Guidelines for Authors

This periodical is a publication of the Academic Publishing and Translation Directorate of Al-Qassim University. Its purpose is to provide an opportunity for scholars to publish their original research.

Manuscripts will be published in on of the following platforms:

1) **Article:** It should be original and has a significant contribution to the field the field in which the research was conducted.
2) **Review Article:** A critical synthesis of the current literature in a particular field, or a synthesis of the literature in a particular field during an explicit period of time.
3) **Brief Article:** A short article (note) having the same characteristics as an article.
4) **Forum:** Letters to the Editor, comments and responses, preliminary results or findings, and miscellany.
5) **Book Reviews**

The Editorial Board will consider manuscripts from all fields of knowledge. A manuscript may be submitted in either Arabic or English, and, if accepted for publication, it may not be published elsewhere without the written permission of the Editor-in-Chief.

General Instructions

1. **Submission of manuscripts for publication:** Papers must be presented in final page format, along with a compact disk (CD) containing the contribution executed on a PC using MS Word or any updated version of it. Pages are to be numbered consecutively and are to include all illustrative material, such as tables and figures, in their appropriate places in the text. If the author does not follow these guidelines, the paper is likely to be rejected or delayed.
2. **Abstracts:** Manuscripts for articles, review articles, and brief articles require both Arabic and English abstracts, using not more than 200 words, in single column (12 cm wide), for each version.
3. **Keywords:** Each article must (may) have keywords before both abstracts (English and Arabic) and they should not exceed 10 words.
4. **Tables and other illustrations:** Tables, figures, charts, graphs and plates should be planned to fit the Journal's page size (12 cm x 19 cm incl. running heads). Line drawings are to be presented on high quality tracing paper using black India ink. Copies are not permitted for use as originals. Line quality is required to be uniform, distinct, and in proportion to the illustration. Photographs may be submitted on glossy print paper, in either black and white, or color, or made by using Adobe Photoshop. Tables and other illustrative material must include headings or titles, and captions for figures.
5. **Abbreviations:** The names of periodicals should be abbreviated in accordance with *The World List of Scientific Periodicals.* e.g., *et al., J. of Food Sci.*

For weights and measurements, and where appropriate, abbreviations rather than words are to be used, e.g., cm, mm, m, km, cc, ml, g, mg, kg, min, %, etc., Fig.

Latin abbreviations such as: *op. cit., loc. cit., ibid.,* are to be in italic (if they are used).

6. **References:** In general, reference citations in the text are to be in the standard (Author, Date) style. Under the "References" heading at the end of the manuscript all references are to be presented alphabetically by the author's last name in the following fashion:

a) Periodical citations in the text are to be in the standard (Author, Date) style. Periodical references are to be presented in the following form: author's surname followed by a given name and/or initials (bold), the title of the article between quotation marks (" "), title of the periodical (in italic), volume number, issue number between brackets (if available), year of publication between brackets and pages.

Example:

Hicks, Granville. "Literary Horizons: Gestations of a Brain Child." *Saturday Review,* Vol. 45, No. (6), (1982), 2-23.

b) Book citations in the text are to be in the standard (Author, Date) style. Book references are to include the following: author's surname followed by a given name and/or initials (bold), title of the book (in italic), city of publication, publisher, year of publication between brackets and the number of pages (if available).

Example:

Daiches, David. *Critical Approaches to Literature.* Englewood Cliffs, New Jersey: Prentice-Hall, Inc., (1983), 350 p.

7. **Content Note or Footnote:** A content note or footnote is a note from the author to the reader providing clarifying information.

A content note is indicated in the text by using a half-space superscript number (e.g. … books[3] are …). Content notes are to be sequentially numbered throughout the text. A reference may be cited in a content note by the use of the standard (Author, Date) style in the same way they are used in the text.

Content notes are to be presented below a solid half-line separating them from the text of the page in which the footnote is mentioned (in single column). Use the same half-space superscript number assigned in the content note(s) in the text to precede the content note itself.

8. **Proofs:** No changes, additions or deletions will be allowed in the pageproof stage.
9. **Opinions:** Manuscripts submitted to the Journal for publication contain the author's conclusions and opinions and, if published, do not constitute a conclusion or opinion of the Editorial Board.
10. **Offprints:** Twenty offprints are supplied free of charge to the author.
11. **Correspondence:** All correspondence may be addressed to:

Division Editor
The Journal of Agricultural and Veterinary Sciences
College of Agriculture & Veterinary Medicine
P.O. Box 6622, Buraydah 51452
E-mail: jvmagsc@yahoo.com
Kingdom of Saudi Arabia

12. **Frequency:** 2 per year
13. **Subscription and Exchange:**

E-mail: jvmagsc@yahoo.com

In the Name of ALLAH,
Most Gracious, Most Merciful

Qassim University
جامعة القصيم
Qassim University Scientific Journal
(Refereed Journal)

Volume (8) – NO.(2)

Journal of
Agricultural and Veterinary Sciences

July 2015 – Ramadan I 1436H

Scientific Publications & translation

EDITORIAL BOARD

Deposit No.: 1429/2022

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 83-81 (January 2015/Rabi I 1436H)

Contents

Page

Plant production and protection

Veterinary Medicine

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 85-94 (July 2015/Ramadan 1436H)

Histochemical Analysis of Glycoconjugates in the Lacrimal Gland of the Camel *(Camelusdromedarius)*

Zarroug H. Ibrahim[1*] and Ali B. Abdalla[2]

[1]*College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum, Sudan.*

[2]*Department of Anatomy, Faculty of Veterinary Medicine, University of Khartoum, Sudan.*

Current address : College of Agriculture and Veterinary Medicine, Qassim University, KSA.

[*]*Corresponding Author: Email: zarrougibrahim@hotmail.com*

(Received 2 /2/2015; accepted 7/5/2015)

Abstract. The mammalian lacrimal gland secretes an aqueous solution in which different chemical substances are present, including proteins and carbohydrates. The histochemical investigations on characterization of camel lacrimal secretion are limited. This study aimed to determine the histochemical properties of the dromedary camel lacrimal gland. Glandular samples of twenty healthy adult camels of both sexes were collected at Omdurman slaughterhouse, Sudan. The camel lacrimal gland was compound with tubulo-acinar mixed secretory units, having serous and mucous cells. Best's carmine reactionwas negative, whereas a strong positive reaction to periodic acid-Schiff's (PAS) diastase resistant was observed in most of the secretory units; the reactions to alcian blue (AB pH 2.5) and aldehyde fuchsin (AF) were strong in the glandular tubules and weak or negative in the acini. Goblet cells in the epithelium of the interlobular ducts were strongly PAS, AB pH2.5 and AF positive.Both acini and tubules showed positive reactions to acid and alkaline phosphatases especially in their apical parts. These results suggest that the predominant acini secretion was serous which contained mainly neutral mucopolysaccharides, whereas the tubules secretion was mucous thatcontained sulfated and carboxylated acid mucopolysaccharides. Moreover, the different staining properties of the camel lacrimal secretion reflect various physiological functions.

Keywords:Glycoconjugates, Histochemistry,Lacrimal Gland,Dromedary.

1. Introduction

The mammalian lacrimal fluid cleans and moistens the anterior part of the eyeball; its excess leaves the eye through the larimal puncta in the upper and lower eyelids to moisten the nasal mucosa. The lacrimal gland, in most domestic mammals, is compound tubule-alveolar in structure with clusters of secretory units (Burkitt*et al*, 1999; Bacha and Bacha, 2000; Ibrahim *et al*., 2006; Mohammadpour, 2011;Kleckowska-Nawrot et al., 2013).

The glandular excretory ducts open into the superior conjunctiva. Unlike other domestic mammals, there are no puncta lacrimalia, either in the lower, or in the upper eyelid of the camel. The two lacrimal ducts start blindly and open into the lacrimal sac, the enlarged beginning of the nasolacrimal duct. The nasolacrimal duct, which begins as a small osseous duct, opens into the nasal vestibule (Abdalla*et al*., 1970; Ibrahim *et al.*, 2006).

The available histochemical studies on the lacrimal gland in domestic mammals show that the gland is mixed with predominant serous secretion (Sinha and Calhoun, 1966; Kühnel 1968*a*, 1968*b*, 1968*c*; Kuhnel and Scheele, 1979; Dellman and Browun, 1981). However, mucous cells predominate in the pig (Dellman and Browun, 1981) and the dog gland is described as purely mucous (Kühnel, 1968*d*; Martin *et al*., 1988).

In the camel, the lacrimal gland is reported as serous (Abdalla*et al.*, 1970; Awkati and Al-Bagdadi, 1971) and seromucous (Mohammadpour, 2011).

It could be mentioned that the review of the literature reveals little about the histochemistry of the lacrimal gland and its duct system in camels. This study aims to characterize the histochemical secretion of camel lacrimal gland, which may add to our knowledge about the unique structural and physiological characteristics of this animal in its harsh environment.

2. Materials and Methods

Lacrimal glands of fifteen healthy adult male and female camels were removed immediately after being slaughtered at Omdurman slaughter house, Khartoum, Sudan.

For paraffin sections, small pieces of tissue were fixed in 10% formalin and Bouin's fluid. The fixed specimens were dehydrated in ascending grades of ethyl alcohol; they were cleared in several changes of xylene, and impregnated and embedded in paraffin wax in accordance with the procedure of Culling (1974). Tissues were blocked and sections, 5-7 µm thick, were cut in a rotary microtome. Some sections were stained by haematoxylin and eosin (H&E) stain for general histology. Histochemical analysis of glandular mucopolysaccarides was conducted using Best's carmine aldehyde fuchsin, periodic acid-Schiff's (PAS) and alcian blue (AB pH2.5) stains as described by Culling (1974).

For frozen sections tissue samples were immediately cut into small pieces about 3-4 mm thick to ensure complete and rapid freezing in liquid nitrogen (-170 °C). Sections 8µm thick were cut by a Slee cryostat, maintained at -20 °C, picked up on clean cover slips and fixed with cold acetone (0-4 °C) in Colombia jars in the cryostat for 30-60 minutes as described by Bancroft (1990). The acetone fixed specimens were processed and stained following the method described by Bancroft (1990) to investigate acid phosphatase and alkaline phosphatase enzymes.

3. Results

The histological and histochemical results of the camel lacrimal gland showed no gender differences.

The general histology showed that the camel lacrimal gland was compound tubulo-acinar in structure. The secretory units were mixed, having serous acini and mucous tubules secretory units; the serous aciniappeared to be predominant (Fig. 1).

The histochemical results of the camel lacrimal gland, which are summarized in table (1), are herewith given:

A. Mucopolysaccharides:

Best's carmine (BC)

The lacrimal gland and its duct system reacted negatively to Best's carmine stain.

Periodic Acid-Schiff's (PAS)

Both acini and tubules were strongly PAS positive, especially in the apical part of the epithelium (Fig.2). A moderate reaction was observed in the, blood vessels and connective tissue septa. Many goblet cells in the interlobular ducts showed very strong PAS positive reactions; their epithelial cells appeared with weak PAS reaction (Fig. 3).

PAS after Salivary Amylase Treatment

The reaction to PAS with or without salivary amylase showed the same results throughout the different parts of the lacrimal apparatus.

Aldehyde Fuchsin (AF)

The glandular tubules and intra and interlobular ducts showed strong aldehyde fuchsin positive reactions, especially in the apical part of their epithelial layer (Fig.4). The reaction was weak or negative in the acini. A weak reaction was observed in the connective tissue capsule, glandular septa and blood vessels.

Alcian Blue

The glandular tubules were strongly positive to alcian blue pH2.5 reactions especially in their apical epithelium, whereas the acini and connective tissue

appeared with weak or negative reaction (Fig. 5). The apical epithelium and goblet cells of the interlobular ducts were moderately alcian blue (pH 2.5) positive; melanin granules were also observed in the duct epithelium (Fig. 6).

Phosphatase Enzymes

Acid Phosphatase (ACP)

Many acini and tubules of the gland reacted strongly to acid phosphatase enzyme (Fig.7); the reaction was confined to the apical portions of the epithelial cells. The intensity of the reaction varied from lobule to lobule and from one secretory unit to another. In the ducts, glandular capsule, trabeculae, and interstitium the reaction was mild.

Alkaline Phosphatase (ALP)

Many glandular secretory units showed a strong reaction in the apical portion of epithelial cells (Fig. 8). Basally, the reaction was weak. The intensity of the reaction varied between the secretory units and even between cells of the same secretory unit. The duct system, capsule, septa and interstitium reacted weakly to alkaline phosphatase.

Table (1). Some histochemical reactions in the lacrimal gland.

Glandular tissue	Histochemical reactions					
	BC	PAS	AF	AB (pH 2.5)	ALP	ACP
Secretory units						
i.Tubules	-	+++	+++	+++	+++	+++
ii.Acini	-	+++	-	±	+++	+++
Interlobular ducts						
i.Goblet cells	-	++++	+	++	+	+
ii. Epithelial cells	-	+	+	++	+	+
Blood vessels	-	++	+	±	+	+
Connective tissue	-	++	+	±	+	+

Stains: (BC) = Best's, (PAS) = Periodic acid Schiff's, (AF) = Aldehyde fuchsin, (AB) = Alcian blue, (ALP) = Alkaline phozphatase, (ACP) = Acid phosphatase

Intensity of reaction: (++++) = Very strong,(+++) = Strong, (++) = Moderate, (+) = Weak(±) = Negative to weak (-) = Negative.

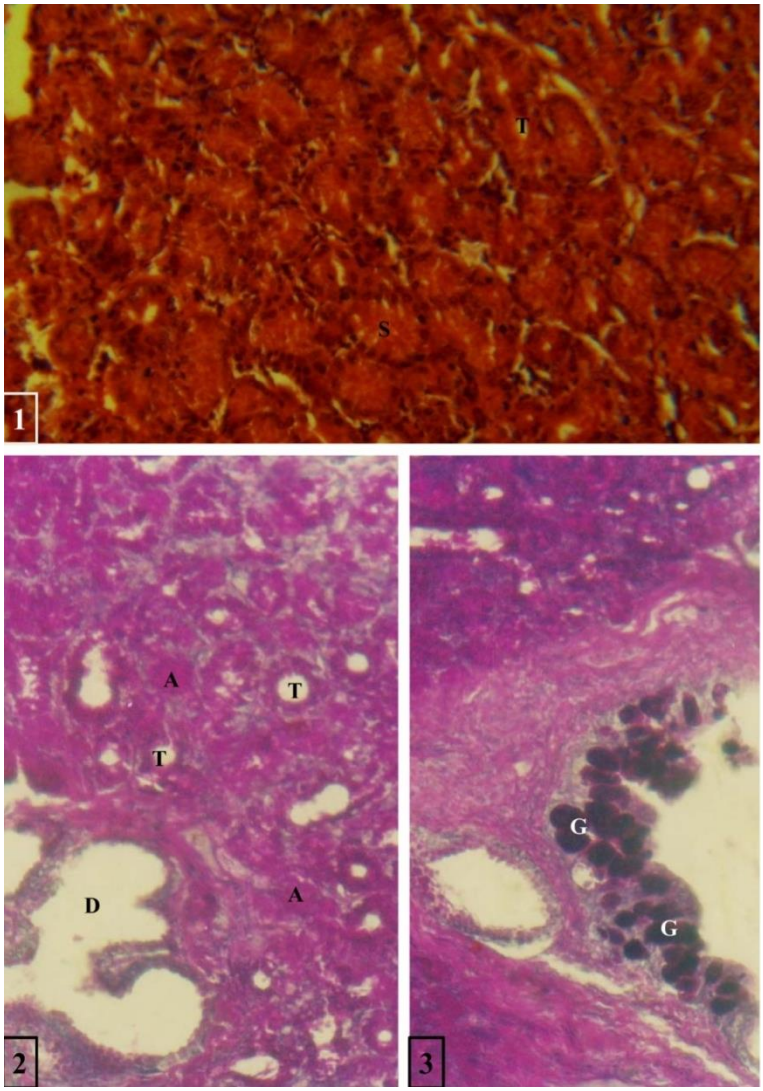


Fig.1: Photomicrograph of camel lacrimal gland showing predominant serous acini (A) and a few mucous tubules (T). Hematoxylin and eosin X100.

Fig.2: Photomicrograph of camel lacrimal gland showing strongPAS positive reaction in the acini (A) and tubules(T). Note the moderate reaction in interlobular duct(D).(PAS)X100

Fig.3: Photomicrograph of glandular interlobular ductshowing strongPAS positive in goblet (G).(PAS)X100.

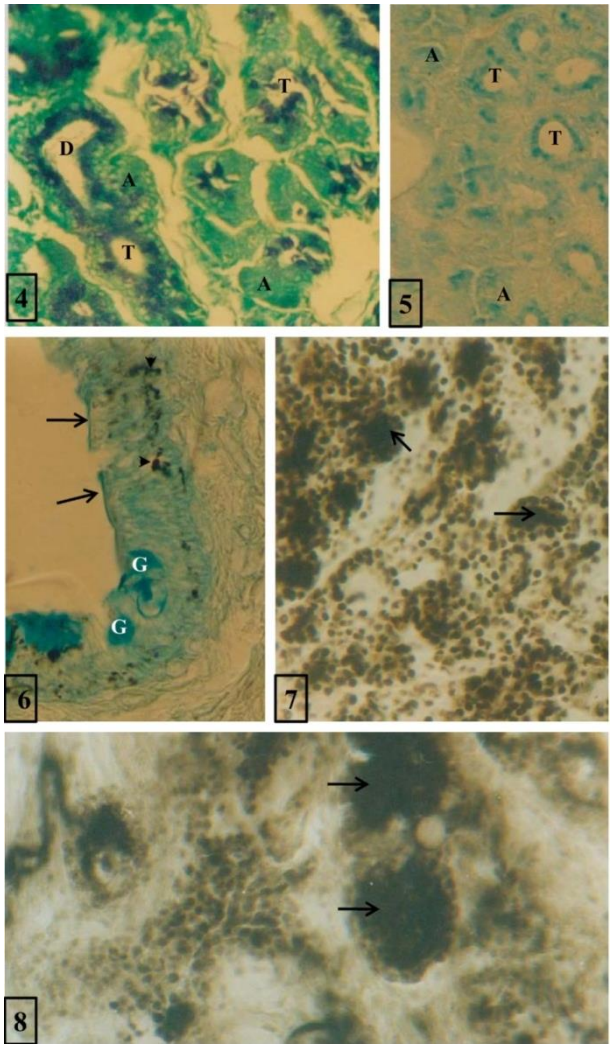


Fig.4: Photomicrograph ofAF reaction with glandular tubules (T) and intralobular ducts (D) showing strong reaction; the acini (A) appear with weak or negative reaction).Aldehyde Fuchsin X100.

Fig.5: Photomicrograph showing glandular tubules (T) with strong positive alcian blue (AB pH2.5) reaction; the acini (A) exhibit weak or negative reaction.Alcian blue (pH 2.5)X250.

Fig.6: Photomicrograph of interlobular duct with strong AB pH2.5 reaction in goblet cells (G) andapical epithelium (Arrows). Note the melanin granules in the duct epithelium (Arrow heads).

