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A Histological Study on the Lacrimal Gland of the Camel *(Camelusdromedarius)*

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ABSTRACT. Unlike other domestic animals the dromedary camel *(Camelusdromedarius)* lacks the lacrimal puncta, and hence, the lacrimal secretion function seems to be confined to the washing and moistening of the anterior surface of the eyeball. This study was conducted with the aim of studying the histological structure of the lacrimal gland of the dromedary camel. The study was performed on lacrimalglandsoffifteen animals obtained from Omdurman slaughter house, Khartoum, Sudan. The results revealed that the lacrimal gland was lobulated compound tubuloacinar with clusters of serous secretory end-pieces which were lined by a simple cuboidal epithelium of pyramidal cells. The dense connective tissue capsule sent connective tissue septa which divided the gland into lobes and lobules. The intralobular ducts were lined by simple columnar epithelium, whereas the interlobular and excretory ducts were lined by stratified columnar or pseudo-stratified columnar epithelium rich in goblet cells, melanin granules and lymphatic infiltration. The lymphocytes were replaced by a well-defined ring of lymphatic nodules around the conjunctival end of the excretory duct. The watery lacrimal secretion is suggested to be helpful in washing and moistening activitiesof the anterior part of the eyeball which is important in the camel dry, hot and dusty environment. Moreover, the increased diffuse and nodular lymphaticsin the excretory ducts might be due toincreased immune response along the draining duct.

Keywords: Lacrimal gland,Dromedary Camel, Histology.

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INTRODUCTION

The main functions of mammalian lacrimal fluid are to clean and to moisten the anterior part of the eyeball; the excess fluid drains through the lacrimal puncta, lacrimal ducts, lacrimal sac and nasolacrimal duct to moisten the nasal mucosa.

The dromedary lacrimal apparatus has not previously been established. While Leese (1927) states that the lacrimal puncta are exceedingly small, Abdalla*et al.* (1970) and Saber and Makady (1987) deny the existence of punctalacrimalia in the camel. Recently, the absence of the lacrimal puncta in the camel is also reported by Ibrahim *et al.* (2006) and Alsafy (2010). Moreover, Awkati and Al-Bagdadi (1971) states that the lacrimal gland in the camel is comparatively less developed than that of the horse or ox.

The lacrimal gland in most domestic mammals is compound tubuloalveolar in structure with clusters of secretory end-pieces arranged in tubules and alveoli which are lined by a simple cuboidal epithelium resting on a layer of myoepithelial cells. The septa, which divide the gland into lobules of different shapes and sizes, come from a connective tissue capsule that surrounds the gland. The interstitial connective tissue surrounding the lobules is composed mainly of reticular fibres and is rich in lymphocytes plasma cells and macrophages (Abdalla*et al.*, 1970; Burkitt*et al*, 1999; Bacha and Bacha, 2000; Ibrahim *et.al*., 2006; Mohammadpour, 2008; Mohammadpour, 2011).

According to Abdalla*et al*. (1970) and Awkati and Al-Bagdadi (1971) the lacrimal gland of the camel is serous in type and it is compound tubular (Abdalla*et al*., 1970) or compound alveolar (Awkati and Al-Bagdadi, 1971). Abdalla*et al.* (1970) state that the secrtetory end-pieces of the gland are lined by pyramidal cells and the superficial layer of the excretory ducts is rich in melanin. The excretory ducts are lined by pseudo-stratified columnar epithelium rich in goblet cells and melanin granules (Abdalla*et al.*, 1970; Awkatiand Al-Bagdadi, 1971).

The present study aimed to investigate the histological characteristics of the lacrimal gland in the dromedary camel and correlate the results with the harsh environmental habitat of this animal.

MATERIALS AND METHODS

Lacrimal glands of fifteen apparently healthy adult camels of both sexes were used in this study. The glands were immediately collected after slaughter at Omdurman slaughter house, Khartoum, Sudan. Each gland was first exposed by careful dissection of the skin and the periorbital structuresat the dorsolateral part of the eyeball and then removed. Small pieces of the gland were fixedinBouin's fluid. The fixed specimens were dehydrated in ascending grades of ethyl alcohol, cleared in several changes of xylene, and impregnated and embedded in paraffin wax according to Culling (1974). Tissues were blocked and sections, 5-7 µm thick, were cut in a rotary microtome.The general histological observations were made on paraffin sections stained with haematoxylin and eosin (H & E) in addition to some

special stains including Van Gesson for the demonstration of collagenous fibres, Aldehyde fuchsin and Orcein for elastic fibres and Gommori´s silver nitrate for reticular fibres (Culling, 1974).

RESULTS

The lacrimal gland was lobulated and covered by a dense connective tissue capsule which rests on a smooth muscle band (Fig.1). The capsule sent connective tissue septa which divided the gland into lobes and lobules of different shapes and sizes. The capsules and septa connective tissues were formed mainly of collagenous fibres and they are rich in adipose tissue. A few reticular and elastic fibers were also observed together with some elastic and smooth muscle fibresandblood vessels (Fig.2). The parenchyma of each lobule consisted of clusters of purely serous secretory units; these units were tubuloacinar in shape (Fig.3). The acini had small lumina, whereas the tubules showed wider lumina. The acini and tubules were lined by a single layer of tall cuboidal cells. The nuclei of these cells were centrally located and they varied in shape from round to oval. The secretory end-pieces interstitial connective tissue consisted mainly of reticular fibres and lymphocytes; the lymphocytes were also observed within the epithelial cells of some secretory end-pieces (Figs. 2, 3).

The epithelial cells of secretory end-pieces rested on layer of myoepithelial (basket) cells which were identified by their elongated shapes and their dark and oval nuclei (Fig.3). The acini and tubules emptied in the intralobular ducts, either directly or through connecting ducts which were lined by a low layer of cuboidal cells (Figs.3, 4). The intralobular ducts opened into the interlobular ducts. The small interlobular ducts were lined by stratified cuboidal or stratified columnar epithelium and the large ones were lined by pseudo-stratified columnar epithelium that contained goblet cells (Figs. 4,5). The interlobular ducts epithelium was surrounded by loose connective tissue in which there were blood vessels and reticular and elastic fibres (Figs .2, 4).

The excretory ducts were lined by stratified or pseudo-stratified columnar epithelium rich in goblet cells, lymphocytes and melanin granules (Figs. 6, 7). The mucosa was folded and surrounded by a lamina propria of made of dense irregular connective tissue which mainly contained collagenous fibres, adipose tissue and some smooth muscle fibres. Many blood vessels were also observed. The initial part of the excretory duct was surrounded by a smooth muscle band on which the lacrimal gland rested (Fig.6). The duct was separated from the smooth muscle band by collagenous fibres and adipose tissue. The wall of the excretory ducts became thicker as they approached the conjunctival end because of the gradual increase in lymphocytic infiltration which was replaced by lymphatic nodules with germinal centres in some sections (Fig. 7). On the other hand, the number of goblet cells and melanin granules, which were mainly observed in the superficial layers of the epithelium, decreased towards the conjunctival end of the ducts.

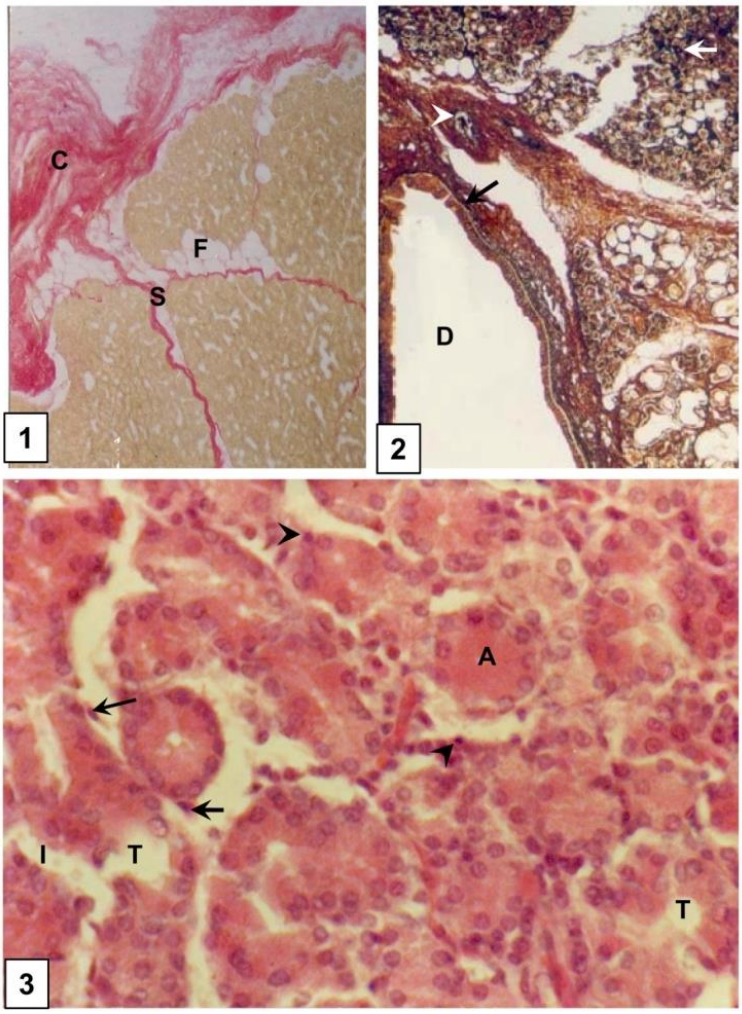

Fig.(1). Camel lacrimal gland covered by a capsule (C) made mainly of dense collagenous connective. The connective tissue septa (S) divide the gland into lobes and lobules tissue. Note the fat cells (F) in the capsule and septa.VanGesson Stain.X40.

Fig.(2). Camel lacrimal gland showing secretory end-pieces interstitial connective tissue consisting mainly of reticular fibres reticular fibres (Arrows) which is also observed in the connective tissue capsule and septa and around the interlobular duct (D) and blood vessels (V). Gomori's Silver Nitrate Stain.X40.

Fig.(3). Camel lacrimal gland serous acini (A) and tubules (T) connected to intralobular duct (I). Note the myoepithelial cells (Arrows) and interstitial lymphocytes (Arrowheads).H&E Stain.X400.

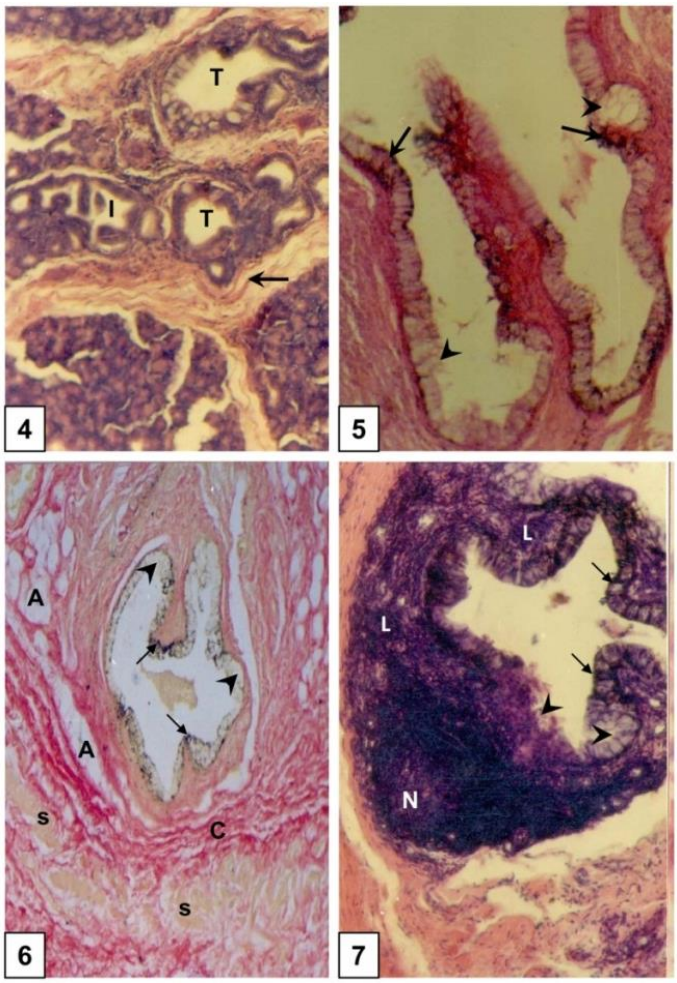

Fig. (4). Camel lacrimal gland showing the intralobular duct (I) connected to secretory end-pieces. The connective tissue septa and wall of interlobular ducts (D) presenta few elastic fibres (Arrows). Orcein Stain.X100.

Fig. (5). Camel lacrimal large interlobular duct with folded mucosa lined by pseudo-stratified columnar epithelium presenting melanin granules (Arrows) and goblet cells (Arrowheads). H&E Stain.X100.

Fig. (6). Camel lacrimal excretory duct with folded mucosa and pseudo-stratified columnar epithelium rich in melanin granules (Arrows) and goblet cells (Arrowheads). The surrounding dense connective contained collagenous fibres (C) and adipose tissue (A) which also separates the smooth muscle band (S) from the duct. Van Gesson Stain.X40.

Fig. (7). Camel lacrimal excretory duct lined by pseudo-stratified columnar epithelium with melanin granules (Arrows) and goblet cells (Arrowheads). Note the diffuse lymphocytes (L) and lymphatic nodule with germinal centre (N). H&E Stain.X100.

DISCUSSION

The present study shows that the lacrimal gland of the camel is lobulated and compound tubuloacinar in structure; the secretory end-pieces which were lined by simple cuboidal epithelium are formed of serous secreting cells. The lacrimal gland in most domestic mammals is also described as compound tubuloacinar in structure (Dellman and Browun 1981; Martin *et al*, 1988; Burkitt*et al.,* 1999; Bacha and Bacha 2000; Shadkhast and Bigham, 2010). However, the dromedary camel gland is found to be compound tubular (Abdalla*et al.* (1970), whereas Awkati and Al-Bagdadi (1971) describe the gland as compound alveolar. The present study confirms the earlier reports of Abdalla*et al.* (1970) and Awkati and Al-Bagdadi (1971) that the secretory end- pieces of the gland are lined by pyramidal cuboidal cells. The present study shows that the lacrimal secretory cells are serous. In many other domestic animals the lacrimal gland is mixed and predominantly serous except in the pig in which mucous cells predominate (Dellman and Brown, 1981). However, Kühnel (1968) claims that the lacrimal gland of the dog is purely mucous. That the lacrimal gland being purely mucous cannot be explained because the major function of the lacrimal secretion is moistening, washing and disinfection of the anterior part of the eye, and this function is not certainly helped by purely mucous secretion.

The present study shows that intralobular ducts are lined by simple columnar cells, whereas the interlobular and ducts excretory ducts are lined by stratified or pseudo-stratified columnar cells rich in melanin granules, goblet cells and lymphocytes. The results of this study clearly confirm the findings of Abdalla*et al.* (1970) and Awkati and Al-Bagdadi (1971). The excretory duct is lined by a stratified columnar epithelium in most mammalian species. However, in the pig, the excretory ducts, at their origin are lined by pseudo-stratified columnar epithelium (kuehnel and Scheele, 1979).

It is clear from the present investigation and the investigations by many authors in the camel (Abdalla*et al.*, 1970; Awkati and Al-Bagdadi, 1971) and other mammalian species including small ruminants (Sinha and Calhoun, 1966), human (Allansmith*et a*l., 1976) and canines (Martin *et al.,* 1988), that lymphocytes, plasma cells, macrophages are of common occurrence in the lacrimal gland interstitial and duct system. In the present study the excretory ducts were rich in diffuse lymphocytes whichwere replaced by a well-defined ring of lymphatic nodules with germinal centres near its conjunctival end.

It can be concluded that the serous secretion of the lacrimal gland is suggested to play an important role in washing and moistening functions of the anterior part of the eyeball of the camel in its dry, hot and dusty environment. Moreover, the increased diffuse and nodular lymphatics might be important in immune response along the excretory ducts.

