	٠.٢٠	٠.٣٧	٠.٢٨
Arachidonic (C _{٢٠:٤} ω _٦)	٠.١٣	٠.١٣	٠.٢٠	٠.١٨	٠.٣٥
Odd carbon number					
Pentaenoic (C _{١٥:٥})	١.٣٦	١.٣٦	١.١٨	١.١٧	٠.٦٩
Heptadecanoic (C _{١٧:٥})	٢.٥٥	٢.٥٣	٢.٣٠	٢.٢٢	١.٥٤
Non identified FA	٠.٠٣	٠.٢٣	٠.١٣	٠.٩١	١.٠٩
Total un SFA	٤٢.٩٣	٤١.١	٤٦.٤٦	٤٩.٠٨	٥٨.٥٢
KS	٠.٨٢٠	٠.٧٥٤	٠.٩٢٨	١.٠٦	١.٥٣

Lipid oxidation

Table (٨) illustrates the peroxide values of prepared beef burgers substituted of beef fat by chickpea flour with different ratio during frozen storage at -٢٠°C for three months. Peroxide value (PV) is a parameter of lipid oxidation and could be used for the evaluation of oils and fat quality.

The obtained results showed that peroxide values of prepared beef burgers was ranged from ١.٢ to ١.٥٧ mill.equiv./Kg lipids. The highest value was found in control sample and the lowest value was in formula (٥). It is worthy to mention that peroxide value was reduced parallel with increasing the replacement rate of chickpea flour. This decreasing of peroxide value is mainly due to the low fat content of prepared beef burgers as shown in the previously results in table (٢). In addition, chickpeas has an antioxidant contents such as polyphenols and flavonoids as illustrated in the previously results in table (٣). Antioxidant could protect against free radical such as reactive oxygen species (Atoui et al., ٢٠٠٥). This decrease of peroxide value may be due to that chickpea contained polyphenols which has antioxidant activity (El-Nahas, ٢٠٠٢).

It is evident from the abovementioned results that peroxide values of all prepared burger samples were sharply increased until reached be-

tween ١٠.١ and ١٠.٩ mill.equiv./Kg lipids. at the end of storage period. The same conclusion was found by (Mehram, Eman, ٢٠١٧ and Ayed, Najlh, ٢٠١٨). Peroxide value was proportionally increases with the same trend, before storing. The highest peroxide value was found in control sample (without addition of chickpea), meanwhile the lowest value of PV was in formula (٣).

The increasing of PV during storage period may be due to the effect of free radical react with oxygen and produced hydroperoxides which are referred to as peroxides (El-Safie, ٢٠٠٠). In addition, frozen storage of meat was affected on the proportions of unsaturated fatty acids and phospholipids. However, the changes of lipids depend on storage time and the type of meat (El-Magoli et al, ١٩٨٢). Also, El-Nahas, (٢٠٠٢) found that chickpeas is a good source of phenolic acids, especially, chlorogenic acid. It is obvious from the results that TBA values of prepared beef burgers were ranged from ٠.١٩٤ to ٠.٢٣٩ malonaldehyde/Kg sample. Control sample (without addition of chickpea) was recorded that highest value of TBA, while formulae (٥) was the lowest value, the higher addition of chickpea flour, the lower value of TBA was noticed. This results may be due to that chickpea is considered as a good source of antioxidant contents as shown in the previously results in table (٣).

The abovementioned results also showed that during frozen storage period, TBA values of beef burgers were gradually increased as the prolonged time proceeded until reached between ٠.٥٠٣ and ٠.٥٤٢. Control sample was recorded the highest value of TBA, meanwhile the lowest value was found in formula (٥) i.e. the higher addition of chickpea, the lower value of TBA was observed. This trend of results was obtained by many authors (Morcy, Hayam, ٢٠١٧ and Al-Ajmi, Najlaa, ٢٠١٨), they found that TBA values were increased during frozen storage of meat products. Oxidation of lipids caused an increase in TBA value during storage period. This increase could be due to instability of the malonaldehyde produced from lipid oxidation, beside microorganisms play an

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important role in decomposition of malonaldehyde (Munoz et al., ١٩٩٨ and Rhee et al, ١٩٩٨).

It is interesting to note that TBA values of all burger samples did not reach to the permissible limits according to the **Egyptian Standard Specifications**, (١٩٩١) which recommended that frozen meat products should not exceed than ٠.٩ mg malonaldehyde/Kg sample. TBA values of prepared beef burgers were decreased as the addition level of chickpea flour increased. Moreover, chickpea has an antioxidative effect.

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Table (٨): Peroxide and thiobarbituric acid values of prepared beef burgers replaced of beef fat by chickpea flour during frozen storage replaced of beef fat by chickpea flour (CF).

