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Ahfad University for Women
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DEVELOPMENT OF LACTOSE-FREE MILK ANALOGUE BASED ON SUNFLOWER SEED KERNEL

Submitted by:

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Dedication

I dedicate this research outcome to the souls of our founder fathers the late Sheikh Babiker Badri and the late Professor Yousif Badri for placing Women's education above every things else. Also for all those who contributed to the continuity of Ahfad University for Women, and also,

To my mother, the first one who taught me how to be patient,

To my father and all family members who always encourage me, and

To my friends who supported me by all means.

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Abbreviations

AOAC : Association of Official Analytical Chemists.

EDTA : Ethylene Diamine Tetra Acetic Acid.

GLM : Generalized Linear Model.

NPU : Net Protein Utilization.

NSI : Nitrogen Solubility Index.

SAS : Statistical Analysis System.

ABSTRACT

Two types of sunflower seed (rain-fed and irrigated sources) were used in developing lactose free milk analogue based on their kernel. Sunflower seed kernels were examined for macronutrients level and the sunflower kernel milk was also assessed for nutrients level as well as subjective acceptability attributes.

The rain-fed sunflower seed kernel was found to contain significantly ($P \leq 0.05$) lower level of fiber (1.45%) and carbohydrate (4.24%), and higher level of protein (34.05%) and fat (57.65%) compared to the irrigated sunflower kernel (3.34, 12.45, 27.00 and 54.9%, respectively). The highest protein solubility of the rain-fed kernel was found in the filtered liquid at PH8 (97.53%), and of the irrigated kernel in the filtered liquid at PH7 (82.10%).

The proximate composition of sunflower kernel milks (rain-fed and irrigated sources) showed more or less similar level of macronutrient except for the carbohydrate level and calories where they were significantly ($P \leq 0.05$) higher in rain-fed kernel milk (4.98% and 71.43 K. cal/100ml, respectively) compared to the irrigated kernel milk (4.13% and 66.25 k. cal/100ml, respectively). With respect to essential mineral elements, the kernel milk was found to contain higher iron (0.09 – 0.12 mg / 100ml) and magnesium (24.00 to 48.00 mg /100 ml) compared to both levels in cow milk.

In acceptability testing of lactose free kernel milk against a control commercial lactose-free milk, sunflower milk sources, the irrigated kernel milk was judged significantly ($P \leq 0.05$) better in flavor, taste, after taste and overall quality while the commercial one was superior ($P \leq 0.05$) only in color and appearance. When the kernel milk was flavored, strawberry flavor was found significantly ($P \leq 0.05$) superior compared to other flavors used.

ملخص البحث

استخدم نوعان من بذور زهرة الشمس (مصادر مطرية ومروية) لتطوير مشابه الحليب خالي من اللاكتوز من لب هكذا بذور. تم فحص لب بذور زهرة الشمس لمستوى العناصر الغذائية الكبرى، كما تم فحص حليب لب البذور لمستوى العناصر الغذائية وعناصر القبول الحسية.

وجد أن لب بذور زهرة الشمس المطري يحتوي معنويا ($P\leq0.05$) على مستويات ألياف (١.٤٥%) ونشويات (٤.٢٤%) أقل ومستويات بروتين (٣٤.٠٥%) ودهن (٥٧.٦٥%) أعلى مقارنة بلب بذور زهرة الشمس المروية (٣.٣٤، ١٢.٤٥، ٢٧.٠٠ و ٥٤.٩%، على التوالي). وجد أيضا أن أعلى نسبة لذوبان البروتين في لب البذور المطرية بعد عملية ترشيح السائل على رقم هيدروجيني ٨ (٩٧.٥٣%) وفي لب البذور المروية بعد عملية ترشيح السائل على رقم هيدروجيني ٧ (٨٢.١٠%).

بالنسبة للعناصر الغذائية الكبرى في حليب اللب فقد اظهر التركيب الكيميائي له تشابه مستويات معظم العناصر ماعدا النشويات والطاقة والتي كانت أعلى معنويا ($P\leq0.05$) في حليب لب البذور المطرية (٤.٩٨% و ٧١.٤٣ كيلو سعر/١٠٠مل حليب، على التوالي) مقارنة بحليب لب البذور المروية (٤.١٣% و ٦٦.٢٥ كيلو سعر/١٠٠مل حليب، على التوالي). أما بالنسبة إلى العناصر المعدنية الموجودة في الحليب فقد أظهرت النتائج بان حليب زهرة الشمس يحتوي على نسبة أعلى من الحديد (٠.٠٩-٠.١٢ مليجرام /١٠٠ مل) والمغنيسيوم (٢٤.٠٠-٤٨.٠٠ مليجرام/١٠٠ مل) من الموجود في حليب الأبقار.

عند إجراء اختبار القبول لحليب لب بذور زهرة الشمس الخالي من اللاكتوز مع الشاهد من الحليب التجاري الخالي من اللاكتوز وجد تميز الحليب المصنوع من لب البذور المروية من حيث النكهة والطعم وإحساس ما بعد الطعم والقبول الكلي للمنتج بينما تميز الحليب التجاري فقط في اللون والمظهر. وعند إضافة بعض المنكهات لحليب لب زهرة الشمس وجد ان نكهة الفراولة هي المميزة معنوياً ($P\leq0.05$) مقارنة بالنكهات الأخرى المستخدمة.

1- INTRODUCTION

Sugars and proteins of cow milk are the most frequent cause of food allergy in infants (Ah-Leung et al., 2007). Hypersensitivity to these compounds may persist through adulthood and can be severe. Studies on large populations of allergic patients showed that most of the patients were sensitized to lactoglobulin, casein, lactalbumin and bovine serum albumin (Kaiser et al., 1990; Host et al., 1992; Wal et al., 1995). Different clinical symptoms of the milk protein allergy have been established (El-Algamy, 2007). Data on prevalence of the milk protein allergy differ (depending on the country), while about 1% of the general adult population or 2–3% of children being considered as approximate figures. Lactose intolerance is neither an allergic nor an immune-mediated disease. It results from a reduced capacity to digest lactose which may affect the quality of diet, e.g. low calcium intake. The maldigestion of lactose is due to a reduced lactase activity in the small intestine. Lactose intolerance is very common among Asian, South American, and African people. Of the world's population, 75% is estimated to be lactase-deficient, with the most common form primarily affecting adults. Lactase activity naturally falls from infantile level to adult levels between the age of 3 and 5 years in 75% of the world's population, while 25% of the population appears to maintain infantile levels of lactase in adulthood (Scrimshaw and Murray, 1988).

There is no unambiguous relation between milk protein allergenicity and its heat processing: Boiling milk for a few minutes (2.5 or 10 minutes) results either in no difference or in a reduction of about 50–66 % of the positive reactions as compared to raw milk. This situation led to an effort to find new ways of food production in order to offer suitable foods to patients suffering from milk protein allergy or lactose intolerance, and whose choice of food is restricted. One possibility is to use vegetable raw materials (e.g. oilseeds, cereals….etc) in the production of dairy-like foods, (e.g. sunflower, soy, rice and groundnut).

Sohn and Sohn (1996) stated that, there are several suggested alternatives to cow's milk such as sunflower seed kernel milk, soybean milk, rice milk and nuts milk. Sunflower milk is relatively low in protein unless the seed is treated with papain (Ferber and Cooke, 1979) or the PH is increased (Hagenmaier, 1974). The color of sunflower milk tends to be dark because of the presence of chlorogenic acid, and it has more tendencies to cream than soymilk. Some work is needed in this line.

1.2 Justification

Many people suffer from taking lactose containing foods particularly mammalian milks. Such phenomenon is characterized by uncomfortable symptoms such as gas, cramping, bloating and diarrhea. Others with true milk allergies can't drink cow's milk at all, so they turn to plant-based milks to get their nutrition. They can use milk alternative which are also called non-dairy beverage prepared from oil seeds such as sunflower seed kernel, groundnut or soybean.

1.3 Objectives of study:

1.3.1 General objective

To study the suitability of irrigated and rain-fed sunflower seed kernel for making lactose free milk intended for lactose intolerant human subjects.

1.3.2 Specific objective

Developing commercial lactose free milk based on vegetable raw material.

2- LITERATURE REVIEW

2.1 Mammals milk

Milk is defined as the secretion of the mammary glands, its primary natural function being nutrition of the young. Milk of some animals, especially cows, buffaloes, goats and sheep is also used for human consumption , either as such or in the form of a range of dairy products (Walstra *et al.,* 2006)

2.1.1 Milk general composition

The biological reason for mammals producing milk is to supply the offspring with nutrients. In order to meet the nutrient requirements of their particular offspring, the composition of milk varies between different mammals. Table 1 shows the average composition of milk from different species that is used in human consumption.