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Animal production

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Different Types of Luteal Sub-function in Relation to Pregnancy Rate In Nelore Cattle

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ABSTRACT. This study was carried out to determine different types of luteal sub-function in relation to pregnancy rate in Nelore cattle. Fifty seven cows were used. Blood samples were collected twice per week for determining progesterone concentration within estrous cycle. Seven progesterone profiles were found normal progesterone profiles, delayed progesterone profiles, insufficient progesterone profiles, short luteal phase, long luteal phase, basal progesterone profile, and non-response to prostaglandin $F_{2\alpha}$ treatment. Normal progesterone profiles (52.8 %) were higher than abnormal progesterone profiles. Therefore, pregnancy rate was significant higher in normal progesterone profile (45.6 %) compare to other abnormal progesterone profiles. In conclusion, Different progesterone profiles were observed in individual animal after AI. In addition to, it was observed that the post insemination progesterone profile has an effect on the pregnancy rates in Nelore cattle.

Keywords: progesterone profiles, pregnancy rate, artificial insemination, Nelore cattle

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INTRODUCTION

In Malaysia, most of the beef herds are pasture-fed and mating is done naturally by mixing bulls with cows at a ratio of 1: 10. Since estrus detection is not widely practiced. One of the reproductive challenges encountered in pasture-based cattle is repeat breeders. Repeat breeder cows have a long calving interval and are usually not treated. However, scientific information with regards to reproduction of Zebu cattle is rather limited compared with that of temperate cattle. The information available (Gordon, 1996) does not suggest that there is any great difference in the reproductive physiology between *B. taurus*and *B. indicus* cattle. Zebu cattle, although well adapted to the tropics and sub-tropics, characteristically show delayed puberty and extended postpartum periods (postpartum anoestrus) compared with temperate breeds. It has been reported that there are differences in the hypothalamic, pituitary and ovarian relationships between Zebu and temperate cattle. These differences may account for differences in fertility between the two species even when similarly fed and managed (Abeygunawardena and Dematawewa, 2004).

The importance of P_4 in the survival of the early embryo and maintenance of pregnancy in cattle has been reported (Oyedipe*et al*., 1986). Therefore, progesterone assays may find useful application in monitoring occurrence of cyclic activities, early pregnancy and repeat breeders in cattle (Rekwot*et al.*, 2000). Low reproductive rates, failures in estrus detection and high frequencies of abnormal post breeding luteal phases are among the major problems leading to reductions in herd fertility and difficulties in reproductive management (Garcia, 1990). Different types of abnormal post breeding luteal phases could be investigated by hormonal radioimmunoassay which can provide practical values in monitoring ovarian activity in Nelore cows. The objectives of the present study were to: (1) investigate the types and incidence of luteal sub-function after insemination; (2) study the relationship between post-insemination luteal function and pregnancy rate.

MATERIALS AND METHODS

Animals and Experiment Protocol

Fifty seven Nelore cows of at least three years of age were selected for the study. These cows were healthy, had calved down at least once before and were cycling normally. This herd was grazIing on *Brachiariadecumbens* pasture and supplemented with commercial concentrates of palm kernel cake at the rate of 2 kg/cow/day. All cows were inserted with controlled internal drug release device (CIDR® device; interAg, New Zealand) for 15 days. This device is readily coated with 1.38 g of progestagen in a silicon rubber elastomer. After 15 days, the CIDR was removed and non-pregnant cow was administered intramuscularly with 2 mL (500 µg) of cloprostenol (Estrumate®, Schering-Plough Animal Health). Cloprostenol was injected within 9 - 19 days after removal of CIDR. Twenty-four hours after treatment, estrus

was observed for all cows. The cows were examined for pregnancy diagnosis by using ultrasound (Aloka SSD-500, Japan) using a 5 MHz transrectal probe.

EstrusDetection and AI

Cows were observed visually for behavioral estrus twice daily 1 hour at 0800h and 1600h until end of the experiment. The signs observed for estrus were clear mucus discharge, congested vulva, mounting and standing to be mounted. After behavioral estrus was observed, artificial insemination (A.I.) was performed immediately and repeated at 12 hours interval in all cows with frozen-thawed Nelore semen. A 0.25 ml straw containing 20×10^6 sperm/mL was used for insemination of these cows.

Blood sample

Blood samples for hormone assay were collected every three to four days (twice per week) beginning at day of CIDR insertion, until end of study. Ultrasound was done to cows that did not show estrus after A.I. Following collection, labeled blood samples were immediately placed in ice (3˚C) and centrifuged for 15 min at 1340 x g. The plasma was transferred into a 2 ml polypropylene tube and stored at –20 ^{O}C until assay.Plasma progesterone concentration was measured using a commercial radioimmunoassay (RIA) kit (Diagnostics Product Corporation, USA).

Statistical Analysis

Data were analyzed using a statistical software SPSS release 12.0. Reproductive parameters in these two treatments were compared by Chi-square.

RESULTS

From our research findings, based on the peak progesterone concentration and the day of onset of luteal phase, seven different progesterone profiles were identified and namely; normal progesterone profile, delayed progesterone profile, insufficient progesterone profile, short luteal phase, long luteal phase, basal progesterone profile, and non-responsive corpus luteum (Figure 1).Percentage of cows with normal and abnormal progesterone profiles in the first and second estrous cycles after treatment is shown in Table (1).

In our study, normal P_4 profile is where progesterone concentration reached 1.0 ng/ml within 4-7 days after insemination and ≥ 2.0 ng/ml thereafter (Figure 1a). Out of the 87 progesterone profiles, 46 (52.8 %) were normal; 29 (33.3 %) of the first estrous cycle and 17 (56.6 %) of the second estrous cycle.

Delayed progesterone profile is where progesterone concentration reached 1.0 ng/ml after Day 7 and ≥2.0 ng/ml thereafter (Figure 1b). Sixteen cows (18.3 %) showed delayed progesterone profile, 10 (17.5 %) of the first estrous cycle and 6 (36.0 %) of the second estrous cycle.

Insufficient progesterone profile is where progesterone concentrations reached 1.0 ng/ml within 4-7 days after insemination and remained <2.0 ng/ml

thereafter (Figure 1c). Two cows (2.2 %) showed insufficient progesterone profile, 1 cow (1.7 %) in the first estrous cycle and 1 cow (3.3 %) in second estrous cycle.

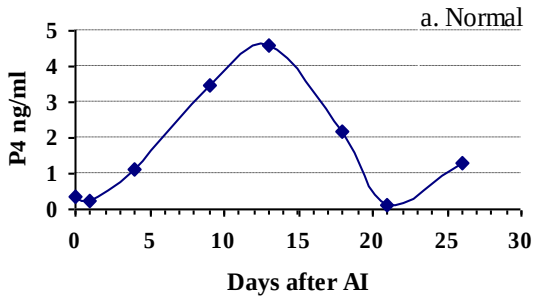
Short luteal phase is where progesterone concentration reached 1.0 ng/ml within 4-7 days after insemination and remained ≥ 2.0 ng/ml for only 5 days (Figure 1d). Four cows (4.5 %) showed short luteal phase, 1 cow (1.7 %) in the first estrous cycle and 3 cows (10.0 %) in the second estrous cycle.

Long luteal phase is where progesterone concentration reached 1.0 ng/ml within 4-7 days and remained ≥ 2.0 ng/ml for more than 25 days (Figure 1e). Four cows (4.5 %) showed long luteal phase, 3 cows (5.2 %) in first estrous cycle and 1 cow (3.3 %) in second estrous cycle.

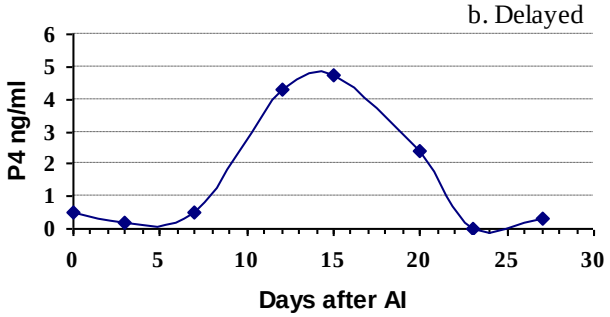
Basal progesterone profile is where progesterone concentrations remained <1.0 ng/ml throughout the estrous cycle (Figure 1f). Eleven cows (12.6 %) showed basal progesterone profile, 9 cows (15.7 %) in first estrous cycle and 2 cows (6.6 %) in second estrous cycle.

Four cow (4.5%) did not response to the $PGF_{2\alpha}$ treatment, whereby the progesterone concentrations remained >1.0 ng/ml after the $PGF_{2\alpha}$ injection (Figure 1g).

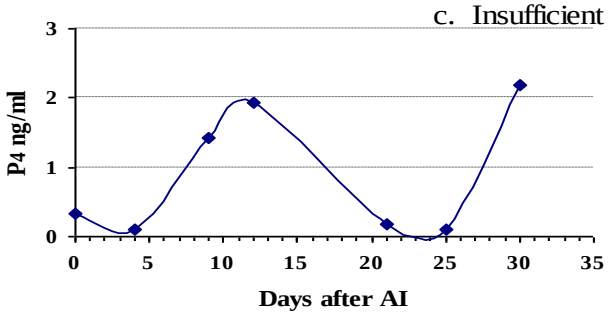
Thus, in the first estrous cycle, 28 (49.1%) out of 57 cows had abnormalities of progesterone profiles were significant higher than in the second estrous cycle, 13 (43.3 %) out of 30 cows had abnormalities of progesterone profiles. Twenty one cows (45.6 %) were pregnant in the normal luteal phase, while 3 (18.7 %) were pregnant in the delayed luteal phase. The difference was statistically significant (P<0.05) (Table 1).



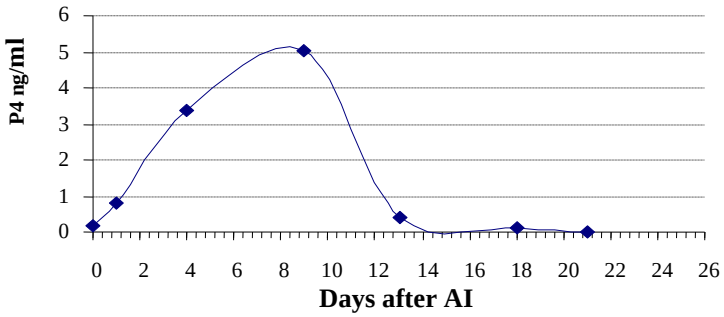
b. Delayed

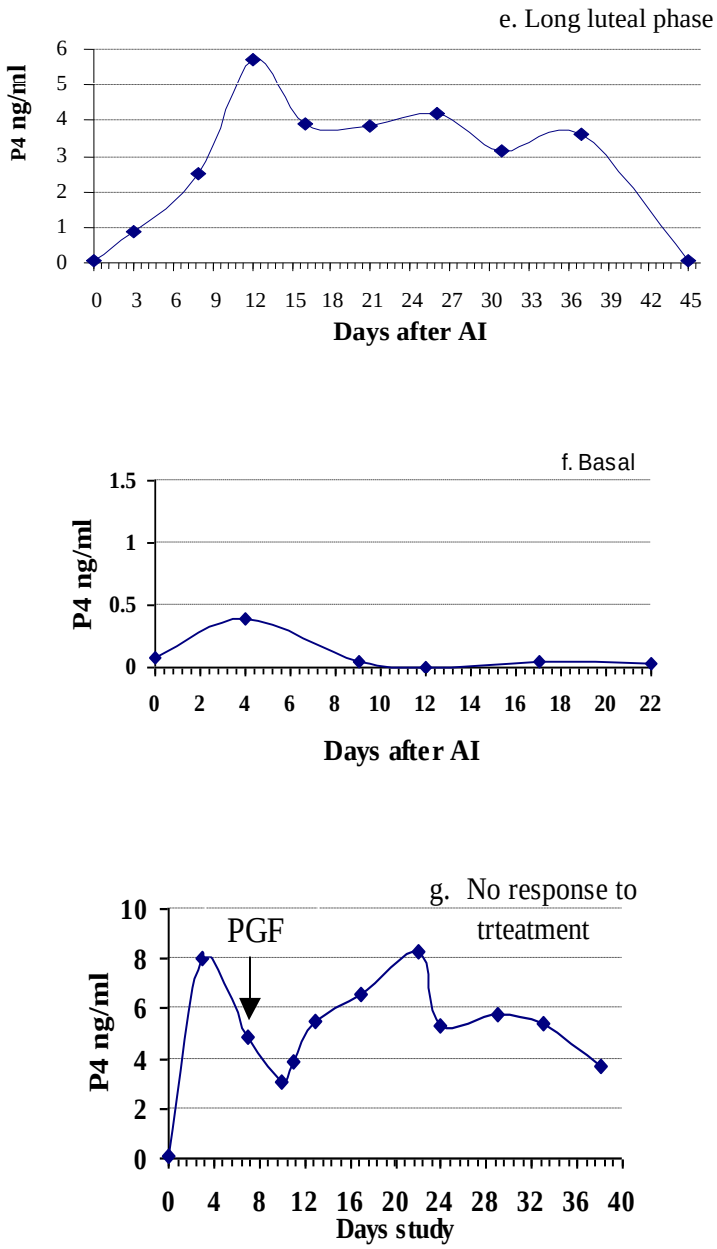


c. Insufficient



d. Short luteal phase





Day 0 is day of AI

Figure (1). Progesterone profiles obtained from different cows after AI.

Table (1). Percentage of cows with normal and abnormal progesterone profiles in the first and second estrous cycles after treatment

Post-AI P4 profile	No. of profiles (%)	First oestrous cycle (%)	Second oestrous cycle (%)	Pregnant (%)
Normal	46 (52.8)	29 (33.3)	17 (56.6)	21/46 (45.6)[a]
Delayed	16 (18.3)	10 (17.5)	6 (36.0)	3/16 (18.7)[b]
Insufficient	2 (2.2)	1 (1.7)	1 (3.3)	0 (0.0)
Short luteal phase	4 (4.5)	1 (1.7)	3 (10.0)	0 (0.0)
Long luteal phase	4 (4.5)	3 (5.2)	1 (3.3)	0 (0.0)
Basal	11 (12.6)	9 (15.7)	2 (6.6)	0 (0.0)
Non-response	4 (4.5)	4 (7.0)	0 (0.0)	0 (0.0)
Total	87	57	30	24/62 (38.7)

[a, b]: Values with different superscripts in the same column differ significantly at P<0.05; chi square.

Table (2) shows total number of cows with normal and abnormal progesterone profiles in first and second oestrous cycles. Normal progesterone profiles were higher (52.8 %) than in abnormal progesterone profiles (47.2 %). However, the differences for abnormal and normal progesterone profiles were not statistically significant.

Table (2). Total of cows with normal and abnormal progesterone profiles in first and second oestrous cycles

	Normal P_4 profiles (%)	Abnormal P_4 profiles (%)	Total
Number of P_4 profiles	46 (52.8)[a]	41 (47.2)[a]	87

[a, b]: Values with different superscripts in the same row differ significantly at P<0.05; Chi-square.

DISCUSSION

In the present study, the normal progesterone profile was recorded in first and second estrous cycle. The increase in the progesterone concentrations a few days (4-7 days) after estrus indicates ovulation and subsequently formation of corpus luteum (Karg, 1981). Progesterone concentration elevates for approximately 2 weeks and is the hallmark of a normal estrous cycle (Hommeida*et al*., 2004). Any deviation from this pattern is likely to be associated with the reduced fertility (Rekwot*et al*., 2000). Therefore, high percentage of pregnancy rate indicates that a herd of cows has hormonal P_4 profile.

Delayed and insufficient progesterone productions were recorded in our study. The luteotrophic hormone (LH) support the corpus luteum for secretion of progesterone (Baird, 1992). Corpus luteum secreting subnormal amounts of progesterone may result from ovulation of immature follicles (Hunter, 1991) or from partial withdrawal of luteotrophic support during the luteal phase (Southee*et al*., 1988). Delayed and insufficient progesterone profile can lead to a stronger luteolytic signal and thus may predispose to a higher incidence of embryo loss (Mann and Lamming, 1995). These abnormal progesterone profiles were also associated with decline in pregnancy rate due to asynchrony between uterus and embryo (Hommeida*et al*., 2004). In cows with normal gestation there is an increase in progesterone concentration in the early postovulatory 15-17 days after AI with a high progesterone concentration during the luteal phase. In addition, the embryos of these cows produced larger amounts of interferon tau (INF-τ) that would alter the dynamics of $PGF_{2\alpha}$ secretion, and therefore the pregnancy was more likely to be maintained (Mann and Lamming, 2001).