Fig.7: Photomicrograph oflacrimal gland showing strong acid phosphatase reaction concentrated in the apical epithelial cells (Arrows).

Fig.8: Photomicrograph oflacrimal gland with strong alkaline phosphatase reaction especially in the apical epithelial cells (Arrows).

4. Discussion

Secretory cell granules are histochemically classified into neutral or acidic mucosubstances according to their staining properties with PAS, AB and AF (Schakelford and Klapper, 1962; Culling, 1974). Most of the epithelial cells of secretory units and those of interlobular and excretory ducts contained neutral mucopolysaccharides as they stained strongly with PAS method. Staining with AB (pH 2.5) proved the presence of strong positive granules in some secretory cells and epithelial cells of interlobular and excretory ducts, indicating presence of acid sulfated acid mucopolysacharides. AF staining showed blue colouration in some epithelial cells of secretory units and duct system, which indicated a low concentration of carboxylated acid mucopolysaccharides. According to Mohammadpour, (2011) both acidic and neutral glycoproteins in the camel lacrimal gland with different staining patterns.Best's carmine negative reaction together with the lack of change after in PAS stain after amylase digestion suggests the absence of glycogen in the camel lacrimal gland. It could be mentioned that these different staining properties of the lacrimal gland secretion shown in present study suggest various physiological functions.

Previous studies on the camel lacrimal gland considered its secretion as serous in camel (Abdalla *et al.*, 1970;Awkati and Al-Bagdadi, 1971). The same findings were reported in the roe deer (Kleckowska-Nawrot *et al*., 2013). (Schakelford and Klapper (1962) described the secretory cell as serous if it contains neutral glycoconjugates i.e. PAS positive and AB and AF negative. On this basis the secretory units of the camel lacrimal gland in this study could be considered as predominantly serous. In other domestic mammals the lacrimal gland was generally found to be predominantly serous (Dellman and Brown, 1981). It becomes clear from this study that the lacrimal secretion of the gland of the camel is mixed i.e. it contains both serous and mucous portions. The predominant serous secretion comes from the secretory units; a considerable amount of mucous secretion comes from the goblet cells of the glandular duct system. In other domestic mammals, the surplus lacrimal secretion leaves the eye through the lacrimal puncta which leads to the nasolacrimal duct to contribute to moistening of the nasal cavity. This function is impossible in the camel because it lacks the lacrimal puncta (Abdalla*et al.*, 1970; Awkati and Al-Bagdadi, 1971; Ibrahim *et al.*, 2006). Therefore, the function of the lacrimal fluid in the camel is confined to the washing and moistening of the anterior part of the eyeball. This feature is probably helpful in preservation of water content in the camel body under its hot circumstances.

In this study, the activity of acid and alkaline phosphatases has been detected in the apical portions of the secretory cells. However, Sinha and Calhoun (1966) claim that the alkaline phosphatase activity is strong in the vicinity of the basement membrane of the secretory cells of the lacrimal gland of small ruminants and the reaction for acid phosphatase is weak. The process of acid and alkaline phosphatases near the basement membrane can be explained by saying that there is an exchange of materials between the secretory cells and blood capillaries in the interstitial connective tissue.

5. References

Abdalla, O., Fahmy, M.F.A. and Arnautovic, I**.**"Anatomical study of the lacrimal apparatus of the one-humped camel"**.** *Acta. Anat*., 75, (1970), 638-650.

Awkati, A. and Al-Bagdadi, F."Lacrimal gland of the camel". *Am. J. Vet. Res*., 32, (1971), 505-510.

Bacha, W. and Linda Bacha.*Color Atlas of Veterinary Histology*. 2nd ed. Lippincott William and Wilkins, (2000).

Bancroft, J. D. An *introduction to Histochemical Technique*. London, Butterworths. (1990).

Burkitt, G.H., Young, B. and Heath, J. W. *Wheatears Functional Histology*. 3rd ed. Churchill Livingstone,(1999).

Culling, C.F.A.*Handbook of Histopathological and Histochemical Technique*. 3rd ed. London: Butterworths,(1974).

Dellmann, H.D. and Brown, E.M**.***Textbook of Veterinary Histology*. Second Edition. Philadelphia:Lea and Febiger,(1981).

Ibrahim, Z.H., Abdalla, A.B. and Osman, D.I. A. "A gross Anatomical Study of the Lacrimal Apparatus of the Camel (*Camelusdromedarius*)". *J.Sci.Technol*,. 9,(2006), 1-8.

Ibrahim Z.H and Abdalla, A.B. "A morphometric study of the lacrimal apparatus of the camel (*Camelus dromedarius*) ". *J.Sci.Technol*. 8, (2007), 40-45.

Kleckowska-Nawrot, J., Maryczi, K**.**, Czogala, J., Kujawa, M., Janeczek, K.M. Chroszcz, A and Brundnickiz, W. 2013. " Morphology of the lacrimal gland and the superficial gland of the third eyelid of Roe deer (*Capreolus capreolus L*. "). Pak. Vet. J., 33: 139-144.

Kühnel, W."Comparative histological, histochemical and electron-microscopical studies on lacrimal glands". III. Sheep. *Z. Zellforsch.,* 86, (1968_a),430-43.

Kühnel, W."Comparative histological, histochemical and electron-microscopical studies on lacrimal glands". III. Sheep. *Z. Zellforsch.,* 87, (1968_b), (1):31-45.

Kühnel, W."Comparative histological, histochemical and electron-microscopical studies on lacrimal glands". V. Cattle. *Z. Zellforsch.*,87, (1968_c), 504-25.

Kühnel, W."Comparative histological, histochemical and electron-microscopical studies on lacrimal glands". IV. Dog. *Z. Zellforsch.*, 88, (1968_d), 23-38.

Kuehnel, W., and Scheele, G."Zur Feinstrukturder Glandula lacrimalis des Schweines (Sus scropha L)". Anat. Anz., 145, (1979), 87-106.

Martin, C.L., Munnell, J. and Kaswan, R."Normal ultrastructure and histochemical characteristics of canine lacrimal glands". *Am J Vet Res.*,49, (1988), 1566-72.

Mohammadpour, A.A."Histochemistry of dorsal lacrimal gland in camel (*Camelusdromedarius*)". *J. Camel. Prac. Res.*, 18,(2011), 1-3.

Schakelford, J.M. and Klapper, C.E."Structure and carbohydrate histochemistry of mammalian salivary glands". Am. J. Anat., **111**, (1962), 25-47.

Sinha, R. D. and Calhoun, M. L. "A gross, histologic and histochemicalstudy of the lacrimal apparatus of sheep and goats". *Am. J. Vet. Res.*. **27**, (1966), 1633-1640.

تحليل نسيجي كيميائي للجلايكوجين المرتبط في الغدة الدمعة للإبل وحيدة السنام

زروق حسن إبراهيم[1]* وعلي بشير عبدالله[2]

[1] *كلية الطب البيطري، جامعة السودان للعلوم والتكنلوجيا، السودان*

[2] *كلية الطب البيطري، جامعة الخرطوم، السودان*

* *كلية الزراعة والطب البيطري، جامعة القصيم، المملكة العربية السعودية*

zarrougibrahim@hotmail.com

ملخص البحث. الغدة الدمعية في الثدييات تفرز محلولا مائيا يحتوي على العديد من المواد الكيميائية كالبروتينات والكربوهيدريتات. الدراسات الكيميائية النسيجية محدودة فيما يخص مميزات السائل الدمعي للإبل وحيدة السنام. هدفت هذه الدراسة لمعرفة المميزات الكيميائية النسيجية للغدة الدمعية للإبل وحيدة السنام. اجريت الدراسة على عينات غدية لعشرين من الإبل جمعت بمسلخ ام درمان في السودان. بينت نتائج الدراسة ان الغدة الدمعية في الإبل عنيبية–انبوبية مركبة ذات وحدات إفرازية مختلطة بها خلايا مصلية ومخاطية. كان تفاعل الوحدات الافرازية سلبيا مع صبغة بست كارمين، بينما تفاعلت معظم الوحدات الافرازية بقوة مع صبغة حمض البيريودك–شيف (PAS) المقاوم لتريم الدياستيز. كان التفاعل قويا مع صبغة الشين بلو(ABpH2.5) والدهايد فكسين(AF) في النبيبات الغدية وضعيفا او سلبيا في العنيبات. الخلايا الكاسية في القنوات الافراغية والبينية أظهرت تفاعلات قوية مع صبغات PAS وAF و AB (pH2.5).أظهرت كل من النبيباتوالعنيبات تفاعلا إيجابيا مع انزيمي الفوسفتيز الحمضي والفوسفتيز القاعدي خاصة في الجزء القمي للظهارة. بينت هذه النتائج ان افراز العنيبات الغالب في الغدة الدمعية للإبل مصليويحتوي على عديد السكريات الميوسي المتعادل وان افراز الانيبيات حمضي يشتمل على عديد السكريات الميوسي الحمضي بشقيه الكبريتي والكاربوكسيلي. كذلك فان اختلاف الخواص الصبغية لإفراز الغدة الدمعية في الإبل قد يكون انعكاسا للعديد من الوظائف الفسيولوجية.

الكلمات المفتاحية: دراسة تحليل نسيجي كيميائي ، الغدة الدمعية، الابل

Animal production

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 97-110 (July 2015/Ramadan 1436H)

Chitinolyticenzymes, classification and applications: An Overview

Mehana E.E[1], Zeitoun M.M.[2] and Mohammed A. Alnassar[3]

[1]*Dept. Pathology, College Medicine, Qassim University, KSA.*

[2] *Dept. Animal Production, Faculty of Agriculture, Alexandria University, Egypt.*

[3] *College of Medicine, Qassim University, KSA.*

(Received 21 /12/2014; accepted 4/6/2015)

ABSTRACT Chitinases are a group of enzymes capable of degrading chitin directly into low molecular weight-products. Chitinases have been isolated from different microorganisms, such as fungi and bacteria. Chitin is an insoluble natural polymer, commonly present among crustacean. Fish scales are one of the most common sources of chitin. A Crustacean and fish scales waste are ideal as row material for chitin production. Fish scales set variety of nutrition, health care, substance-in one. About 130 million tons of fish wastes is generated each year in the (***Wekipedia, 2013***). More than 80.000 (***Wekipedia, 2013***) metric tons of chitin is obtained per year from the marine waste. Chitin is closely associated with minerals, lipids and pigments. Chitinolytic enzymes have wide range of applications such as, preparation of single cell protein, preparation of pharmaceutically important chito-oligosaccharides and N-acetyl D-glucosamine. They also play an important physiological and ecological role in ecosystems as recyclers of chitin by generating carbon and nitrogen sources. They are recently used as immuno-stimulants in fish aquaculture. In this review, the occurrence and structure of various types of chitin as well as the, sources of chitinases were discussed classification and their mode of action. Besides, the updated information about the biomedical applications of the chitinases were considered

Keywords: Applications, Chitin, Chitinases, Fish, Mode of action.

Introduction

Chitin is a linear insoluble home polymer of unbranched chains ofß-1-4-linked sugar (N-acetyl glucosamine, (GleNAC) residues, whereas chitosan contains glucosamine residues. Chitin forming linkages with glucan, protein and other polymers(Deshpande, 1986)The name of "chitin" is derived from the Greek name word " chiton ", meaning a coat of mail, and was apparently first used by Bradconnot in 1811 (You, 1999). Chitinases catalyze the hydrolysis of chitin, an unbranched polymer of ß-1,4-N-acetyl glucosamine. In recent years, soil-born microorganisms that produce chitinases are considered as potential biocontrol agents against fungi. Chitin is the second most abundant polymer in nature, after cellulose. Chitin is present in insects, crustaceans and in most fungi, plants, vertebrate's but prokaryocytes don't contain chitin. Chitinases, however are synthesized by a vast array of organisms, including those who are not composed of chitin (Fig.1-3).

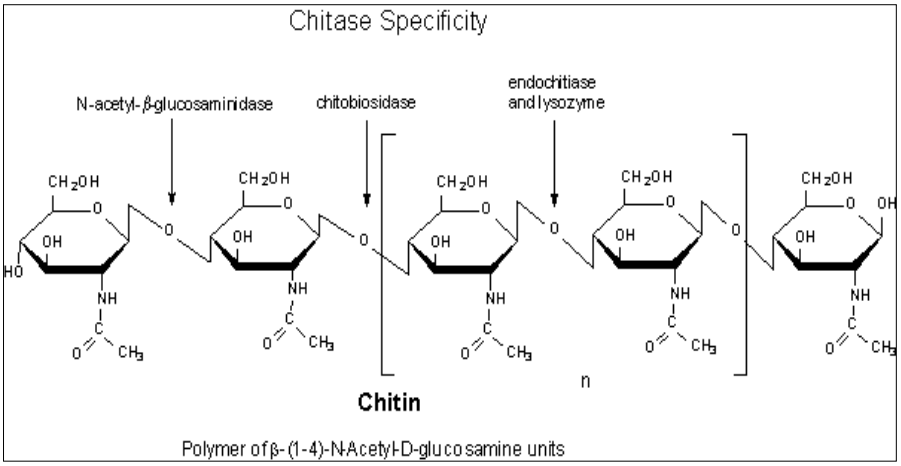


Fig. (1): Chitinase
EC# 3.2.1.14
Synonyms: 1,4-β-poly-N-acetylglucosaminidase

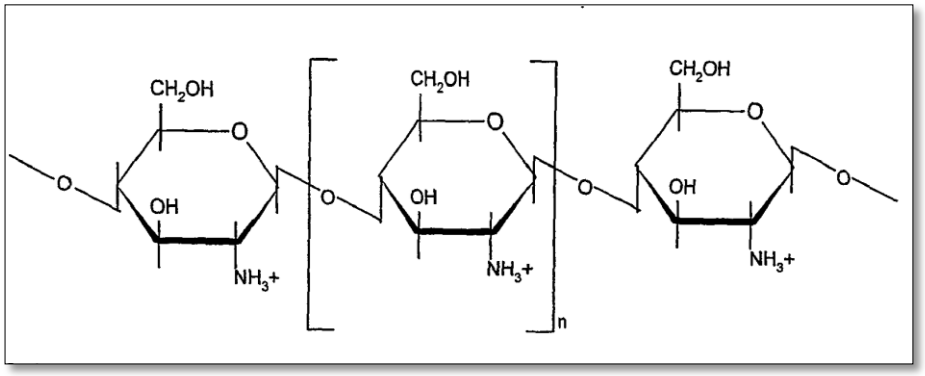


Fig. (2): Chitosan

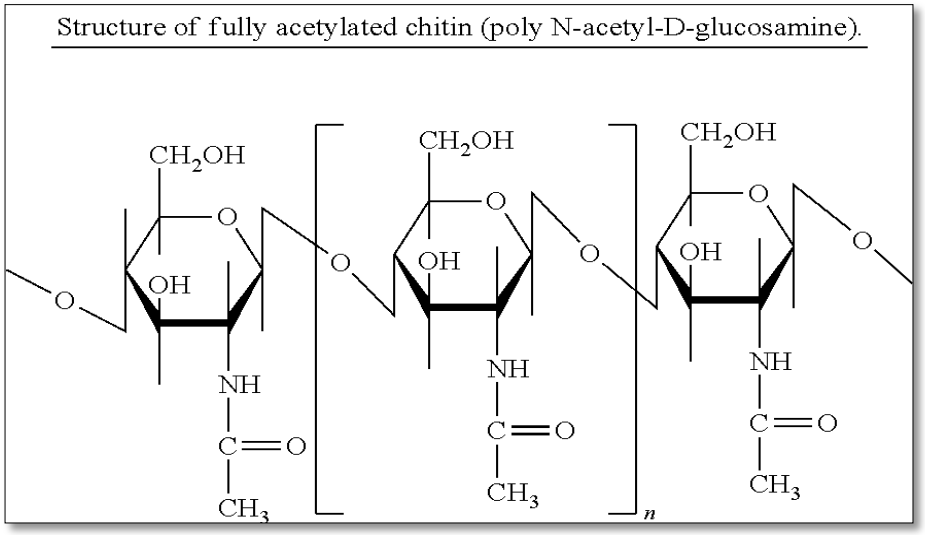


Fig. (3)

Chitinases are digestive enzymes that breakdown glycosidic bonds in chitin (Jolles and Muzzarelli, 1999). As chitin is a component of the cell walls of fungi and exo-skeletal elements of some animals (including worms and arthropods), chitinases are generally found in organisms that either need to reshape their own chitin or dissolve and digest the chitin of fungi or animals (Sami et al ., 2001). The chitinolytic activity was higher in the gastric tissue of marine fishes as these found in the gastric contents of *Entmopterusspinax*, *Coryphaenoidesrupestris* which

indicated that the chitinasesare produced mainly by the digestive glands (Fange *et al.*, 1997 and Pandey, 2010). The chitinolytic system helps in completing the enzymatic hydrolysis of chitin to free N-acetyl glucosamine (GLcNAc) and this action is well known as synergistic and consecutive (Deshpande , 1986and Shaikh and Deshpande, 1993). Chitin is the second most abundant natural biopolymer and the main structural component of invertebrate exoskeleton and cell wall of filamentous fungi (Verna, 2008).Chitinases (EC 3.2.1.14) area group of enzymes capable of degrading chitin directly into low molecular weight-products. They have been isolated from different microorganisms, such as fungi and bacteria. A novel chitinase-producing bacterium C4 was isolated from the soil samples. Molecular identification showed that strain C4 should belong to the genus Sanguibacter (Tao *et al.*, 2005). Chitin is an insoluble natural polymer, commonly present among crustacean. Fish scales are one of the most common sources of chitin. Crustacean and fish scales waste are ideal as row material for chitin production. Chitinase is an enzyme produced by variety of microorganisms, which can digest the high molecular weight-chitin and convert them into low molecular weight and eco-family products (Saranya and Tha, 2013). Macrophages can synthesizelarge amounts of chitotriosidase (Human chitinases) upon an appropriate stimulus, such as the massive lysosomallipid accumulation that occurs in macrophages of Gaucher patients (Hollak *et al*, 1994). Chitotriosidase is largely secreted as a 50-kDa active enzyme containing C-terminal chitin binding-domain (Renkema *et al.*, 1997 and Tjoelker *et al.*, 2000).The major difference between human chitotriosidase and the mouse acidic chitinase is revealed by comparison of RNA expression patterns. Although human chitotriosidase mRNA is mainly found in lymph nodes, bone marrow and lungs, the mouse acidic chitinase mRNA is predominantly found in stomach, sub maxillary glands and at a lower level in the lungs(Rolf *et al.*, 2001).Chitin is closely associated with minerals, lipids and pigments (Park and Kim, 2010). Crustacean and fish scale waste are ideal as row material for chitin production. Fish scales, therefore set variety of nutrition and health care substance-in one, which are successfully isolated from the common carp fish (*Cyprinuscarpio L*) (Zaku *et al.*, 2011). In nature, two major types of chitin occur, which are characterized by an antiparallel (α- chitin) or a parallel (ß-chitin) arrangement of N-acetylglucosamine chains. After cellulose, chitin is the second most abundant polymer found in biosphere (Tharanathan and Kittur , 2003). Like cellulose, chitin is an abundant biopolymer that relatively resistant to degradation (Akaki and Duke, 2005).Chitin represents 22-44 % of fungal cell walls (Muzzarelli, 1977 and Muzzarelli *et al.*, 1994).Chitin is part of complex structures with other organic and inorganic compounds. In arthropods chitins are covalently linked to proteins and tanned by quinines, in fungi it is covalently linked to glucans, while in bacteria chitin is diversely combined according to Gram (+/-) classification. Dueto the fact that some individuals are allergic to crustaceans, the presumed relationship between allergy and the presence of chitin in crustaceans has been investigated. Fukamizo, 2000, support the fact that chitin depresses the development of adaptive type-2 allergic responses. Crabs, shrimp, prawn and lobster contain chitins as well as

chitosans of all grades. Once these compounds purified, they should not be considered as "crustacean derivatives" because the isolation procedures have removed proteins, fats and other contaminants to such an extent as to allow them to be classified as chemicals regardless of their origin (Muzzarelli, 2010). Degradation of exogenous chitin present in the cell walls of dead hyphal fragments or inthe exoskeleton of dead arthropods, cell wall remodeling during the fungal hyphal life cycle, which includes putative roles ofchitinase during hyphal growth, branching, hyphal fusion and autolysis, and competition and defense against other fungi or arthropods in the fungal habitat (Verna, 2008). An immunostimulant is a natural occurring compound that modulates the immune system by increasing the host's resistance against diseases that in most circumstances are caused by pathogens, polysaccharides (chitosan, chitin, lentinan, schzophyllan) and oligosaccharides were used as immune stimulants(***Ian and Roy, 2005)***..Chitin and Chitosan are polysaccharides forming the principle component of crustacean insect exoskeletons and the cell walls of certain fungi.Rainbow trout injected with chitin showed stimulated macrophages activities and increased resistance to *V.anguillarum* infection (***Sakai et al, 1992***).Yellow tail injected with chitin alone showed increased protection against the bacteria of Pastereulla *piscicda*.Also, ***Kawakami et al, 1998***)showed immune stimulatory effects in brook trout injected with or immersed in chitosan solution.Increases were observed in the immunological parameters ofblood(i.e. NBT, potential kill activity, myeloperoxidase and total immunoglobulinsconcentration) in rainbow trout injected or immersed in chitsonsolution (***Anderson et al., 1995***).