Table 2.1. Average composition of milk from different species

Species	**% Component**					
	Water	**Fat**	**Casein**	**Whey protein**	**CHO (Lactose)**	**Ash**
Human	87.1	4.5	0.4	0.5	7.1	0.2
Cow (*Bos taurus*)	87.3	3.9	2.6	0.6	4.6	0.7
Zebu (*Bos indicus*)	86.5	4.7	2.6	0.6	4.9	0.7
Sheep	82.0	7.2	3.9	0.7	4.8	0.9
Goat	86.7	4.5	2.6	0.6	4.3	0.8
Camel	86.6	4.5	2.7	0.9	4.5	0.8
Buffalo (*Bubalus bubalis)*	82.5	7.5	3.6	0.7	4.8	0.8
Horse	88.8	1.9	1.3	1.2	6.2	0.5

Adapted from Walstra *et al*. (1999) and Akers (2002).

The principal chemical components or groups of chemical components are those present in the largest quantities. Of course, the quantity (in grams) is not paramount in all respects. For example, vitamins are important with respect to nutritive value; enzymes are catalysts of reactions; and some minor components contribute markedly to the taste of milk (Walstra *et al*., 2006).

2.1.1.1 Fat

Akers (2002) stated that the main component in milk fat is triglycerides, which consist of three fatty acids attached with ester bonds to a glycerol molecule. These fatty acids either originate from the cow's diet or from de novo synthesis in the epithelial cells. The fat content in milk usually ranges between 3.8 and 4.9%.

2.1.1.2 Proteins

Milk proteins are synthesized from amino acids derived either from blood or synthesis in the epithelial cells. The mammary specific proteins consist mainly of caseins (80%) whey proteins (20%). The total protein content ranges between 3.0 and 3.6% (Vickie *et al.*, 2003)

2.1.1.3 Lactose

Sjaastad *et al.* (2003) reported that the most common carbohydrate in milk is lactose, which is specific to mammalian milk. The lactose content of milk is usually in the range 4.6-4.8%. Akers (2002) concluded that lactose is synthesized in the Golgi apparatus and consists of galactose and glucose linked together. The enzyme lactose synthase, which is essential for lactose synthesis, is derived from galactosyl-transferase and α-lactalbumin..

2.1.1.4 Minor components in milk:

In addition to the main components of milk (water, fat, protein and lactose), there are many other compounds present in milk (Walstra *et al., 1999)*. Milk contains numerous different components with low molecular weight such as calcium, potassium, sodium, chloride, magnesium, zinc, phosphates and citric acid. Milk is a major source of vitamin A and vitamin B in particular, but all vitamins are present in milk, although some in small concentrations e.g. vitamin C (Walstra & Jenness, 1984).

Milk contains many different types of enzymes, both native enzymes excreted by the mammary gland and enzymes of microbiological origin. The function of many of the enzymes in milk is not yet fully known, but some have antimicrobial function. Lactoperoxidase and lysozyme are examples of enzymes acting against bacteria (Walstra *et al*., 1999).

2.1.2 Nutritional value of milk:

Milk is an excellent source of major nutrients essential for human development including: proteins, carbohydrate, fat, minerals and vitamins especially A, D, E and K. Milk protein is rich in essential amino acids making milk and milk products very important constituents of the human diet. Carbohydrate in milk is in the form of lactose, which is difficult to digest in lactose intolerance individuals. Lactose intolerant individuals, especially young children cannot enjoy the nutritional the nutritional benefits of consuming milk. Clinical signs of lactose intolerance include bloating and gas production as a result of lactose breakdown of undigested lactose by bacteria in the lower portion of the gastrointestinal (GI) tract. Consumption of fermented milk has been shown to suppress clinical signs of lactose intolerance with enhanced nutritional and health benefits (Branca and Rossi, 2002).

2.2 Vegetable milk

The production of vegetable ‘‘milk” using legumes and oil seeds is an old technology that dates back to the 13th century (Smith and Circle, 1997). In time the technology has been improved to include development of vegetable alternatives to dairy milk especially in the formulation of infant foods because they are high in protein, minerals and vitamins. The major legumes that have been used in vegetable milk production include soybean, cowpea, winged bean, groundnut and melon seeds or ‘‘agushie” (Nelson *et al.,* 1976; Senayah, 1993).

The term "milk" in relation with "sunflower milk", "soy milk", "oat milk", "rice milk", "almond milk" or other plant based "milks" is legally and scientifically debated. Thus, especially in Europe, the term "milk" is commonly replaced by the term "drink". It can be argued that the term "milk" should only be used for soy based milk alternatives if enriched with the essential amino acid methionine as well as iron, zinc, calcium and vitamins (Van Winckel *et al*., 2011).

Current renewed interest in vegetable milk and products made using it is because of the growing awareness of the nutritional benefits of plant-based foods by health conscious consumers (Diarra *et al.*, 2005). Consequently sunflower, soy and peanut milk have been used in a variety of milk-based products including coffee creamers (Mulando and Resurreccion, 2006), and chocolate milk drink (Deshpande *et al.*, 2008). Legumes and oil seeds have characteristics that make it convenient to combine two or more to obtain an acceptable product. In particular, peanut milk is known to be high in energy, whereas cowpea milk on its own has low energy (Akinyele and Abudu, 1990). Combining the two effectively removed these limitations (Asiamah, 2005). Furthermore, it has been demonstrated that cowpea milk has a strong beany flavour while peanut–cowpea composite blends have minimal beany flavour (Nadutey, 1999). A composite product made using peanuts and cowpeas as sources of nutrients such as protein, dietary fibre, folate and other vitamins and minerals would be an ideal dairy milk substitute. Vegetable milk made from peanut and cowpea blends could be dehydrated to produce an inexpensive dry milk powder. Indeed Chandrasekhara *et al.* (1964) reported spray drying of peanut milk combined with other sources of milk, and the powder could be reconstituted in water.

2.2.1 Alternatives to dairy Products

Non-dairy milk drinks are mostly based on soy, but often almonds, coconut milk, oat or rice are used as base materials. Nowadays, a wide range of yoghurts, desserts, creams, sauces, cheeses, ice-cream and other products are often made from soya. Non-dairy cheese can also be based on tapioca or arrowroot flour for example (Bachmann 2001).

2.2.1.1 Soymilk

Sirtori *et al*.(1993) reported that soybeans are known to have beneficial health effects, and consumption in the western world has been rising for the past few decades. The consumption of soy foods has been linked to the prevention and treatment of chronic diseases, potentially lowering cancer mortality rates, and reducing the risk of heart disease due to the cholesterol lowering effect of soy proteins (Messina *et al*., 1994; Kennedy, 1995).

Currently the main types of soy products relatively high in soy protein and which utilize the majority of the bean are soymilk and tofu. Although these products have been manufactured for centuries in East and South Eastern Asia, the physicochemical nature of soymilk particles, their colloidal behaviour and their interactions in soymilk are not well understood. The use of soymilk as a base ingredient in other foods has great potential, due the high nutritional value of soymilk and its relatively simple production process (Liu, 1997).

The production of soymilk consists of grinding soaked beans with water, removing some of the insoluble fiber, heat treating the slurry, and homogenizing the mix, not necessarily in this exact order. The final composition of soymilk is around 8-10% total solid, including 3.6% protein, 2% fat, 2.9% carbohydrates, and 0.5% ash, and it depends on processing conditions and soybean variety used (Liu, 1997). Heat treatment of soymilk is necessary for several reasons: to achieve microbial safety, enhance shelf-life and colloidal stability, enhance flavor and nutritional value by denaturing trypsin inhibitors, haemagglutinins, saponins and other antinutritional compounds (Rackis, 1974; Kwok and Niranjan, 1995; Iwuoha and Umunnakwe, 1997; Kwok *et al*., 2002).

Soymilk provides a plentiful and inexpensive supply of protein and calories. It is considered as a suitable economical substitute for cow's milk and an ideal nutritional supplement for lactose-intolerant population. Additionally, soybean is a rich source of isoflavone, which are reported to have beneficial estrogenic effects with potential bioactive antioxidant properties. Asian populations, with their intake of soy derived isoflavones, are known to have the lowest incidence of osteoporosis, menopausal symptoms, mortality from cardiovascular disease, and cancer (Rekha and Vijayalakshmi, 2008). Jacobsen *et al*. (1998) reported that frequent consumption (more than once a day) of soymilk was associated with 70 % reduction of the risk of prostate cancer. The association was upheld when extensive adjustments were performed. Furthermore, the possibility that phytoestrogens (e.g., daidzein, genistein) may offer a natural alternative to conventional hormone replacement therapy (HRT) for the prevention of bone loss due to estrogen deficiency associated with loss of ovarian function during the menopause (Setchell and Cassidy, 1999). Soy protein is correlated with significant decreases in serum cholesterol and the risk of heart disease. There has been suggested by Carroll *et al*. (1995) that the presence of factors in soy protein can counteract the effects of hypercholesterolemic amino acids. Substitution of soy protein for animal protein in the diet reduced the concentration of serum cholesterol in humans. The difference effects of dietary proteins on serum cholesterol concentrations in humans and in rabbits are primarily due to changes in LDL cholesterol, and the hypercholesterolemia produced by dietary casein which associated with down-regulation of hepatic LDL receptors. Furthermore, phytic acid content in soybean has also been associated with reduced risk of cancer, reducing inflammation and minimizing diabetes (Vucenik and Shamsuddin, 2003; Yoon *et al*., 1983; Sudheer *et al.,* 2004). However, some evidences have been reported that taking soy products associated with health risks and phytic acid-containing soybeans is also criticized for reduction of mineral availability.