A short luteal phase was observed in first and second estrous cycles. These cows, with a short luteal phase, failed to maintain pregnancy. Rekwot*et al.,* (2000b) reported that, the short estrous cycle of 11–17 days were observed in 12.8 % of Bunaji cattle were involved in an intensive artificial insemination program. Even though, fertilization, early embryo development and transport of embryo into the uterus appeared normal (Hommeida*et al*., 2004). In previous studies (Kafi and McGowan, 1997; Taponen*et al*., 2003), failure to maintain pregnancy may be associated with premature luteolysis and this factor was considered as a major reason to poor fertility. This earlier development of luteolytic mechanism would not allow enough time for the embryo to develop and thus, to produce sufficient amount of INF-τ to adequately block luteolysis. The embryo toxic effect may be due to the release of oxytocin and $PGF_{2\alpha}$ from the regressing CL and thus, stimulating further release of $PGF_{2\alpha}$ (Seals *et al*., 1998). Weems *et al*. (1998) reported that significant quantities of $PGF_{2\alpha}$ and PGE released from Day-14 CL of cycling cows cultured *in vitro*. Silvia *et al.* (1991) have suggested that a “positive-feedback loop” occurs between uterine $PGF_{2\alpha}$ and luteal oxytocin. Administration of oxytocin is known to increase concentrations of $PGF_{2\alpha}$ and shorten the estrous cycle length in cattle (Seals *et al*., 1998). Therefore, the secretion of $PGF_{2\alpha}$ due to release of oxytocin from the CL may be having a negative effect on embryonic survival and shorten the estrous cycle in cattle.

A long luteal phase was observed in first and second oestrous cycles. In previous studies, prolonged luteal phases were found in 15.7% of crossbred Brown Swiss x Nelore cows following natural service cows (Garcia, 1990) and 38.5% of Bunaji cattle were involved in an intensive artificial insemination program(Rekwot*et al*., 2000 b). Santos *et al*., (2004) suggested that when CL was maintained and return to estrus was delayed beyond Day 24 of luteal phase, it could point to embryonic losses occurring after Day 16 of gestation. If it is assumed that all of the prolonged luteal phases observed here are indicative of embryonic death, then such mortality

would have to be considered as one of the most deleterious factors affecting conception rate. On other hand, in some studies (Cooke and Benhaj, 1989; Lamothe-Zavaleta*et al*., 1991) suggested that a higher level of cortisol during the mid luteal phase was implicated prolongation estrous cycle length in sheep and cow. High level of cortisol during mid luteal phase may be due to an acute inhibitory effect on the ability of oxytocin to produce and release uterine $PGF_{2\alpha}$ as a consequence of oxytocin-induced motility (Cooke and Benhaj, 1989).

Basal P_4 profile was observed in 11 cows, 4 cows. One cow in first estrous cycle which had cystic follicle (size >30 mm) observed during ultrasound scanning continued to show estrus for 4 days after treatment. The appearance of cystic follicle following the basal progesterone profile has probably been due to the stress imposed to the cows (Hollenstein*et al*., 2006). The stress reactions of cows during the rectal palpation/insemination (Waiblinger*et al*., 2004; Mohamed Ali *et al.*, 2014) and acute restraint (Hollenstein*et al.*, 2006; Mohamed Ali *et al.*, 2014) as well as repeated blood collection, ultrasonograph examination and heavy handling which could have contributed to stress in those cows. Increase cortisol and progesterone secretion from the adrenal cortex and decrease pituitary LH release (Hollenstein*et al*., 2006) cause persistence of follicle that can adversely affect the early growing follicles and their subsequent selection for ovulation (Liptrap *et al*., 1989).

The rest of the 10 cows have a basal progesterone profile which suppressed growing corpus luteum resulting in anoestrous. In one study by Garcia, (1990), periods of acyclicity in crossbred brown Swiss x Nellore cattle following natural service (basal progesterone levels for periods ≥15 d) were found in 5.8% of the cases. However, since the early CL in ruminants is LH-independent, whereas the mid-luteal phase CL requires LH for luteotrophic support (Baird, 1992). In addition, β-adrenergic stimulation is important in functional regulation of the newly-formed CL (Kotwica*et al.*, 2002). The total number of β-receptors (expressed per tissue weight of whole CL) was correlated ($r = 0.76$, $P < 0.004$) with progesterone plasma concentrations and was higher on days 8 and 12 than on days 4 or 16 of the estrous cycle. Noradrenaline (NA) can interact with both α- and β-adrenergic receptors, although its stimulatory effect on luteal steroidogenesis is mediated by β-receptors (Kotwica*et al*., 1991). Thus, NA stimulated the secretion of progesterone during all stages of CL development in heifers (Kotwica*et al*., 2002). Conversely, infusion of a β-blocker in mid-cycle in heifers reduces progesterone secretion by 20–30% (Kotwica*et al*., 2002). Therefore, long-term stress has been found to inhibit LH release (Breen and Karsch, 2004) and this also decreases concentrations of β-receptors (Kotwica*et al*., 2002). In these events, growing corpus luteum and progesterone secretion were impaired and may be accompanied with basal progesterone profile.

Incomplete luteolysis or non-response of corpus luteum to treatment was observed in 2.5 % and 6.3 % of treated cows in first estrous cycle. This observation was also reported by Pinheiro*et al*., (1998) who indicated a high percentage of Nelore females possessing an active CL not responding to usual doses of $PGF_{2\alpha}$.

Delayed decrease or no decrease in P_4 after injection of prostaglandin was the most frequent P_4 deviation and was due to either incomplete luteolysis and/or excessive production of P_4 from adrenal glands than the corpus luteum (Callesen*et al.,* 1988). Alternatively, the failure of response following luteolytic doses of $PGF_{2\alpha}$ was suggested to be due to refractoriness of the CL in individual cows due to rapid metabolism (Peters1984; Chauhan *et al*., 1986). However, in some studies (Duchens*et al*., 1994; Bage*et al*., 2000), it was noted that supra basal plasma progesterone concentrations measured during estrus were marginally elevated (0.5–1.0 ng/ml vs. <0.5 ng/ml normal values) and could either be a consequence of an incomplete preovulatory luteolysis or caused by an increased progesterone release from the adrenal glands. The increased levels of progesterone are known to delay or inhibit the onset of estrus, inhibit the preovulatory LH-surge by negative feedback on the hypothalamus (Duchens*et al*., 1994; Bage*et al*., 2000).

Chronic glucocorticoid treatment prevented luteolysis by blocking $PGF_{2\alpha}$ production (Wang *et al.,* 1993). In study by Wang *et al.* (1993), dexamethasone has been shown to severely impair PGE_2 and $PGF_{2\alpha}$ production in ovarian cell desperate. Glucocorticoids inhibit uterine $PGF_{2\alpha}$ production in the rat (Johnson and Dey 1980; Dey*et al*., 1982) and the cow (Dobson *et al.,* 1987) and such treatment extended luteal function in the latter species (Dobson *et al*., 1987). In the bovine ovary, leucocytes, T-lymphocytes, and macrophages significantly increase during regression of the CL (Penny *et al*., 1999). During late regression, 70% of all proliferating cells in the bovine CL are CD-14-positive macrophages (Bauer *et al*., 2001) which are considered a characteristic of lipopolysaccharide-induced macrophage activation. Therefore, the involution of the CL is an inflammatory-like condition (Neuvians*et al*., 2004). Pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) (Fairchild-Benyo and Pate, 1992), interleukin 1β (IL-1β) (Nothnick and Pate, 1990), and interferon gamma (IFNγ) (Fairchild-Benyo and Pate, 1992) are involved in bovine luteolysis. In mid cycle bovine luteal cells, TNFα, IL-1β, and IFNγ may serve a role in stimulating production of $PGF_{2\alpha}$ that was required for corpus luteum regression (Silva *et al*., 2000; Neuvians*et al*., 2004). Glucocorticoids are well-known immunosuppressive agents. Significantly, glucocorticoids suppress the activation/infiltration of leukocytes and inhibit the secretion and action of cytokines (Wang *et al*., 1993). So that, high level of cortisol at $PGF_{2\alpha}$ injection and during luteolysis after $PGF_{2\alpha}$ injection may cause inhibition of immune cell-derived cytokines and reason for incomplete luteolysis or non-response to $PGF_{2\alpha}$ treatment in Nelore cattle.

CONCLUSION

Different progesterone profiles were observed in individual animal after AI. Additionally, it was observed that the post insemination progesterone profile has a significant effect on the pregnancy rates in tropic regions.

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Evaluation of the Male and Female Sex Steroid Hormones Residues in Eggs, Milk and their Productsin Alqassim Region

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ABSTRACT. The residues of the sex steroid hormones were determined in the milk, eggs and their products. Sixty five samples of eggs (Baladi and Pure Breeds), milk (liquid, powder and condensed), cheese (soft, semi-soft and hard), ice cream, chocolates, butter (local and imported) and yoghurt were used in addition to 97 processed samples were used in this study. Five grams of each sample was taken out after homogenizing the sample in an electric blender, mixed with 10 ml hexane in a conical flask (100 ml) and incubated under shaking in a shaking water bath for 2 hours (30ºC). The sample was poured off into a clean centrifuge tube and centrifuged (3000 rpm/15 minutes, 5°C). The supernatant was aspirated and poured into a clean labeled glass tube. The steroid extract was then exposed to a weak stream of nitrogen gas while samples were incubated on a warm (25ºC) metal block until the whole liquid was evaporated. The precipitate was reconstituted in 5 ml phosphate buffer saline (PBS, *p*H 7.2). Hormone determinations of progesterone (P_4), estradiol17β (E_2), testosterone (T) and androstenedione (A_2) were conducted by commercial ELISA kits. Results exhibited that the highest residues of the four hormones were detected in fresh eggs followed by milk due to the condensed and powder milk, then cheeses. However, the lowest levels of the four hormones existed in yoghurt. Butter, sweets, ice creams and bakeries contained intermediate levels of the four hormones. Also, baladi eggs contained less residues of the four hormones than in white eggs. Also imported food products (i.e. milk, butter, cheese) contained higher residues of the four hormones than their local counterparts.Despite the detection of some residues of the four sex steroid hormones in the food products studied, these levels still far below the maximum allowable levels approved by WHO guidelines in this respect. In conclusion, the consumers especially the children and adolescents must not exceed consuming more than 3 eggs per day and it is better to consume local fresh milk than the imported condensed milk to avoid the accumulation of such hormone residues in their bodies which might affect their future sex life.

Keywords: Testosterone, progesterone, estradiol 17β, androstenedione, eggs, milk products

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INTRODUCTION

Hormones are essential chemicals in the body for its growth, development and functions. Hormones coordinate early brain growth, sexual development and other important sequencing that takes places in human bodies. The body makes its own hormones according to its requirements. However, exogenous hormones, those originating outside the body, i.e. birth control, estrogen replacement therapy, pharmaceuticals, pollutants, etc clearly can disrupt the body hormone function and sometimes causing grievous harm, as with diethylstilbestrol (DES) (Colborn et al., 1996).

On average, U.S. dairy cows produce six times more milk than they did a century ago (Oransky, 2007). Most of this increase is attributable to selective breeding. Because milk production is regulated hormonally, some researchers speculate that the breeding for higher milk production has selected for endocrine variants, and this in turn, may have altered hormonal micro constituents in milk (Remesar*et al*., 1999).

Cows are typically milked early in their pregnancies and then allowed to dry up during late pregnancy. Some researchers speculate that simultaneous pregnancy and lactation is more common now than in past years and that the contemporary practice of milking cows into late pregnancy has boosted estrogen levels in the milk (Hartmann *et al.*, 1998). However, there has been little research into whether contemporary milk supplies have a higher proportion of milk from pregnant cows than in previous years. It is nonetheless an important question because dairy cow pregnancy status and stage affect estrogen and progesterone levels in milk (Hartmann *et al.*, 1998).

Application of hormones to animals may serve a number of purposes such as increased food production, medical treatment, or improved reproductively. It has been suggested that dairy products that contain hormones (such as IGF-I) could increase breast cancer risk (Moorman & Terry, 2004).

Due to the nature of steroids as fat-soluble, the hen eggs contain high levels of these hormones. Moreover, some poultry farms were practicing the addition of contraceptive pills in the chicken feedstuffs to accelerate the weight gain within shorter periods.

Recently, most kids, adolescents and even adults consume lots of fast and ready-made diets containing products of animal origin. Few articles revealed that the excess consumption of cow's milk and dairy products might have negative impacts on the human health. Also, large sector of people consume imported dried and condensed milk, cheese and other eggs-containing products. These together with the appearance of the obesity in youngsters and the subsequent sub fertility in the newly wedding couples have drawn the attention of the researchers to monitor two of the main ingredients (i.e. eggs and milk). Since these two ingredients mainly contribute in most foodstuffs ingested by the consumers, the efforts were directed toward monitoring

residues of testosterone, estradiol 17 ß, progesterone and androstenedione in the raw and manufactured products.

MATERIALS AND METHODS

Sample Collection

A hundred sixty two samples were purchased from local markets in Al-Qassim region, central of Saudi Arabia, we were chosen the sixty five samples were used in this study, some products were prepared in the University kitchen and the others samples were provided from the poultry unit in the experimental farm. Samples involved all available types of soft and semi-soft cheeses, liquid and powder milk, fresh eggs, ice cream, chocolates, milk/egg-containing desserts, yoghurt, butter and baked recipes prepared by the use of milk and eggs or their derivatives in the Faculty of Agriculture educational kitchen.

Foodstuff Steroid Extraction

Five grams solid material (or 5 milliliter in case of liquid) was taken, blended in a blender to obtain homogenous samples. Ten milliliters of 95% hexane were added in a conical flask to each sample for steroid extraction and samples were left in a shaking water bath (30^0C) for two hours. The sample was then poured off into a labeled clean glass tube and centrifuged (3000 rpm/15 minutes/5°C). The supernatant was poured off into another clean glass labeled tube which was inserted into a warm metal block (25^0C) and exposed to a weak stream of nitrogen gas for evaporating the solvent and precipitating the hormones. The dried samples were hence reconstituted in five milliliters phosphate buffer saline (PBS, *p*H 7.2). The final results were attributed to a concentration of hormone in one ml equivalent to one gram of the original sample.

Enzyme Immunoassays (EIAs) of Steroid Hormones in Foodstuffs

Hormones were determined by commercial kits (E2, T and P4, Human Gesellschaft fur Biochemica und DiagnosticambH, Germany).

Estradiol 17β (E2) was determined according to Ratcliffe*et al.* (1988), testosterone (T) was determined according to Rassaie*et al.* (1992), progesterone (P4) was determined according to Radwanska*et al.* (1978) and androstenedione (A) (DRG Diagnostics, GmbH, Germany) was determined according to Mango *et al.* (1986). All determinations were performed in duplicates to verify the intra-assay coefficient of variation which values were 3.5, 4.7, 5.2 and 6.1% for P4, E2, T and A, respectively. Each hormone was determined in one assay, therefore no interassay coefficient of variations was considered.

Data Processing and Analysis

Differences in the values of the four hormones between milk, eggs and their products were analyzed by the general linear model of SAS (SAS, 2000).Mean

comparisons were achieved by the Duncan's Multiple Range Test (DMRT, Steel and Torrie, 1980). The significance level was considered at ≤ 0.05.

RESULTS AND DISCUSSION

Eggs contained the highest (P<0.05) concentrations of the four hormones (Fig. 1-4). Egg levels of testosterone (Fig. 1), estradiol 17β (Fig.2), progesterone (Fig.3) and androsteneione (Fig. 4) were 5.96 ng/g, 204.3 pg/g, 13.8 ng/g and 2.14 ng/g eggs, respectively. Eggs contain between 1.7 and 6.3 times of testosterone than other products. Likewise, eggs contain between 1.3 and 9.9 folds higher estradiol 17ß than other products. The contemporary values were 1.2-3.2 in case of progesterone and 4.8-16.6 in case of androstenedione. Contrariwise, yoghurt showed the lowest concentrations of T, E2 and P4. The yogurt values of T, E2, P4 and A2 were 0.95ng /g, 20.7 pg /g, 4.3 ng/g and 0.43 ng /g, respectively. It has been found that egg yolk is rich in fats which involves these steroid hormones. The boiling process reduces the moisture content in the egg, therefore this concentrates the hormonal levels per gram as compared with their values in the raw fresh eggs.