Formulae Storage	(١) Control	(٢) ٤% (CF)	(٣) ٨% (CF)	(٤) ١٢% (CF)	(٥) ١٥% (CF)
* Peroxide values					
Zero time	١.٥٧	١.٤٢	١.٣٠	١.٢٢	١.٢٠
١- month	٥.٧	٥.٥	٥.٤	٥.٢	٥.١
٢- months	٧.٨	٧.٧	٧.٥	٧.٣	٧.٠
٣- months	١٠.٩	١٠.٧	١٠.٦	١٠.٣	١٠.١
** Thiobarbituric acid values					
Zero time	٠.٢٣٩	٠.٢٢٧	٠.٢١٠	٠.٢٠١	٠.١٩٤
١- month	٠.٣٨٢	٠.٣٧٠	٠.٣٥٤	٠.٣٣٧	٠.٣٣١
٢- months	٠.٤٤١	٠.٤٣٣	٠.٤٢٥	٠.٤١١	٠.٤٠٢
٣- months	٠.٥٤٢	٠.٥٣٣	٠.٥٢٥	٠.٥١٤	٠.٥٠٣

* Results expressed as mg malonaldehyde/kg sample.

** mg malonaldehyde/kg sample

Microbiological changes

Total bacterial count, psychrophilic bacteria, spore forming bacteria and fungi; yeasts of prepared beef burgers replaced of beef fat by chickpea flour with different percentage during frozen storage at -20°C for ٣ months. The obtained results showed that total bacterial count was ranged from ٢.٠×10^5 to ٢.٩٧×10^5 cfu/g, control sample was recorded the highest number of bacteria and the lowest number was in formula (٥). It is evident from the results that total bacterial counts of all prepared beef burgers were decreased as the prolonged time proceeded until reached between ٥.٥×10^4 and ٧.٨×10^4 cfu/g at the end of storage. The higher level of chickpea, the lower number of total bacteria was observed. The same trend of results are in agreement with those noticed by (Emam, ٢٠٠٣, Mehram, Eman, ٢٠١٧ and Al-Ajmi, Najlaa, ٢٠١٨). Freezing process reduced the growth of spoilage and pathogenic bacteria which present on meat surfaces (Sebranek et al, ٢٠٠٥ and Gammarielo et al., ٢٠١٤). However, the growth of microorganisms leading to meat

spoilage and reduced shelf-life (**Sharon, ٢٠١٦**). The obtained results indicated that total psychrophilic bacteria of prepared beef burgers was ranged from ٣.٧×١٠^٤ to ٤.٣×١٠^٤ cfu/g. The higher number was found in control sample and the lower was in formula (٥). However, the high level of chickpea flour caused a decrease of total psychrophilic bacteria in both at zero time and during frozen storage period. This decrease of total psychrophilic bacteria as the addition of chickpea flour may be due to the reduction of moisture content of prepared burgers as shown in the previously results in table(٤). During frozen storage period, total psychrophilic bacterial counts of all samples was slightly increased upon storage period until reached between ٥.٣×١٠^٤ and ٦.٥×١٠^٤ cfu/g. The higher addition of chickpea flour, the lower number of psychrophilic bacteria was observed. The same conclusion was found by **Emam, ٢٠٠٣; Mehram, Eman, ٢٠١٧ and Al-Ajmi, Najlaa, (٢٠١٨)**.

The obtained results illustrated that spore forming bacteria of prepared beef burgers was ranged from ٣.٠×١٠^١ to ٤.١×١٠^١ cfu/g. The highest number was found in control sample and the lowest one was in formulae (٥). This decrease of total spore forming bacteria due to the addition of chickpea flour may be due to the reduction of moisture content during processing as shown in the previously results in table (٣). Most of enterobacteria which present in meat come from fecal contamination (**Du et al., ٢٠٠١**). The obtained results also revealed that spore forming bacteria of all prepared beef burgers were deceased during frozen storage period until reached between ٢.٤×١٠^١ and ٣.١×١٠^١ cfu/g. control sample was recorded the highest number of spore forming bacteria, meanwhile formula (٥) was the lowest number. The same trend of results are in agreement with those obtained by **Emam, (٢٠٠٣), Morcy, Hayam, (٢٠١٧) and Mehram, Eman, (٢٠١٧)**.

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The results revealed that total fungi and yeasts was ranged from 2.26×10^2 to 3.41×10^2 cfu/g. Control sample was recorded the highest number of total fungi and yeasts, while formula (٥) was the lowest number. The decrease of total fungi and yeasts as a result of addition chickpea flour could be attributed that the reduced of moisture content as shown in the previously results in table (٣).

During frozen storage, total fungi and yeasts was slightly decreased as the prolonged time proceeded until reached between 7.3×10^1 and 1.12×10^2 cfu/g. The higher levels of chickpeas, the lower number of total fungi and yeasts was observed. The decrease of total fungi and yeasts during frozen storage was confirmed by **Emam, (٢٠٠٣)**; and **Ayed, Najlh, (٢٠١٨)**.

Fungi and yeasts are resistant to freezing, frozen storage and also more tolerant to reduced water activity (**Jay, ١٩٩٦**).