2.2.1.2 Peanut milk

Peanut (*Arachis hypogaea L*) is a major source of edible oil and protein meal and therefore considered to be highly valuable in human and animal nutrition. Peanut is also a good source of antioxidant, such as p-coumaric acid, that may be contributing factors to potential health benefits of their consumption (Talcot *et al.,* 2005, Duncan *et al.,* 2006). Since peanut has such potential health benefits, therefore its consumption especially in the developing countries, should be increased. It is necessary to develop of peanut processing into other useful and edible products.

It has been well known that peanut milk and peanut milk products have nutritional benefits because of their extreme richness in protein, minerals and essential fatty acids such as linoleic and oleic acids, which are considered to be highly valuable in human nutrition. It is extensively used in India and other developing countries by vegetarians and more recently by children allergic to cow milk proteins (Kouane *et al,* 2005). Peanut milk is the water extract of peanut that is an inexpensive source of protein and calories for human consumption. Such as soymilk, it is seen as a low-cost substitute for dairy milk for the developing countries. Being free of cholesterol and lactose, peanut milk is also a suitable food for lactose-intolerant consumers, vegetarians and milk-allergy patients.

Peanut milk may be produced by soaking and grinding full-fat raw peanuts with water to get a slurry, subject to filtration Many ways of producing peanut milk have been done by various researchers (Beuchat and Nail, 1978, Bucker *et al*., 1979, Isanga and Zhang, 2009). The variation in peanut to water ratio used for peanut milk extractionan affects the peanut milk composition. However, in all cases, this low cost milk has high protein content.

The current interest in peanut milk and peanut milk products is motivated by the fact that dairy and dairy products are always priced too high for the low income earness. Another factor, no less important, is the growing awareness of the nutritional benefits of vegetable proteins in low cholesterol diets by health conscious people (Kouane *et al*., 2005).

Fermentation of peanut milk may serve as one possibility that can increase the consumption of this valuable crop, and hence improve protein availability and consumption (Sunny-Roberts *et al*, 2004). Several researchers have studied the fermentation of peanut milk by lactic acid bacteria in laboratory scale (Beuchat and Nail, 1978, Bucker *et al.,* 1979, Lee and Beuchat., 1991, Sunny-Roberts *et al.,* 2004, and Isanga and Zhang, 2009). The growth of lactic acid bacteria in peanut milk depends on a number of factors such as strain of lactic acid bacteria, availability of nutrition, and fermentation temperature and time.

2.2.2 Sunflower seed in Sudan

Skoric (1992) reported that the sunflower (*Helanthus annus L.*) is one of the major oil seed crops. Although it originated in United State, its utilization has been predominantly European. In recent year, sunflower has become an established crop in many countries throughout the world including Sudan (Mohamed, 2010). Result from experimental trails and large-plot trails indicate that satisfactory yields (0.5-0.8 metric tons per feddan) can be obtained from several varieties and hybrids of sunflower grown on the central clay plains of the Sudan. Further study revealed that sunflower could be grown successfully in a large area with 600-800 mm total annual rainfall on the chromic vertisols. Biological properties of sunflower and its climatic and soil requirements indicate that, Damazin, Gedaref, Renk and Kadugli are suitable areas for rain-fed cultivation. Blue Nile, White Nile, Suki and Schemes are regions potentially favourable for sunflower growing with supplementary irrigation. Sunflower seed may be dehulled and eaten without processing. The dehulled seed may be roasted in oil and salted or used as nutmeats and in candy salads and bakery goods. The seed may also be salted in the shell and eaten directly as in case of peanut (Ibrahim, 2001).

2.2.3 Composition of sunflower seed kernel

Grompone (2005) found 55% oil in sunflower kernel and around 40% in seeds, whereas Canibe *et al*. (1999), studying different sunflower genotypes during the period 2004/2005, reported that their oil contents ranged from 29.5 to 50.2%, depending on the region and agricultural conditions.

Crude protein content of whole and dehulled seeds varied from 15 to 20%. Protein and oil contents of dehulled samples were higher than those of whole seeds because the fraction of hulls varied from 16 to 45% of the seed weight and it was mainly built of cellulose and lignin with low levels of oil and proteins (Grompone, 2005). Crude fiber contents in dehulled samples of the range between 10.28 and 11.25 % , and a small reduction of fiber was probably due to an incomplete dehulling.

Hull contents in varieties were 41.4 and 40.5%. Hartman et al. (1999) found that the hull content of Brazilian sunflower varieties ranged from 39.3 to 57.5%. Pedrosa *et al*. (2000) reported the hull content in Spanish sunflower varieties varying from 20.26 to 23.75% and Singh *et al*. (1999) found the hull content in Indian sunflower varieties varying from 22 to 28%.

Moisture of samples was below 10%, within the limit described by Grompone (2005) as necessary to avoid enzymatic reactions. The crude protein content of defatted sunflower seeds varied from 28.4 to 36.2%, while the fiber content ranged from 15 to 27%, depending on the method.

The nutritional values of defatted sunflower seeds reported by Stringhini *et al*. (2000) for protein, detergent acid and neutral detergent fiber were 27.36, 31.68 and

42.15%, respectively. These values seem to be related to dehulled seeds. Furlan *et al*. (2001) reported protein and crude fiber contents of 34.0 and 21.7%, respectively. Singh *et al*. (1999) reported values of acid detergent fiber and lignin of defatted sunflower meal (with hull) varying from 27 to 32% and from 9 to 13.6%, respectively.

The results of fiber content found in literature vary according to the processing method but also to differences in the analytical procedure, which also depends on the analytical conditions at hydrolysis. For example, acid detergent-sensitive fiber measures lignin and cellulose whereas detergent-neutral fiber measures also hemicelluloses content (Van Soest, 1967).

2.2.4 Nutritional value of sunflower seed kernel

WHF (2013) concluded that the sunflower seeds kernel are the richest natural food source of vitamin E (31-35mg). A regular intake of sunflower will promote protection from ageing, free radicals and skin cell damage, as vitamin E is a powerful antioxidant. Also the sunflower kernels are low in saturated fat (5g) and a good source of monounsaturated (9.5g) and polyunsaturated (33g), mainly in the form of omega-6, (30g) with a trace of omega-3. The protein content of sunflower seeds is complete in all essential amino acids and they supply 23% protein and are the 10^{th} best protein food with 58% use able protein (NPU). The supply of minerals, especially magnesium (354mg), is very good with the copper value (1.8 mg) also abundant and vital for blood development, skin healing, nerve fiber protection and cartilage repair. For a big natural vitamin B_1 boost, sunflower seeds provide 2.3 mg plus B_2 (0.3 mg) and B_3(4.5mg).

Koch (2011) stated that the supply of phosphorus in sunflower kernel (700 mg) is most beneficial for the brain, nerve and bones, and in combination with the abundant supply of silicon (554 mg) also essential for the brain, nerve and bone. The supply of calcium (354 mg) is good and the supply of potassium (700-900 mg) all added up to promote strong muscular action and proper digestion. The selenium content of the kernel is very good at 59 mcg (70-80 mcg is the daily requirement). This combined with the exceptional vitamin E content makes the sunflower seed a potent antioxidant. Selenium works with vitamin E to protect against free radicals and promote DNA repair and also to induce apoptosis, or the self-destruction of cancerous cells. Sunflower kernels are an excellent source of zinc (5mg), essential to fight infections and for body healing. Also, sunflower seeds are ideal for the reproductive system in combination with the abundant vitamin E content. The supply of manganese (2 mg) and iron (7 mg) are further proof that the sunflower is the brightest supplier of surprising sun-filled health benefits (WHF, 2013).

2.2.5 Sunflower seed protein

Anisimova *et al.,* 2002 reported that the sunflower meals are a rich source of highly soluble protein. An important advantage of sunflower protein products is that,, to date, no toxic component has been reported. Sunflower protein products are deficientin lysine and isoleucine. However, they are rich in other essential amino acids, especially methionine and cystine. Sunflower protein concentrate and isolate, which employ alkaline treatment, give a very dark color due to oxidation of chlorogenic acid.

Saeed and Cheryan (1988) stated that the sunflower proteins possess poor water solubility; they were reported to be 15-30% soluble between pH3 and 6. There was no sharp minimum solubility at the isoelectric point and a broad range minimum solubility was found between pH 3 and 6. Good protein extractability (over 90%) was observed at pH 10.

Differences in protein solubility between sunflower albumin, globulin were reported, and a contribution of the individual fractions to the variations of the functional properities was found. The albumin fraction was almost completely soluble above pH7 and exhibited high solubility at acids pH's with minimum solubility of 50% at pH 5.0. The protein solubility if albumin fraction was about 70% higher at the acidic end of the curve and in the neutral pH regions compared to sunflower. The globulin fraction was completely soluble in the neutral pH range, however , in the acid pH region, solubility of globulin fraction was markedly lower than albumin (Canella *et al.,* 1985).