However, it is clear that the bacterial fermentation processes in yoghurt might have a reduction effect on the steroidal turnover leading to less levels of these hormones. Also, the high percent of moisture in yoghurt might play a vital role in reducing these residues. The fermentation process leads to a reduction of the lactose content of milk and an increase in lactic acid (Keszei*et al.*, 2010). The cholesterol-lowering effect of yoghurt in the body might also be considered in this aspect (Pigeon *et al.*, 2002).

It has been found that butter contains the second highest level of T (3.54 ng/g), followed by milk (3.09 ng/g) and cheese (2.61 ng/g). On the contrary, ice cream, sweets such as chocolate, and pastries manufactured with milk and eggs contained 2.02, 1.11and 1.31 ng T/g, respectively. These values represent between 18-33% of the T content in eggs.One study found that almost 47% of estrogens intake in a standard human diet came from dairy products (Moorman and Terry, 2004; Ganmaa and Sato, 2005). Milk and dairy products have been estimated to account for approximately 60-80% of the estrogens and progesterone consumed in the average U.S. diet overall (Hartmann *et al.*, 1998).While the content of butter estradiol 17ß the approached 21% of that in the eggs and contained about 46% of the progesterone found in eggs and about 17% androstenedione of the content of the eggs.

The content of cheese of T, E2, P4 and A2 amounted to 44%, 31%, 56% and 15%, respectively of their contemporaries in eggs. One notable change in dairy production occurred in 1993 when the FDA approved the use of recombinant bovine growth hormone (rBGH), also known as recombinant bovine somatotropin (rBST) (Gandhi and Snedeker, 2000). The use of rBGH is not approved in Canada or the European Union. While rBST is still used to maximize milk production in dairy

cattle, less than 30% of U.S. dairy cattle are now treated and that number is decreasing (Gandhi and Snedeker, 2000).

For the naturally occurring hormones; estradiol 17β (CVM, FDA 1994 b), progesterone (CVM, FDA 1994 a), and testosterone (CVM, FDA 1983), FDA has set the allowable incremental increases in hormone levels above those normally present (Table 1).

Table (1). The maximum allowable levels of the E2, P4 and T in muscles, liver, kidney and fats

Hormone	Muscle	Liver µg/kg (ppb)	Kidney	Fat
Estradiol 17 β	0.12	0.48	0.36	0.24
Progesterone	3	12	9	6
Testosterone	0.64	2.6	1.9	1.3

Despite the high detected levels of the four sex hormones in eggs, relative to milk and other products, there still these levels below the maximum allowable levels stated by the CVM/FDA (1994a) as shown in Table (1). The World Health Organization (WHO) guidelines for these hormones confirm that the values obtained in the current study are still in the average allowable levels for safe human uses (FAO/WHO, 1999). The approved US-FDA guidelines, so far depend on acceptable residue levels in tissue from hormone-treated animals on the presumably highly overestimated production rates in prepuberal children given in the JECFA report (Anderson and Skakkebaek, 1999). In conclusion, this study confirmed that the consumers, especially the children, adolescents and pregnant women must not exceed consuming more than 3 eggs per day. Moreover, due to the intensive existence of these hormones in the imported powdered and condensed milk, it is advised that consumers must consume the fresh local milk to avoid the cumulative effects of such hormones in their bodies.

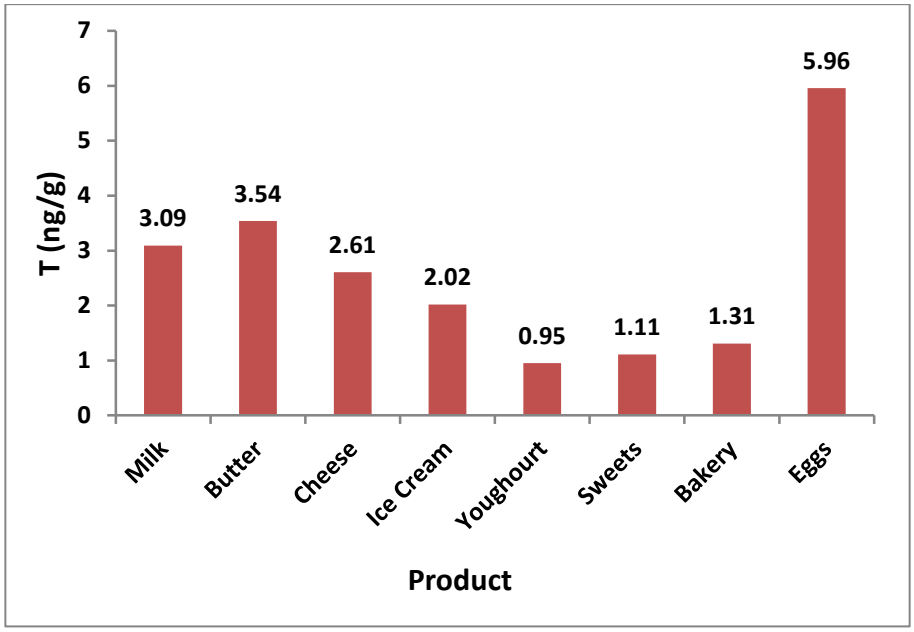

Figure (1). Levels of Testosterone in Milk, Eggs and Their Products.

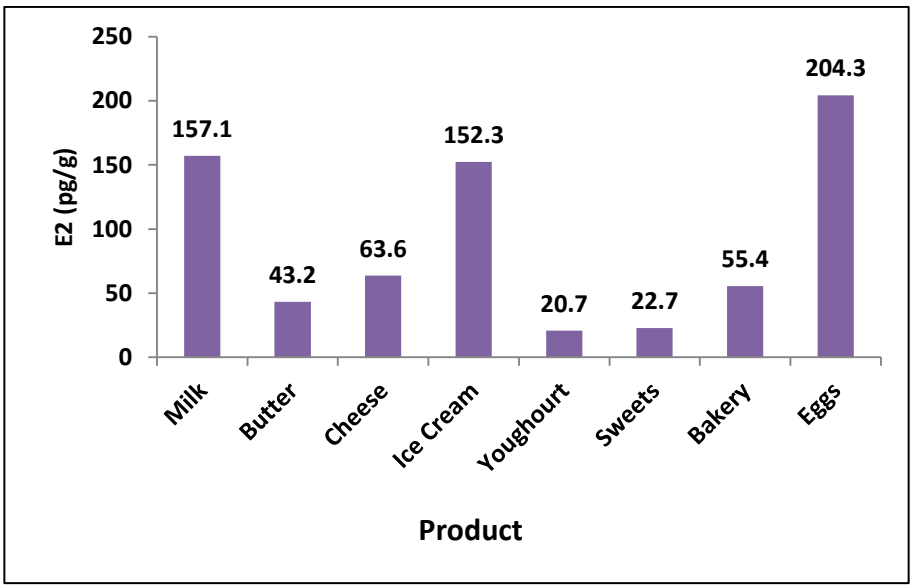

Figure (2). Levels of Estradiol 17βin Milk, Eggs and Their Products.

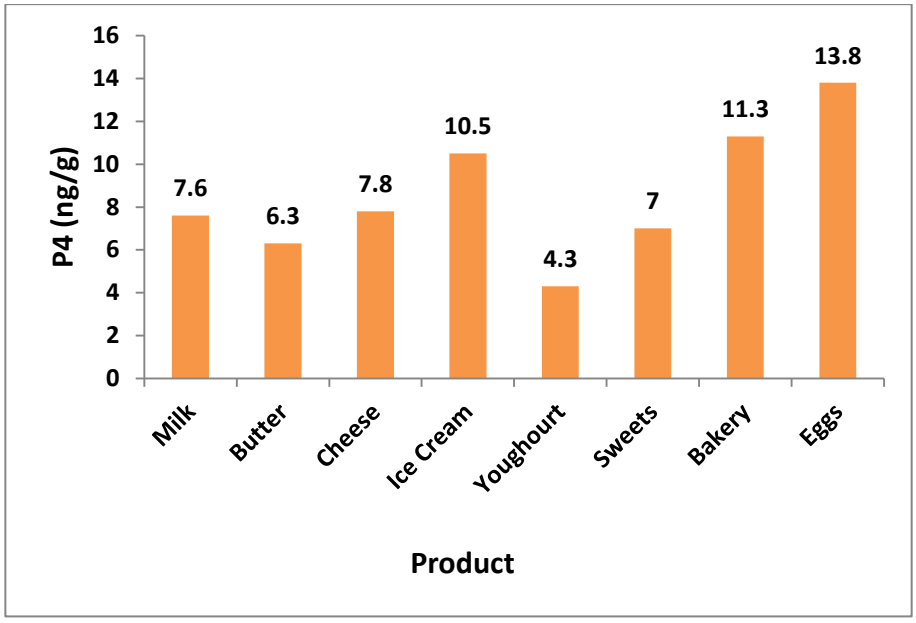

Figure (3). Levels of Progesterone in Milk, Eggs and Their Products.

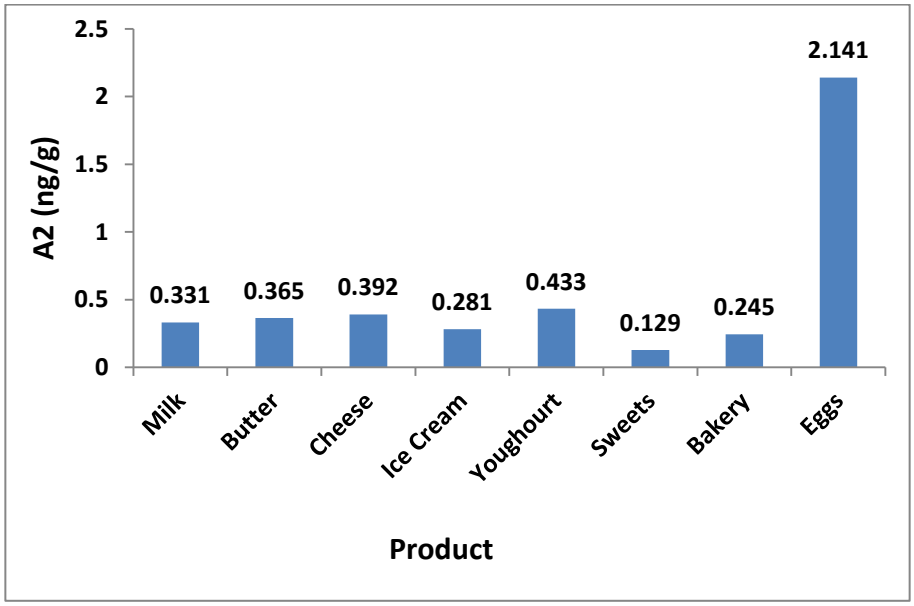

Figure (4). Levels of Androstenedionein Milk, Eggs and Their Products.

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Physico-chemical Characteristics of Egyptian Ben Seed Oil (*moringaoleifera*lam.) Extracted by Using Aqueous Enzymatic Technique

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ABSTRACT. *Moringaoleifera* Lam. is a multipurpose tree cultivated to use as vegetable spice for cooking and cosmetic and as a medicinal plant. Owing to the use of these seeds and its oil as food, some characteristics were studied. The seeds oil of the Egyptian ben (*Moringaoleifera*) was extracted by an aqueous-enzyme-assisted technique using an olive two-phase decanter mill. The enzyme was chosen following a preliminary study conducted on the enzymatic extraction which showed protease to be the best enzyme with the highest oil recovery value. Chemical and physico-chemical characteristics and fatty acid compositions of the extracted oil were determined. The mature seeds contained 40.84% (crude protein), 33.48% (crude fat), 11.55% (carbohydrate), 9.96% (crude fiber) and 4.17% (ash) as dry matter. The chemical and physico-chemical characteristics of the extracted oil were:refractive index (20°C), 1.4665; density (20°C), 0.9122 g/cm³; smoked point, 207˚C; color (1-in. cell) 3.6 R - 35 Y; saponification value, 194.4; unsaponifiable matter, 0.58%; acidity (as oleic acid), 1.4%; total Phenols, 466.8 ppm and total Tocopherols, 71 ppm. The oxidative stability of *Moringaoleifera* seed oil was determined and found to be, Peroxide value, 5.44 meqO2/Kg; Thiobarbituric acid, 0.874 malonaldhyde/kg oil; Rancimate induction period, 304.8 hr and Specific extinction at 232nm, 1.3720 and 270 nm, 0.1018. Results obtained also showed that, the Moringa oil is highly unsaturated 77.61 %, due to its high content of oleic acid (72.29%). These results strongly suggested the potential use of Moringa oil as high quality edible oil and for industrial applications.

***Keywords*:** Aqueous enzymatic extraction, Fatty acid composition,Moringa seed oil, Physico-chemical characteristics, Oxidative stability.

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INTRODUCTION

Consumption of edible oils has grown with the increase in world population. The increasing health awareness and consciousness amongst consumers made the food industry more discriminating in the types of oil they use for food applications. Many circumstances have focused attention on high-oleic vegetable oils, which have been demonstrated to reduce the risk of coronary heart disease. The demand for high-oleic oils is increasing but there are only a few known sources available. *Moringaoleifera*seed oil, which is naturally high-oleic oil, therefore, presents a great opportunity for the oil industry for meeting this ever-increasing demand, (Abdulkarim; 2006).

MoringaoleiferaLam. is the most widely cultivated, known and utilized species belong to the family Moringaceae that includes 13 species of trees and distributed in sub-Himalayan ranges of India, Sri Lanka, North Eastern and South Western Africa, Madagascar and Arabia (Fahey, 2005; Mughal *et al*., 1999; Anwar and Bhanger, 2003). This rapidly-growing tree also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree, was utilized by the ancient Romans, Greeks and Egyptians (Fahey, 2005). The Moringa tree is well-regarded as a fast growing tree in the tropics and subtropics with great economic value for the food and medicinal industries (Makkar and Becker, 1999). It is cultivated to be used as a vegetable (leaves, green pods, flowers, roasted seeds), for spice (mainly roots), for cooking and cosmetic oil (seeds) and as a medicinal plant (all plant organs) (Ramachandran*et.al*, 1980; Jahn, 1996). *Moringaoleifera* seed kernels contain oil that is commercially known as "Ben oil" or "Behen oil". *Moringaoleifera* seed oil (yield 30-40% by weight). It is characterized by a pleasant tasting, highly edible, a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads and in the manufacture of perfume and hair care products (Tsaknis*et al*., 1999). It is also been used by watchmakers for illumination and lubrication of delicate mechanisms. The oil was reported to be resistant to rancidity and used extensively in the process of effleurage (a process of extracting perfumes by exposing absorbents to the exhalations of flowers) (Ndabigengeser and Narasiah, 1998). *Moringaoleifera* seed oil resembles olive oil in its fatty acid composition and therefore, can be used as a possible substitute to the expensive olive oil (Ramachandran*et al*., 1980). The characteristics of *Moringaoleifera* seed oil can be highly desirable especially with the current trend of replacing polyunsaturated vegetable oils with those containing high amounts of monounsaturated acids (Corbett, 2003). High oleic acid vegetable oils have been reported to be very stable even in highly demanding applications like frying (Warner and Knowlton, 1997). The press cake obtained after oil extraction has water soluble proteins that act as effective coagulants for water purification (Foidl*et al*., 2001).Moringa seed oil content and its physico-chemical properties show a wide variation depending mainly on the species and environmental conditions (Ibrahim *et al*., 1974).

Usually solvents are used to extract fats and oils. Solvents, however, are not environmentally friendly. Enzymes, on the other hand, considered to be a green catalyst, and are often used to improve the efficiency of oil extraction. Rosenthal *etal.*, 1996, have reviewed the use of enzymes in the extraction of oil, protein, and other components from oil-containing seeds and fruits. Also, the advantage of enzymatic extraction over solvent extraction has been highlighted in several previous studies (Abdulkarim*et al*., 2005; Sajid and Farooq, 2008) whom mention, for example, the extraction temperature is lower, explosive solvents not required and harmful waste are not produced. Enzymatic extraction processes are carried out in an aqueous medium, thus phospholipids are separated from the oil so that there is no need for degumming thereby reducing the overall cost of processing of the oil to the final product (Christensen, 1991).