Aquaculture:-

Also known as aqua farming, is the farming of aquatic organisms such as fish, crustaceans, molluscus and aquatic plants..Particular kinds of aquaculture including fish farming, shrimp farming, oyster farming, algaculture (such as seaweed farming) and cultivation of ornamental fish. Global crustacean production reached more than 1.6 million tons in 2003, worth about 9 billion US dollars ***(FAO, 2009)***.. The other 25% is produced mainly in Latin America, where Brazil is the largest producer ***(FAO, 2009)*** .The global annual production of freshwater prawns in 2003 was about 280,000 tons, of which China produced 180,000 tons followed by India and Thailand with 35,000 tons each. Additionally, china produced about 370,000 tons of river crab (***FAO, 2009***) . Aquaculture shellfish include various oyster, mussel and clam species. These bivalves are filter and/or deposit feeders, which rely on ambient primary production rather than inputs of fish or other feed. As such shellfish aquaculture is generally perceived as benign or even beneficial. (**(*Wikipedia-Free encyclopedia, 2013*)**.

Species Distribution:

Chitinivorous organisms include bacteria like *Aeromonas , Bacillus and Vibrio Spp.* among others, which may be pathogenic or detritivorous (***Xio et al ., 2005 and Hunt et al ., 2008).***They attack organisms. living arthropods, zooplankton

or fungi or they may degrade the remains of the Fungi, such as coccidiodesimmitis , also possesderivativechitinases related to their roleasdetritivores and also to their potential as arthropod pathogens. Although mammals don't produce chitin, they have two functional chitinases (chitotriosidase -CHIT1and acidic mammalian chitinase-AM case) as well as chitinase –like proteins (such as YKL-40) that have high sequence similarity but lack chitinase activity.

Sources of Chitinases

I- Marine or Aquatic Chitinases :-

Chitin is obtained from marine invertebrates,, shellfish , fishes , insects, fungi and algae.(***Fange et al ., 1997 ; Gutowska et al ., 2004 ; Muzzarelli , 2010 ; Park and Kim , 2010 ; Zaku et al ., 2011 ; Wikipedia , 2013 and Zhang et al ., 2013 .***) .

II-Fungal Chitinases

Degradation of exogenous chitinases present in fungal cell walls of dead hyphal fragments , cell wall remodeling during lifecycle , which includes putative roles of chitinases during hyphal growth , branching , hyphal fusion and autolysis.

(Muzzarelli et al ., 1994 ; Rachel and Ilan , 1998 ; Springer , 2001 ; Tharanathan and Kiltur , 2003 ; Tao et al ., 2005 ; Chuan , 2006 ; Nopakarn et al ., 2007 ; Shakhbazau and Kartel , 2008 ; Verna , 2008 ; Wikipedia , 2013 and Zhang et al ., 2013) .

III-Microbial Chitinases (A&B):

A-Bacterial Chitinases :-Chitinivorous organisms include bacteria like Aeromonas , Bacillus, Vibrio, among others, which may be pathogenic or detritivorous.

(Godrich , 1975 ; Patil et al ., 2000 ; Tao et al ., 2005 ; Xiao et al ., 2005 ; Hunte et al ., 2008 ; Chang et al ., 2010 ; Saranya and Tha , 2013 ; Springer , 2013 ; and Wikipedia , 2013).

B-Parasitic Chitinaseslike the protozoa andC-Insect Chitinases.

(Muzzarelli et al ., 1994 ; Rache and Ilan , 1998 ; Jolles and Muzzarelli , 1999 ; Sami et al ., 2001 ; Nopakarn et al ., 2007 ; Park and Kim , 2010 ,Saranya and Tha, 2013;and Wikipedia, 2013).

IV-Human Chitinases :

(***Hollak et al ., 1994 ; Renkema et al ., 1997 ; Tjoelker et al ., 2000 and Rolf et al ., 2001 and Wikipedia , 2013***) .

V- Mammalian Chitinases :

Chitinases have been isolated from stomach of certain mammals, including humans .Chitinases activity can also be detected in human blood and possibly cartilage.

(Rolf et al ., 2001 ; Eurich , 2009 and Wikipedia , 2013) .

VI-Plant Chitinases (Including aquatic plants):

The proposed role of plant chitinases is a defense mechanism against chitin-containing organisms .It has been observed that the plant chitinases inhibit the growth of fungal hyphae ***(Rachel and Ilan , 1998) .***

Global Production of Chitinases

Ten percent of the global landings of aquatic products consists of organisms rich in chitinousmaterial (10-55 % on dry weight). About 130 million tons of fish wastes is generated each year in the world. More than 80,000 metric tons of chitin is obtained per year from the marine waste (***Prameela et al ., 2010 and Wikipedia , 2013)*** . More than 80000 metric tons of chitin are obtained per year from the marine waste.***(Muzzarelli, 1977 and Subasinghe, 1995).***

Chitinolytic Enzymes: Mode of action, structure andclassifications:-

Table (1) showing Somechinitolytic enzymes and their mode of action *(Patil et al., 2000).*

Chitinolytic Enzymes	**Mode of Action**
Chitinase 1,4-β-poly-N-acetylglucosaminidase EC 3.2.1.14	Random hydrolysis of the chitin
Earlier classification-chitobiase EC3.2.1.29;β-D-Acetylglucominidase EC 3.21.30	Hydrolysis of terminal non-reducing sugar
Present classification β-N-Acetylthexosaminidase EC3.21.52	Successive removal of sugar unit from the non-reducing end.
Chitobiohydrolase	Successive removal of dimer sugar from the non-reducing end

Based on amino acids sequence similarity of chitinases from various organisms, five classes of chitinases have been proposed. These classes can be grouped into two families of glycosyl hydrolases, family 18 and 19 (***Henrissat, 1993***). Chitinases from classes I,II and IV are of plant origin and make up the family 19 glycosyl hydrolases (***Sahi and Monocha, 1993 and Hamel et al ., 1997).***These chitinases share a homologous catalytic domain in addition to the signal peptide found in all the class I chitinases consisted of cysteine-rich region to the catalytic domain(***Perrakis et al., 1993***).Class II chitinases, found mainly in dicotyledons. Class V chitinases, also identified mainly in dicotyledons, comprise a group of extracellular chitinases that share 41-47 % sequence identity with class I chitinases in the catalytic domain and also contain cysteine-rich regions resembling chitin-binding domains.However, class IV chitinases are smaller because of deletions in both domains (***Collinge et al., 1993***).Phylogenetic analysis of chitinases from classes I,II and IV suggests a large evolutionary distance between chitinases of class

IV and those of class I and II, suggesting a remote divergence between these plant and fungal in origin (***Hamel et al ., 1997)***.Together with class V chitinases they make up the family 18 glycosyl hydrolases (***Henrissat, 1993)***.Chitinases are expressed by plants in response to assorted environmental stimuli such as fungal challenge and osmotic pressure or developmental stage,such as fruit ripening process induced by ethylene (***Yun et al., 1996 and Busam et al., 1997).***The chemical structure of chitin is similar to that of cellulose with 2-acetamido-2-deoxy-β-D-glucose (NAG) monomers attached via β linkage. Chitosan is the decathylated (to varying degrees) from of chitin, which unlike chitin, is soluble in acidic solutions (***You, 1999).*** Chitinolytic enzymes have catalytic and substrate binding mechanisms different from those of lysozymes. Based on the X-ray crystals structures of chitinases and their complexes with substrate analogues, the catalytic mechanisms discussed based on the relative locations of catalytic residues to bound substrateanalogous. Resembling the lysozyme catalytic center, family 19 chitinases, family 46 chitinases, and family 23 lysozymes have two carboxyl group at either side of the catalytic center, which are catalytic reaction of the enzymes taking place through a single displacement mechanism. In family 18 chitinases, one can identify only one catalytic carboxylated as a proton donor, but not the second catalytic carboxylate whose function and location are similar to those of AsP52 in lysozyme (***Fukamizo, 2000***).

Measurements of Enzyme Activity:-

Chitinases stimulate macrophages by interacting with receptors on the macrophage surface that mediate the internalization of chitin particles to be degraded by lysozyme and N-acetyl-beta-glucosamminidase .The macrophages produce cytokines and other compounds that help in inflammation process , antibacterial , antifungal and antiviral roles did .

There exist several methods for the estimation of chitinases activities, some of which are;

1- H^3-Chitin and Fluorogenicsubstrateused for the estimation of hydrolyzing endo

andexo-chitinases, both specific soluble and insoluble substrate are available

(***Cabib, 1988 and Young, 1999)***.

2- Radioactive measurements are sensitive methods and give reproducible results.

3-Dye spectrometry in which soluble and insoluble-chitin substrates labeled with dyes

using dye spectrometer.

4-Late clearing assay in which acarboxy methyl-substrate soluble chitin covalently linked with Ramazol Brilliant Violet 5Ris suitable for screening of chitionlytic microorganisms and for detection of chitinase activity by plate clearing

assay***(Hood 1991***).The method is essential based on the precipitation of non-hydrolyzed chitin by HCl.

5-PAGE (polyacrylamide gel electrophoresis)(***Trudel and Asselin, 1989***)

6-Paper Chromatography and colorimetric method can be used to follow the hydrolysis of chitin.

7-MASS C^{13}–NMR spectroscopy (Hydrolysis of the colloidal chitin by the chitinase mixture procedure by Myrotheciumverrucaria. (***Rajamohanan et al ., 1996***).

Biomedical Applications of ChitinolyticEnzymes

Chitinases (EC 3.2.1.14) have an immense potential.Chitinolytic enzymes have wide-ranging applications such as;Preperation of pharmaceutically important chitooligosaccharides and N-acetyld-glucosamine.Preperation of single cell-protein .Isolation of protoplasts from fungi and yeasts.Control of the pathogenic fungi.Treatment of chitinouswastes.Control of malaria transmission ***(Neetu et al., 2006***)Application of chitinous products in foods and pharmaceutical as well as processing aids has received considerable attention in recent years as exotic synthetic compounds are losing their appeal (***You , 1999)***.Chitinases also play important physiological and ecological roles in ecosystems as recycler of chitin by generating carbon and nitrogen sources (***Rachel and Ilan, 1998)***.Considered potential biocontrolagents against fungi and nematodes which cause diseases of agricultural crops (***Rachel and Ilan, 1998)***.Fungi chitinases have physiological role in cell division , differentiation and nutritional role (***Saranya and Tha, 2013***).Chitinase genes isolation were also promising role in protection plants(***Saranya and Tha, 2013***).Chitinases play roles in nutrition and parasitism (Fungi, protozoa and invertebrates). Chitinases involved in the defense mechanism of plants and vertebrates .Used for biological control of insect pests "Baculovirus" and mosquito control.Chitinase serum activity of human also described.In defense against fungal pathogens (***Escott et al., 1996 and Aerts et al., 1996***).An enzyme, chitotriosidase is used as a marker of Gaucher disease " lysosomal storage disorder(***Aerts et al., 1996***).Used in food industry to remove the unwanted tannins and for producing gallic acid which is used as a preservative and effective in recovery of tannase enzyme. (***Ohtakara , 1988***)..Chitin used in coast-saving and environmentally friendly way in aquaculture, crustacean shells (e.g. shrimp-shell meal), which is supplemented into aquaculture as food after degradation by chemical method (***Zhang et al., 2013).***The dietary chitin supplemented at 5 % enhances the grass shrimp (Penaeusmondon) growth ***(Shiau and Yu, 1998***).

Chitinase isolated from Aeromonasveronii CD3 used as a new strategy to control the myxozoan disease in fish, which caused a serious damage in aquaculture, through its capacity to damage the shell valve of myxospores(***Liu et al ., 2011)***.Chitin microparticles had a beneficial effect in preventing and treating the

histopathological changes in the air ways of asthmatic mice.Chitin depresses the development of adaptive type2 allergic responses (***Muzzarelli, 2010)***.Stomach chitinases have an indirect digestive role in breakdown pre exoskeletonandthehind gut chitobiase chitin dimmers into absorbablemonomers of NAG. High chitinase activities in 13 different species of marine fishes were measured in stomach and stomach contents, lower but significant in intestinal tissues and contents. The chitinolytic enzymes have the ability to breakdown chitin into nutritive monomers due to the relationship between fish and the enteric bacteria that release the extracellular chitinases. This relationship could further be defined by a proposed nutrients recycling idea (***Magdalena, 2002 and Gutowska et al ., 2004***).

Conclusion

Finally, chitinases have many beneficial biomedical applications in the fields of medicine, agriculture,aquaculture, environment, etc.This article highligten the importance of the usability of recycling one of the critical problem of disposing fish scales and crustacean which negatively affect the environment. These wastes can be benefited of in the field of pharmaceutical industry to produce chitinases, thatmight be used as immune stimulants for fish to enhance the immunity against many bacterial, viral and parasitic diseases.

References

Aerts, JMFG, Boot, RG, and Renkema, GH. (1996). Chitotriosidase: a human macrophage chitinase that is a marker of Gaucher disease manifestation. In: Muzzarelli RAA, editor. Chitin Enzymology, Vol. 2 AtecEdizioniGrottammare;, pp3-10 .

Akaki,C, and Duke, GE.(2005). Apparent chitin digestibility in Eastern screech Owl (Otusasio) and the American kestrel (Falco sparverius). Journal Experimental Zoology, 283(4-5): 387-393.

Anderson DP,Siwicki,AK, and Rumsey, GL. (1995). Injection or immersion delivery of selected immunostimulants to Trout demonstrate enhancement of non-specific defense mechanisms and protective immunity. In: Shariff M, Subsighe RP, Arthur JR. Diseases in Asian Aquaculture, Vol.110.

Busam, G,kassemyer,H, and Matern, U. (1997) : Differential expression of chitoinases in Viltsvinfer L responding to system acquired resistance activators of fungal challenge : Plant Physiology , 115: 1029-1038 **.**

Cabib, E. (1988). Assay for chitinase using tritiated chitin. In: Wood WA, Kellogg SI, editors, Methods in Enzymology. Vol. 161. San Diego, CA: Academic Press. , pp.424-6**.**

Chang, WT, Ming, LC, and Wang, SL. (2010). An antifungal chitinase produced by Bacillus subtilis using chitin wastes as a carbon source. World Journal of microbiology and Biotechnology, 26(5): 945-950**.**

Chuan,LD. (2006). Review of fungal chitinases. Mycopathologia, 161(6). 345-360.

Collinge, DB,Ktagh, KM, Nielsen, KK, and Vad, RK.(1993). Plant chitinases. Plant Journal, 3:31-40**.**

Deshpande, M V. (1986). Enzymatic degradation of chitin & its biological applications .Journal of Science Research, 45: 273-81**.**

Escott, GM, Walters, CE, Ingham, E, and Adams, DJ. (1996). Expression of chitinase activity during monocyte differentiation. In: MuzzarelliRAA, editor, Chitin Enzymology Vol.2 Grottammare: European .Chitin Society, p 11-20.

Eurich, K. (2009). Potential role of chitinase 3 –like -1in inflammation –associated carcinogenic changes of epithelial cells.World J. Gastroenterology , 15(42): 5249-5259 **.**

Fange, R., Lunbdald, J and Slettengren, K. (1997). Chitinolytic enzymes in the digestive system of Marine Fishes . Marine Biology, 53, 317-321.

Fukamizo, T. (2000). Chitinolytic enzymes catalysis , substrate binding , and their application . Current protein and Peptide Science, 1(1): 105-124.

Gutowska, MA, Drazen, JC, and Robinson, BH. (2004). Digestive chitinolytic activity in marine fishes of Monterey Bay, California. Comparative Biochemistry and Physiolology A. Nov, 139 (3): 351-8**.**

Hamel, F, Bovin, R, Temblay, C, and Bellemare, G. (1997). Structural and evolutionary relationships among chitinases of flowering plants. J. Mol. Evol. , 44: 614-626.

Henrissat, BB. (1993). New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochemistry Journal, 293: 781-788.

Hollak, C.E., Weely, V, and Aerts , JMF. (1994). Marked elevation of plasma chitotriosidase activity: a novel hallmark of Gaucher disease. J.Clin.Invest. 93: 1288-1292.

Hunt, DE, Gevers, D, and Polz, MF. (2008). Conservation of the chitin utilization pathway in the Vibrionacea. Applied and Environmental Microbiology, 74(1): 44-51**.**

Ian, B. and Roy, A.D. (2005). The use of immunostimulants in fish larval aquaculture. Fish and Shellfish Immunology, 19:457-472.

Jolles, P. and Muzzarelli, RA. (1999). Chitin and Chitinases. Basel: Birkhauser. , ISBN: 3-7643 -5815-7.