Nitrogen solubility profiles of sunflower protein in water and salt solutions were similar. Sunflower exhibited a U-shaped pH-solubility profile with minimum solubility at pH 5 (Rossi and Germondari 1982). The sunflower protein solubility considerably increased in 1.0M NaCl and 0.5M $CaCl_2$ in the range 4 to 7 with an increase in NSI to 76% in 0.5M $CaCl_2$ and 55% in 1.0M NaCl. These pH conditions are present in several food products. The increase in NSI was observed up to 0.75M NaCl, and there was no NSI increase at higher concentrations. High solubility in salt solutions is important for the practical use of these proteins.

2.2.6 Sunsational sunflower non dairy beverage

Sunsational is a delicious non dairy beverage made of lightly roasted sunflower kernels grown in the USA. (Appendix I), the sunflower beverage delivers a rich, creamy taste packed with nutrition. Sunsational is high in fiber (4%), carbohydrates (10%), sugars (6%) and low in protein (2%) and calories (80kcal) and high in vitamin E and A, and antioxidants, low in calorie, soy free, gluten free, tree nut free and free of lactose. The colour of product is off white due to presence of antioxidants (phenolic acid and chlorogenic acid) (http://sunsationalnondairy.com/).

The root of *Sunsational Sunflower Non Dairy Beverage* go back to 2007. By May of 2009, an actual formula for the seed milk was established. The basic concept of how to make these alternative beverages, bunch of different types of bulk seeds along with other ingredients spread out all across the counter, then fired up the blender, broke out the cheesecloth and started making seed milk (http://sunsationalnondairy.com/).

3- MATERIALS AND METHODS

3.1 Materials

Two samples of sunflower seed, irrigated and rain-fed, were purchased from Sennar and Sudanese Arab Company for Vegetable Oils, respectively. The chemical reagents used were donated by Ahfad University for Women, Food Science and Technology Lab. The flavors used were purchased from Al-Tasamh Market in Al-Maomaora Street.

3.2 Methods

3.2.1 Preparation of sunflower seed kernel

Sunflower seeds were cleaned and freed from foreign matter and broken seeds. The sunflower seeds hulls were separated manually by hands. Pure sunflower kernels were then stored at room temperature (30 ± 5 ^{0}C) until use.

3.2.2 Preparation of sunflower seed kernel milk

Fig. 1 illustrates the flow diagram showing steps involved in preparation of the sunflower seed kernel milk. Such steps briefly involved seed dehulling (kernel separation), kernel grinding and blending in water, clarification of milk using two methods (centrifugation or filtration), then thermal treatment of the kernel milk (boiling at 90 ^{0}C for 10 minutes) before cooling the end product and keeping it refrigerated for different uses (e.g. analysis, flavouring…..etc.).

Clean sunflower seed

↓ Dehulling (manual)

Sunflower seed kernel

↓ Grinding (mixer)

Ground kernel

↓ Blending in water (30gm/100ml water)

Sunflower seed kernel milk

↓ Filtration using cheesecloth

Clear kernel milk

↓ **Addition of flavor**

↓ Heat treatment (boiling round 90 ^{0}C for 10 minutes)

Ready to use kernel milk with flavor

↓ Heat treatment (boiling round 90 ^{0}C for 10 minutes)

Ready to use kernel milk without flavor

Fig 1: Flow diagram showing sunflower seed kernel milk process with and without flavors

3.2.3 Physiochemical characteristics

3.2.3.1. Proximate analysis of sunflower seed kernel and milk

3.2.3.1.1 Determination of moisture content in sunflower seed kernel

Moisture in sunflower seed kernel was determined according to the AOAC method (2000). Two grams of sunflower seed kernel powder were weighed accurately in clean preheated moisture dish of known weight by using sensitive balance. The uncovered sample and dish were kept in an oven at 103 ± 2 ^{0}C and let to stay for five hours. The dish was covered and transferred to a desiccators and weighed after reaching room temperature. The loss of weight was calculated as percent of sample weight and expressed as moisture content.

<u>Calculation</u>

$$\text{Moisture content (\%)} = \frac{Wt1 - Wt2}{\text{sample } Wt} \times 100$$

Where:

Wt1 = weight (g) of sample + dish before oven drying

Wt2 = weight (g) of sample + dish after oven drying

3.2.3.1.2 Determination of ash content in sunflower seed kernel and milk

Total ash in sunflower seed kernel and milk was determined according to the AOAC method (2000). Two grams of sunflower seed kernel powder and 5 ml of sunflower seed kernel milk were weighed in empty crucible for known weight. The sample was heated in a Muffle-Furnace at 550 ^{0}C until its weight is constant. The ash residue was kept in a desiccators at room temperature then re-weighed. The process was repeated until constant weight was obtained.

% Ash content was calculated using the following equation:

$$\text{Ash content (\%)} = \frac{Wt1 - Wt2}{\text{sample } Wt} \times 100$$

Where:

Wt1 = weight (g) of sample with ashed sample

Wt2 = weight (g) of empty crucible

3.2.3.1.3 Determination of crude protein in sunflower seed kernel and milk

The determination of crude protein in sunflower seed kernel and milk was carried out according to the AOAC (2000) methods. A 0.2 gram of kernel or 2 ml of milk, plus 0.4 gram of catalyst mixture (potassium sulfate + cupric sulfate 10:1 by wt), and 7 ml concentrated nitrogen free sulphuric acid, were mixed in a kejeldahl flask (100 ml). The mixture was digested until it became clear, then cooled, diluted, and placed in the distillation apparatus. Fifteen milliliters of 40% NaOH solution were added and the mixture was heated and distilled until 50 ml were collected in a 100 ml conical flask. The ammonia evolved was received in 10 ml of 2% boric acid solution plus 3-4 drops of universal indicators (methyl red and bromo cresol green). The trapped ammonia was titrated against 0.02N HCl. The percentage (g/100g or ml) of protein was calculated by using an empirical factor to convert nitrogen into protein as follows:

$$\%\ \text{Nitrogen content} = \frac{TV \times N \times 14.00 \times 100}{1000 \times \text{Wt of sample}}$$

$$\%\ \text{Protein content} = \%\ \text{nitrogen content} \times 6.25$$

Where:

TV= Actual volume of HCl used for titration (ml HCl – ml blank)

N= Normality of HCl

14.00= Nitrogen factor

1000= to convert from mg to gram

6.25= Constant factor for sorghum and legumes

3.2.3.1.4 Determination of fat content in sunflower seed kernel

Fat content in sunflower seed kernel was determined using the method of the AOAC (2000). A dry empty extraction flask was weighed, about 2 g of sample was weighed and placed in a filter paper, then placed in extraction thimble free from fat and covered with cotton wool. The thimble was placed in an extractor.

Extraction was carried out for 6 hours with petroleum ether, after that the apparatus was carefully dismantle and the solvent was evaporated to dryness in an air-oven. The flask with extracted fat was cooled and weighed.

Calculation

$$\%Fat = \frac{W2 - W1}{Wt} \times 100$$

Where:

W1 = weight (g) of extraction flask

W2 = weight (g) of extraction flask with oil

W3 = weight (g) of sample

3.2.3.1.5 Determination of fat content in sunflower seed kernel milk

The fat content in sunflower seed kernel milk was determined by the Gerber method (AOAC, 2000). Then ml of sulphuric acid (density 1.815 gm/ml) was poured carefully into a clear dry Gerber tubes, 10.29 ml of sunflower seed kernel milk were added to the sulphuric acid, and then 1 ml of amyl alcohol was added to the tube followed by addition of distilled water, the contents in the tubes were thoroughly mixed till no white particles were seen. The tubes were centrifuged at 1100 rpm for 5 minutes. The butyrometeric were then placed in a water bath at 70 ^{0}C for about 4 minutes. The fat column separated was read and taken as percent fat in sunflower seed kernel milk

3.2.3.1.6 Determination of fiber content in sunflower seed kernel

The fiber content was determined using method of AOAC (2000). The two grams of an air dried fat-free sunflower kernel, were transferred to dry 600 ml beaker. The sample was digested with 200 ml of 1.25 % (0.26 N) sulphuric acid for 30 minutes, and the beaker was periodically swirled. The contents were removed and filtered through Buchner funnel, and washed with boiling water. The digestion was repeated using 200 ml of 1.25 % (0.23 N) NaOH for 30 minutes, and treated similarly as mentioned earlier. After the last washing the residue was transferred to ashing dish, and dried in an oven at 105 ^{0}C overnight then cooled and weighed. The dried residue was ignited in a muffle furnace at 550 ^{0}C to a constant weight, and allowed to cool, and then reweighed.

Calculation

$$\%Fiber\ content = \frac{(W2 - W1)}{W3} \times 100$$

Where:

W1 = weight (g) of crucible

W2 = weight (g) of crucible with ashed sample

W3 = weight (g) of the original sample

3.2.3.1.7 Determination of carbohydrates in sunflower seed kernel and milk

Carbohydrates were calculated by difference according to AOAC (2000).