Currently, much effort is being devoted to develop and introduce commercial enzymes for aqueous enzymatic oil extraction (Sajid and Farooq, 2008). This technology has been developed to extract oil on a laboratory and pilot scale from many oil-bearing materials. So the aim of this study is to evaluate the physico-chemical characteristics of *Moringaoleifera* seed oil extracted by using enzymatic aqueous extraction methods.

MATERIALS AND METHODS

Materials

Moringaoleifera seeds

The *Moringaoleifera* seeds (Egyptian Varity) were obtained from Desert Research Center, El-ShaikhZwaid experimental station, North Sinai desert, Egypt. The *Moringaoleifera* pods obtained in dry state of mature fruits which chilled manually by hand to obtain seeds then stored at room temperature until analysis and oil extraction.

The Protease from *Bacillus sp*.

The protease from *Bacillus sp.* is highly soluble in detergents at a wide range of pH and temperatures and with a declared activity of 16 Anson units per gram (AU/g) was donated by NovozymesBagsvaerd, Denmark.

Methods

Preparation of*Moringaoleifera*seeds

The obtained *Moringaoleifera* seeds were crushed twice using grinder then stored in deep freezer at -18˚C ±2 until analysis. Some of *Moringaoleifera* seeds were taken and used to separate shells from kernels by hand then every part grinded alone then stored at -18˚C ±2 until analysis.

Aqueous enzyme extraction of *Moringaoleifera* seeds oil

Dried *Moringaoleifera* seeds were soaked in water for 24 hr, and then crushed to a fine paste by inner hammer crusher then the obtained *Moringaoleifera* seeds paste was mixed by stirring the paste slowly and continuously in mixing units (consisting of semi-cylindrical with an outer chamber through which heated water circulates) with distillated water at a ratio of 1:6 w/v. The mixture was then gently boiled for 5 minute and allowed to cool down to room temperature. Two percent v/w of protease from *Bacillus sp*. was added and the mixture incubated at 45˚C for 24 hour with continuous stirring or movement by means of a spades inside the mixing unit (malaxer) (Abdulkarim*et al*., 2005). Afterwards the paste is pumped into an industrial decanter where the emulsion phase (seed cake) is separated from the solid phase. The emulsion was then decanted into a separating funnel and allowed to separate into the oil and water layer which drained off to obtain the oil (Aparna*et al.*, 2002). The obtained oil was stored at -18˚C until it was analyzed.

***Moringaoleifera*seed cake**

The *Moringaoleifera* seed cake wascollected then sun dried and milled to a fine powder and stored at -18˚C until it was analyzed according to (Sajid and Farooq, 2008).

Proximate analysis

The *Moringaoleifera* seeds, seed shell, seed kernel and seed cake were analyzed for moisture, total ash, crude protein (N×6.25), and crude fiber and oil contents according to the methods described by A.O.A.C. (2000). Total carbohydrate was determined by difference.

Analysis of Extracted *Moringaoleifera* seeds oil

Physical parameters

Specific gravity, Refractive index, Smoked point of extracted oil were determined according to the methods described by A.O.A.C. (2000).

Viscosity was determining using Brookfiled viscometer DVIII Ultra Programmable Rheometer (A.O.A.C., 2000).

Color were determine using Lovibondtintometer (Model E) where the oil sample were placed into an 5.25 inch cell up to three quarter full and the color was determined by achieving the best match with the standard colors scales (yellow, red and blue), (A.O.C.S., 1985)

Chemical parameters

Free fatty acids, Saponification number, Unsaponfication matter, Total phenols and Total tocopherols of extracted oil were determined according to A.O.A.C. (2000).

Oxidative stability

Peroxide Valuewas determined according to the methods described by A.O.A.C. (2000).

The specific extinction at 232 nm and 270 nm were determined according the methods described by A.O.C.S. (1991) where 1% solution of extracted oil in cyclohexane was measured in 1 cm cell at 232 nm and 270 nm using Hitachi U-3210 Spectrophotometer (Hitachi Ltd. Tokyo, Japan).

The Thiobarbituric acid value (TBA) of the extracted oil was determined according to Pearson, (1976) by dissolving a known weight oil sample in carbon tetrachloride (10ml) followed by the addition of 10 ml of 0.67% TBA reagent dissolved in 50% acetic acid. The mixture was then transfer to a reparatory funnel and the aqueous layer was drawn into a test tube which immersed in boiling water bath for 30 minute. The developed color was then measured at 532 nm against a blank reagent. The TBA value was calculated by using the following equation:

TBA value as mg malonaldehyde / kg oil = 7.8 × O.D

Where O.D is the absorbance of the developed color at 532 nm.

Rancimat induction periodof the extracted oil was determined according toTsaknis*et al.,* (1999) by using Rancimate apparatus (Metrohom) 679 Ltd., CH-9100 Herisa, Switzerland. The Rancimat comprises of control unit and the wet sections containing 6 reaction vessels. About 2.5 gm of the tested oil were weighted into each of the six reaction vessels which exposed to a stream of atmospheric oxygen (20L/ hr) at 100±0.2˚C. The volatile decomposition products (mainly organic acids) were trapped in a measuring vessel filled with distilled water (60 ml) and continuously detected with a conductivity curve and results which are outputted on a built-in-printer of the control unit. The induction time is the time needed to reach the break point of this curve (point of greatest curvature).

The break point is designed as the interaction point of the two extrapolated straight parts of the curve. The induction time was then designated, i.e., the time of the start of the experiment and the intersection point.

Determination of fatty acids composition

The fatty acids composition of the extracted oil was determined by Gas-Liquid chromatography (GLC) according to A.O.A.C. (1990). The sample was injected into Perkin-Elmer gas liquid chromatography Model 8310 with graphic printer Model 100 was used for detecting the fatty acids under the following operator conditions:

* Detector: Dual flame ionization, temperature at 250˚C.
* Column: Coiled stainless steel (1.8m (length of colum) × 0.3mm), peaked with 15% DEGA chromosorb W, Aw (80-100 mech).
* Carrier gas: helium at flow rate 2ml/min.
* Oven temperature program:

Initial temperature: 100˚C (Hold for 1 min).
Rate 5˚C / min to 200˚C (Hold for 1 min).
Rate 6˚C / min to 250˚C (Hold for 1 min).

Statistical analysis

The different data were exposed to proper statistical analysis according to Statistical Analysis User' s Guide (SAS 1988). Duncan multiple rang at 5 % level of significance was used to compare between means.

RESULTS AND DISCUSSION

Proximate analysis

The proximate chemical analysis of *Moringaoleifera* seeds were analyzed according to their potential food value where it can be eaten as green, roasted, powdered and steeped for tea or used in curries Gassenschmidt*et al.*, (1995). Thus as it was reported that *Moringaoleifera* seeds are also sometimes eaten without any previous treatment, considerable caution should be exercised in the use of this seeds as food since the nature of the adverse factor is presently unknown Oliveira and Silveira (1999).

Therefore, the proximate chemical analysis for *Moringaoleifera* seeds, seed kernel and seed shell were studied.

Results obtained in Table (1) shows that the moisture content of seeds, seed kernel and seed shell shows to be 5.45%, 5.72 and 7.75%, respectively, which are generally low and this is an indication that they can be stored for a long time without the development of moulds, (Anhwange*et al.*, 2004). Also this value was fall below the 15% moisture content required as safe storage limit for plant food materials as reported by Sena*et al.*, (1998).

Table (1).Proximate analysis of *Moringaoleifera* seeds, seed kernel, seed shell and seed cake

Proximate analysis (%)	**Seeds**	**Kernels**	**Shell**	**Seed cake**
Moisture content	5.45b	5.72b	7.75a	5.82b
Crude protein #	40.84b	42.29a	7.72c	42.50a
Fat #	33.48b	40.87a	3.63d	10.25c
Crude fiber #	9.96b	1.55c	6.24a	2.88c
Ash content #	4.17a	2.85b	5.43a	3.72b
Total carbohydrate # *	11.55c	12.44d	76.98a	40.65b

: Dry weigh basis.

*: Calculated by differences.

Mean values in the same row bearing the same superscript do not differ significantly ($P \leq 0.05$).

*Moringaoleifera*seeds, seed kernel and seed shell contained 40.84%, 42.29% and 7.72% of crude protein, respectively, which was higher than those data obtained by Maher, (2006) who found that the crude protein content of *Moringaoleifera* seeds and seed kernel was 27.2% and 34.6%, respectively, but was quietly agree with the seed shell crude protein contents which found to be 7.02%. On the other hand, our result of seed protein content was found to be in accordance with the protein percent (40.31%) obtained by Anhwange*et al.*, (2004).

Also, it could be found that, the percent of crude protein in *Moringaoleifera* seeds was higher than those data reported by Bullock *et al.*, (1989) and Ranhotra *et al*., (1996) for important grain legumes and cereals which contain, in general, 18 to 25% and 7.8 to 22.8% dry matter crude protein content, respectively.

Furthermore, the obtained data of the same table evident that, the oil contents of seeds, seed kernel and seed shell was 33.48%, 40.87% and 3.63%, respectively. The highest percent of oil make *Moringaoleifera*seeds as distinct potential for the oil industry. These results are quite agreement with those obtained by Farooq and Umer, (2007) and Abdulkarim*et al.*, (2005) whom found that the oil percent of *Moringaoleifera* seeds was 34.8% and 30.8%, respectively. Also they mentioned that the variation in oil yield may be due to the differences in variety of plant, cultivation climate, harvesting time of the seeds and the extractions methods.

Therefore, it could be reported that, the percent of oil in *Moringaoleifera* seeds was higher than the oil contents of soybean varieties which ranged from 14.9% to 22% as reported by Leffel and Rhodes (1993). On the other hand, the oil content of *Moringaoleifera* seeds was comparable to that of the other oil seeds such as sunflower seed oil (45.5%) as mentioned by Alza and Fernandez, (1997) and rapeseed (43.7%) as reported by Zhou *et al.*, (1990).

So it could be concluded that the tested *Moringaoleifera* seed kernel contained a much higher contents of protein and oil. Thus, *Moringaoleifera* seed kernel could be potentially a good source of protein and oil, which should be exploited to determine if they are commercially viable and utilized towards human nutrition field.

Crude fiber and Ash content of *Moringaoleifera* seed were 3.47% and 4.17% respectively. The values of these parameters were quite agreement with those reported with Nzikou*et al.*, (2009) whom found that crude fiber and ash content of *Moringaoleifera* seed from Congo-Brazzaville was 3.2% and 4.2 %, respectively.

In the same time, it could be reported that, *Moringaoleifera* seed cake considered to be a good source of protein (42.29%) which could be added to poultry feed as a source protein and of calories then it may be replaced of soybean meal in the local poultry industry as reported by Farooq*et al.*, (2006).

Physical parameters

The results of physical parameters of the extracted *Moringaoleifera* seed oil (MOO) were shown in Table (2).

Table (2).Physical parameters of extracted *Moringaoleifer*a seeds oil.

Physical parameters		*Moringaoleifera*oil
Specific density (20˚C)		0.9122 ± .012
Refractive index (20˚C)		1.4665 ±0.0
Viscosity (cP)		11776 ±3.06
Smoked point (˚C)		207 ±2.52
Color units*	Y	35
	R	3.6

*Lovibond using 5.25 inch cell.

Values are expressed as means ± standard deviation.

Regarding to the results of specific density of MOO at 20˚C presented in Table (2), it was found to be 0.9122. These obtained values are in close agreement with those obtained by Tsaknis*et al.*, (1999) that mentioned that density value of *Moringaoleifera* oil was 0.9182. On the other hand our value was higher than those data reported by Sajid and Farooq, (2008) who mentioned that density values were 0.866.

The refractive index is considered as one of the most important parameters used for estimation of the degree of unsaturation as well as its correlation with iodine value El-Sheikh (1993). From Table (2), refractive index of MOO at 20˚C was 1.4665, which was in agreement with those values obtained by Sajid& Farooq, (2008) and Maher, (2006).

As given in Table (2), the viscosity value of extracted MOO was 11776 cP and this is much higher than the value of 85 CP, obtained by Abdulkarim*et al.*, (2006).The smoked point of the tested MOO was found to be 207˚C which were slightly higher than that found by Maher, (2006) which was 198˚C but was found to be in accordance with the value (203˚C) reported by Anwar and Bhanger, (2003), These differences of viscosity and smoked point values may be due to the differences in variety of plant type and cultivation climate.

The color of the edible oil is one of the most considerable commercial important physical characteristics. In Table (2) shows that, the MOO color was (35 Y, 3.6 R) and this is agree in their yellow units and higher in their red units than those reported in literature (Lalas and Tsaknis, 2002; Anwar and Bhanger, 2003; Tsaknis*et al.*, 1999). The intensity of the color of vegetable oils depends mainly on the presence of various pigments such as chlorophyll, which are effectively removed during the degumming, refining and bleaching steps of oil processing. Vegetable oils with minimum values of color index are more suitable for edible and domestic purposes Farooq *et al.*, (2006).

Chemical parameters

The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Therefore, the free fatty acids (% as oleic acid), saponification number, unsaponification matter, total phenols and total tocopherols were determined and data were shown in Table (3):

The contents of free fatty acids (% as oleic acid) of the MOO was 1.4% and these were found to be in line with the value obtained by both Abdulkarim*et al.*, (2005) and Lalas and Tsaknis, (2002) whom found that the free fatty acids (% as oleic acid) were 1.13% and 1.12%, respectively. This can be attributed to the water added during the extraction of the oil which enhanced the action of lipolytic enzyme Sengupta and Gupta, (1970).

Also the obtained results in Table (3) illustrated that the saponification number of MOO was 194.7 mg KOH/g oil which were within the permissible levels (188-194 mg KOH/g oil) for the edible oil sunflower reported in the Egyptian Standard Specifications (1993).

Table (3).Chemical parameters of extracted *Moringaoleifer*a seeds oil

Chemical parameters	*Moringaoleifera oil*
Free fatty acids (%)	1.4 ±0.17
Saponification number (mg KOH/g oil)	194.7 ±1.0
Unsaponification matter (%)	0.58 ±0.03
Total Phenols (ppm)	466.8 ±2.0
Total Tocopherols (ppm)	71.0 ±0.89

Values are expressed as means ± standard deviation.

According to Egyptian Standard Specifications (1993-2000), the unsaponification matter content should not exceed 1.5% in the edible oil. The content of the investigated MOO was 0.58% which found to be well in line with that obtained by Abdulkarim *et al*., (2005) whom found that the unsapnofiction matter was 0.59%, on the other hand it found to be lower than the value of 0.71% obtained by Sajid and Farooq, (2008).

Total phenols content considered to be one of the most important quality parameter due to its higher activity as antioxidant. Thus, it was important to determine this parameter in our investigated MOO which was 466.8 ppm as gallic acid and which found to be very much higher than the value reported by Fouad, (2005) who mentioned that total phenols content of olive oil extracted by centrifugal system was 210 ppm as gallic acid. No previous reports on the total phenol content of MOO are available in literature which with the data of present analysis could be compared.

Tocopherols is considered to be a natural antioxidant compounds and hence knowledge of the edible oils content of tocopherols can provide us information about their oxidative state especially the content of α-tocopherol which exhibits the highest vitamin E activity Rossell, (1991). Therefore, total tocopherols content were determine as α-tocopherol and found to be 71 ppm which was in harmony with those data (72.76 ppm) obtained by Sajid and Farooq, (2008). On the other hand, our result was so much higher than the value (15.38 ppm) reported by Lalas and Tsaknis, (2002) as α-tocopherol.

Oxidative stability

The oxidative stability of extracted *Moringaoleifera* seed oil was determined by measuring the Peroxide value (PV), Thiobarbituric acid (TBA), Specific extinction at 232 and 270 nm and Rancimate induction period. The obtained results were tabulated in Table (4) as following:

Table (4). Oxidative stabilityof extracted *Moringaoleifer*a seeds oil.

Oxidative stability Parameter	*Moringaoleifera* oil
Peroxide value (meqO2/Kg)	5.44 ±0.1
TBA value (malonaldhyde/kg oil)	0.874 ±0.02
Specific extension at 232 nm	1.3720 ±0.013
Specific extension at 270 nm	0.1018 ±0.01
Rancimate at 100˚C (hr)	304.8

Values are expressed as means ± standard deviation.