Kawakami H, Shinohora, N, and Sakai, M. (1998). The non-specific immunostimulation and adjuvant effects of Vibrio anguillarumbacterin, M-glucan , Chitin or Freund,s Complete adjuvant in Yellowtail

Seriolaquinqueradiata to Pasteurellapiscida infection. Fish Pathology., 33: 287-292 .

Liu,Y, Zhou, Z, Miao, W, Zhang ,Y, and Yao, B. (2011). A chitinase from AeromonasVeronii CD3 with potential used to control Myxozoan disease. Plsone. 6(12): e 29091.doi:10.1371.

Magdalena, G, (2002). Chitinase activity of fishes with varying depth distributions. The Evergreen state College, Drazen. Microbiology Review. 11: 317-338.

Muzzarelli, RA, Ilari, P, Taris, R, Dubini, B, and Xia, W. (1994). Chitosan from Absidiacoerulea. Carbohydrates Polymers, 25: 45-50.

Muzzarelli,RA. (2010). Chitin and Chitosans as immunoadjuvants and non – allergic drug carriers. Marine Drugs. 21(8) 292-312.

Muzzarelli, RA. (1977). Chitin. Oxford: Pergamon press.

Neetu, D, Rupinder, T, and Singh, HG. (2006). Biotechnological aspects of chitinolytic enzymes: a review Applied Microbiology and. Biotechnology. 71: 773-782.

Nopakarn, R, Shigerkazu, Y, Abhinya, P, Massoru, W, and Takashi, T. (2007). Purification of Aspergillus SP. SI-13 chitinases and their role in saccharification of chitin in mash of solid –sate culture with shellfish waste .J. Bioscience and Bioenginer. , 103 (6): 535-541**.**

Ohtakara, A. (1988). Viscometeric assay for chitinase. In: Wood WA, Kellogg ST, editors. Methods in Enzymology, Vol. 161. San Diego, CA : Academic press, pp.426-30 .

Pandey, A. (2010). Chitinase enzymes in fish. Springer shops by Google. 2010; 435.

Park, B, and Kim, M. (2010). Applications of chitin and its derivatives in biological Medicine . International Journal of Molecular Science, 11, 5152-5164.

Patil, RS; Vandana, G, Mukund, V and Deshpande, E. (2000). Chitinolytic enzymes: an exploration. Enzyme and MicrobiolTechnology, 26: 473-483.

Perrakis, A, Wilson, KS, Chet,I, and Vorgias, CE. (1993). Phylogenetic relationships of chitinases. In: chitin Enzymology. Muzzarelli RAA, editor, .Ancona: European Chitin Society, 217-232.

Prameela, K, Murali, CH, and Hemalatha, KPJ. (2010). Bio-Efficiency of Paediococcusacidilactici ATCC 8042 for recovery of chitin and cartnoids in fermentation of Shrimp Biowaste. International J. Chem. tech. Research., 2(4): 1924-1928.

Rachel, C K and Ilan, C (1998). The molecular biology of chitin digestion Journal of Biotechnology, 9: 270-277.

Rajamohanan, PR; Ganapathy, S; Vyas, PR; and Ravikumar, A. (1996). Solid state CP/MASS C^{13}-NMR spectroscopy: a sensitive method to monitor enzymatic hydrolysis of chitin. J. Biochem. Biophys. Methods, 31: 151-63.

Renkema, GH, Boot, RG, Donker, KW, and Aerts, AO. (1997). Synthesis, sorting, and processing into distinct isoforms of human macrophage chitotriosidase.Eur. J. Biochem ., 244 , 279 -285 .

Rolf, GB. Edward, F., Blommaar, TC., Moe, C, Aerts, FG. (2001). Identification of a novel acidic Mammalian chitinases Distinct from Chitotriosidase. J.Biol. Chem., 276: 6770-6778.

Sahi, AS, and Monocha, MS. (1993). Chitinases of fungi and plants: their involvement in morphogenesis and host parasite interaction. FEMS

Sami, L., Pusztahelyi, E, Grallert, AKI (2001). Autolysis and aging of *Penicillium chrysogenum* cultures under carbonstarvation :chitinase production and fungal effect of allosamidin. The journal of General and Applied Microbiology, 47(4): 201-211.

Saranya, N. and Tha, T. (2013). Production and some properties of chitinase from Pseudomonas putida isolated from fish waste dumping soil. Online International DisplinaryJournal , III(V):Sept-Oct.

Shaikh, SA. and Deshpande, MV. (1993). Chitindyticenzymes : their contribution to basic and applied research. World J MicrobiolBiotechnol, 9 : 468-75 .

Shakhbazau, UA, and Kartel, NA. (2008). Chitinases in bioengineering research.Russain Journal of Genetics. , 44(8): 881-889 .

Shiau,SY, Yu and YP.(1998). Chitin but not chitosan supplementation enhances growth of grass shrimp. Penaeusmonodon. Journal of Nutrition , 128(5): 908-912.

Springer, L. (2001). Effect of chitin sources on production of chitinases and chitinosanse by streptomycesgriseus HUT 6037 .Biotechnology and Bioprocess Engineering. , 6(1): 18-24.

Springer, L. (2013). Microbial chitinases : Natural sources , Mutagenesis , and direct evolution to obtain thermophilic counterparts. Thermophilic Microbes in Environmental and Industrial Biotechnology., 649-669.

Subasinghe, S. (1995). The development of Crustacean and mollusk industries for chitin and chitosan resources. In: Zakaria MB, Wan Muda WM, Abdullah MP, editors, Chitin and Chitosan. Malaysia: Penerbit University Kebangsoan, pp. 27-37.

Tao, Y; Jin, H., Long, Z., Zhang, L. and Liu, S. (2005). Purification of Microbiology , 55 (3): 213-218 .

Tharanathan, RN. And Kittur,FS. (2003). Chitin –the undisputed biomolecule of great potential. Critical Review in Food Science and Nutrition, 43: 61-87.

Tjoelker, LW, Gosting, LF and Gray PW. (2000). Family 19 chitinases Streptomyces thermoviolances OPC-520 .J.Biol. Chem. 275:514-520

Trudel, J. and Asselin, A. (1989). Detection of chitinase activity after polyacrylamide gel electrophoresis. Anal Biochem. , 178: 362-6 .

Verna,S. (2008). Chitinase of filamentousfungi : a large group of diverse proteins with multiple physiological functions. British Mycological Society, 22 : 36-42 .

Wikipedia-Free encyclopedia, 2013.PP.1-8 .

Xio X, Yin, X, and Sun, L. (2005). Chitinase genes in lake sediments of Ardley Island , Antarctica " Applied and Environmental Microbiology ,71: 7904-7909.

You, JJ. (1999) : Trends in Food Science &Technology ,10(2) : 37-51 .

Zaku, SG, Emmanuel, SA, Aguzue, OC, and Thomas, SA. (2011). Extraction and Characterization of chitin; a functional biopolymer obtained from scales of Common carp (Cyprinuscarpio L.) : A lesser known source.African Journal of Food Science , 5,478-483.

Zhang, Y, Zhou, Z, Liu, Y, Cao, Y, and Ringo, E. (2013). Highly–yield–production of a chitinase from AeromonasVeronii B565 as a potential feed supplement for warmwater aquaculture. Appl. Microbiol .Biotechnol., June18 .

Food Science and Human Nutrition

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 113-126 (July 2015/Ramadan 1436H)

Quality characteristics and rheological properties of Barhi date (*Phoenix dactylifera* L.) jams with addition of carrot and pumpkin purees

Mohamed G. E. Gadallah[1,2], Ahmed A. Al-Hassan[1] and El-Sayed A. Abd El-Hady[1*]

[1] *Food Science and Human Nutrition Department, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia*

[2] *Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

[*] *Corresponding author e-mail: (saelhady@yahoo.com)*

(Received 2 /4/2015; accepted 3/6/2015)

ABSTRACT. Sensory evaluation, color parameters, mineral determination, rheological properties and nutritional value of Barhi date jam prepared with substituted by carrot or/and pumpkin purees at concentrations 25, 50 and 75% were studied. The lowest jam sample in lightness (34.86) and the highest in yellowness (51.71) which prepared by Barhi date 25%: carrot 75%. In addition, panelists results showed that the best jam sample in overall acceptability (35.7) was jam prepared with Barhi date 25% : carrot 75% followed by jam prepared with Barhi date 50% : pumpkin 50% (33.9). Caloric values of Barhi date jam samples containing carrot and pumpkin purees recorded the values ranged between 398.87 k. cal. for jam sample containing Barhi date 50%: pumpkin 50% to 379.17 k. cal. for the control jam sample. It was clearly appeared that the control jam sample had significant ($P \leq 0.05$) increase in potassium content (101.36 ppm) followed by 95.00 and 86.98 ppm which recorded by Barhi date 25%: pumpkin 75% and Barhi date 50%: carrot 25%: pumpkin 25%, respectively. Calcium content (14.25 ppm) was significant ($P \leq 0.05$) increase in jam sample prepared by Barhi date 50%: carrot 25%: pumpkin 25%, followed by 11.68 ppm which given by Barhi date 50%: carrot 50%. The relationship between shear stress (τ) and shear rate (γ) for all prepared date jam samples were nonlinear, and the flow behavior index of all prepared date jams were less than unity indicating a non-Newtonian behavior of the samples, with a pseudo-plastic type. Jam prepared from Barhi date 25%: carrot 75% had higher plastic viscosity value being 58.43 (Pa.s) followed by 49.04 (Pa.s) which given by jam sample prepared by Barhi date 50%: carrot 50% at temperature (20ºC). The highest apparent viscosity was found for jam sample prepared from Barhi date 25%: carrot 75% followed by Barhi date 50%: carrot 50% when compared to control date jam and other prepared jam samples. Finally, our results indicated that jam prepared from Barhi date 25%: carrot 75% is the best sample in color properties, texture, viscosity and sensory acceptability followed by jam prepared from Barhi date 50%: pumpkin 50%.

***Keywords*:** Barhi date jam, carrot, pumpkin, sensory evaluation, rheological parameters

INTRODUCTION

Date palm (*Phoenix dactylifera* L., family Arecaceae) is the important and famous tree for its delicious fruits. Due to date fruit's nutritional, distinct medicinal properties and economic, it is a common food source for most of people in Middle East and around the world (Vinson *et al*., 2005). Three different palm dates forms are used being: (i) khalal (fresh, hard ripe, coloring stage), (ii) rutab (crisp to succulent or ripe stage), and (iii) tamri (soft pliable, full ripe stage) (Vayalil 2012). They are mainly consumed as dried fruits (60%-70%) at rutab (semi-ripe) or fresh fruits (30%- 40%) in the tamer stages (fully ripe) with little or no processing (Ali *et al*., 2012).

Kingdom of Saudi Arabia was the third country for date production around the world. The annual production in 2013 was 1.065 million ton, representing about 12.5% of the total world production of date fruits (FAOSTAT, 2013). The increasing production of dates in Kingdom of Saudi Arabia and the limited amount of processed dates enhanced the endeavor of incorporating dates or date paste in food products.

Barhi date is a mid-season cultivar extensively cultivated in the Saudi Arabia. Barhi dates are different than other cultivars in which fruit are marketed and consumed fresh at the mature full yellow (bisir) stage as a crispy apple-like fruit due to low contents of soluble tannins (Botes and Zaid, 1999). At the rutab stage (ripening), fruit become softer and sweeter but their market value decreases. Parn *et al*., (2015) mentioned that date-based fruit bars may be expected to fulfill the requirements of health conscious consumers. It is anticipated that this novel food product will attract better market ability at the international level.

Jams are important food products which are prepared and preserved by sugar at a high concentration (Kordylas, 1990). In Saudi Arabia, no date jam has been developed for the market.

Banga, (1984) repoted that carrot (*Daucus carota* L.) is one of the most important vegetable crops cultivated in moderate climate regions. Carrot originates from the wild plants growing in south western Asia and Europe. Yellow and orange carrot types were derived by selection process from red carrots. Regarding to carrot nutritional value, it have been ranked tenth in terms among 38 other fruits and vegetables of their nutritional value, and seventh for their contribution to nutrition (Alasalvar, *et al*., 2005).

Carrots, a good source of carotene, are usually used either fresh or processed into juices, beverages, jams, baby foods and fiber products (Lombrana and Dias, 1985). The benefits of carotenes, including its conversion in the body into vitamin A and the unique biological effects of carotenes as antioxidant activity, stimulation of the immune response and reducing the risk of cancer (Olson, 1988 and Benoist, *et al*., 2001).

Pumpkins, which are the different species fruits of the genus *Cucurbita*, are growing worldwide for their seeds and pulps for human nutrition, either for preparation of foods such as purees, jellies, jams, and syrups, or direct consumption.

Pumpkin pulps has huge amounts of carotenoids, which are pigments that give leaves, flowers, and fruits coloration that ranges from yellow to red (Oliver and Palou, 2000). In addition to pro-vitamin A activity of some carotenoids, such as *β*-carotene, *β*-cryptoxanthin and α-carotene, studies have also mentioned that the carotenoids consumption lowers the risk of cardiovascular and degenerative diseases, cataracts, macular degeneration as well as certain types of carcinomas (Rao and Rao, 2007).

Therefore, the aim of this study was to evaluate the effect of adding carrot and pumpkin purees on chemical composition, color parameters, sensory properties, minerals contents and rheology parameters of Barhi date jams and to extent the shelf life of Barhi date fruits which considers the important variety grown in Qassim region, Saudi Arabia.

MATERIAL AND METHODS

Materials:

Barhi date fruits (*Phoenix dactylifera* L.) were brought during the summer season of 2013 from the Agricultural and Veterinary Research Station of the College of Agriculture and Veterinary Medicine, Qassim University, Burydah, Saudi Arabia. Carrot, pumpkin and commercial grade sucrose were purchased from the local market, Burydah, Saudi Arabia. Food grade pectin and citric acid were purchased from Loba Chemie, LTD, India.

Methods

Preparation of Barhi date jams:

After sorting, the Barhi dates were pitted and the fleshes were washed and air dried over eight hours before grinding. Also, carrots and pumpkin were washed, peeled and cut into small pieces (2 cm thick slices). Barhi dates, carrots and pumpkins were first blanched separately in boiling potable water with a ratio 1:5 (w:w) for 10 min. After cooking, Barhi dates, carrots and pumpkins were homogenized with the blanching water for 2- 3 min in using a food mixer obtaining Barhi dates paste, carrots and pumpkins purees.

For control jam sample contained 100% Barhi dates, one kilogram of Barhi date paste was mixed with 500 g of sucrose sugar, gently heated in an open air with manually stirred until the total soluble solids (TSS) content reached 65 Brix. Then pectin was added (5 g/kg of Barhi date: sugar mixture) followed by the addition of citric acid (3 g/kg of added sugar). The heating was continued for a few minutes until the TSS reached about 68%. Finally, the prepared date jam was hot poured into clean dry glass jars with screw caps. Samples were immediately cooled to room temperature and stored until further analysis (Besbes *et al*., 2009). Samples of carrot and pumpkin jams were prepared by replacing 25, 50 and 75% of Barhi date paste with carrot or/and pumpkin purees and different amounts of sugar, citric acid and pectin as mentioned in Table (1).

Table (1): Recipe of prepared Barhi date jams and mixed with carrot and pumpkin purees.

Jam samples	Ingredients / gram					
	Barhi date	Carrot	Pumpkin	Sugar	Citric acid	Pectin
Control (Barhi date 100%)	1000	0	0	500	1.5	7.5
Barhi date 25%: carrot 75%	250	750	0	875	2.6	9.4
Barhi date 25%: pumpkin 75%	250	0	750	875	2.6	9.4
Barhi date 50%: carrot 50%	500	500	0	750	2.3	8.8
Barhi date 50%: pumpkin 50%	500	0	500	750	2.3	8.8
Barhi date 50%: carrot 25%: pumpkin 25%	500	250	250	750	2.3	8.8

Proximate chemical composition:

Moisture, crude protein (N x 6.25), lipids (ether extract) and ash contents of raw materials and prepared jams were determined according to the methods of the AOAC (2000). Nitrogen free extract (NFE) were determined by difference. Total soluble solids were determined by a digital Abbe Refractometer, AOAC (2000). Caloric values (k. cal.) of prepared dates jams were calculated with following equation, caloric values = (crude protein (g) x 4) + (lipids (g) x 9) + (NFE (g) x 4).

Color determination of prepared date jams:

Prepared date jams color was determined using spectrophotometer (Minolta Color Reader CR-10, Minolta Co. Ltd., Japan) according to the method described in Francis (1983). Color coordinates X, Y and Z were converted to corresponding Hunter L*, a* and b* color coordinates according to formula given by manufacturer. The vertical coordinate "L" is lightness from 0 (total light absorbance and therefore completely black) through grey (50) to 100 (complete light reflectance); the horizontal coordinate "a" is greenness/redness, from -60 (green) through grey to +60 (red); orthogonal horizontal coordinate "b" is yellowness from -60 (blue),to +60 (yellow) indicates for yellowness.

Sensory evaluation of prepared date jams:

Prepared Barhi date jam samples and its mixtures with carrot and pumpkin purees were coded with different numbers and submitted to sensory evaluation by fifteen member semi trained panelists from colleagues at College of Agriculture and Veterinary Medicine, Qassim University. The panelists were asked to rate each sensory attribute using the control Barhi date jam as the basic for evaluation. Jams were evaluated for appearance (10), color (10), odor (10), taste (10) and overall acceptability (40) according to Besbes *et al*., (2009).

Minerals determination of prepared jams:

The mineral contents of the prepared date jam samples, including potassium, calcium, ferrous, cupper and zinc, were determined using an Atomic Absorption Flame Emission Spectrophotometer (Perkin-Elmer Model AA-6200 from Shimadzu 7000, Japan) as reported by AOAC, (2000).

Rheological properties of prepared jams:

Rheological properties of date jam samples were determined by the Brookfield digital rheometer model DV-III+ (Brookfield Engineering Lab., INC., Middleboro, USA) at 20 °C according to method described by Hegedusic *et al*., (1995). The Brookfield small sample adapter and Sc4 -14 spindle were used. The data were analyzed using the Bingham plastic and Power law mathematical models, respectively as follows:

These models are: $\tau = \tau_o + \eta\gamma$ and $\tau = k\gamma^n$

where: τ = shear stress (Pa), τ_o = yield stress (Pa), η = plastic viscosity (Pa s), γ = shear rate (s^{-1}), k = consistency coefficient (Pa s^n) and n = flow behavior index (dimensionless).

The apparent viscosity (Pa s) was measured at 10 rpm.

Statistical analysis

Data were expressed as the means ± SE. Statistical analysis was carried out using the PROC ANOVA followed by Duncan's Multiple Range Test with $p \leq 0.05$ being considered statistically significant to compare between means according to Snedecor and Cochran (1980). All procedures were triplicate using Statistical Analysis System program (SAS, 2000).