Carbohydrates without fiber = 100 – (Ash% + moisture% + crude protein% + oil %)

3.2.3.2 Determination of total solids in sunflower kernel milk

Total solids of kernel milk were determined by the AOAC method (2000). Five grams of milk sample were placed into a clean dried flat-bottom dishes, heated in a steam bath for 10-15 minutes and transferred to an air oven for 3 hours at 103±2C. The dishes after drying were placed in desecrator to cool and reweighed. The total solids content per 100 ml of milk were calculated for the following equation:

$$\%TS = \frac{W1}{W2} \times 100$$

Where:

W1: weight of sample after drying

W2: weight of sample before drying

3.2.3.3 Nitrogen solubility of kernel protein at different pH values

Nitrogen solubility of both rain-fed and irrigated sunflower kernels were determined at different pH values (5, 6, 7 and 8) by the procedure of Hagenmaier (1972), modified by Quinn and Beuchat (1975) with a slight modification. Twenty grams material were suspended in 500 ml distilled water and blending for 15 minutes before the desired pH was maintained by addition of 1N HCl or 1N (clarification procedure). The suspension was shaken for another 45 minutes at room temperature, and readjustment of pH, part of sample centrifuged at 3000 rpm for 20 minutes at room temperature and other part not centrifuged, then soluble nitrogen in the supernatant was estimated by the micro-kjeldahl method. Percent protein extracted was calculated with reference to the total amount of protein in the sample.

Calculation

$$\text{Soluble protein} = \frac{T \times N \times Tv \times 14 \times 6.25 \times 100}{a \times b \times 1000}$$

Where:

T = Titer reading

N = Normality of acid (0.02 N)

Tv = Total volume of aliquot extracted

14 = each ml of hydrochloric acid is equivalent to 14 mg nitrogen

a = volume of ml of aliquot taken for digestion

b = weight of (gm) of sample of sunflower kernel extracted

1000 = conversion of mg into gm

6.25 = Protein factor

$$\text{\% Solubility} = \frac{\text{Soluble protein} \times 100}{\text{Crude protein of the sample}}$$

3.2.3.4 Determination of mineral matter in sunflower kernel milk

Minerals in kernel milk were extracted from the samples by dry ashing method that was described by Chapman and Pratt (1982). The amount of iron was determined using atomic absorption spectroscopy (Perkin-Elmer 2380). The calcium and magnesium were determined by titration. Ammonium vandate was used to determine phosphorus along with ammonium molybdate method of Chapman and Pratt (1982). Potassium content was determined by flame photometer (CORNIGEEL) according to AOAC (2000).

3.2.3.4.1 Determination of Potassium content in milk

Potassium content of extracted sunflower milk was determined according to AOAC (2000) using flame photometer (corning 400). One milliliter of the extract was taken and diluted in a 50 ml conical flask with distilled water. The standard solutions of the KCL and NaCl were prepared by dissolving 2.54, 3.33 g of KCl and NaCl, respectively. Ten ml of this solution were taken and diluted with 1000 ml distilled water to give a 10 ppm concentration. The flame photometer was adjusted to zero using distilled water as a blank and to 100 using standard solution.

$$\%K = \frac{[F.R \times D.F \times 100]}{10^3 \times S \times 10}$$

Where:

F.R = Flame photometer reading

D.F = Dilution factor

S = Sample weight

3.2.3.4.2 Determination of phosphorous content in milk

The determination of phosphorus content was carried according to the method of Chapman and Pratt (1982). Two milliliters of the extract were pippetted into a 50 ml volumetric flask. Ten milliliters of ammonium molybdate-ammonium vanadate reagents (22.5 g of $WH_4)_6$ MO_7O_{24} $4H_2O$ in 400 ml distilled water + 1.25 g ammonium vanadate in 300 ml boiling water + 250 ml conc. HNO_3, then diluted to volume. The density of the color was read after 30 minutes at 470 nm using a spectrophotometer (Corning, 259). A standard curve of different KH2PO4 concentration was plotted to calculate the ion phosphorus concentration.

$$\text{Phosphorus \%} = \frac{\text{Ash dilution} \times \text{Reading curve}}{\text{Oven dry weight of sample} \times 10^3} \times 100$$

3.2.3.4.3 Determination of calcium and magnesium content in milk

Calcium and magnesium in kernel milk were carried out according to the method of Pearson (1976) using titration procedure. Calcium and magnesium were obtained together by taking 2 ml of the extract in 50 ml flask. 20 ml of distilled water, 10 drops of buffer, and 3 drops of erochrome black were added to the extract giving a brown color. The mixture was then titrated against 0.01 N EDTA (Ethylene Diamine Tetra Acetic acid) until a blue color was given indicating the end point. The magnesium content was obtained by subtracting the calcium content from the calcium plus magnesium content. The formula of calculating mineral content was as follows:

$$\text{Minerals } (Ca^{++} + Mg^{++}) = \frac{[T.R \times N(EDTA) \times D.F \times M.Wt \times 10]}{10^6 \times S \times 2\text{Valence}}$$

Where:

T.R = titration reading

N(EDTA) = normality of EDTA

D.F.= dilution factor

M.wt = molecular weight of the element estimated

S= sample weight

3.2.3.4.4 Determination of calcium content in kernel milk

Two milliliters of ash solution which prepared from kernel milk was placed in a 50 ml conical flask. Ten milliliters of distilled water were then added to the contents in the flask. 3-4 drops of 4N NaOH were added with small amount of peroxide indicator (0.5g of ammonium purpurate was mixed with 100g of powdered K_2SO_4) giving color. The contents of the flask were titrated with 0.01 N EDTA (ethylene Diamine Tetra Acetic acid) until a violet color, indicating the end point, was obtained.

Calculation:

$$\%Ca = \frac{[T.R \times N(EDTA) \times D.F \times M.Wt \times 10]}{10^6 \times S \times 2 \times Valence}$$

Where:

T.R = titration reading

N(EDTA) = normality of EDTA

D.F.= dilution factor

M.wt = molecular weight of the element estimated

S= sample weight

Values were then converted into mg/100 milk milk.

3.2.4 Acceptability of kernel milk

Samples of kernel milk (beverages) prepared from rain-fed and irrigated seed kernels in water containing 12.33 % total solids and 11.33 % total solids, respectively, were tested for acceptability against commercial lactose free milk formula. Eight assessors from Ahfad University for Women were asked to rank the three beverages.

Quality attributes judged beverages included colour (appearance), flavor, after taste, and overall quality of the product (Appendix Form II). Sum of ranks were then statistically analyzed using Ihekoronye and Ngoddy (1985) Tables at $P \leq 0.05$ (Appendix III).

3.2.5 Statistical analysis

All chemical analyses were performed in three replicates and the results were statistically analyzed using the GLM procedure with SAS (2004) software. Duncan's multiple comparison procedure was used to compare the means. A probability of $p \leq 0.05$ was used to establish the significance differences among treatments.

4- RESULTS AND DISCUSSION

4.1 Ratios of sunflower seed kernel to husk (hull)

Table (4.1) shows ratios (as%) of kernel to husk separated from rain-fed and irrigated sunflower seed. The weight of 100 seeds (g) of both sources are similar, however, the percent weight of the kernel separated from the irrigated seed (72.75%) was significantly ($P \leq 0.05$) higher compared to percent weight of kernel separated from rain-fed seed (67.95%). Lower husk is eventually given by irrigated seed (27.25%). High kernel weight in oilseeds in general and sunflower seed in particular reflects good agricultural practices. It was obvious that uncontrolled irrigation (rain-fed) may lead to depression in seed quality. Hartman *et al.*(1999) and Pedrosa *et al.*(2000) reported the hull content in sunflower seed as 39.3 to 57.5% and 20.26 to 23.75, respectively.

4.2 Proximate composition of sunflower seed kernel

Table (4.2) shows proximate composition of the sunflower seed kernel calculated on dry-matter basis. The moisture content of each seed is shown at the bottom of the table. The kernel separated from rain-fed seed is characterized by lower ($P \leq 0.05$) fiber and carbohydrates, and higher ($P \leq 0.05$) protein and fat compared to the kernel separated from irrigated seed. Grompone (2005) reported moisture, protein, and fiber values in sunflower seed kernel as 10%; 28.4 to 36.2%; and 15 to 27%, respectively.

4.3 Protein solubility at different pH values

Table (4.3a and 4.3b) (Figs.2 and 3 {Appendix IV}) show the solubility (%) of sunflower kernel proteins at different pH values (5, 6, 7 and 8) as affected by method of clarification (centrifugation and filtration).