The commonly used methods for measuring the auto-oxidation statues of any edible oil are the formation of the peroxides and (TBA) test, Basuny, (1990). The first parameter considered to be an indicator for the extent of forming the hydroperoxides (the primary products of lipid oxidation) but peroxide value alone is not a suitable parameter to assess the extent of fats and oils deterioration because of the faster breaking down rate of the hydroperoxides than its formation to form the secondary oxidation products (responsible for the off flavors of rancid oil) which measured by TBA test.

Results in Table (4) showed that the peroxide value of MOO was 5.44 meqO2/Kg, this value was higher than those value reported by Sajid and Farooq, 2008; Farooq and Umer, 2007; Maher, 2006. However, the peroxide value of MOO was lower than the recommended value which should not exceed 15meqO2/Kg for human consumption as reported by Egyptian Standard Specifications (1993-2000).

Results of TBA analysis presented in table (4) demonstrated obviously that TBA value of MOO were 0.874 as mg malonaldhyde/kg. This value was higher than the value reported by Maher (2006) as he found that, TBA of the extracted MOO was 0.026 as mg malonaldhyde/kg. On the other hand, our value was greatly much lower than the critical value which must not be exceeding 10 mg malonaldhyde/kg oil for the edible purpose as reported by Almandoes*et al*. (1986) and Schmedes and Holmer (1989).

Ranalli*et al*. (2000) mentioned that, the specific extinction values at 232 and 270 nm measure the level of primary and secondary oxidation, respectively. From table (4), it could be noticed that, the measured values at 232 (related to peroxide value results) were 1.3720, this value is in accordance with those obtained by Farooq and Umer (2007) who mentioned that the conjugated fatty acids diene (at 232 nm) of MOO was 1.38, while it was slightly lower than the value of 1.803 found by Maher (2006) for extracted MOO.

With regards, the specific extinction at 270 nm (related to TBA results) was found to be 0.1018 and this value supposed to be much lower than the values obtained by both of Farooq and Umer (2007) and Sajid and Farooq (2008). However, our conjugated fatty acids triene (at 270 nm) value was lower than the recommended critical level which should not exceed 1.0 for the edible oils as reported by Egyptian Standard Specifications (2000).

The induction period, which is characteristic of the oxidative stability of the edible oils and fats (Anwar *et al.*, 2003), was measured by Rancimate method at 100˚C. The Rancimate induction period can be useful because it act as “screening” test and eliminate the possibility of introducing lower stability oils into the production area (Morton and Chidley, 1988).

As indicated in Table (4) the induction period of MOO was found to be 304.8 hr. we can attributed this results to the highly content of phenols as mentioned above. Also, our present result was found to be very much higher than those reported by Hisham (2005) who found that the induction period of the extracted MOO was 190.26 hr.

Fatty acids composition

It is well known the knowledge of the fatty acids composition of new vegetable edible oil can give us an expecting for its possibility uses for edible or industrial purpose. Also the physico-chemical characteristic of lipids largely depends upon their fatty acids profile (Basuny, 1990). So the fatty acids composition of MOO was determined by GC and the results were tabulated in Table (5).

Table (5). Fatty acids composition of *Moringaoleifer*a seeds oil.

Fatty acid composition		Determined value (%)
Myristic	C14:0	0.07
Palmitic	C16:0	7.79
Palmitoleic acid	C16:1	1.78
Stearic	C18:0	4.08
Oleic acid	C18:1	72.29
Linoleic	C18:2	1.01
alpha-linolenic	C18:3	0.34
Arachidic	C20:0	3.59
Eicosanic acid	C20:1	2.19
Behenic acid	C22:0	6.87
Lignoceric	C24:0	1.14
Total saturated fatty acids		22.39
Total unsaturated fatty acids		77.61

From data in Table (5) we can conclude that the total saturated fatty acids were 22.40%, the major saturated fatty acids was Palmitic acid with 7.79% followed by Behenic acid with a substantial amount 6.87%. Therefore, the MOO considered to be used as a natural source of behenic acid, which has been used as an oil structuring and solidifying agent in margarine, shortening and foods containing semi-solids and solid fats, eliminating the need to hydrogenate the oil, (Abdulkarim*et al.*, 2005).

The total unsaturated fatty acids were 77.61 %, the dominant unsaturated fatty acid was Oleic acid with 72.29 % and this percent was slightly higher than the percent of 70.0% obtained by Abdulkarim*et al.* (2005). These make it desirable in terms of nutrition and high stability cooking and frying. Also the higher intake of good fats (monounsaturated/oleic) is associated decrease risk of coronary heart diseases (Corbett, 2003). In addition, high-oleic oils have low saturated fatty acids levels. Therefore, high-oleic oils can viewed as a healthy alternative to partially hydrogenated vegetable oils, (Abdulkarim*et al.*, 2005).

CONCLUSION

From the above results, it could be concluded that the *Moringaoleifera* seed oil exhibit as a good higher oxidative state and stability and healthy oil, this is due to the higher level of monoenoics fatty acids, particularly oleic cid, which is less prone

to the oxidation than polyenoics fatty acids and also to the presence of high percent of phenols compounds. Also, *Moringaoleifera* seed oil has the potential to become a new source of high-oleic acid oil and its full potential should be exploited. It contains high monounsaturated to saturated fatty acids ratio and might be an acceptable substitute highly monounsaturated oils such as olive oil in diets. The production of such useful oil like *Moringaoleifera* oil could be economic benefit to the native population of the areas, where the tree is cultivated.

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Effect of Organic Acids Pretreatments on Physicochemical Properties and Shelf Life of Refrigerated BoltiFish *(Tilapia nilotica)*

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ABSTRACT. Quality of whole Nile Bolti fish (*Tilapia nilotica*) stored under refrigeration at 4±1°C after dipping in aqueous solutions (2 liter of dipping solution: 1 kg of fish) of acetic acid (AA, 1%), citric acid (CA, 3%) and mixture of (1% AA+ 3% CA) for 5 minutes was investigated. Changes in physicochemical parameters (chemical composition, pH, thiobarbituric acid values (TBA), total volatile basic nitrogen contents (TVB-N), water holding capacity (WHC) and plasticity) were evaluated during 12 days of storage. The data revealed that, the suggested treatments were significantly affected the chemical composition, especially for the lipids and protein contents. Sample dipped in acetic acid (1%) had significantly lower pH value (5.95), at the end of storage time than the control and other treated samples. The highest values of WHC during storage were given by fish samples treated with CA followed by AA and its combination with CA. Significant reduction in TBA concentrations and total TVB-N in fish samples treated with selected organic acids was assessed when compared to control. In conclusion, the results found that acetic acid and citric acid solutions, especially in combinations, can be utilized as safe organic preservatives for Boltifish under refrigerated storage up to 12 days.

Keywords:Bolti fish, acetic acid, citric acid, refrigerated storage, physicochemical properties

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INTRODUCTION

Fish is one of the most highly perishable food products, due to its biological composition, the low salt content, the high level of moisture, protein and free amino acids and the high pH and water activity (Hernández *et al*., 2009). Fresh fish spoilage process will start within 12 h of their catch in the high ambient temperatures of the tropics (Berkel*et al*., 2004). Rigor mortis is the process through which fish loses its flexibility due to stiffening of fish mussels after few hours of its death (Adebowale*et al*., 2008). The quality of fish degrades, due to a complex microbiological, physiological and enzymatic breakdown of major fish molecules that lead to its deterioration (Ghaly*et al*., 2010). Degradation of fish protein muscles have been related to proteolytic activity which results in protein solubilization(Opara*et al*., 2007). Many indices have been used for the assessment of fish quality during storage, such indices comprise changes in the microbial population chemical changes, including total volatile basic nitrogen and thiobarbituric acid contents, which show a good correlation with sensory analysis (Elena-Ciornea*et al*., 2009; Hélène*et al*., 2010).

Different methods have been used for extending fish products shelf life such as cold storage and antioxidants (Rostamzad*et al*., 2011a). Organic acids could be used as antimicrobial compounds, it must qualify as being safe according to the specific country's legislation (Ghaly*et al*., 2010). Low molecular weight organic acids, such as acetic, lactic, and citric acid have been used in a dip treatment to improve sensory attributes and extend the shelf life of fish because of their availability (Ray and Bhljnia, 2008, García-Soto *et al*., 2013). Elnimr (2011) investigated that, pre-treatment of Bolti fish by acetic acid (5%) for a period of 15 min resulted in marked reduction of heavy metals in fish muscles, he suggested that, the reduction of heavy metals in fish pre-treated with acetic acid could be due to formation of insoluble acetate salts of these metals. The present study was aimed to evaluate the quality of Egyptian Bolti fish by treated with organic acids, and their effect on the physicochemical properties of fish during refrigerated storage.

MATERIALS AND METHODS

Materials

Fresh Nile Bolti fish (*Tilapia nilotica*) were collected through July 2012 from General Authority for Fish Resources Development, Cairo, Egypt. The average weight of each one was about 170 g. Fish samples were put in ice box and immediately transferred to Food Science Department, Faculty of Agriculture, Ain Shams University for further treatments.

Methods

Preparation and treatment of fish samples

Fish samples were washed by tap water, eviscerated, scaled, rewashed and drained, then divided into three groups which were separately immersed for 5 minutes in an aqueous solutions (2 liter of dipping solution : 1 kg of fish) containing citric acid (3% w/v) or acetic acid (1% v/v) or mixing solutions from (3% w/v) citric acid and (1% v/v) acetic acid. After pre-treatments, each group of samples (triplicate) were immediately drained well and packed in polyethylene bags, labeled and refrigerated at 4±1°C, while control samples were immersed in distilled water only (Taheri*et al*., 2012).

Proximate chemical composition

Bolti flesh samples were analyzed for moisture, protein, fat, and ash contents according to the methods of AOAC (2005).

Change in pH

Change in pH values of the fish homogenates were measured according to the method of Suvanich*et al*., (2000).

Water holding capacity and plasticity

Thefilter press method described by Eide*et al*., (1982). was followed for Water holding capacity (WHC) and plasticity (cm^2/ 0.3 g sample) evaluation.

Thiobarbituric acid:

Thiobarbituric acid (TBA) values (mg malonaldehyde/kg sample) were determined according to Siu and Draper, (1987) by multiplying the absorbance at 532 nm by 7.8.

Total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) content (mg N/100 g sample) was performed using Macrokjeldahl procedure according to Harold *et al*., (1987).

Statistical analysis

Obtained data were statistically analyzed using the Statistical Analysis System according to User's Guide of Statistical Analysis System (SAS, 2000) by one-way analysis of variance (ANOVA). Differences among the mean values of the various treatments and storage times were separated using Duncan's Multiple range test at significant level 95 % ($P \leq 0.05$).

RESULTS AND DISCUSSION

Proximate chemical composition

Proximate chemical composition (moisture, protein, lipid, and ash) of treated Bolti fishsamples during refrigerated storage at 4±1°C is listed in Table (1).

It could be observed that, moisture content was around 82.44% at zero time of cold storage among all investigated samples, then subsequently decreased after 12 days of storage by loss rate being 8.5, 5.8, 5.5 and 8.3% for control, AA, CA and AA+CA treated samples, respectively. Samples immersed in citric acid (3%) had the highest moisture content (77.32%) at the end of cold storage time. At zero time, crude protein content of control sample (84.15%) was close to crude protein levels of other treated fish samples (84.05, 83.91, 85.29% for AA, CA and AA+CA treated samples) respectively, such content consequently dropped during refrigerated storage by loss rate being 13.8% in control treatment, 10.5% in AA treatment, 9.2% in CA treatment and 11.4% in AA+CA treatment.El-Shamery, (2010) mentioned that, the decrease of fish moisture content may be due to evaporation of water during storage, and the decrease of protein content may be due to the loss of nitrogen as volatile bases and nitrogenous substance.

The lipid content of different treated samples under investigation was around 9.8% at zero time of storage then significantly ($p \leq 0.05$) increased up to the end of various storage time. These results have gone inversely with those of moisture and protein contents as shown before. Ash content of all investigated samples were about 5% (dry basis) at zero time of storage. However, during cold storage a significant ($p \leq 0.05$) decrease was observed. This may be due to the leaching out of soluble nitrogenous compounds, water soluble vitamins and minerals in the resultant drip, Abd El-Razik (1997).

Table (1). Proximate chemical composition (% on dry weight basis) of Bolti fish immersed in different organic acids and refrigerated at 4 ± 1°C for 12 days.

		Storage time (days)				
Treatments	**Constituents (%)**	**Zero**	**3**	**6**	**9**	**12**
Control	Moisture	82.89±0.25^{Aa}	79.87±0.29^{Bb}	78.12±0.39^{Cc}	76.90±0.30^{Cd}	75.83±0.34^{Be}
	Protein	85.29±0.39^{Aa}	78.88±0.38^{Db}	77.07±0.13^{Dc}	75.02±0.18^{Dd}	72.50±0.27^{Ce}
	Lipid	10.20±0.02^{Ae}	15.36±0.01^{Ad}	18.21±0.04^{Ac}	21.11±0.01^{Ab}	24.28±0.03^{Aa}
	Ash	5.49±0.02^{Aa}	5.08±0.06^{Bb}	4.48±0.02^{Cc}	3.15±0.01^{Dd}	2.86±0.01^{De}
1% acetic acid	Moisture	82.01±0.52^{Ba}	81.01±0.37^{Ab}	79.83±0.06^{Ac}	79.56±0.11^{Ac}	77.24±0.34^{Ad}
	Protein	84.05±0.36^{Ba}	81.04±0.16^{Cb}	79.26±0.36^{Cc}	77.46±0.11^{Cd}	75.22±0.30^{Be}
	Lipid	10.19±0.21^{Ae}	13.44±0.01^{Bd}	15.26±0.05^{Cc}	18.43±0.04^{Bb}	21.03±0.20^{Ca}
	Ash	5.46±0.03^{Aa}	5.38±0.03^{Ab}	4.55±0.03^{Bc}	3.85±0.04^{Ad}	3.08±0.04^{Be}
3% citric acid	Moisture	81.84±0.36^{Ba}	80.22±0.27^{Bb}	78.77±0.36^{Bc}	78.21±0.14^{Bd}	77.32±0.29^{Ae}
	Protein	83.91±0.40^{Ba}	82.31±0.19^{Ab}	80.18±0.29^{Bc}	78.64±0.08^{Ad}	76.18±0.24^{Ae}
	Lipid	9.43±0.01^{Be}	12.20±0.02^{Dd}	14.82±0.01^{Dc}	17.31±0.02^{Db}	19.91±0.01^{Da}
	Ash	5.440±0.06^{Aa}	5.33±0.12^{Aa}	4.60±0.02^{Ab}	3.79±0.03^{Bc}	3.20±0.03^{Ad}
1% acetic acid + 3% citric acid	Moisture	82.987±0.12^{Aa}	80.513±0.46^{ABb}	78.123±0.09^{Cc}	77.373±0.47^{Cd}	76.063±0.35^{Be}
	Protein	84.150±0.23^{Ba}	81.680±0.14^{Bb}	80.650±0.08^{Ac}	77.760±0.17^{Bd}	75.520±0.04^{Be}
	Lipid	9.410±0.02^{Bd}	12.883±0.03^{Cd}	15.550±0.04^{Bc}	18.327±0.01^{Cb}	21.360±0.06^{Ba}
	Ash	4.787±0.04^{Ba}	4.440±0.05^{Cb}	3.573±0.02^{Dc}	3.503±0.02^{Cd}	2.993±0.03^{Ce}

-Means of triplicate ± Standard Deviation (SD).

- Different capital letters in the same column indicate significant differences ($p \leq 0.05$).

- Different small letters in the same raw indicate significant differences ($p \leq 0.05$).

- Control samples were organoleptically rejected after 6 days of storage.

Changes in pH values

The relation between immersing of Bolti fish samples in organic acids and storage time at 4±1°C on the pH values are shown in Table (2).