RESULTS AND DISCUSSION

Proximate chemical composition of Barhi dates, carrot and pumpkin:

Proximate composition of Barhi date, carrot and pumpkin used in preparation of jam samples are determined and the results are presented in **Table (2)**. The obtained data indicated that carrot had significant ($P \leq 0.05$) high in moisture content (86.77%) followed by Barhi date, while pumpkin sample recorded the lowest moisture content (10.61%). No significant difference could be observed in crude protein between pumpkin and carrot (7.40 and 6.80%, respectively). It could be observed that, Barhi date had significant ($P \leq 0.05$) low in ether extract and ash which recorded 0.14 and 3.98%, respectively, meanwhile, the highest ash content (8.31%) was found by carrot sample with significant higher than other samples. For nitrogen free extract, Barhi date recorded the highest value (92.60%) followed by carrot and pumpkin which being 83.38 and 82.77%, respectively. The results are in accordance with Miller *et al*., (2002) and Habiba and Mehaia (2008). Assirey (2015) reported that the important date varieties grown in Saudi Arabia have a high content of sugar (71.2–81.6%) and low concentrations of protein (1.72–4.73%) and lipids (0.12–0.72%). Dates were rich in sugar and fiber deserving to be used for value-added product formulation (Barreveld, 1993 and Besbes *et al*., 2009)

Table (2): Proximate composition (% on dry weigh basis) of Barhi dates, carrot and pumpkin.

Sample	Moisture	Crude protein (N x 6.25)	Ether extract	Ash	Nitrogen free extract*
Barhi date	22.15 ± 0.25[b]	3.28 ± 0.37[b]	0.14 ± 0.45[c]	3.98 ± 0.36[c]	92.60 ± 0.56[a]
Carrot	86.77 ± 0.35[a]	6.80 ± 0.35[a]	1.51 ± 0.25[b]	8.31 ± 0.44[a]	83.38 ± 0.55[b]
Pumpkin	10.61 ± 0.20[c]	7.40 ± 0.49[a]	3.78 ± 0.35[a]	6.05 ± 0.26[b]	82.77 ± 0.65[b]

*NFE: Calculated by differences, Data are the mean ± SE, n = 3,

Means having the same letter within each property are not significant difference at p≥0.05.

Proximate composition of prepared Barhi dates jams with carrot and pumpkin purees:

Table (3) show proximate composition and caloric values of Barhi date jam (control sample) and jams prepared with replacing 25, 50 and 75% of date pastes by carrot or/and pumpkin purees. The obtained data revealed that the highest moisture content (25.63%) was given by jam prepared from Barhi date 25%: carrot 75% without significant (P≥0.05) differences with other jam samples. This result could be attributed to the high moisture content of used carrot.

Crude protein of prepared date jams was lower than main sources ranged from 6.67% for Barhi date 50%: pumpkin 50% jam sample to 3.53% for control date sample. This could be explained by the addition of sugar in the formulation and the involvement of the proteins in Maillard reaction during heat processing. Indeed, the formation of volatile nitrogen-containing compounds, during the Maillard reaction, could cause a small reduction in the protein content (Pearson 1976).

Data also indicated that significant (P≤0.05) increase was found in fat content (ether extract) 2.79% which recorded by jam prepared from Barhi date 50%: pumpkin 50% and the lowest fat content (0.13%) was given by control Barhi date sample. Replacing 75 and 50% of Barhi date paste with carrot puree resulted in significant (P≤0.05) increase in ash content being 5.90 and 5.67%, respectively compared to other jam samples and control jam which recorded the lowest ash content 4.03%, the higher value of ash content in used carrot could be explain this results.

No significant (P≥0.05) difference was observed in nitrogen free extract between control jam sample and jam prepared from Barhi date 25%: pumpkin 75% which given 90.97 and 90.42%, respectively. On the other side, Barhi date 50%: carrot 50% jam sample had the lowest nitrogen free extract (86.10%) followed by (86.77%) for Barhi date 50%: pumpkin 50% jam sample. These results are in accordance with Habiba and Mehaia (2008) and Besbes *et al*., (2009).

Finally, the caloric values of Barhi date jam samples containing carrot and pumpkin purees recorded the caloric values ranged from 379.17 k. cal. for the

control jam sample to 398.87 k. cal. for that containing Barhi date 50%: pumpkin 50%.

Table (3): Proximate composition (% on dry weigh basis) and caloric values (k.cal) of prepared Barhi dates jams with carrot and pumpkin purees.

Jam samples	Moisture	Crude protein (N x 6.25)	Ether extract	Ash	Nitrogen free extract	Caloric values
Control (Barhi date 100%)	25.00±0.36$^{a}_{b}$	3.53±0.43^{c}	0.13±0.01^{e}	4.03±0.24^{c}	90.97±0.22^{a}	379.17±0.83$_{e}$
Barhi date 25% : carrot 75%	25.63±0.46^{a}	6.13±0.20^{a}	0.85±0.21$^{c}_{d}$	5.90±0.53^{a}	87.52±0.94^{c}	382.25±0.71$_{d}$
Barhi date 25% : pumpkin 75%	24.83±0.52$^{a}_{b}$	4.77±0.26^{b}	0.58±0.09$^{d}_{e}$	4.83±0.37^{b}	90.42±0.35$^{a}_{b}$	385.98±0.69$_{c}$
Barhi date 50% : carrot 50%	25.07±0.18$^{a}_{b}$	6.00±0.36^{a}	2.23±0.30^{b}	5.67±0.41^{a}	86.10±0.49$^{c}_{d}$	388.47±0.63$_{b}$
Barhi date 50% : pumpkin 50%	24.70±0.15$^{a}_{b}$	6.67±0.44^{a}	2.79±0.12^{a}	4.77±0.17^{b}	86.77±0.70$^{c}_{d}$	398.87±0.50$_{a}$
Barhi date 50% : carrot 25%: pumpkin 25%	24.07±0.18^{b}	4.60±0.28^{b}	1.27±0.21^{c}	5.43±0.41^{a}	88.70±0.86^{b}	384.63±0.39$_{c}$

Data are the mean ± SE, n = 3,

Means having the same letter within each property are not significant difference at p≥0.05.

Color attributes of prepared date jams with carrot and pumpkin:

Color is an important characteristic for jam because it, together with texture and aroma, contributes to consumer preference. It depends on physicochemical characteristic of used materials and on operating conditions applied during cooking**.** The L*, a* and b* value for prepared jam samples are summarized in **Table (4)** and used Barhi dates, carrot and pumpkin purres are shown by **Fig. (1)**, also prepared date jam samples with carrot and pumpkin are shown by **Fig. (2).** In general, a lower L* value indicate a darker jam, a* positive value is associated with jam redness, whereas higher b* value leads to higher jam yellowness.

It could be noticed that no significant (P≥0.05) difference was found in L^{*} (lightness) between control (jam prepared with Barhi date only) and jam prepared with Barhi date 50%: pumpkin 50% which recorded 47.31 and 47.36, respectively. Contrary, the lowest jam sample in lightness (34.86) and the highest one in yellowness (51.71) which prepared by Barhi date 25%: carrot 75% and it was the best jam sample in sensory properties determined by the panelists Table (5). These results could be attributed to high content of carotenoids in carrot which leads to formation a strong orange color in prepared jams. Barhi date jam (control sample) showed the lowest yellowness (b^{*}) being 42.18 comparing to all other samples which contained carrot or pumpkin and these results were logically. Similar findings

are in accordance with Habiba and Mehaia (2008); Besbes *et al*., (2009); Renna, *et al*., (2013) and Kamiloglu, *et al*., (2015).

Table (4): Color properties of prepared Barhi date jams with carrot and pumpkin purees.

Jam samples	Color characteristics		
	L*	***a****	***b****
Control (Barhi date 100%)	47.31 ± 0.93^{a}	3.78 ± 0.41^{e}	42.18 ± 1.16^{e}
Barhi date 25%: carrot 75%	34.86 ± 0.83^{d}	10.77 ± 0.25^{c}	51.71 ± 0.74^{a}
Barhi date 25%: pumpkin 75%	43.99 ± 0.88^{b}	6.66 ± 0.41^{d}	45.05 ± 0.33^{cd}
Barhi date 50%: carrot 50%	37.72 ± 0.59^{c}	18.12 ± 0.49^{a}	48.15 ± 0.71^{b}
Barhi date 50%: pumpkin 50%	47.36 ± 0.34^{a}	4.17 ± 0.21^{e}	43.18 ± 0.21^{de}
Barhi date 50%: carrot 25%: pumpkin 25%	42.93 ± 0.82^{b}	13.56 ± 0.34^{b}	46.79 ± 0.53^{bc}

Data are the mean ± SE, n = 3,

Means having the same letter within each property are not significant difference at p≥0.05.

Fig. (1): Barhi date fruits, carrot and pumpkin

Fig. (2): Prepared Barhi date samples.

Sample No. 1: control jam (Barhi date 100%)
Sample No. 2: jam prepared from Barhi date 25%: carrot 75%
Sample No. 3: jam prepared from Barhi date 25%: pumpkin 75%
Sample No. 4: jam prepared from Barhi date 50%: carrot 50%
Sample No. 5: jam prepared from Barhi date 50%: pumpkin 50%
Sample No. 6: jam prepared from Barhi date 50%: carrot 25%: pumpkin 25%

Sensory properties of prepared Barhi date jams with carrot and pumpkin:

Sensory properties (appearance, color, odor, taste and overall acceptability) of Barhi date jam and those date jams prepared by substitution with different ratios of carrot and pumpkin purees were evaluated and analytical data are tabulated in **Table (5).** The obtained data indicated that no significant (p≥0.05) differences were detected between control and other samples in appearance, color, odor and taste.

Jam sample which containing Barhi date 25% : carrot 75% had the highest score from the panelists being 9.0, 8.9, 9.2 and 8.6 for appearance, color, odor and taste, respectively. On the other hand, control jam prepared from Barhi date fruits only recorded the lowest scores in appearance, color and odor which being 7.8, 8.0 and 8.0, respectively comparing to other treatments. The same trend was observed for the overall acceptability; where, the jam prepared with Barhi date 25%: carrot 75% had significant ($p \leq 0.05$) higher score (35.7) followed by jam prepared with Barhi date 50% : pumpkin 50% (33.9), while the lowest overall acceptability (31.8) was recorded by jam prepared by Barhi date fruits (control sample), similar observations are in accordance with Renna, *et al*., (2013). These findings are in harmony with those obtained by Habiba and Mehaia (200**8)** who found that the overall acceptability scores indicated that organoleptically acceptable carrot jam can be prepared with date paste from Sukary and Wannanah pastes replacing up to 50% and 25% of the added sugars, respectively. Besbes *et al*., (2009) showed that Allig and Kentichi jams presented a higher overall acceptability. However, quince jam (the most consumed in Tunisia) and Deglet Nour jams did not show any significant differences. The use of dates may also be attractive to consumers as a positive alternative to conventional fruit in jam production.

Table (5): Sensory properties of prepared Barhi date jams with carrot and pumpkin purees.

Jam samples	Sensory characteristics				
	Appearance (10)	Color (10)	Odor (10)	Taste (10)	Overall acceptability(40)
Control (Barhi date 100%)	7.8 ± 0.66^{a}	8.0 ± 0.55^{a}	8.0 ± 0.71^{a}	8.0 ± 0.45^{a}	31.8 ± 0.60^{b}
Barhi date 25%: carrot 75%	9.0 ± 0.45^{a}	8.9 ± 0.51^{a}	9.2 ± 0.37^{a}	8.6 ± 0.24^{a}	35.7 ± 0.24^{a}
Barhi date 25%: pumpkin 75%	8.2 ± 0.49^{a}	8.4 ± 0.40^{a}	8.6 ± 0.50^{a}	7.8 ± 0.58^{a}	33.0 ± 0.37^{b}
Barhi date 50%: carrot 50%	8.4 ± 0.51^{a}	8.4 ± 0.24^{a}	8.6 ± 0.37^{a}	8.2 ± 0.49^{a}	33.6 ± 0.24^{ab}
Barhi date 50%: pumpkin 50%	8.4 ± 0.24^{a}	8.9 ± 0.51^{a}	8.8 ± 0.49^{a}	7.8 ± 0.24^{a}	33.9 ± 0.51^{ab}
Barhi date 50%: carrot 25%: pumpkin 25%	8.2 ± 0.37^{a}	8.8 ± 0.49^{a}	8.4 ± 0.51^{a}	8.0 ± 0.55^{a}	33.4 ± 0.37^{b}

Data are the mean ± SE, n = 10,

Means having the same letter within each property are not significant difference at $p \geq 0.05$.

Minerals composition of prepared Barhi date jams:

Table (6) shows minerals content (potassium, calcium, ferrous, cupper and zink) of Barhi date jams and its mixtures with carrot or/and pumpkin. It was clearly appeared that the control sample which prepared from 100% Barhi date fruits had significant ($P \leq 0.05$) increase in potassium content (101.36 ppm) followed by 95.00 and 86.98 ppm which recorded by Barhi date 25%: pumpkin 75% and Barhi date 50%: carrot 25%: pumpkin 25%, respectively. On the other hand, jam sample prepared from Barhi date 25%: carrot 75% was the lowest selected sample in potassium content (72.32 ppm).

These data could be attributed to the high content of potassium in Barhi date, so jam samples which contain lower amounts of date lead to contain lower amounts of potassium. Contrary, data showed that calcium content (14.25 ppm) was significant ($P \leq 0.05$) increase in jam sample prepared by Barhi date 50%: carrot 25%: pumpkin 25%, followed by 11.68 and 10.07 ppm which given by Barhi date 50%: carrot 50% and Barhi date 25%: pumpkin 75%, respectively. No significant ($P \geq 0.05$) difference was found in ferrous, cupper and zink contents between control sample and all other treatments. These results are agreement with those of Abdelmoneim *et al.* (1983); Sawaya *et al.* (1983) and Habiba and Mehaia (2008).

Assirey (2015) mentioned that the predominant mineral in dates is potassium, Dates are nutritious and can play a major role in human nutrition and health. In addition, dates are an important nutritional source of minerals and free amino acids.

Table (6): Mineral content of prepared Barhi date jams with carrot and pumpkin purees.

Jam samples	Mineral content (ppm)				
	Potassium	Calcium	Ferrous	Cupper	Zinc
Control (Barhi date 100%)	101.36±11.08^{a}	9.55±0.35^{c}	0.852±0.11$_{a}$	0.178±0.01^{a}	0.129±0.01^{a}
Barhi date 25%: carrot 75%	72.32±8.21^{c}	9.25±0.66^{c}	0.557±0.02$_{a}$	0.146±0.02^{a}	0.228±0.06^{a}
Barhi date 25%: pumpkin 75%	95.00±1.73^{ab}	10.07±0.73^{bc}	0.642±0.06$_{a}$	0.145±0.02^{a}	0.156±0.02^{a}
Barhi date 50%: carrot 50%	79.00±2.09^{bc}	11.68±0.65^{b}	0.647±0.19$_{a}$	0.149±0.01^{a}	0.126±0.03^{a}
Barhi date 50%: pumpkin 50%	76.72±4.75^{bc}	9.43±0.52^{c}	0.584±0.08$_{a}$	0.128±0.01^{a}	0.132±0.01^{a}
Barhi date 50%: carrot 25%: pumpkin 25%	86.98±6.43^{abc}	14.25±0.04^{a}	0.677±0.11$_{a}$	0.199±0.05^{a}	0.190±0.02^{a}

Data are the mean ± SE, n = 3,

Means having the same letter within each property are not significant difference at $p \geq 0.05$.

Rheological properties of Barhi dates jam with carrot and pumpkin:

The obtained rheological results of Barhi date jams without or with carrot and pumpkin are given in **Table (7)** and **Fig. (3).** It could be observed that the relationship between shear stress (τ) and shear rate (γ) for all date jam samples were nonlinear, also the flow behavior index (n) of all prepared date jams were less than unity indicating a non-Newtonian behavior of the samples, with a pseudo-plastic type.

The obtained data showed that date jam is a pseudo-plastic with yield stress like many other fruit pulps and purees such as mango pulp (Bhattacharya, 1999) and peach and papaya purees (Guerrero and Alzamora, 1998).

As seen, jam prepared from Barhi date 25%: carrot 75% had higher plastic viscosity value being 58.43 (Pa s) followed by 49.04 (Pa s) which given by jam sample prepared by Barhi date 50%: carrot 50% at temperature (20ºC).

The flow-behavior index values ranged from 0.36 which recorded by jam sample prepared with Barhi date 25%: carrot 75% to 0.13 which given by jam prepared by Barhi date 50%: pumpkin 50% at 20 ºC. The observed changes in flow behavior may also be attributable to changes in the colloid state of the pulp components (especially pectin). In addition, consistency coefficient values ranged from 23.35 to 27.43 (Pa s^n) for selected Barhi date jams with carrot and pumpkin. Jam sample prepared from Barhi date 25%: pumpkin 75% had the highest value of yield stress 233.15 (Pa) compared to control jam sample which recorded the lowest yield stress 197.05 (Pa).

The differences in pectin fractions in Barhi dates, carrot and pumpkin must be taken into account because polymer interactions play the major role in flow behavior of the fruit products (Van Buren 1991). The results of apparent viscosity were illustrated by **Fig. (3)**, it could be noticed that the highest apparent viscosity was found for jam sample prepared from Barhi date 25%: carrot 75% followed by Barhi date 50%: carrot 50% when compared to control date jam and other treatments. Finally, we suggested that addition of carrot pulp at 75% and 50% to date jam resulted in announced increase in product apparent viscosity and flow behavior index.

Table (7): Rheological properties of Barhi date jams with carrot and pumpkin purees (at 20 ºC).