Table (٩): Microbiological changes of prepared beef burgers replaced of beef fat by chickpea flour (CF) during frozen storage (cfu/g)

Formulæ Storage	(١) Control	(٢) % (F)	(٣) % (CF)	(٤) % (CF)	(٥) % (CF)
Total bacterial count					
Zero time	2.97×10^5	2.72×10^5	2.51×10^5	2.30×10^5	2.0×10^5
١- month	1.65×10^5	1.42×10^5	1.3×10^5	1.12×10^5	1.0×10^5
٢- months	9.1×10^4	7.3×10^4	6.5×10^4	6.0×10^4	5.8×10^4
٣- months	7.8×10^4	7.0×10^4	6.3×10^4	6.1×10^4	5.5×10^4
Psychrophilic bacteria					
Zero time	4.3×10^4	4.1×10^4	4.0×10^4	4.0×10^4	3.7×10^4
١- month	5.2×10^4	5.0×10^4	4.5×10^4	4.1×10^4	4.0×10^4
٢- months	5.6×10^4	5.3×10^4	4.9×10^4	4.6×10^4	4.5×10^4
٣- months	6.5×10^4	6.1×10^4	5.7×10^4	5.2×10^4	5.3×10^4
Fungi and Yeasts					
Zero time	4.1×10^1	3.5×10^1	3.2×10^1	3.0×10^1	3.0×10^1
١- month	4.0×10^1	3.6×10^1	3.1×10^1	3.1×10^1	2.8×10^1

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٢- months	٣.٣ x 10 ^١	٣.٢ x 10 ^١	٣.٠ x 10 ^١	٢.٧ x 10 ^١	٢.٥ x 10 ^١
٣- months	٣.١ x 10 ^١	٣.٠ x 10 ^١	٢.٧ x 10 ^١	٢.٤ x 10 ^١	٢.٤ x 10 ^١
Spore forming bacteria					
Zero time	٣.٤١ x 10 ^١	٣.١٢ x 10 ^١	٢.٩٣ x 10 ^١	٢.٦٥ x 10 ^١	٢.٢٦ x 10 ^١
١- month	٣.٢١ x 10 ^١	٢.٨٧ x 10 ^١	٢.٤٥ x 10 ^١	٢.١١ x 10 ^١	٢.٠١ x 10 ^١
٢- months	٢.٥٦ x 10 ^١	٢.٠١ x 10 ^١	٩.٧ x 10 ^١	٨.٥٠ x 10 ^١	٨.٠١ x 10 ^١
٣- months	١.١٢ x 10 ^١	١.٠ x 10 ^١	٧.٩ x 10 ^١	٧.٦ x 10 ^١	٧.٣ x 10 ^١

Sensory evaluation

Generally, all prepared burger formulae were recorded highly acceptable score for color, texture, taste, odor and overall acceptability by the panelists in either at zero time or at the end of storage period. Statistical analysis of the obtained data showed that there were no significant ($P \leq 0.05$) difference among all prepared beef burgers in the score of color, odor and overall acceptability in either after processing or during subsequent frozen storage period. Meanwhile, there were significant difference in both texture and taste.

Table (١٠): Organoleptic evaluation score of burgers substituted of beef fat by chickpea flour (CF) during frozen storage.

Formulae Storage	(١) Control	(٢) % (CF)	(٣) % (CF)	(٤) % (CF)	(٥) % (CF)
Color score					
Zero time	٨.٨	٨.٧	٨.٦	٨.٤	*٨.٢
١- month	٨.٦	٨.٥	٨.٦	٨.٣	*٨.٠
٢- months	٨.٤	٨.٣	٨.٢	٨.٠	*٧.٥
٣- months	٨.٢	٨.٠	٨.١	*٧.٧	*٧.٣
Odor score					
Zero time	٩.٠	٩.١	٩.٠	٩.٠	٩.٢
١- month	٨.٩	٨.٨	٨.٩	٨.٨	٨.٧
٢- months	٨.٧	٨.٥	٨.٥	٨.٦	٨.٦
٣- months	٨.١	٨.١	٨.٢	٨.١	٨.٣
Texture score					

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Zero time	٩.١	٨.٧	٨.٤	*٨.٢	*٧.٧
١- month	٨.٥	٨.٢	٨.٠	*٧.٩	*٧.٤
٢- months	٨.٣	٨.٠	٧.٩	*٧.٤	*٧.٢
٣- months	*٧.٣	*٧.١	*٧.١	*٧.٠	*٦.٨
Overall acceptability score					
Zero time	٨.١	٨.٤	٨.٥	٨.٨	*٩.٠
١- month	٨.٤	٨.٤	٨.٥	٨.٧	٨.٨
٢- months	٧.٨	٨.٣	٨.٤	٨.٥	*٨.٧
٣- months	٧.٦	*٧.٥	*٧.٥	*٧.٤	*٧.٥

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