The highest protein (nitrogen) solubility was obtained with rain-fed kernel (97.53%) at pH8 when the liquid or blend was filtered, whereas the minimum protein solubility was recorded with the same rain-fed kernel (17.17%) at pH6 when the blend

Table (4.1). Ratios (%) of kernel and husk separated from rain-fed and irrigated sunflower seed

Sunflower seed source	Weight of 100 seeds (g)	Kernel		Husk	
		Weight (g)	%	Weight (g)	%
Rain-fed	5.15 ±1.34 [a]	3.50±1.34 [a]	67.95±1.34 [b]	1.65±1.34 [a]	32.05±1.34 [a]
Irrigated	4.75±0.21 [a]	3.46±0.21 [a]	72.75±0.21 [a]	1.29±0.21 [a]	27.25±0.21 [b]

* Any two means values having different superscript letter (s) in the same column are significantly ($P \leq 0.05$) different

Table (4.2). Proximate composition of sunflower seed kernel (% Dry matter)*

Sunflower kernel source	Parameter				
	Ash %	**Protein %**	**Fiber %**	**Fat %**	**Total carbohydrate%**
Rain-fed	4.06±0.01 [a]	34.05±0.21 [a]	1.45±0.21 [b]	57.65±0.20 [a]	4.24±0.01 [b]
Irrigated	5.65±1.60 [a]	27±1.00 [b]	3.34±0.03 [a]	54.90±0.14 [b]	12.45±4.30 [a]

*Moisture content of rain-fed kernel was 4.4±0.14 and of irrigated kernel was 12.5±0.71

Any two mean values having different superscript letter (s) in the same column are significantly (P≤0.05) different.

was centrifuged. It was also clear that for rain-fed kernel, both pH values of 7 and 8 were suitable for extraction of most of the proteins provided that the blend is filtered (80.36; 97.53%, respectively) and not centrifuged. With the irrigated kernel, it seems that only pH7 was able to extract levels of proteins higher than 80% by filtration. Worth-mentioning that the pH of rain-fed kernel blended in water alone was 8 compared to a pH of 7 recorded for irrigated kernel blended in water alone as well. This would suggest the mere use of water only to prepare a sunflower seed kernel milk analogue without a need for re-adjusting pH of blend to improve protein solubility. Saeed and Cheryan (1988) reported low solubility of protein of sunflower kernel in the range of 15-30% at acidic pH between 3 and 6.

4.4 Total solids (%) of filtered milk analogue prepared from sunflower seed kernel as affected by level of material extracted.

Table (4.4) shows total solids (%) of filtered milk analogue prepared from sunflower seed kernel as affected by level of material extracted. It was obvious that the more material (kernel) used, the more total solids are extracted in the milk analogue. The trend of extractability of the solids in milk is more or less similar in kernels from the two seed sources. Since the filtered milk is going to be further boiled, the picture of total solids in the final milk product is yet to be assessed after boiling.

4.5 Changes in total solids (%) of a boiled sunflower kernel milk analogue as affected by level of material and clarifying method

Table 4.5 (a and b) shows total solids (%) of sunflower kernel milk analogue (vegetable milk) prepared in water as affected by level of material (kernel weight) and clarifying method. Since the objective of making vegetable milk is to obtain level of solids more or less similar to the natural cow milk solids (~ 13%), it was obvious that levels below 30 g kernel / 100 ml milk analogue were not enough to produce such required solids. Referring to both tables (6 (a and b)) level of required solids close to 13% may be obtained from rain-fed kernel using 30 g material in 100 ml blend and filtrated (12.33 %), whereas values close to 12% may be obtained from irrigated kernel using 30 g material in 100 ml blend and filtrated as well. The latter preparations were made and taken for further tests (acceptability).

Table (4.3a) Solubility (%) of rain-fed sunflower seed kernel protein at different pH values as affected by method of clarification

PH value	Centrifuged liquid (milk)	Filtered liquid (milk)
5	30.22±5.83 [eg]	56.32±1.94 [d]
6	17.17±2.91 [h]	61.82±1.94 [c]
7	29.53±2.91 [ef]	80.36±2.91 [b]
8	37.09±1.94 [e]	97.53±1.94 [a]

* Any two mean values having different superscript letter in columns and rows (s) are significantly ($P \leq 0.05$) different

Table (4.3b) Solubility (%) of irrigated sunflower seed kernel protein at different pH values as affected by method of clarification

PH value	Centrifuged liquid (milk)	Filtered liquid (milk)
5	27.43±0.04 [e]	79.6±1.68 [a]
6	31.05±0.07 [d]	73.23±5.19 [ab]
7	36.3±0.42 [c]	82.1±5.5 [a]
8	27.43±0.04 [e]	65.9±4.14 [b]

* Any two mean values having different superscript letter in columns and rows (s) are significantly ($P \leq 0.05$) different

Table (4.4). Total solids (%) of filtered milk analogue prepared from sunflower seed kernel as affected by level of material extracted.

Seed sources	Level of material extracted (g/100ml)		
	20	25	30
Rain-fed	11.41±1.20 c	15.66±0.33 b	17.12±0.45 a
Irrigated	9.91±0.36 c	14.23±0.45 b	16.07±0.12 a

Mean values having different superscript letter(s) in all rows and columns are significantly (P≤0.05) different.

Table (4.5a): Changes in total solids (%*) of a boiled ** milk analogue * prepared from a kernel separated from rain-fed seed**

Milk clarifying method	Level of kernel (g) in vegetable milk (100 ml)		
	20	**25**	**30**
Centrifugation	3.67±1.53 ^{ef}	7.00±2.36 ^{cd}	10.50±1.06 ^{ab}
Filtration	6.00±2.01 ^{ce}	8.67±1.20 ^{bc}	12.33±1.86 ^{a}

* Mean values having different superscript letter(s) in all rows and columns are significantly (P≤0.05) different

** Boiling at 90 ^{0}c for 10 minutes.

*** As affected by level of material and clarifying method

Table (4.5b): Changes in total solids (%*) of a boiled ** milk analogue * prepared from a kernel separated from irrigated seed**

Milk clarifying method	Level of kernel (g) in vegetable milk (100 ml)		
	20	**25**	**30**
Centrifugation	4.33±0.58 ^{de}	4.67±0.58 ^{cd}	6.33±2.12 ^{bc}
Filtration	6.00±0.7 ^{b}	7.33±2.3 ^{ab}	11.33±1.15 ^{a}

* Mean values having different superscript letter(s) in all rows and columns are significantly (P≤0.05) different

** Boiling at 90 ^{0}c for 10 minutes.

*** As affected by level of material and clarifying method

4.6 Changes in levels of total solids (%) and protein (%) in a filtered kernel milk analogue as affected by the boiling process.

Table 4.6 shows changes in total solids (%) and proteins (%) in a filtered kernel milk analogue as affected by the boiling process. The boiling process has reduced significantly ($P \leq 0.05$) levels of total solids and proteins in the kernel milk irrespective of the kernel source. After boiling, the proteins in the kernel milk were significantly ($P \leq 0.05$) reduced from 9.97% in the rain-fed seed kernel to 1.74%, and from 6.65% in the irrigated seed kernel to 1.97%. the boiling process seemed to have affected solubility of both suspended solids and proteins. The stability of proteins in sunflower kernel milk or beverage shall remain a technological issue need to be handled during production of such products at a larger scale.

4.7 Proximate composition of sunflower seed kernel milk

Table (4.7) shows composition of sunflower seed kernel milk from two different seed sources. Such vegetable milk is characterized by high level of fat (> 4.50%) and low level of protein (< 2.00%), compared to mammals milk. Sunflower milk proteins being low and on how to improve them either by treatment with the enzyme papain (Ferber and Cook,1979), or by increasing pH (Hagenmaier, 1974).

4.8 Mineral content of sunflower kernel milk

Table (4.8) shows mineral matter in sunflower seed kernel milk compared to respective values in cow milk. It was obvious that the vegetable milk (kernel milk) is characterized by presence of iron (0.09-0.12 mg/100ml), although low level, compared to complete absence of such element in animal milk. Magnesium in vegetable milk was also found higher (24.00-48.22mg/100ml) compared to level in cow milk (12.00 mg/100ml). cow milk has an advantage over vegetable milk in levels of minerals such as calcium, phosphorus, and potassium. Koch (2011) and HWF (2013), reported values for iron (7mg), calcium (354mg), potassium (700-900mg), phosphorus (700mg), and magnesium (354mg) in sunflower seed kernel. Level of iron in the final vegetable milk depends upon method of extraction of the soluble solids of the material.

Table (4.6). Changes in levels of total solids (%*) and protein (%) in a filtered kernel milk analogue as affected by the boiling process**

Source of kernel	T.S(%)		Protein (%)	
	Before boiling	After boiling	Before boiling	After boiling
Rain-fed seed	17.12±0.45 ^{a}	12.33±1.86 ^{b}	9.9±0.75 ^{a}	1.74±0.17 ^{b}
Irrigated seed	16.07±0.12 ^{a}	11.33±1.15 ^{b}	6.6±0.21 ^{a}	1.97±0.07 ^{b}

Mean values having different superscript letter(s) in same rows are significantly (P≤0.05) different

* Extraction of 30g kernel in 100ml water.