Jam samples	Rheological properties			
	Plastic viscosity η (Pa.s)	Yield stress τ_o (Pa)	Consistency coefficient k (Pa.s^n)	Flow behavior index n
Control (Barhi date 100%)	35.62 ± 2.78	197.05 ± 6.55	23.35 ± 0.76	0.25 ± 0.04
Barhi date 25%: carrot 75%	58.43 ± 5.70	198.05 ± 13.45	25.60 ± 1.79	0.36 ± 0.02
Barhi date 25%: pumpkin 75%	34.59 ± 9.46	233.15 ± 46.75	26.51 ± 5.47	0.23 ± 0.02
Barhi date 50%: carrot 50%	49.04 ± 0.22	226.10 ± 17.30	27.43 ± 1.91	0.29 ± 0.03
Barhi date 50%: pumpkin 50%	18.03 ± 0.45	226.20 ± 4.50	24.41 ± 0.49	0.13 ± 0.00
Barhi date 50%: carrot 25%: pumpkin 25%	31.99 ± 1.04	200.75 ± 2.45	23.36 ± 0.09	0.23 ± 0.01

Data are the mean ± SE, n = 3,

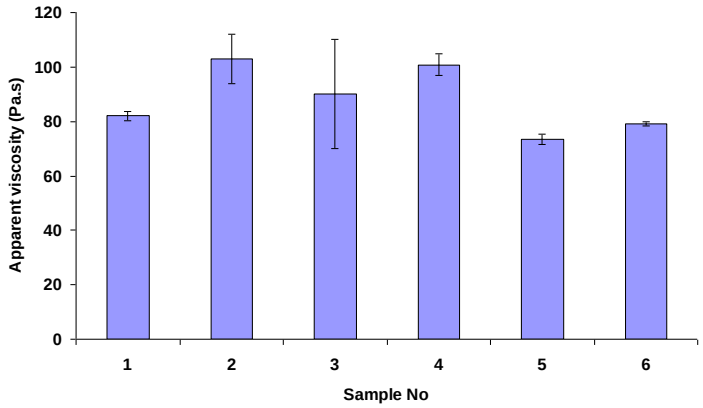


Fig. (3): Apparent viscosity (Pa.s) of prepared Barhi date jams with carrot and pumpkin

Sample No. 1: control jam (Barhi date 100%)
Sample No. 2: jam prepared from Barhi date 25%: carrot 75%
Sample No. 3: jam prepared from Barhi date 25%: pumpkin 75%
Sample No. 4: jam prepared from Barhi date 50%: carrot 50%
Sample No. 5: jam prepared from Barhi date 50%: pumpkin 50%
Sample No. 6: jam prepared from Barhi date 50%: carrot 25%: pumpkin 25%

CONCLUSION

Results from this study clearly showed that the control jam sample which prepared from only Barhi date had significant increase in potassium content followed by jam sample prepared from Barhi date 25%: pumpkin 75%. Calcium content was significant increase in jam sample prepared by Barhi date 50%: carrot 25%: pumpkin 25% compared to control jam and other samples. Our findings confirmed that the flow behavior index of all prepared date jam samples were less than unity indicating a non-newtonian behavior, with a pseudo-plastic type. Addition of carrot pulp at 75% and 50% to date jams resulted in improvement of jam texture by increase in product apparent viscosity and flow behavior index. It could be concluded that the lowest jam sample in lightness and the highest one in yellowness which prepared by Barhi date 25%: carrot 75% and it was the best jam sample in sensory properties and in overall acceptability by the panelists followed by jam prepared from Barhi date 50%: pumpkin 50% compared to control Barhi jam and other samples.

REFERENCES

Abdelmoneim, I. M.; Hamad, A. M. and Kahtani, M. S. (1983). "Date Varieties for Jam Production." First Symposium on Date Palm, King Faisal University, Al-Hassa, Saudi Arabia, 496-502.

Alasalvar, C.; Al-Farsi, M.; Quantick, P. C.; Shahidi, F. and Wiktorowicz, R. (2005). Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to eat shredded orange and purple carrots. Food Chem., 89, 69–76.

Ali A.; Waly M. I.; Essa M. M.; Devarajan S. (2012). Nutritional and medicinal value of date fruit. In: Manickavasagan A, Essa MM, Sukumar E, editors. Dates: production, processing, food, and medicinal values. New York: CRC Press; pp. 361-375.

AOAC. (2000). The Official Methods of Analysis of the Association of Official Analytical Chemists International. 17th ed., Vol. II, Gaithersburg, MY, USA: Food Composition, AOAC International.

Assirey, E. A. (2015). Nutritional composition of fruit of 10 date palm (*Phoenix dactylifera* L.) cultivars grown in Saudi Arabia. J. Taibah University for Science 9: 75–79.

Banga, O. (1984). Carrot. In: Evolution of crop plants. (Simmonds N.W. 3th.ed.) Longman, London. pp: 291-293.

Barreveld W. H. (1993). Dates palm products, FAO agricultural services bulletin No. 101.

Benoist, B. ; Martines, J. and Goodman, T. (2001). "Vitamin A supplementation and the control of vitamin A deficiencies: conclusions." Food Nutr. Bull., 22, 3: 335-337.

Besbes, S.; Drira, L.; Blecker, C.; Deroanne C. and Attia H. (2009). Adding value to hard date (*Phoenix dactylifera* L.): Compositional, functional and sensory characteristics of date jam. Food Chem., 112: 406–411.

Bhattacharya, S. (1999). Yield stress and time-dependent rheological properties of mango pulp. J. Food Sci., 64: 1029-1033.

Botes, A. and Zaid, A. (1999). The economic importance of date production and international trade, in: Zaid A., Arias E.J. (Eds.), Date palm cultivation, FAO Plant Production and Protection, 156, 45–57.

FAOSTAT (2013). Food and Agriculture Organization of the United Nations. FAO. Statistical databases. http://faostat.fao.org.

Francis, F. J. (1983). Colorimetry of foods. In Peleg, M. and E.B. Bagly. Physical Properties of foods. pp 105-123. The AVI publishing company Inc. Westport, Connecticut, USA.

Guerrero, S. N. and Alzamora, S. M. (1998). Effect of pH, temperature and glucose addition on flow behavior of fruit purees: II. Peach, papaya and mango purées. J. Food Eng., 37: 77-101.

Habiba R. A. and Mehaia M. A. (2008). Improving Carrot Jam Characteristics and Its Nutritional Value by Using Date Paste Instead of Sugar. Journal of Agricultural and Veterinary Sciences, Qassim University, 1, 1: 13-18.

Hegedusic, V.; Lovric T. and Parmac A. (1995). Influence of phase transition (freezing and thawing) on thermophysical and rheological properties of apple puree – like products. ACTA – Aliment, 22: 337-349.

Kamiloglu, S.; Pasli, A. A.; Ozcelik, B.; Camp, J. V. and Capanoglu E. (2015). Color retention, anthocyanin stability and antioxidant capacity in black carrot (*Daucus carota*) jams and marmalades: Effect of processing, storage conditions and in vitro gastrointestinal digestion. J. functional foods, 13: 1–10.

Kordylas, J. M. (1990). Processing and Preservation of Tropical and Subtropical Foods. Hong Kong: McMillan Education Ltd.

Lombrana, J. I. and Dias, I. M. (1985). "Rheological and Chemical Changes in Stored Carrot Juice." Canadian Instit. Food Sci. Technol., 18, 3: 213-219.

Miller, C. J.; E. V. Dunn and I. B. Hashim (2002). Glycemic index of three varieties of dates. Saudi Med J; 23, 5: 536-538.

Oliver, J. and Palou, A. (2000). Chromatographic determination of carotenoids in foods. J. Chromatography A, 881, 543–555.

Olson, J. A. (1988). "Biological Actions of Carotenoids." Presented at the Annual Meeting of the Federation of American Societies for Experimental Biology, Las Vegas, NV,

Parn, O. J.; Bhat, R.; Yeoh, T. K. and Al-Hassan A. A. (2015). Development of novel fruit bars by utilizing date paste. Food Bioscience, 9: 20 – 27.

Pearson, D. (1976). The chemical analysis of foods. London, New York: Churchill-Livingstone. pp. 142–151.

Rao, A.V. and Rao, L. G. (2007). Carotenoids and human health. Pharmacological Research, 55: 207–216.

Renna, M.; Pace, B. M.; Santamaria, P.; Serio, F. and Gonnella M. (2013). Comparison of two jams making methods to preserve the quality of colored carrots. LWT - Food Science and Technology 53: 547-554.

SAS (2000). Statistical Analysis System. SAS User' s Guide (Version 8.1) SAS institute Inc., CARY, NC. USA.

Sawaya, W. N.; Khatchadourian, H. A.; Khalil J. K. and Mashadi A. S. (1983). "Processing of Three Major Saudi Arabian Cultivars into Jam." J. Food Sci. Technol., 20, 4: 149-152.

Snedecor, G. W. and Cochran, W. G. (1980). Statistical Methods 7th Ed. Iowa State University Press, Ames, Iowa.

Van Buren, J. P. (1991). Function of pectin in plant tissue structure and firmness. In: Walter, R. H. (ed.), The Chemistry and Technology of Pectin, Academic Press, New York, pp. 1-22.

Vayalil P. K. (2012). Date fruits (*Phoenix dactylifera* L.): an emerging medicinal food. Crit Rev. Food Sci. Nutr.; 52: 249-271.

Vinson J. A.; Zubik L.; Bose P.; Samman N and Proch J. (2005). Dried fruits: excellent in vitro and in vivo antioxidants. J. Am. Coll. Nutr.; 24: 44-50.

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 127-142 (July 2015/Ramadan 1436H)

Thermal Behavior of Selected Pig Meat - Biopolymer Mixtures for Halal Purposes: A review

M. Abd Elgadir[1*], Mohamed E. S. Mirghani[2*] andMousa H. M.[1]

[1]*Department of Food Science &Human Nutrition, College of Agriculture &Veterinary Medicine, Qassim University, Al-Qassim, Kingdom of Saudi Arabia*

[2]*Department of Biotechnology Engineering, Kulliyyah (Faculty) of Engineering International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, P. O. Box 10 Gombak, 50728 KL, Malaysia*

[*]*Email: Email: m.saeed@qu.edu.sa*

(Received 18 /2/2015; accepted 13/6/2015)

ABSTRACT. The presence of biopolymers in meat–biopolymer mixtures could influence thermal characterization of the system. This article reviews different ranges of functions that biopolymers perform in pig meat–biopolymer mixtures for halal issues. Effect of selected biopolymers such as starch, protein and hydrocolloids are summarized. The results revealed that the presence of different types of biopolymers (starch, protein and hydrocolloid) in the pig meat - biopolymer mixtures, the mixtures behaved differently from when they are present individually in a single phase. This could assist in understanding these characteristics for halal food issue.

***Keywords*:** Pig, food system, collagen content, differential scanning calorimetry (DSC).

INTRODUCTION

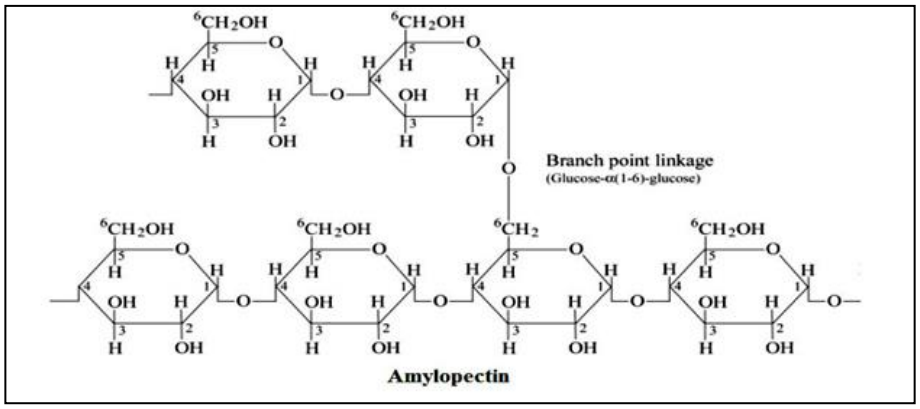
Halal meat authentication is a major issue in many places such as Asia, Germany, Switzerland, Greece, United Kingdom, Spain, France, Russia, Sweden, Italy, South and North America (Meza-Márquez*et al*., 2010) and Saudi Arabia. Numerous methods have been proposed for detection of Halal meat adulteration such as gas chromatography (GC) (Farag*et al*., 1983), high pressure liquid chromatography (HPLC) (Marikkar*et al*., 2005), differential scanning calorimetry (Coni*et al*., 1994), electronic nose (Che Man *et al*., 2005), and DNA-based methods (Aida *et al*., 2007).

Several studies have been reported to investigate thermal properties of pig meat as well as its by products for many purposes (Chen and Hsieh, 2002; Doerscher*et al*., 2004; Voutila*et al*., 2007; Dàvila*et al*., 2007;.Voutila *et al*., 2008; Choi *et al*., 2011). Biopolymers in food systems are important because of their great influences on food properties and formulation (Tolstoguzov, 2003). They interact in several ways in food to produce various attributes that impact on quality, texture, and stability (Okechukwu and Rao 1998). The understanding of such properties will lead to improvements in the formulation of biopolymers-based foods (Turgeon *et al*., 2003). Many researchers have reported that when two or more biopolymers are mixed together in food systems, the mixtures behaved differently from when they are present individually in a single phase (Turgeon *et al*., 2003, de Kruif and Tuinier 2001, Zaidul*et al*., 2007, Ragaee and Abdel-Aa, 2006, Marcoa and Rosell, 2007, Ryan and Brewer, 2007 and Wang *et al*., 2009). Many studies investigated thermal behaviours of different biopolymer food based systems such as starch-water interactions (Tananuwong and Reid, 2004), protein mixtures (Hendrix *et al*, 2000, BaezaandPilosof, 2002; Galani and OwusuApenten, 2000 and Li *et al*., 2007), starch - meat complexes (Jeng-Yune*et al*., 2003) amylose-lipid complexes (Tufvesson*et al*., 2003) and protein- hydrocolloids interaction (Kyaw*et al*. 2004). The impact of pig meat – biopolymer mixtures was not much studied. This review paper highlights the thermal behavours of selected studies on pig meat – biopolymer mixtures.

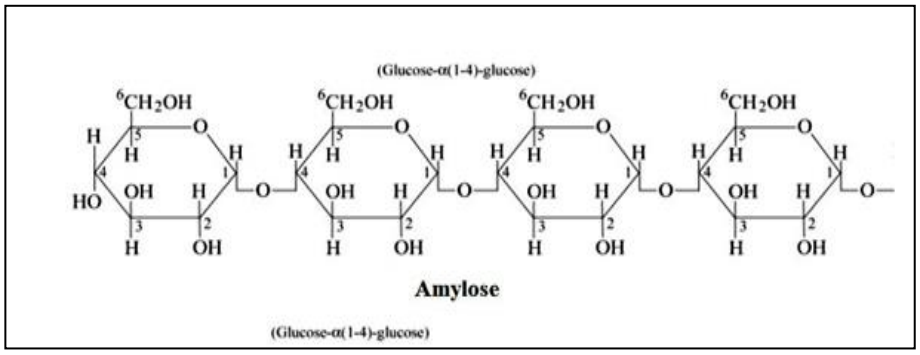
Impact of starch

Starch is recognized as an important foods biopolymer. It is not a uniform material but described as a compose of two types of glucose polymers: a branched polymer of glucose termed amylopectin and a linear chain molecule termed amylose (Figure 1) A. Native starch consists normally of approximately 25% amylose and 75% amylopectin. The starch industry includes extraction and refining starches from seeds, roots and tubers, by wet grinding, sieving and drying. In food products starch occurs normally in combination with other biopolymers, present as indigenous constituents of ingredients such as cereals, fruit, vegetables and meat, or added as thickeners, stabilizers and gelling agents. Several studies investigated presence of starch in the food systems (Nayouf*et al*., 2003, Ravindra*et al*., 2004, Shim and Mulvaney, 2001, Verbeken*et al*., 2004, Yang *et al*., 2004 and Zaleska*et al*., 2001). The functionality of starch may vary due to differences in botanical origin (Li and Yeh, 2003). It is used to improve different characteristics of meat from

several sources. It is used as filler in surimi and used to increase the firmness of products (Verrez-Bagnis*et al*., 1993) and to enhance the strength of the gel (Kim and Lee, 1987). Pre-gelatinized starch is well recognized as firmness and water holding capacity of surimi reducer (Chung and Lee, 1991). Starch added in the chopping process of turkey bologna was found to be effective in reducing cooking loss and purge loss while not increasing product hardness (Dexter *et al*, 1993). Modified starch was used to improve the texture of low fat frankfurters (Yang *et al*., 1995) and comminuted scalded sausages (Pietrasik, 1999).



(A)



(B)

Fig. (1). Structure of (A) amylose and (B) amylopectin

Source: Whistler and Daniel (1985)

Starch added in meat emulsions resulted in forming strong heat induced protein network (Carballo *et al*., 1996). Starches added on batters were influenced by starch type, water-to-starch ratio, presence of other compounds (lipids), or processing factors (Dexter *et al*., 1993). When starch is heated within food system above the gelatinization temperature in presence of excess water, irreversible swelling of the granules occurs along with a concomitant changed of structural order but granules still maintain their identity (Hug-Iten*et al*., 2001). The gelatinization changes include granule swelling due to absorption of moisture in the amorphous regions of the granule, leaching of small molecular weight polymers including amylose, loss of the crystalline order and the consequent loss of birefringence, leaching of larger molecular weight polymers from the granule including fragments of amylopectin and, finally, starch solubilization (Sakonidou*et al*., 2003).

The functions of three starches (potato, corn, and rice) on the formation of starch and pork ham batter composite during heating were investigated (Li and Yeh, 2002). Starch (30%, based on weight of pork ham) was added with adjusting water content of f 76±2 %. The materials were mixed to form a uniform mass called starch/meat composite (SM composite). Thermal properties were measured by DSC at scanning temperature was increased from 25 to 120^{o}C at 5C/min. The onset temperature (T_o), peak temperature (T_p), and heat of phase transition (AH) were determined. The results showed that the pork ham batter exhibited two endothermic peaks with T_{pl}at 65.2^{o}C and T_{p2}, at 78.2^{o}C which fell in the range of typical transitions of meat (Findlay and Barbut, 1990). The first onset temperature was 54^{o}C, which concurred with the major increase in rigidity development beginning in the range of 50 - 58^{o}C for comminuted pork (Foegeding 1988). It was noticed that compared to control T_o and T_p increased in 4-5^{o}C for corn and rice starch and 1.8^{o}C for potato starch. The values of AH for control pork ham batter, potato starch/pork, corn starch/ pork and rice starch/pork mixtures were 3.7 ± 0.4, 10.2 ± 0.2, 9.1 ± 0.2 and 8.0 ± 0.5, respectively. The authors concluded that the meat protein and starch underwent the phase transition independently during heating. The effects of different types of starches on DSC thermal characteristics of starch/pork meat complexes were investigated in *Yian-jiao-pi* (traditional Chinese food). NaCl and $NaHCO_3$ content were adjusted to be 1% and 0.1% (based on weight of pork ham), respectively, by adding a solution of NaCl (25%) and $NaHCO_3$ (3%). The addition of NaCland $NaHCO_3$ was to simulate the traditional method of making *Yian-jiao-pi* and resulted in a pH of 6.2±0.1. Starch (30%, based on the weight of pork ham) was added with adjustment of water content. Ten types of starches were added to the complexes which include potato, tapioca, waxy corn, sweet potato (TNu17), sweet potato (TNu57), high amylose corn, corn, rice, pea and mung bean. The materials were mixed to form a uniform mass and were called the SM complex in this study. The gelatinization temperature and heat of gelatinization of starches in the complexes added with NaCl and NaHCO3 were determined using a DSC. The result revealed that the presence of NaCl dose T_o by 2.5 - 4.5^{o}C and T_p by 3 - 5.3^{o}C due to the repression on swelling of starch granules (Bello-P_erez and Paredes-L_opez,

1995). Except that for potato starch (only 1.4°C increase in T_o and1.7°C increase in T_p) was recorded. The heat of gelatinization was also increased, except that for rice with a slight decrease of 0.3 J/g.

Impact of protein

Proteins are known as linear polymers contain 20 different L-α- amino acids. They generally found as globular proteins which are aqueous solutions of acids, soluble in water or salts bases. They function widely in living systems exist as either and serve as the main animal tissues structural materials (Scope, 1994). To form fibers, generally fibrous proteins fully extended and associated closely with each other in parallel structures through hydrogen bonding. In the other hand, globular proteins fold into complicated spherical structures held together by a combination of hydrogen, ionic, hydrophobic and covalent (disulfide) bonds (Scope, 1994). Figure (2) shows the different protein types of structure in solution (Tornberg*et al*., 1990).