Data indicated that, the initial pH value of the control (6.58) could be the same with that of 3% citric acid (6.41), while samples treated with 1% acetic acid (5.82) or mixing of 1% acetic acid + 3% citric acid (5.75) had significantly ($p \leq 0.05$) lower pH. Storage time had a significant ($P \leq 0.05$) effect on the pH values for each of the control as well as, CA and AA+CA treated samples, the pH values were significantly decreased by 0.32 - 0.49 unit at the third day of cold storage, followed

by a significant increase after 12 days of storage. According to Aycicek*et al*., (2004), the decrease in pH values may be attributed to the relationship between post-mortem of fish and the formation of lactic acid from break down of glycogen by soluble muscle enzymes. While, Increasing pH values with the progression of cold storage may be due to the production of volatile basic compounds as a result of protein breakdown or bacterial deamination of amino acids with ammonia production (Hassan *et al*., 1999, Kilinc and Cakli, 2005).

Results also verified that, samples dipped in acetic acid (1%) had significantly ($P \leq 0.05$) lower pH value (5.95), at the end of storage time than the control and other treated samples. These results are in agreement with (Haghparast*et al*., 2010).

Table (2). Effect of organic acids and storage time on pH values of refrigerated Bolti fish.

Storage time (days)	pH values			
	Control	**1% acetic acid**	**3% citric acid**	**1% acetic acid+3% citric acid**
Zero	6.58 ± 0.38^{Aa}	5.82 ± 0.11^{Ba}	6.41 ± 0.15^{Aa}	5.75 ± 0.08^{Bb}
3	6.09 ± 0.24^{Ab}	5.83 ± 0.29^{ABa}	6.05 ± 0.24^{Aab}	5.43 ± 0.10^{Bc}
6	6.42 ± 0.15^{Aab}	6.08 ± 0.24^{ABa}	5.92 ± 0.22^{Bb}	5.76 ± 0.15^{Bb}
9	6.75 ± 0.11^{Aa}	6.14 ± 0.14^{Ba}	6.16 ± 0.17^{Bab}	6.24 ± 0.19^{Ba}
12	6.82 ± 0.13^{Aa}	5.95 ± 0.11^{Ca}	6.21 ± 0.12^{Bab}	6.29 ± 0.13^{Ba}

- Means of triplicate ± Standard Deviation (SD).

- Different capital letters in the same column indicate significant differences ($p \leq 0.05$).

- Different small letters in the same raw indicate significant differences ($p \leq 0.05$).

Water holding capacity (WHC)

Water holding capacity (WHC) values for different treated Bolti fish samples which determined as area of released water in (cm^2/ 0.3g sample), were determined during refrigerated storage at (4±1°C for 12 days) and the results are given in Table (3).

It could be noticed that, the WHC of all samples progressively decreased ($p \leq 0.05$) with the increase of outer zones, resulted from secretion of water from samples. The highest values of WHC during storage appeared for fish samples treated with CA followed by AA-treated samples and its combination with CA, this may be related with fish sample pH. According to Gonçalves and Ribeiro, (2008), when the muscular pH reaches to isoeletric point of the proteins, WHC decreased. This is possibly due to fish protein denaturation or aggregation which subsequently lead to reduce water binding abilities of protein, or to the biochemical changes associated with cooling (Offer and Knight, 1988).

Table (3). Water holding capacity (WHC) of Bolti fish samples immersed in different organic acids and storage for 12 days at 4±1°C

Storage time (days)	WHC (cm^2/ 0.3 g sample)			
	Control	1% acetic acid	3% citric acid	1% acetic acid+3% citric acid
Zero	$7.50±0.30^{Ae}$	$6.63±0.40^{Be}$	$6.43±0.25^{Bd}$	$6.50±0.20^{Bd}$
3	$8.56±0.31^{Ad}$	$7.26±0.31^{Bd}$	$7.10±0.36^{Bc}$	$7.26±0.25^{Bc}$
6	$9.50±0.30^{Ac}$	$8.10±0.30^{Bc}$	$7.46±0.42^{Cc}$	$8.16±0.21^{Bb}$
9	$10.43±0.21^{Ab}$	$9.06±0.31^{Bb}$	$8.10±0.46^{Cb}$	$10.16±0.35^{Aa}$
12	$10.96±0.25^{Aa}$	$9.80±0.20^{Ca}$	$8.90±0.10^{Da}$	$10.43±0.15^{Ba}$

- Means of triplicate ± Standard Deviation (SD).
- Different capital letters in the same column indicate significant differences ($p \leq 0.05$).
- Different small letters in the same raw indicate significant differences ($p \leq 0.05$).

Plasticity

Data given in Table (4) show the plasticity values of differently immersed Bolti fish samples during cold storage at 4±1°C. Significant ($p \leq 0.05$) decremental pattern in sample's plasticity during cold storage was observed. This might be explained on the basis of denaturation and / or aggregation of protein during cold storage, as well as the tightening of fish muscle because of evaporation (Madkour*et al*., 2000). On the other hand, samples treated with CA showed higher plasticity than other treatments which may be due to the effect of citric acid in retarding the formation of lipid oxidation products that able to denature proteins and consequently reduce the flesh tenderness.

Table (4). Plasticity of Bolti fish samples immersed in different organic acids and storage for 12 days at 4±1°C

Storage time (days)	Plasticity (cm^2 /0.3 g sample)			
	Control	1% acetic acid	3% citric acid	1% acetic acid+3% citric acid
Zero	$5.10±0.26^{Aa}$	$4.63±0.15^{Ba}$	$5.23±0.25^{Aa}$	$4.86±0.21^{ABa}$
3	$4.90±0.10^{Aa}$	$4.33±0.21^{Bb}$	$4.70±0.20^{ABb}$	$4.30±0.30^{Bb}$
6	$3.73±0.15^{Bb}$	$3.83±0.15^{Bc}$	$4.33±0.15^{Ac}$	$3.96±0.25^{Bb}$
9	$3.00±0.20^{Bc}$	$3.26±0.12^{ABd}$	$3.50±0.10^{Ad}$	$3.30±0.20^{ABc}$
12	$2.43±0.12^{Cd}$	$2.83±0.15^{Abe}$	$3.07±0.12^{Ae}$	$2.77±0.12^{Bd}$

- Means of triplicate ± Standard Deviation (SD).
- Different capital letters in the same column indicate significant differences ($p \leq 0.05$).
- Different small letters in the same raw indicate significant differences ($p \leq 0.05$).

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) values of Bolti fish samples immersed in different organic acids during 12 days of storage at 4±1°C are given in Table (5).

Data showed that TBARS values of all samples were significantly (P≤0.05) increased with the storage time, and control samples recorded the highest TBA levels at the end of storage up to 12 days in compared to treated samples. Results also indicated that, TBARS after 6 days of storage were above 3.50 for the control (3.74), whereas those fish immersed in CA (3%) or mixing of AA (1%) + CA (3%), of aqueous solutions did not reached the same level by the 12 days of storage. Khidhir (2014) found that control sample had higher (P≤0.05) Thiobarbituric acid (TBA) values (4.065 mg malonaldehyde/ kg) as compared with fish fillets treated with sodium lactate.

However, after 12 days of cold storage, it could be noticed that, TBA values for different organic acid treated samples were lower than the maximum level which is 4.5 mg malonaldehyde/ kg as proposed in ES: 3494/ 2005. It is deduced that, the combination of acetic acid (1%) with citric acid (3% w/v) solutions was more effective for TBARS decreasing during refrigeration of Bolti fish till the end of storage time when compared with control and other samples. Results of TBA values of the present work suggesting that citric acid (3%) has a potent antioxidant effect more than AA (1%). Rostamzad*et al*., (2011b) mentioned that citric acid and its salts are widely known for their role as chelators and acidulants.

Table (5). Changes in TBA values of treated Bolti fish during storage for 12 days at 4± 1°C

Storage time (days)	TBA values (mg malonaldehyde/kg)			
	Control	1% acetic acid	3% citric acid	1% acetic acid+3% citric acid
Zero	1.08 ± 0.05^{Ae}	0.92 ± 0.04^{Be}	0.90 ± 0.05^{Be}	0.79 ± 0.08^{Ce}
3	1.99 ± 0.04^{Ad}	1.72 ± 0.07^{Bd}	1.51 ± 0.06^{Cd}	1.49 ± 0.06^{Cd}
6	3.74 ± 0.08^{Ac}	2.11 ± 0.06^{Bc}	1.80 ± 0.09^{Cc}	1.74 ± 0.06^{Cc}
9	4.50 ± 0.08^{Ab}	2.77 ± 0.11^{Bb}	2.60 ± 0.06^{Cb}	2.46 ± 0.07^{Cb}
12	5.57 ± 0.09^{Aa}	4.11 ± 0.07^{Ba}	3.33 ± 0.05^{Ca}	3.22 ± 0.07^{Ca}

- Means of triplicate ± Standard Deviation (SD).
- Different capital letters in the same column indicate significant differences (p≤ 0.05).
- Different small letters in the same raw indicate significant differences (p≤ 0.05).

Total volatile basic nitrogen (TVB-N)

The changes in total volatile basic nitrogen(TVB-N) values of the control and other treated Bolti fish samples during refrigerated storage at 4±1°C are shown in Table (6).

It could be noticed that, the initial values of TVB-N in different investigated treatments were ranged between 11.01 to 11.99 mg/100g muscle, reaching a value of 15.87, 14.79, 14.33, and 13.44 mg N/100 g by day 3 for each of the control, AA, CA and AA+CA treated samples, respectively. With the progression of cold storage, a significant increase in TVB-N was noticed; these increasing might be attributed to the breakdown of nitrogenous substances as a result of microbial activity and any autolytic enzymes found naturally in fish tissus (El-Shamery, 2010). By the end of the storage time (12 days), a significantly ($P \leq 0.05$) higher value of 29.54 mg/100 g was detected in TVB-N for control comparing with those in the different treated samples. Nevertheless, the TVB-N values in the different samples analyzed, throughout the storage time, were all below the maximum value of 30 mg N/100g flesh specified by the Egyptian standards ES: 3494/ 2005 for chilled fish.

Table (6).Changes in TVB-N of Bolti fish immersed in different organic acids and storage for 12 days at 4± 1°C.

Storage time (days)	TVB-N (mg/100g sample)			
	Control	1% acetic acid	3% citric acid	1% acetic acid+3% citric acid
Zero	11.99 ± 0.21^{Ae}	11.71 ± 0.58^{ABe}	11.15 ± 0.29^{Be}	11.01 ± 0.21^{Be}
3	15.87 ± 0.49^{Ad}	14.79 ± 0.43^{Bd}	14.33 ± 0.53^{BCd}	13.44 ± 0.50^{Cd}
6	22.40 ± 0.50^{Ac}	19.27 ± 0.43^{Bc}	18.39 ± 0.57^{BCc}	17.64 ± 0.56^{Cc}
9	27.21 ± 0.35^{Ab}	23.52 ± 1.06^{Bb}	22.54 ± 0.50^{BCb}	22.17 ± 0.63^{Cb}
12	29.54 ± 0.56^{Aa}	24.92 ± 0.84^{Ba}	24.64 ± 0.64^{Ba}	23.19 ± 0.43^{Ca}

- Means of triplicate ± Standard Deviation (SD).
- Different capital letters in the same column indicate significant differences ($p \leq 0.05$).
- Different small letters in the same raw indicate significant differences ($p \leq 0.05$).

CONCLUSIONS

Pretreatment of Bolti fish with acetic acid (1%), citric acid (3%) and mixture of (1% acetic acid + 3% citric acid) and refrigeration storage at 12 days was evaluated. could be concluded that the highest values of WHC during storage were appeared for fish samples treated with citric acid followed by acetic acid treated samples and its combination with citric acid. The mixture of acetic acid with citric acid solutions

is effective for TBARS decreasing of Bolti fish during storage time when compared to other samples. Therefore acetic acid and citric acid can be utilized as safe organic preservatives for fish under refrigerated storage, but effects were enhanced in combinations.

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Plant production and protection

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Impact of Soil Salinity on Agriculture in Arid Regions

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ABSTRACT. Soil salinity is one of the most brutal environmental factors limiting the productivity of crop plants. Salinity occurs in areas that have one of three criteria; shallow groundwater, bad drainage system or poor quality of irrigation water. In salt affected soils, there are three main types of soils; Saline soils, Sodic soils and Saline-sodic soils. Salinityaffects crop growth by osmoticinfluencesand specific iron toxicities. There is a varying impact of soil salinity and irrigation water on the proportion of production for different crops. In this review**,** some general information is presented on how salinity affects plant growth and how different measurements of salinity in solution cultures and field studies can be reconciled to a common basis.Commonsaline soil remediation techniques to treat salinity include physical removal, leaching, and/or subsurface drainage to reduce groundwater elevations

Keywords: soil, salinity, remediation, arid regions, irrigation

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INTRODUCTION

Salt-affected soils occur in all continents and under almost all climatic conditions. Their distribution, however, is relatively more extensive in the arid and semi-arid regions compared to the humid regions. In arid and semi-arid regions, the concentration of salt-related ions in both water and soil tends to be moderate or relatively high. Water and soil salinity are higher in arid regions where the rate of chemical weathering is higher for arid and semi-arid areas than for humid areas because the temperatures of air and soil are higher. Also, evapotranspiration rates in arid and semi-arid regions exceed those of humid regions because the solar radiation in the former is greater and the air there is drier. Naturally, there are different types of solid salts in the soil profile, in addition to the presence of soluble salts in the water used for irrigation. Due to the high potential of evaporation in arid environment, salts accumulate in soil surface along with shortage water to leach salts from the soil profile. It makes the soil salinity a problematic on the agricultural sector.

Salinity commonly occurs in irrigated soil because of the accumulations of soluble salts introduced from the continuous use of irrigation water containing high or medium quantity of dissolved salts. Accumulation of excess salts in the root zone resulting in a partial or complete loss of soil productivity is a worldwide phenomenon. This review will cover the causes of soil salinity, types of salt-affected soils, measuring of salinity, problems for both agriculture sector and plant production, and soil salinity treatment.

The causes of soil salinity

There are three key mechanisms of soil salinity:

• Soil salinity occurs in regions that have a shallow groundwater where soluble salts in groundwater rise by capillary action into the rooting zone.

• Bad drainage system allowing salts to stay in the plant root zone, andincreasing of salts quantity by rain, irrigation, rock erosion and windtransport.

• Poor quality of irrigation water (high salinity) in areas used irrigation system for agriculture, which is relatively has low hydraulic conductivity and/or high evaporation rates, is a major reason for salts accumulation in the plant root zones.

Salt-affected soils

In salt-affected soils, three main types of soils have been coined by Szabolcs(1974):

A) Saline soils - The soluble salts are chiefly NaCl and Na_2SO_4. The saline soils contain appreciable quantities of Cl^-, SO_4^{--}, Ca^{2+} and Mg^{2+}. These soils contain sufficient neutral soluble salts causing negative effect on growth of most crop plants. They also contain enough soluble salts that injure plants. They are characterized by white or light brown crusts on the surface. Saline soils usually have an electrical conductivity (EC) of more than 4 mmho cm^{-1}. The calcium and magnesium salts are

at a high enough concentration to offset the negative soil effects of the sodium salts. The pH of saline soils is generally below 8.5. The normal desired range is 6.0 to 7.0. Leaching the salts from these soils does not increase the pH of saline soils.

In fact, soils are considered saline when the $EC_e > 4$. When $4 < EC_e < 8$, the soil is called slightly saline. When $8 < EC_e < 16$, it is called (moderately) saline, and when $EC_e > 16$, it is called severely saline (Richards, 1954).

B)Sodic soils – These soils contain Na^+ salts capable of alkaline hydrolysis, mainly Na_2CO_3. Previously, these soils have also been termed as 'Alkali. Alkali soils may or may not contain excess soluble salts.

Sodic soils are low in soluble salts but relatively high in exchangeable sodium. Sodic soils are unsuitable for many plants because of their high sodium concentration, which may cause plant rooting problems, and because of their high pH, which generally ranges from 8.5 to 12.0.

These high sodium levels disrupt both the chemical and physical composition of soil clays. As a result, the soil surface has low permeability to air, rain and irrigation water. The soil is sticky when wet but forms hard clods and crusts upon drying. This phenomenon may not occur in very sandy soils because they lack clay content.

C)Saline-sodic soils are similar to saline soils, except that they have significantly higher concentrations of sodium salts relative to calcium and magnesium salts.