** Boiling at 90 ^{0}c for 10 minutes

Table (4.7). Proximate composition of sunflower seed kernel milk

Milk Type	Parameter				
	Fat %	Ash %	Protein %	**Total carbohydrate%	Calories (kcal/100ml)
Rain-fed kernel milk	4.95±0.07 [a]	0.66±0.21 [a]	1.74±0.17 [a]	4.98 [a]	71.43 [a]
Irrigated kernel milk	4.65±0.21 [a]	0.58±0.06 [a]	1.97±0.07 [a]	4.13 [b]	66.25 [b]

Any two mean values having different superscript letter (s) in the same column are significantly ($P \leq 0.05$) different

Table (4.8). Levels of mineral matter in sunflower kernel milk* compared to levels in cow milk

Mineral (mg/100ml)	Sunflower kernel milk		Cow milk **
	Rain-fed	Irrigated	
Iron * **	1.17	0.98	0.00
Calcium	8.00	12.00	123.00
Potassium	98.40	89.60	141.00
Magnesium	24.00	48.00	12.00
Phosphorus	76.30	75.90	95.00

* Filtered milk

** Cited from Fox and McSweeney (1998)

*** Fe was calculated from Flame Atomic Absorption Spectrophotometer

4.9 Acceptability of Sunflower Milk Beverage

Table (4.9) shows sum of ranks concluded from acceptability of milks prepared from sunflower seed kernel from different sources (rain-fed and irrigated) against commercial lactose-free milk. The commercial lactose-free milk was superior ($P \leq 0.05$) only in colour (appearance) since it was slightly whitish compared to the creamy colour the sunflower kernel-based milk has. The milk based on irrigated kernel has no significant ($P \leq 0.05$) in flavor, after taste and the overall quality compared to the commercial formula. The milk prepared from the rain-fed kernel was poor in appearance, taste, after taste and the overall quality

4.10 Acceptability of irrigated sunflower kernel milk beverage containing different flavors

Table (4.10) shows acceptability of sunflower kernel milk beverage containing different flavors. It was obvious that the kernel milk beverage flavored with strawberry was significantly ($P \leq 0.05$) superior and most favorite for assessors with respect to colour, flavor, taste, after taste, and hence the overall quality of the drink. Banana-flavored beverage came second in preference order. Assessors seemed not to accept chocolate flavor in vegetable milk beverage.

Table (4.9). Acceptability * of sunflower milk beverage

Milk type	Color (appearance)	Flavor (odor+taste)	Taste	After taste	Overall quality
Rain-fed kernel milk	23 c	17 a	21 c	21 c	21 c
Lactose-free milk	8 b	19 a	14 c	12 a	14 a
Irrigated kernel milk	16 a	12 a	13 a	15 a	13 a

* Any two sum of ranks having different superscript letter are significantly ($P \leq 0.05$) different

Table (4.10): Acceptability of sunflower kernel milk from irrigated sources beverage containing different flavours

Type of flavoured kernel milk beverage	Color (appearance)	Flavor (odor+taste)	Taste	After taste	Overall quality
Milk without flavor (control)	26 c	25 a	23 a	23 a	26 c
Milk with straw berry flavor	13 b	12 b	11 b	12 b	9 b
Milk with banana flavor	17 a	24 a	23 a	22 a	21 a
Milk with chocolate flavor	24 a	19 a	23 a	23 a	24 a

Any two sum of ranks having different superscript letter are significantly ($P \leq 0.05$) different

5- CONCLUSIONS AND ROCOMMENDATIONS

5.1 Conclusions

From an attempt made to develop lactose-free milk based on sunflower seed kernel dehulled from rain-fed and irrigated seeds, the following points can be concluded;

- The rain-fed sunflower kernel contains higher levels of protein and fat compared to the irrigated sunflower kernel which was found to contain higher levels of fiber and carbohydrates.
- Solubility of proteins in sunflower seed kernel was found to differ according to seed source. The filtered liquid was found to contain more soluble proteins compared to the centrifuged liquid.
- pH8 was found to yield highest level of soluble proteins in rain-fed seed kernel, whereas pH7 gave highest level of protein solubility in irrigated seed kernel.
- Milk based on irrigated sunflower kernel was found to be more acceptable in flavor, taste, aftertaste, and overall quality than both milk based on rain-fed seed kernel and a commercial lactose-free milk.
- When the sunflower seed kernel milk was flavored, assessors judged irrigated sunflower seed kernel milk with strawberry flavor superior compared to the other flavors used.
- Sunflower milk based on different kernels has similar levels of macronutrients except for total calories and carbohydrates which are higher in rain-fed kernel milk.
- With respect to micronutrients in kernel milk, the levels of iron and magnesium were found higher in sunflower seed kernel milk compared to cow milk.

5.2 Recommendations

A vegetable milk was developed based on sunflower seed kernel. The outcome of such work helped in outlining following recommendations;

- Sunflower seed kernel can be used from both rain-fed and irrigated seed sources to prepare lactose-free vegetable milk or beverage for special dietary uses.

- The technology of making kernel milk needs to be further tackled along the chain of production (e.g extraction, filtration, stabilization of suspended solids, thermal treatments, flavoring,……etc). Such technology usually covers areas like supplementation and shelf life of end products.

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Appendices

Appendix I

Sunsational

Non Dairy Sunflower Beverage

"Sharing a dairy free experience with a delicious taste all of its own"

- *New beverage made from sunflower kernels*
- *Delicious taste*
- *Great source of fiber (good for digestion)*
- *Great source of folate (improves cell function)*
- *Excellent source of Vitamin A (great for skin)*
- *Omegas 6 & 9 (good for heart, lowers cholesterol)*
- *Contains chlorogenic acid (regulates blood sugar levels)*
- *Contains complex plant protein and aminos*
- *Free of lactose, gluten, soy and nuts*
- *Sourced and made in USA*
- *Non GMO sunflower seeds*
- *Kosher certified*
- *More antioxidants than a glass of green tea!*

Vanilla [8-5209100301-5]
Ingredients: purified water, sunflower kernels, organic evaporated cane juice, potassium citrate, natural vanilla extract, sunflower lecithin, carrageenan, natural flavor, sea salt, xanthan gum

Original [8-5209100300-8]
Ingredients: purified water, sunflower kernels, organic evaporated cane juice, potassium citrate, sunflower lecithin, carrageenan, natural flavor, sea salt, xanthan gum

Nutrition Facts
Original and Vanilla

Serving Size 8 oz. (240mL)
Servings Per Container: about 4

Amount Per Serving	
Calories 80	Calories from Fat 30
	% Daily Value*
Total Fat 3.5g	**5%**
Saturated Fat 0.5g	**3%**
Trans Fat 0g	
Cholesterol 0g	**0%**
Sodium 90mg	**4%**
Total Carbohydrate 10g	**3%**
Dietary Fiber 4g	**16%**
Sugars 6g	
Protein 2g	**4%**
Vitamin A	20%
Vitamin C	0%
Calcium	8%
Iron	4%
Folate	15%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300mg	375mg
Dietary Fiber		25mg	30mg

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

www.sunsationalnondairy.com

Appendix II

Ahfad university for women

School of health science

M.S.C in Human Nutrition

Form used for acceptability test of the sunflower milk:-

Sample NO………………... **Date……………...**

Please. **Kindly examine samples of sunflower milk presented to you ,and rank them for acceptability parameters mentioned on form**

Note:

Number 1 for the best in quality and No. 4 to the least in quality. Do not repeat the rank order

Sample code	Appearance and color	flavor	Taste	After taste	Overall quality
S					
U					
N					
F					

Appendix III

Rank totals

Rank totals required for significance at the 5% level ($P \leq 0.05$). The four figure blocks represent: lowest insignificant rank sum, any treatment–highest insignificant rank sum, any treatment. Lowest insignificant rank sum, predetermined treatment–highest insignificant rank sum, predetermined treatment.