The physical and chemical properties of proteins depend on relative amounts of amino acid residues component and their placement along a chain of protein polymer (*Gutteridge and Thornton, 2005).* The ability of binding partners to induce conformational changes in proteins enables construction of complex networks enormously.

However, in muscle foods, myofibrillar proteins are fully stretched and closely associated with each other in parallel structures (Iwata *et al*., 2000) and their levels greatly affect emulsion stability, texture, as well as microstructure of the system (Youssef and Barbut, 2010).

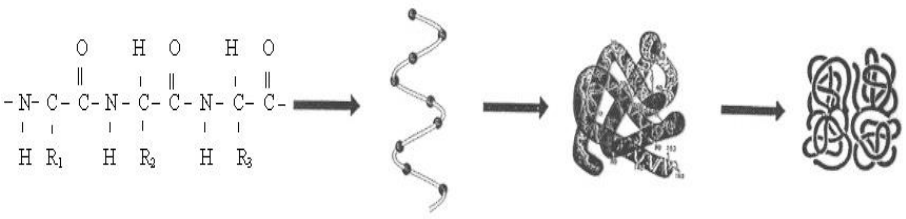


Fig. (2). The different types of proteins structures in solution.

Source: Tornberg*et al*. (1990).

Thermal treatment is one of the most important factors influencing gelation properties of myofibrillar protein because heat is required at pH values above 5.3 for denaturation and unfolding of protein molecules necessary for gel formation (Sun and Holley, 2011). The optimal temperature for the heat-induced gelation of myosin

at pH 6 was 60 to 70^{o}C (Ishioroshi*et al*., 1979). Camou*et al*. (1989) have proposed that a slow heating rate may allow more favorable protein–protein interactions to occur, producing a stronger, better-ordered 3-dimensional gel. Smith and O'Neill (1997) reported that an increased in muscle food gel strength when the heating rate was reduced from 5 to 1^{o}C/min. it was found that when muscle food was subjected to a slower heating rate (0.55 ◦C/min), the texture of low- and high-fat muscle food possessed greater hardness, cohesiveness, springiness, and chewiness than those heated at 1.10 and 1.90^{o}C/min, regardless of the fat level. The authors concluded that this was due to the fact that the gel was more fully formed. A heating rate of 12^{o}C/h produced stronger myosin gels than heating at 50^{o}C/h or heating at a constant temperature (Foegeding*et al*., 1986) and the strongest gels were formed when 70^{o}C was reached, supporting the data previously reported. This agreed with the studies that conducted by Xiong and Blanchard (1993). Camou and Sebranek (1991) found similar results using gels formed from *Pale, Soft, Exudative pork meat* (PSE) pork. Fernández-Martín*et al*. (2009) studied muscle food using differential scanning calorimetry and have found that the typical trace for muscle proteins gave three values main endothermic transitions at around 62.4°, 69.5 and 80.7°C. These were considered to be due to the thermal denaturation of myosin (first and second), sarcoplasmic and connective - tissue proteins (second) and actin (third) as it was earlier reported by Stabursvik and Martens (1980). They found that the DSC-trace gave an enthalpy of denaturation of 16.4 J/g, similar to literature values which agreed with that of the literature (Stabursvik*et al*., 1984).

During heat treatment of meat severe structural changes, which influence the textural characteristics of the end product, are taking place (Tornberg, 2005). These changes associated with thermal denaturation of the meat proteins that initiates changes in structural features of the meat matrix and a simultaneous redistribution of the myowater, which is reflected in loss of water (cooking loss) (Micklander*et al*., 2002). Thermal studies on meat have demonstrated three denaturation steps that have been ascribed to myosin denaturation (40 - 60°C), sarcoplasmic protein and collagen denaturation (60 – 70°C temperature) and actin denaturation (80°C) (Stabursvik and Martens, 1980, Stabursvik*et al*., 1984, Bertola*et al*.,1994). Bertram *et al*. (2006) studied a direct relationship between thermal denaturation of specific proteins/protein structures and heat - induced changes in water mobility during heating of pork. However, typical transition temperatures for muscle proteins were found at range from 43 to 67 °C for myosin and its subunits, 67 to 69 °C for sarcoplasmic proteins, and 71 to 83°C for actin (Smyth *et al*., 1996). The study investigated muscles *longissimus dorsi* from three pigs, which were offspring of a Duroc/Landrace boar cross-bred with Landrace/Yorkshire sow At 24 h post-mortem an approximately 8 cm broad chop was cut from the anterior end of *M. longissimus dorsi* from the right side of each carcass. The samples from each animal were heated to 25, 40, 45, 50, 55, 60, 65, 70 and 75 °C, respectively, for 30 min in the NMR probe using heated air followed by DSC measurements. The samples (0.3 - 0.5 g) were placed into the DSC sample cells and placed in the DSC together with

an empty reference cell. DSC scans were recorded from 3 to 90 °C with a heating rate of 1 °C/min. The DSC thermograms revealed endothermic transitions at 54, 65 and 77 °C, probably reflecting the denaturation of myosin, sarcoplasmic proteins together with collagen and actin, respectively.

Non-meat proteins are added to food systems in order to control cost, improve fat stabilization and bind water (Hsu and Sun, 2006). Soy proteins are common non-meat additives as an examples used by the meat industry (Youssef and Barbut, 2011). Whey proteins contain numerous disulphide bonds and proline amino acids (Kurt and Zorba, 2005). When they heated above 70°C, they have the ability of forming thermally induced gels (Langley and Green, 1989).

This can positively affect meat products' stability and texture. Whey proteins are surface-active globular proteins and can be adsorbed at the fat/water interface where they unfold, and potentially can help stabilize the fat globules within a food matrix (Sun, *et al*., 2007). Such functional properties make whey proteins a useful additive in the production of meat emulsions and therefore whey is commonly used to improve emulsification, water binding and texture. Lin and Mei (2000) indicated that soy proteins can improve emulsifying capacity and stability of meat products. Fukushima (2004) reported that soy proteins are composed of the two major proteins: β- conglycinin (7S) and glycinin (11S). It has been reported that the 7S and 11S denature around 70°C and 90°C, respectively which prevent regular soy proteins to undergo sufficient structural changes during common meat products preparation (Zhang *et al*., 2004).

Impact of hydrocolloids

Hydrocolloids or gums have are used widely in food systems as additives for many reasons such as food texture improvement (Armero and Collar, 1996). They could be able to slow down the retrogradation properties of starches (Davidou*et al*., 1996), extend overall quality of products during time, increase moisture retention and replace gluten-substitutes as gluten-free breads formulation (Toufeili*et al*., 1994).Upon addition of nonmeat ingredients, thermal denaturation changes of meat proteins that occur may indicate shifts in interaction between meat proteins and nonmeat ingredient or the physical state (Donatus and Xiong, 2001). In biological systems, interactions between proteins and hydrocolloids occur widely. These components are added into foods in order to improve their functional properties. The understanding of the mechanisms involved in proteins – hydrocolloids interactions is an important (Stainsby, 1980). In low-fat meat products, hydrocolloids are used as water binders and have been reported to affect thermal transition temperatures of meat proteins (Shand*et al*., 1994). It was found that possible protein - carbohydrate interactions as it was investigated earlier in gelling systems of xanthan-myofibrillar protein (Xiong and Blanchard, 1993). Good examples of hydrocolloids that utilized in meat mixtures are konjac gum, flaxseed gum, carrageenan gum, curdlan gum, xanthan gum and locust bean gum (Tarté, 2008). Carrageenan improves texture, consistency, water retention, and slice ability of meat products when combined with

added brine in high levels (Bater*et al*., 1992). Carrageenan is added at quantities of range from 0.5 to 0.8% in order to improve syneresis control and slice ability. This could be possible in many regions of the world where from 8 to 12% starch is added to produce economical products. Earlier, it was reported that a concentration of 1.5% kappa carrageenan in a 38% added ingredient cured pork ham, highest cook yield for cook-in-bag products was exhibited Mills (1995). DeFreitas*et al*., 1997 utilized ground pork and meat myofibrillar protein (MP) gels as model systems to study thermal transition temperatures by differential scanning calorimetry. The results revealed that the addition of up 2% of κ, ι, or λ carrageenan (CGN) to meat myofibrillar protein (MP) caused a very slight change in thermal denaturation of the meat proteins. The authors noticed three transition temperatures the samples of ground pork, which were (59.4°C), related to the characteristic of myosin protein, (67.8°C) related to characteristic of sarcoplasmic proteins and (82.4°C) related to the characteristic of and actin protein. This result suggested that molecular interactions between CGN and meat proteins did not occur so, no major shifts in transition temperatures reported by the added CGN to the system. Chen *et al*. (2007) investigated thermal properties of flaxseed gum (FG) - salt-soluble pork meat protein mixture (SSPMP). FG in concentrations of 0% and 2% were mixed with SSPMP in concentration of 2%.The mixture was evaluated using differential scanning calorimetry (DSC) method for thermal properties. The DSC was set at heating rate of 10^{o}C /min. and over a temperature range of 25 –100^{o}C and the samples were scanned. The results showed that the DSC thermograms of the meat without FG gave three major peaks at temperatures of 58.4^{o}C (*T*M1), 66.6^{o}C (*T*M2) and 81.9^{o}C (*T*M3), respectively. Transitions temperature for porcine were assigned earlier at 58^{o}C and 66^{o}C for its myosin protein and 78^{o}C for its actin protein (Stabursvik*et al*. 1984). In the other hand three peaks were derived from pork muscle which represented myosin protein at 59^{o}C, sarcoplasmic proteins at 66.5^{o}C and actin protein at 81^{o}C (Quinn *et al*. (1980). It was observed that the addition of 2% of FG to meat protein caused slightly increase in transition temperature of the system. The addition of 2% FG to the mixture of meat-salt (MS), increase the value of both TM1 and TM3 by 1.2^{o}C and 1.6^{o}C, respectively. The results indicated that slightly higher temperature are needed to denatured meat protein in the presence of FG, which explained that there might be an interaction between FG meat protein. The addition of FG in concentration of 2% to SSPMP caused an increase in the transition temperatures of *T*SSPMP1 and *T*SSPMP2 by 1.9^{o}C and 5.9^{o}C 2% FG, respectively. This result suggested that the shifts in SSPMP transition temperatures would be the likely indicator of interactions between polysaccharide and protein in the presence of FG. The authors concluded that denaturation of the meat protein needs slightly higher temperature in the presence of FG. Curdlan gum (CUDG) is recognized as a thermo-gelable bacterial polysaccharide. It forms two types of gels depending on its heating temperature characteristics (Harada *et al*., 1987). One is formed when its aqueous suspension is heated to a temperature of 60°C and then cooled below 40°C; the resulting gel is known as a low-set gel. The other type of gels is formed by heating the aqueous suspension to a temperature of slightly less

than 80°C; and gel namely a high–set gel is resulted. Funami, (1998) investigated the thermal properties of pork–carrageenan and pork–CUDG in concentrations of 3% (W/W) in aqueous suspension using DSC method. A temperature range from 5°C to 70, 75 or 80°C at a heating rate of l°C/min and held for 60 min. were used in the DSC device for the measurement of the mixtures. A stable baseline was not established at the beginning and end of each DSC measurement so, the thermal curves could be clearly noticed over the range of 30 to 70 or 75°C. Using this method, a single endotherm was observed for both suspension individually, with a T_p at 54.5°C for CUDG and at around 57.3°C for carrageenan during the first scan at each final temperature. The value of *A*H1 for CUDG was found to be much smaller than the *A*H1 for carrageenan, and the *A*H2 value for CUDG decreased by -50 – 60% of its AH1. At the same time the AH2 for carrageenan decreased by only 10% of its AH1. The ratio of AH2/AHl decreased with increasing heating temperature for CUDG, but it remained almost the same for carrageenan. However, gelling enthalpies of various concentrations of CUDG aqueous suspensions using DSC (Hirashima*et al*. 1995). It was observed that the ratio AH2/AHl decreased gradually at first heating temperature, then rapidly when heating temperatures reached around 70°C. This result suggesting that CUDG formed thermo–irreversible gel or heat-stable gel above 70°C. Thermal curve for the control system showed three apparent transition temperatures with peaks at 48.6, 57.7 and 66.3°C, respectively. The shapes of the DSC curves and the T_p values for the meat/ CUDG and meat – carrageenan pastes were found to be almost identical compared to those of control system.

CONCLUSION

The present paper investigated thermal behavior of pig meat – biopolymer mixtures in several research works. The information provided would assist both food technologists and meat formulators to collect valuable data about the behavior of pig meat - biopolymer mixtures when they are thermally treated. An understanding of capabilities and limitations of pork meat - biopolymer mixtures could also give processors a higher probability of success for halal issues.

REFERENCES

Aida A.A., Che Man Y.B., Raha A.R., Son R. (2007). Detection of pig derivatives in food products for Halal authentication by polymerase chain reaction–restriction fragment length polymorphism. Journal of the Science of Food and Agriculture, 87: 569 - 572.

Armero E., Collar C. (1996). Antistaling additives, flour type and sourdough process effects on functionality of wheat doughs. Journal of Food Science, 61: 299 - 303.

Baeza R.I., Pilosof A.M.R. (2002). Calorimetric studies of thermal denaturation of β-lactoglobulin in the presence of polysaccharides. Lebensmittel-Wissenschaft und-Technologie,**35:** 393 - 399.

Bater B., Descamps O., Maurer A. J. (1992). Quality characteristics of hydrocolloid-added oven roasted turkey breasts. Journal of Food Science,57: 1068 - 1070.

Bello - Pérez L.A., Paredes - López O. (1995). Starch and amylopectin: effect of solutes on their calorimetric behavior. Food Chemistry, 59: 243 - 7.

Bertola N.C., Bevilacquia A.E., Zaritzky N.E. (1994). Heat-treatment effect on texture changes and thermal denaturation of proteins in beef muscle. Journal of Food processing and Preservation,**18**: 31 - 46.

Bertram H.C., van den Berg F., Wu Z., Andersen H. J. (2006). NMR relaxometry and differential scanning calorimetry during meat cooking. Meat Science, 74: 684 - 689.

Camou J. P., Sebranek J. G., Olson D. G. (1989). Effect of heating rate and proteinconcentration on gel strength and water loss of muscle protein gels. Journal of FoodScience, 54: 850 - 854.

Camou J. P., Sebranek J. G. (1991). Gelation characteristics of muscle proteins from pale, soft, exudative PSE pork. Meat Science, 30: 207 - 220.

Carballo J., Femandez P., Barreto G., Solas M. T., Colmenro F. J. (1996). Morphology andtexture of bologna sausage as related to content of fat, starch andegg white. Journal of Food Science, 61: 652 - 655.

Che Man Y.B., Gan H.L., NorAini I., Nazimah S.A.H., Tan, C.P. (2005). Detection of lard adulteration in RBD palm olein using an electronic nose. Food Chemistry, 90: 829 - 835.

Chen H. H., Xu S. Y., Wang, Z. (2007). Interaction between flaxseed gum and meat protein. Journal of Food Engineering, 80: 1051 - 1059.

Chen, F. C., Hsieh Y. H. P. (2002). Porcine troponin I: a thermostable species marker protein. Meat Science, 61: 55 - 60.

Choi Y.S., Choi J.H., Han D.J., Kim H.Y., Lee M. A., Kim H.W., Jeong J.Y., Kim C.J. (2011). Effects of rice bran fiber on heat-induced gel prepared with pork salt-soluble meat proteins in model system. Meat Science, 88: 59 - 66.

Chung K. H., Lee C. M. (1991). Water binding and ingredient dispersion pattern effect on surimi gel texture. Journal of Food Science, 56, 1263 - 1266.

Coni E., Pasquale M.D., Cappolelli P., Bocca A. (1994). Detection of animal fats in butter by SC: A pilot study. Journal of the American Oil Chemists' Society, 71: 807 - 810.

Davidou S., Le Meste M., Debever E., Bekaert D. (1996). A contribution to the study of staling of white bread: Effect of water and hydrocolloid. Food Hydrocolloids, 10: 375 - 383.

Dàvila E., Parés D., Cuvelier G., Relkin P. (2007). Heat-induced gelation of porcine blood plasma proteins as affected by pH.Meat Science, 76: 216 - 225.

de Kruif C.G., Tuinier, R. (2001). Polysaccharide protein interactions. Food Hydrocolloids,**15**: 555 - 563.

DeFreitas Z., Sebranek J.G., Olson G.G., Carr J.M. (1997). Carrageenan effects on thermal stability of meat proteins. Journal of Food Science, 62: 544 - 547.

Dexter D. R., Sofos J. N., Schmidt G. R. (1993). Quality characteristics of turkey bologna formulatedwith carrageenan, starch, milk andsoy protein. Journal of Muscle Foods, 4: 207 - 223.

Doerscher D. R., Briggs J. L., Lonergan SM. (2004). Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar protein gels. Meat Science, 66: 181-188.

Donatus E.N.A., Xiong Y.L. (2001). Effects of carrageenan on thermal stability of proteins from chicken thigh and breast muscles. Food Research International, 34: 247 - 253.

Ensor S.A., Sofos J.N., Schmidt, G.R. (1991). Differential scanning calorimetric studies of meat protein-alginate mixtures. Journal of Food Science, 56: 175 - 182.

Farag R.S., Abo-raya S.H., Ahmed F.A., Hewedi F.M., Khalifa H.H. (1983). Fractional crystallization and gas chromatographic analysis of fatty acids as a means of detecting butter fat adulteration. Journal of the American Oil Chemists' Society, 60: 1665 - 1669.

Fernández-Martín F., López-López I., Cofrades S., Colmenero F. J. (2009). Influence of adding Sea Spaghetti seaweed and replacing the animal fat with olive oil or a konjac gel on pork meat batter gelation. Potential protein/alginate association. Meat Science, 83: 209 - 217.

Findlay C.J., Barbut S. (1990). Thermal analysis of meat. In: Harwalker U.R., Ma C.Y. (Eds.), Thermal analysis of food, Elsevier Applied Science, New York, USA, pp. 93 - 125.

Foegeding E. A, Allen C. E., Dayto W. R. (1986). Effect of heating rate on thermally formed myosin, fibrinogen and albumin gels. Journal of Food Science, 51: 104 - 108.

Foegeding E.A. (1988). Thermally induced changes inmuscle proteins. Food Technolology, 42: 58 - 64.

Fukushima D. (2004). Soy proteins. In: Yada R. Y. (Ed.), Proteins in Food Processing, Woodhead Publishing Ltd., Cambridge, England, pp. 123 - 145.

Funami T., Yada H., Nakao Y. (1998). Thermal and rheological properties of curdlan gel in minced pork gel. Food Hydrocolloids, 12: 55 - 64.

Galani D., OwusuApenten R.K. (2000). Owusu revised equilibrium thermodynamic parameters for thermal denaturation of β-lactoglobulin at pH 2.6. ThermochimicaActa**, 363:** 137 - 142.