Sodicity is one of the most important types of salinity which occurs when Na+ is more than 15% of the exchangeable cation (Rozemaand Flowers 2008)**.**Sodicity changes soil physical properties by destroying soil structure, reducing the permeability and porosity of soils (Rengasamy*et al*., 2003)**.**Sodic soils constitute about 50% of the world's salt soil (Martinez-Beltran and Manzur, 2005). Saline-sodic soils typically have an EC of less than 4 mmho cm^{-1}, and the pH is generally below 8.5. The exchangeable sodium percentage is more than 15 percent of the cation exchange capacity (CEC). CEC is a measure of the soil's capacity to hold cations, namely, calcium, magnesium, potassium, sodium, hydrogen and aluminum. Increase of CEC is more problematic to removal and remediation of the salt problem. Water moves through these soils as much as it does in saline soils, although, the steps for correcting saline-sodic soil are different. Simply leaching the salts from this soil will convert it from saline-sodic to sodic soils.

Measuring salinity

Soil salinity is measured as the salt concentration of the soil solution in terms of g/l or electric conductivity (EC) in deci-Siemens per meter (dS/m). The international system (SI) unit of EC is dS/m. Salinity is also measured as mmhos/cm (mM) which is vastly used in laboratory experiments.

Electrical conductivity (EC) is used to measure total dissolved solids (TDS) in both water and soil solution with unit and it is possible to estimate TDS from EC, for example, TDS in parts per million (ppm) is equal to 640 multiple EC in (ds/m or mmhos/cm).

Also, the salinity can be more easily measured without centrifugation, in a 2:1 or 5:1 water:soil mixture (in terms of g water per g dry soil) than from a saturated paste. The relation between EC_e and $EC_{2:1}$ is about 4, hence: $EC_e = 4\ EC_{1:2}$ (ILRI, 2003). In fact, there are some factors affecting the value of electrical conductivity (EC). One of them is the temperature which affects the electrical conductivity value (EC) by increasing when the temperature is increased. Also, the value of electrical conductivity (EC) is affected by the types of ions; for example, the value of electrical conductivity (EC) for sodium chloride solution is higher than the value of (EC) for calcium sulfate solution. On the other hand, the rate between the electrical conductivity (EC) and salts concentration is not linear all the time especially in higher salts concentration (Hanson, 1979; Rhoades, 1972; Ayers and Westcot, 1985).

Salinity problems for both agriculture sector and plant production

Impact of agriculture sector

A considerable amount of land in the world is affected by salinity which is increasing day by day. Including soil salinization, about 15% of the total land area of the world has been degraded by soil erosion (Wild, 2003). For example, 357 million hectares of dry-land cropping are affected by salinity in the Australasia (Szabolcs, 1989). In Bangladesh, the estimates indicate that Bangladesh has about 2.8 million ha of land affected by salinity and poor quality water. (Chanratchakool, 2007) In addition to soil deterioration, the cost of salinity is estimated to be about12 billion US$a year (Ghassemi*et al*., 1995). Soil salinity was increasing worldwide, for example, 45 million hectares or roughly 20% of irrigated land are affected by increasing salt content, this is recorded in many regions around the world as India, Pakistan, China, Syria, Iraq and Australia (Aslam and Prathapar, 2006; Gupta and Abrol, 2000; Chengrui and Dregne, 2001; Sarraf, 2004; Rengasamy, 2006). For agricultural sector, soil salinity can leave desertification of soil permanently. On the other hand, direct costs of increasing salinity to agricultural producers include:

- Reduced farm income.
- Reduced water quality for stock, domestic and irrigation use.
- Damage and reduced life of farm structures such as buildings, roads, fences and underground pipes and services.
- Reduced productivity of agricultural land.
- Animal health problems, e.g. saline water supply.
- Farm machinery problems (bogging, rusting).

- Breakdown of soil structure, increased erosion and nutrient loss.
- Loss of beneficial native flora and fauna.
- Decreased land value.

Increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% loss of cultivable lands by the middle of the twenty first century (Mahajan and Tuteja, 2005)**.** Finally, further soil salinization influence roughly 831 million hectares worldwide (Fig.1).

Impact of soil salinity on yield production

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil.

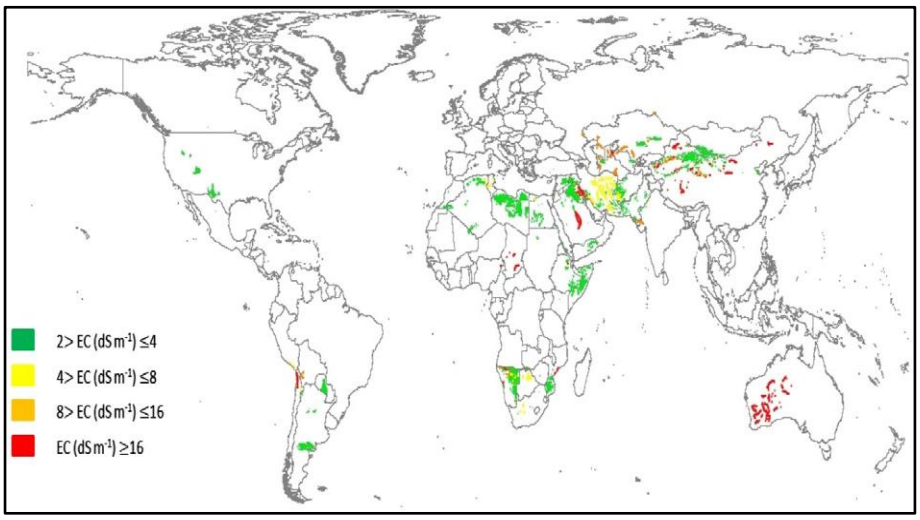

Fig.(1). Global distribution of saline soils using data from the Harmonized World SoilDatabase (Findell and Eltahir, 2003).

Soil salinity affects crop growth by osmotic and specific ion toxicities of specific ion. Generally, the difference between concentration of soil water and root cell of plant allows water to move from or into plant root, where the concentration of salts in the root cell must be higher than that in soil solution to allow water to move from soil into root of plant. Normally, the concentration of solutes in the root cell is higher than that in the soil water and this difference allows water to move freely into the plant root. But as the salinity of the soil water increases, the difference in concentration between constituents in the soil water and those in the root lessens, initially making the soil water less available to the plant. Salinity can also affect

crop growth through the effect of chloride, boron, and sodium ions on plants by specific-ion toxicities, which occurs when these constituents in the soil water are absorbed by the roots and accumulate in the plant's stems or leaves. Often, high concentrations of sodium and chloride are synonymous with high salinity levels. High sodium and chloride concentrations can be toxic to woody plants such as vines, avocado, citrus, and stone fruits. Boron is toxic to many crops at relatively low concentrations in the soil. Often, the result of specific-ion toxicity is leaf burn, which occurs predominately on the tips and margins of the oldest leaves. Boron injury has also been observed in deciduous fruit and nut trees as "twig die back". This occurs in species where the boron absorbed by the plant can be mobilized via complexes with polyols. For more information see Brown and Shelp (1997).

Using saline water or water with high boron concentrations for sprinkler irrigation can also injure leaves. Like chloride and sodium, boron can be absorbed through the leaves and can injure the plant if it accumulates to toxic levels. The crop's susceptibility to injury depends on how quickly the leaves absorb these constituents, which is related to the plant's leaf characteristics and how frequently it is sprinkled rather than on the crop's tolerance to soil salinity. Plants with leaves that have long retention times, for example - such as vines and tree crops - may accumulate high levels of specific elements even when leaf absorption rates are low. Plant sensitivity to salinity also depends on the plant growth stage (i.e. germination, vegetative growth, or reproductive growth). Many crops such as cotton, tomato, corn, wheat, and sugar beets may be relatively sensitive to salt during early vegetative growth, but may increase in salt tolerance during thelater stages. Other plants, on the other hand, may respond in an opposite manner. Research on this matter is limited, but if salinity during emergence and early vegetative growth is below levels that would reduce growth or yield, the crop will usually tolerate more salt at later growth stages than crop salt tolerance guidelines indicate.

Soil salinity treatment

The most common saline soil remediation techniques include physical removal, leaching, and/or subsurface drainage to reduce groundwater elevations. Soil profiles are naturally heterogeneous; it may not be necessary to remediate all of soil in a site in order to support plant growth. If salts are not inherently phytotoxic to the target species, plant germination or growth may be improved by maintaining higher water content (reducing osmotic stress) or providing access to non-saline water in a segment of the soil profile.

Physical removal

Physical removal, the scraping of the soil surface to remove the accumulated salts, and flushing water over the surface to remove salt crusts have limited uses. Scraping large areas results in requirements for excavation and disposal of large volumes of soil and the method has had only limited success (Abrol*et al*., 1988). It does not address salinity present in the subsoil, since only the topsoil is removed. It is also expensive to remove and dispose of large volumes of soil.

Leaching

Leaching is the most commonly used procedure and the only practical method for removing salts from the root zone of the soil profile (Abrol*et al*., 1988). Leaching is accomplished by surface irrigation in excess of evapotranspiration (ET) demand with water of a relatively low EC on the soil surface and allowing it to percolate. The ability to leach water through the soil profile is dependent on good drainage through the root zone. Leaching is most effective when the salty leached groundwater is discharged through subsurface drains that can carry the leached salts out of the restoration area. However, leaching is possible when there is sufficiently high aquifer transmissivity and natural drainage. Leaching during the summer months is less efficient because large quantities of water are lost by evapotranspiration (i.e. the amount of water percolated through the rooting zone is reduced), and furthermore the water that percolates has an elevated EC due to evapoconcentration.

The discharge of salty drainage water may pose environmental problems to downstream areas. The environmental hazards must be considered very carefully and, if necessary mitigating measures must be taken. If possible, the drainage must be limited to wet seasons only, when the salty effluent inflicts the least harm.

Crop Tolerance

Mass (1984) reported that crop tolerance to salinity ranges widely from the very salt-sensitive bean to the highly tolerant barley and cotton. The U.S. Salinity Laboratory in Riverside has been testing the salt tolerance of crops since it was established in 1937. Table (1) shows nearly 70 crops, which will be useful in predicting responses on saline soils.

Table (1). Impact salinity of soil (EC_e) and irrigation water (EC_w) on the proportion of production for some crops

Crops	100%		50%		0%	
	EC_e	EC_w	EC_e	EC_w	EC_e	EC_w
Barley	8.0	5.3	18	12	28	19
Cotton	7.7	5.1	17	12	27	18
Sugar beet	7.0	4.7	15	10	24	16
Sorghum	6.8	4.5	9.9	6.7	13	8.7
Wheat	6.0	4.0	13	8.7	20	13
Soybean	5.0	3.3	7.5	5.0	10	6.7
Cowpea	4.9	3.3	9.1	6.0	13	8.8
Groundnut (Peanut)	3.2	2.1	4.9	3.3	6.6	4.4
Sugarcane	1.7	1.1	10	6.8	19	12
Corn (maize)	1.7	1.1	5.9	3.8	10	6.7

Continue Table (1).

Crops	100%		50%		0%	
	EC_e	EC_w	EC_e	EC_w	EC_e	EC_w
Flax	1.7	1.1	5.9	3.9	10	6.7
Broad bean	1.5	1.1	6.8	4.5	12	8.0
Bean	1.0	0.7	3.6	2.4	6.3	4.2
Zucchini	4.7	3.1	10	6.7	15	10
Broccoli	2.8	1.9	8.2	5.5	14	9.1
Tomato	2.5	1.7	7.6	5.0	13	8.4
Cucumber	2.5	1.7	6.3	4.2	10	6.8
Spinach	2.0	1.3	8.6	5.7	15	10
Celery	1.8	1.2	9.9	6.6	18	12
Cabbage	1.8	1.2	7.0	4.6	12	8.1
Potato	1.7	1.1	5.9	3.9	10	6.7
Corn, sweet	1.7	1.1	5.9	3.9	10	6.7
Sweet potato	1.5	1.0	6.0	4.0	11	7.1
Pepper	1.5	1.0	5.1	3.4	8.6	5.8
Lettuce	1.3	0.9	5.1	3.4	9.0	6.0
Radish	1.2	0.8	5.0	3.4	8.9	5.9
Onion	1.2	0.8	4.3	2.9	7.4	5.0
Carrot	1.0	0.7	4.6	3.0	8.1	5.4
Bean	1.0	0.7	3.6	2.4	6.3	4.2
Turnip	0.9	0.6	6.5	4.3	12	8.0
Sudan grass	2.8	1.9	14	9.6	26	17
Alfalfa	2.0	1.3	8.8	5.9	16	10
Corn (forage)	1.8	1.2	8.6	5.7	15	10
Date palm	4.0	2.7	18	12	32	21
Grapefruit	1.8	1.2	4.9	3.3	8.0	5.4
Orange	1.7	1.1	4.8	3.2	8.0	5.3
Peach	1.7	1.1	4.1	2.7	6.5	4.3
Apricot	1.6	1.1	3.7	2.5	5.8	3.8
Grape	1.5	1.0	6.7	4.5	12	7.9
Almond	1.5	1.0	4.1	2.8	6.8	4.5
Plum, prune	1.5	1.0	4.3	2.9	7.1	4.7
Blackberry	1.5	1.0	3.8	2.5	6.0	4.0
Boysenberry	1.5	1.0	3.8	2.5	6.0	4.0
Strawberry	1.0	0.7	2.5	1.7	4	2.7

Salinity may also vary throughout the season, often increasing with time. Although most crops become more tolerantat later stages of growth, there are some exceptions. Plants are generally most sensitive during the seedling and early vegetative stages of growth. Because of the greater sensitivity at seedling emergence, it is imperative to keep salinity levels in the seed bed as low as possible at planting time. Salt tolerance information usually applies to crops irrigated by surface methods, such as furrow or basin-type flooding. Sprinkler-irrigated crops are subject to damage by both soil salinity and salt spray to the foliage. Salts may be directly absorbed by the leaves, resulting in injury and loss of leaves. In crops that normally restrict salt movement from the roots to the leaves, foliar salt absorption can cause serious problems not normally encountered with surface irrigation systems. The degree of injury depends on weather conditions and water stress. For instance, leaves may contain excessive levels of salt for several weeks without any visible injury symptoms and then become severely burned when the weather becomes hot and dry. Salt tolerance ratings cannot provide accurate estimates of actual crop yields, which depend on many other growing conditions, including weather, fertility, soil type, water stress, insects, and disease. The ratings are useful, however, in predicting how one crop might fare relative to another on saline soils under different cultural conditions. As such, they are valuable aids in managing salinity problems in irrigated agriculture.

It was clear, from the previousdata that most crops do not grow well on soils that contain salts. One reason is that salt causes a reduction in rate and amount of water that plant roots can take up from the soil. Also, some salts are toxic to plants when present in high concentration. The highly tolerant crops can withstand a salt concentration of the saturation extract up to 10 g L^{-1}. The moderately tolerant crops can withstand salt concentration up to 5 g L^{-1}. The limit of the sensitive group is about 2.5 g L^{-1} (Brouwer*et al*., 1985).

In fact, to prevent salts in the soil water from reducing water availability to the plant, the plant cells must adjust osmotically - that is, they must either accumulate salts or synthesize organic compounds such as sugars and organic acids. These processes use energy that could otherwise be used for crop growth. The result is a smaller plant that appears healthy in all other respects. Some plants adjust more efficiently, or are more efficient at excluding salt, giving them greater tolerance to salinity. Plants vary widely in their response to soil salinity.

Some plants, called halophytes, actually grow better under high levels of soil salinity. These plants adjust osmotically to increased soil salinity largely by accumulating salts absorbed from the soil water. Salts accumulate in the root cells in response to the increased salinity of the soil water, thus maintaining water flow from the soil to the roots. The membranes of these plants are very specialized, allowing them to accumulate salts in plant cells without injury. Most crop plants are called glycophytes. They are a plant group that can be affected by even moderate soil salinity levels even though salt tolerance within this group varies widely. Most glycophytes also adjust osmotically to increased soil salinity, but by a process different from that of

halophytes. Rather than accumulating salts, these plants must internally produce some of the chemicals (sugars and organic acids) necessary to increase the concentration of constituents in the root cell. This process requires more energy than that needed by halophytes, and crop growth and yield are therefore more suppressed.

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