No. of reps.	Number of treatments, or samples ranked																		
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2	...	...	....	.....	.....	.....	.....	...	.....	.....	.....	.....	.....	.....	...	.....	....	....	3–39
	.....	.....	.....	3–9	3–11	3–13	4–14	4–16	4–18	5–19	5–21	5–23	5–25	6–26	6–28	6–30	7–31	7–33	7–35
3	.....	.....	.....	4–14	4–17	4–20	4–23	5–25	5–28	5–31	5–34	5–37	5–40	6–42	6–45	6–48	6–51	6–54	7–56
	...	4–8	4–11	5–13	6–15	6–18	7–20	8–22	8–25	9–27	10–29	10–32	11–34	12–36	12–39	13–41	14–43	14–46	15–48
4	.....	5–11	5–15	6–18	6–22	7–25	7–29	8–32	8–36	8–40	9–43	9–47	10–50	10–54	10–53	11–61	11–65	12–68	12–72
	...	5–11	6–14	7–17	8–20	9–23	10–26	11–29	13–31	14–34	15–37	16–40	17–43	18–46	19–49	20–52	21–55	22–58	23–61
5	....	6–14	7–18	8–22	9–26	9–31	10–35	11–39	12–4[illegible]	12–48	13–52	14–56	14–61	15–65	16–69	16–74	17–78	18–82	18–87
	6–9	7–13	8–17	10–20	11–24	13–27	14–31	15–35	17–33	18–42	20–45	21–49	23–52	24–56	25–60	27–63	25–67	30–70	31–74
6	7–11	8–16	9–21	10–26	11–31	12–36	13–41	14–46	15–51	19–55	15–60	19–65	19–71	20–76	21–81	22–86	23–91	24–96	25–101
	7–11	9–15	11–19	12–24	14–28	16–32	18–36	20–40	21–45	23–49	25–53	27–57	29–61	31–65	32–70	34–74	36–78	38–82	40–86
7	8–13	10–18	11–24	12–30	14–35	15–41	17–46	16–52	19–58	[illegible]–63	22–69	23–75	25–80	26–86	27–92	29–97	30–103	31–109	32–115
	8–13	10–18	13–22	15–27	17–32	19–37	22–41	24–46	26–51	28–56	30–61	33–65	35–70	3[illegible]–75	39–80	42–84	44–89	46–94	48–99
8	9–15	11–21	13–27	15–33	17–39	18–46	20–52	22–58	24–64	26–71	27–77	29–83	30–90	32–96	33–103	35–109	37–115	38–122	40–128
	10–14	12–20	15–25	17–31	20–36	23–41	25–47	28–52	31–57	33–63	36–68	39–73	41–79	44–84	47–89	49–95	52–100	54–106	57–111
9	11–16	13–23	15–30	17–37	19–44	22–50	24–57	26–64	28–71	30–78	32–85	34–92	36–99	38–106	40–113	42–120	44–12[illegible]	45–135	47–142
	11–16	14–22	17–28	20–34	23–40	26–46	29–52	32–58	35–64	38–70	41–76	45–81	48–87	51–93	54–99	57–105	60–111	63–117	66–123
10	12–18	15–25	17–33	20–40	22–48	25–55	27–63	30–70	32–78	34–86	37–93	39–101	41–109	44–116	46–124	48–132	51–139	53–147	55–155
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	37–63	40–70	[illegible]–76	47–83	51–89	54–96	57–103	61–109	64–116	68–122	71–129	75–135
11	13–20	[illegible]	[illegible]	22–44	[illegible]	[illegible]	31–68	34–76	36–8[illegible]	[illegible]	[illegible]	45–109	47–118	50–126	53–13[illegible]	55–142	58–151	60–160	63–168
	14–19	18–26	21–34	25–41	2[illegible]–49	33–55	37–62	41–69	45–76	49–83	53–9[illegible]	57–97	60–105	64–112	68–119	72–123	76–132	80–140	84–14[illegible]
12	15–21	18–30	21–39	25–47	28–56	31–65	34–74	37–83	41–91	44–100	47–109	50–118	53–127	56–136	59–145	62–154	65–163	68–172	71–181
	15–21	19–29	24–36	28–44	32–52	37–59	41–67	45–75	50–82	54–90	58–98	63–105	67–113	71–121	76–128	80–136	84–144	89–151	93–159
13	16–23	20–32	24–41	27–51	31–60	35–69	38–79	42–88	45–98	49–107	52–117	56–126	59–136	62–146	66–155	69–165	73–174	76–184	79–194
	17–22	21–31	26–39	31–47	35–56	40–64	45–72	50–80	54–89	59–97	64–105	69–113	74–121	78–130	83–138	88–146	93–154	97–163	102–171
14	17–25	22–34	26–44	30–54	34–64	38–74	42–84	46–94	50–104	54–114	57–125	61–135	65–145	69–155	73–165	76–176	80–186	84–196	88–206
	18–24	23–33	28–42	33–51	38–60	44–68	49–77	54–86	59–95	65–103	70–112	75–121	80–130	85–139	91–147	96–156	101–165	106–174	111–183
15	19–26	23–37	28–47	32–58	37–68	41–79	45–89	50–100	54–111	58–122	63–132	67–143	71–154	75–165	79–176	84–186	88–1[illegible]7	92–208	96–219
	19–26	25–35	30–45	36–54	42–63	47–73	53–82	59–91	64–101	70–110	75–120	81–129	87–138	92–148	98–157	104–165	109–17[illegible]	115–185	121–194
16	20–28	25–39	30–50	35–61	40–72	45–83	49–95	54–106	59–117	63–129	68–140	73–151	77–163	82–174	86–186	91–197	95–209	100–220	104–232
	21–27	27–37	33–47	39–57	45–67	51–77	57–87	63–97	69–107	75–117	81–127	87–137	93–147	100–158	106–166	112–176	118–186	124–196	130–206
17	22–29	27–41	32–53	38–64	43–76	48–88	53–100	58–112	63–124	68–136	73–148	78–160	83–172	88–184	93–196	98–208	103–220	108–232	113–244
	22–29	28–40	35–50	41–61	48–71	54–82	61–92	67–103	74–113	81–123	87–134	94–144	100–155	107–165	113–176	120–186	126–197	133–207	139–218
18	23–31	29–43	34–56	40–68	46–80	51–93	57–105	62–118	68–130	73–143	79–155	84–168	90–180	95–193	100–206	106–218	111–231	116–244	121–257
	24–30	30–42	37–53	44–64	51–75	58–86	65–97	62–108	79–119	86–130	93–141	100–152	107–163	114–174	121–185	128–196	135–207	14[illegible]–218	149–229
19	24–33	30–46	37–58	43–71	49–84	55–97	61–110	67–123	73–136	78–150	84–163	90–176	96–189	102–202	107–216	113–229	119–242	124–256	130–269
	25–32	32–44	39–56	47–67	54–79	62–90	69–102	76–114	84–125	91–137	99–148	106–160	114–171	121–183	128–195	136–206	143–218	151–2[illegible]	158–241

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Appendix IV

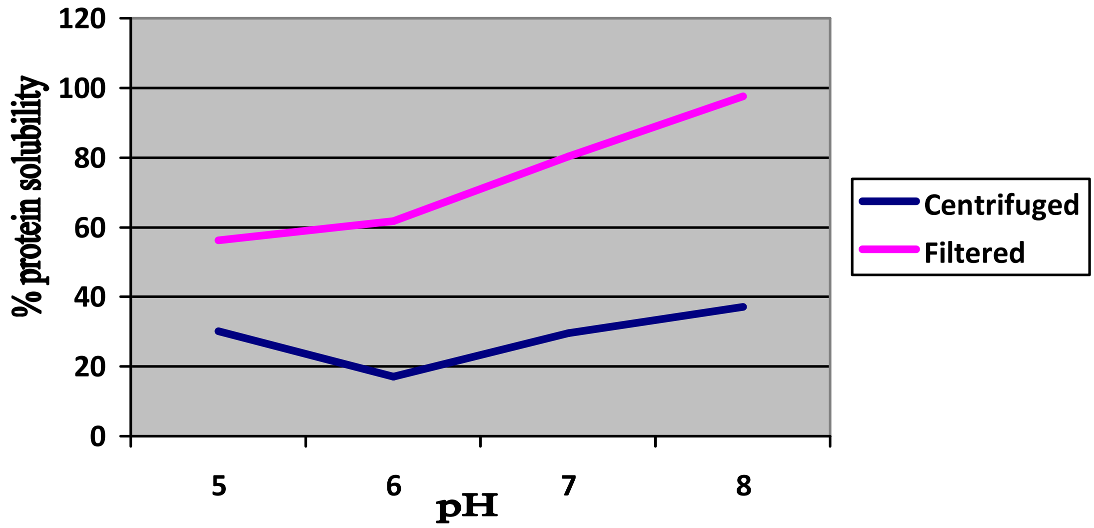

Fig 2: Solubility of rain-fed sunflower seed kernel protein at different pH values as affected by method of clarification

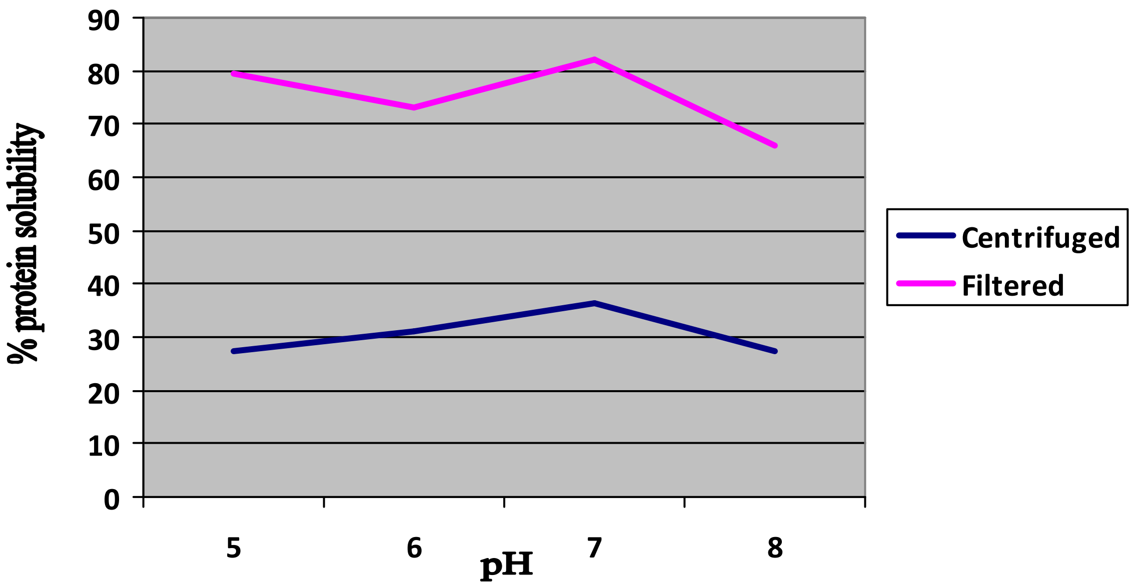

Fig 3: Solubility of irrigated sunflower seed kernel protein at different pH values as affected by method of clarification