Gutteridge A., Thornton J. M. (2005). Understanding nature's catalytic toolkit. Trends in Biochemical Sciences, 30: 622 - 629.

Harada T., Sato S., Harada A. (1987). Curdlan. Bull. Kobe Women's University, 20: 143-164.

Hendrix T., Griko Y.V., Privalov P.L. (2000). A calorimetric study of the influence of calcium on the stability of bovine α-lactalbumin. Biophysical Chemistry,**84:** 27 - 34.

Hirashima M., Takaya T., Nishinari K. (1995). Presented at the 42nd Japanese Society for Food Science and Technology, p. 171 (in Japanese).

Hsu S. Y., Sun L. Y. (2006). Comparisons on 10 non-meat protein fat substitutes for low-fat Kung-wans. Journal of Food Engineering, 74: 47 - 53.

Huffman L. M. (1996). Processing whey protein for use as a food ingredient. Food Technology, 50: 49 - 52.

Hug-Iten S., Esher F., Conde-Petit B. (2001). Structural properties of Starch in bread and bread model systems influence of an antistaling-amylase. Cereal Chemistry, 78: 421 - 428.

Ishioroshi M., Jima K. S., Yasui T. (1979). Heat induced gelatin of myosin: Factors of pH and salt concentrations. Journal of Food Science, 44: 1280 - 1284.

Iwata K., Ishizaki S., Handa A., Tanaka M. (2000). Preparation and characterization of edible films from fish water-soluble proteins. Fisheries Science, **66**: 372 - 378.

Jeng-Yune L., Yeh A.(2003). Effects of starch properties on rheological characteristics of starch /meat complexes. Journal of Food Engineering, 57: 287 - 294.

Kim J. M., Lee C. M. (1987). Effect of starch on textural properties of surimi gel. Journal of Food Science, 52: 722 - 725.

Kurt S., Zorba O. (2005). The effects of different levels of non-fat dry milk and whey powder on emulsion capacity and stability of beef, turkey and chicken meats. International Journal of Food Science and Technology, 40: 509 - 516.

Kyaw Z. Y. (2004). Protein - Hydrocolloids interaction in *keropoklekor*. Ph.D. Thesis University Putra Malaysia Malaysia.

Langley K. R., Green M. L. (1989). Compression and impact strength of gels, prepared from fractionated whey proteins, in relation to composition and microstructure. The Journal of Dairy Research, 56: 275 - 284.

Li J. Y., Yeh A. I. (2002). Functions of starch in formation of starch/meat composite during heating. Journal of Texture Studies,33: 341 - 366.

Li J.Y., Yeh A. I. (2003). Effects of starch properties on rheological characteristics of starch/meat complexes. Journal of Food Engineering,57: 287 - 294.

Li J.Y., Yeh A.I., Fan K.L. (2007). Gelation characteristics and morphology of corn starch/soy protein concentrate composites during heating. Journal of Food Engineering, 78: 1240 - 1247.

Lin K. W., Mei M. Y. (2000). Influence of gums, soy protein isolate, and heating temperature on reduced-fat meat batters in a model system. Journal of Food Science, 65: 48 - 52.

Marcoa C, Rosell C. M. (2007). Effect of different protein isolates and transglutaminase on rice flour properties. Journal of Food Engineering, 84: 132 - 139.

Marikkar J.M.N., Ghazali H.M., Che Man Y.B., Peiris T.S.G., Lai O.M. (2005). Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis. Food Chemistry, 91: 5 - 14.

Meza-Márquez O.G., Gallardo-Velázquez T., Osorio-Revilla G. (2010). Application of mid-infrared spectroscopy with multivariate analysis and soft independent modeling of class analogies (SIMCA) for the detection of adulterants in minced beef. Meat Science, 86: 511- 519.

Micklander E., Peshlov B., Purslow P.P., Engelsen S.B. (2002). NMR cooking: The multiple states of water in meat during cooking. Trends in Food Science and Technology, **13**: 341 - 346.

Mills E. W. (1995). Nonmeat binders for use in cook-in-bag and smoked ham. Journal of Muscle Foods, 6: 23 - 35.

Murugaiah C., Noor Z.M., Mastakim M., Bilung L.M., Selamat J., Radu, S. (2009). Meat species identification and Halal authentication analysis using mitochondrial DNA. Meat Science, 83: 57 - 61.

Nayouf M., Loisel C., Doublier J.L. (2003). Effect of thermomechanical treatment on the rheological properties of crosslinked waxy corn starch. Journal of Food Engineering **59**: 209 - 219.

Okechukwu P. E., AnandhaRhao M. (1998). In: Walter R. H. (Ed.) Rheology of structured polysaccharide systems: starch and pectin, polysaccharide association structures in food, Marcel Dekker, New York, pp. 289 - 328.

Pietrasik Z. (1999). Effect of content of protein, fat andmodified starch on binding textural characteristics, and colour of comminuted scalded sausages. Meat Science, 51: 17 - 25.

Pospiech E., Greaser M.L., Mikolajczak B., Chiang W., Krzywdzińska M. (2002). Thermal properties of titin from porcine and bovine muscles. Meat Science,62:187 - 192.

Quinn J. R., Raymond D. P., Harwalker V. R. (1980). Differentialscanning calorimetry of meat proteins as affected by processingtreatment. Journal of Food Science, 45: 1146 - 1149.

Ragaee S., Abdel-Aa E. M. (2006). Pasting properties of starch and protein in selected cereals and quality of their food products. Food Chemistry, 95: 9 - 18.

Ravindra P., Genovese D.B., Foegeding E.A., Rao M.A. (2004). Rheology of heated mixed whey protein isolate/cross-linked waxy maize starch dispersions. Food Hydrocolloids, **18**: 775 - 781.

Rodriguez M., Oses J., Ziani K., Mate J. I. (2006). Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. Food Research International, 39: 840 - 846.

Ryan K. J., Brewer M. S. (2007). In situ examination of starch granule-soy protein and wheat protein interactions. Food Chemistry, 104: 619 - 629.

Sakonidou E.P., Karapantsios T.D., Raphaelides S.N. (2003). Mass transfer limitations during starch gelatinization. Carbohydrate Polymers,**53**: 53 - 59.

Scope R. K. (1994). Separation by precipitation. In: Scope R.K. (Ed.), Protein purification; Principles and practice,Springer – Verlag, New York, USA, p. 71 - 101.

Shim J., Mulvaney S.J. (2001). Effect of heating temperature, pH, concentration and starch/whey protein ratio on the viscoelastic properties of corn starch/whey protein mixed gels. Journal of the Science of Food and Agriculture,**81**: 706 - 717.

Smith A. B., O'Neill E. (1997). Heat-induced gelation properties of surimi frommechanically separated chicken. Journal of Food Science, 62: 326 - 330.

Smyth A.B., Smith D.M., Vega-Warner V., O'Neill E. (1996). Thermal denaturation and aggregation of chicken breast muscle myosin and subfragments. Journal of Agricultural and Food Chemistry, **44**: 1005 - 1010.

Stabursvik E., Fretheim K., Frøystein T. (1984). Myosin denaturation in pale, soft, and exudative (PSE) porcine muscle tissue as studied by differential scanning calorimetry.Journal of the Science of Food and Agriculture,**35**: 240 - 244.

Stabursvik E., Martens H. (1980). Thermal denaturation of proteins in post rigor muscle tissue as studied by differential scanning calorimetry.Journal of the Science of Food and Agriculture,**31**: 1034 - 1042.

Stainsby G. (1980). Proteinaceous gelling systems and their complexes with polysaccharides. Food Chemistry, 6: 1 - 3.

Sun X. D., Holley R. A. (2011). Factors Influencing Gel Formation by Myofibrillar Proteins in Muscle Foods. Comprehensive Reviews in Food Science and Food Safety,10: 33 - 51.

Sun C., Gunasekaran S., Richards M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. Food Hydrocolloids, 21: 555 - 564.

Tananuwong K., Reid D.S. (2004). DSC and NMR relaxation studies of starch-water interactions during gelatinization. Carbohydrate Polymers,**58:** 345 - 358.

Tarté R. (2008). In: Tarté R. (Ed.): Ingredients in meat products: properties, functionality and applications, Springer – Verlag, New York, USA, pp. 57 - 82.

Tolstoguzov V. B. (2003). Some thermodynamic considerations in food formulation Food Hydrocoloids, 17: 1 - 23.

Tornberg E., Olsson A., Persson K. (1990). The structural and interfacial properties of food proteins in relation to their function in emulsions. In: Larsson K., Friberg S. E., Food Emulsions New York and Basel, USA, pp. 247 - 324.

Tornberg E. (2005). Effects of heat on meat proteins - implications on structure and quality of meat products. Meat Science, **70**: 493 - 508.

Toufeili I., Dagher S., Shadarevian S., Noureddine A., Sarakbi M., Farran M. T. (1994). Formulation of gluten-free pocket-type flat breads: Optimization of methylcellulose, gum arabic, and egg albumen levels by response surface methodology. Cereal Chemistry, 71: 594 - 60.

Tufvesson F., Wahlgren M., Eliasson A- C. (2003). Formation of amylose-lipid complexes and effects of temperature treatment. Starch/Stärke,61 - 71.

Turgeon S. L., Beaulieu M., Schmitt C., Sanchez C. (2003). Protein–polysaccharide interactions: phase-ordering kinetics, thermodynamic and structural aspects. Current Opinion in Colloids and Interface Sciences, **8:** 401 - 414.

Verbeken D., Thas O., Dewettinck K. (2004). Textural properties of gelled dairy desserts containing *κ*-carrageenan and starch. Food Hydrocolloids,**18**: 817 - 823.

Verrez-Bagnis V., Bouchet B., Gallant DJ. (1993). Relationship between the starch granule structure and the textural properties of heat-induced surimi gels. Food structure, 12: 309 - 320.

Voutila L., Mullen A. M., Ruusunen M, Troy D, Puolanne E. (2007). Thermal stability of connective tissue from porcine muscles. Meat Science, 76 : 474 - 480.

Voutila, L., Ruusunen M., Puolanne E. (2008). Comparison of the thermal characteristics of connective tissue in loose structured and normal structured porcine M. semimembranosus, Meat Science, 80:1024 - 1030.

Wang B., Wang L., Li D., Bhandari B., Wu W., Shi J., Chen X. D., Mao Z. (2009). Effects of potato starch addition and cooling rate on rheological characteristics of flaxseed protein concentrate. Journal of Food Engineering, 91: 392 - 401.

Whistler R. L., Daniel J. R. (1985). Carbohydrate. In: Fennema, O. R. (Ed.). Food Chemistry, New York: Marcel Dekker, p. 69.

Xiong Y. L., Blanchard S. P. (1993). Viscoelastic properties of myofibrillar protein and polysaccharide composite gels. Journal of Food Science**, 58**: 164 - 167.

Yang A., Trout G. R., Shay B. J. (1995). Evaluation of carrageenan, isolated soy protein and a modified starch in low fat frankfurters. In: 41st Annual International Congress of Meat Science and Technology (pp. 435 - 436). San Antonio: TX, USA, 20 - 25 August 1995, Proceedings 2.

Yang H., Irudayaraj J., Otgonchimeg S., Walsh, M. 2004. Rheological study of starch and dairy ingredient-based food systems. Food Chemistry**, 86**: 571 - 578.

Youssef M. K., Barbut S. (2010). Physicochemical effects of the lipid phase and protein level on meat emulsion stability, texture and microstructure. Journal of Food Science, 75: 108 - 114.

Youssef M. K., Barbut S. (2011). Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. Meat Science, 87: 54 - 60.

Zaidul I. S. M., Nik Norulaini N. A., Mohd. Omar A. K., Yamauchi H., Noda, T. (2007). RVA analysis of mixtures of wheat flour and potato, sweet potato, yam, and cassava starches. Carbohydrate Polymers, 69: 784 - 791.

Zaleska H., Ring S., Tomasik P. (2001). Electrosynthesis of potato starch - whey protein isolate complexes. Carbohydrate Polymers**, 45**: 89 - 94.

Zhang H., Takenaka M., Isobe S. (2004). DSC and electrophoretic studies on soymilk protein denaturation. Journal of Thermal Analysis and Calorimetry, 75: 719 - 726.

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 143-160 (July 2015/Ramadan 1436H)

Implementation of Hazard Analysis Critical Control Points System (HACCPs) during Extraction Olive Oil using Hydraulic Press Method

El-Kalyoubi[1], M. H., M.M. Abd El-Razik[1,2] , Abou-Zaid, F.O.[3] and Walaa, M. El-Sayed[3]

[1] *Food Science Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.*

[2] *Food Science and Human Nutrition Dept., Agric. and Vet. Med. College, Qassim Univ., Qassim, KSA.*

[3] *Agri. Industrialization Unit, Desert Research Centre, Cairo, Egypt.*

(Received 21 /3/2015; accepted1 3/5/2015)

ABSTRACT. The present work was carried out to investigate the possibility of implementation of HACCP system during olive oil extraction using Hydraulic press method to improve the quality and safety of produced oil. The analysis consisted of detailed studies of oil extraction by Hydraulic press method, the control measures and monitoring procedures for extraction oil are suggested. The microbial profile of tested samples from different processing steps and swabs from worker's hands and equipments surface were used as criteria of sanitation practices in this work. Aerobic bacterial count, yeast & mold count, coliform group and spore forming bacterial counts were used to identify the contamination sources by potential microbial hazards presenting in the various extraction steps of oil. The obtained results showed that, there were different biological, chemical and physical hazards in the raw material used, at the same time, contamination from equipments and the lack of adequate personal hygiene practice for workers in the extraction plants were identified as others sources of hazards. A personal hygiene training, good manufacturing practices (GMPs), cleaning and sanitation procedures as well as the rearrangement of the layout of the extraction plant under investigation could improve quality and safety of olive oil extracted. HACCP system can be also used to control the safety and quality of olive oil extraction plant.

***Keywords*:** GMP - Hazard - HACCP – Hydraulic press – Hygienic practice - Quality – Olive oil - Safety.

INTRODUCTION

Olive (*Olea europea* L.) belongs to family *Oleaceae* is considered as one of the most important fruit crops for human. Olive fruits are accepted by world health organization as an important source of nutrition, makes a significant contribution to the economy and the agriculture of olive cultivating nations. According to the International Olive Oil Council (IOOC) 97% of the world's olive production occurs in Mediterranean countries. Nowadays, the biological and nutritional values of virgin olive oil and its tangible effects on human health are widely became known, (Alarcón de la Lastra, *et al*., 2001). So olive oil, which has been a staple delectable food in the Mediterranean area for thousands of years, has become more popular than ever in many countries, (Ranalli, *et al*., 2003). Virgin olive oil is defined as the oil obtained from the olive fruit through physical procedures, which including the following processing steps: washing, grinding, mixing, pressing, decantation, centrifugation or filtration, without being mixed with oils of another nature. The quality of virgin olive oil is directly related to the quality of the fruit from which it is extracted, (José and Yousfi, 2006). Olive oil extraction process is affected by handling operation and various practices during manufacturing which may sometimes cause some risks and damage that effective of the quality of extracted olive oil. So it was interest to follow HACCP system during extraction processes of olive oil to identify these risks and how they could be controlled to produce a high quality, healthy and safe olive oil product. Nowadays, with the progress of food quality and safety systems, HACCP system has become synonymous with food safety. It is a worldwide recognized systematic and preventive approach that addresses biological, chemical and physical hazards through anticipation and prevention rather than through inspection and testing of end-product, (Gandhi, 2008). Essentially, HACCP is a system that identifies and monitors specific hazards (biological, chemical, or physical) that can adversely affect the safety of the food product. This hazard analysis serves as the basis for establishing critical control points (CCPs). CCPs identify as those points in the process that must be controlled to ensure the safety of the food product. Further, critical limits are established that document the appropriate parameters that must be met at each CCP. Monitoring and verification steps are included in the system, again, to ensure that potential risks are controlled. The hazard analysis, critical control points, critical limits, and monitoring and verification steps are documented in a HACCP worksheet plan. Seven principles have been developed which provide guidance on the development of an effective HACCP plan.

So, the present work was carried out to investigate the possibility of implementation of HACCP system during olive oil extraction by Hydraulic press method to improve the quality and safety of produced oil.

MATERIAL AND METHODS

Material:

Olive fruits:

The olive fruits (*Olea Europea L.*) of Picual cultivar from cultivation season of 2010- 2011 was obtained from Marsa Matrouh governorate, Egypt, were used in olive oil production during investigation.

Sampling:

Samples were taken from the olive oil extraction process plant by Hydraulic press method; those including olive fruits, water, and olive paste from different steps during extraction process. Different samples were selected randomly, put into sterile plastic bags and bottles, then transported to the laboratory in the same day of sampling as the period between taking the samples and analysis does not exceed 24 hours those especially for microbiological analysis,, in an insulated and refrigerated box. The samples from processing surfaces and hands of extraction plant workers were taken by swab method according to Stinson and Tiwari (1978).

Methods:

Olive oil extraction:

The different steps of olive oil extraction using Hydraulic press method were presented in Fig. (3). The processing steps were fruits receiving, cleaning to remove leaves, washing fruits, crushing and olive paste malaxation.

Microbiological analysis:

Olive fruits and paste:

Different samples were examined for total aerobic bacterial count, (ISO 4833, 2003), spore forming bacterial and yeast & mold counts, (ISO 21527, 2008), coliform group, (ISO 4832, 2006).

Water samples:

Water samples from investigated extraction plant were examined for total aerobic bacteria count and coliform group.

Swab samples:

Swab samples from workers hands, utensils and equipments in the investigated extraction plant were tested for total aerobic bacterial count and coliform group.

Different samples were aseptically removed from the plastic bags or bottles, then 10 g (solid sample) or 10 ml (water sample) from each was homogenized in 90 ml of sterile diluents (0.1% peptone water) with a Stomacher for 30 Sec. Serial dilutions were prepared in peptone water (0.1%) and 1ml aliquots were plated in each specific medium and incubated at different temperatures as listed in Table (1).

For aerobic spore forming bacterial count, serial dilutions of different samples were pasteurized at 80°C for 10 min and 1 ml aliquots were plated in the medium as listed in Table (1).

Table (1). Microbiological media and incubation conditions used for microbiological analysis.

Microbiological analysis	Incubation		
	Time (h)	Temp (°C)	Growth medium
Total aerobic colony count	48	37	Plate count agar
Aerobic spore forming bacterial	48	37 and 55	Plate count agar
Yeast and mold count	72	25	Potato dextrose agar
Coliform group	24 – 48	37	Vilot red bile agar

Air samples:

Settling plates were carried out to evaluate the microbiological load of air in different locations through extraction olive oil processing line under investigation. The sterilized Petri plates (Standard size of 90 mm diameter) which contained about 15 ml of the selective media were distributed through different locations on processing lines and then left to exposure to air for 15 min. After exposure, plates were incubated according to the appropriate procedures then, the colonies were counted as colony forming unit (cfu) per each area**,** (A.O.A.C, 2007).

Olive oil characteristics:

Acidity (% as oleic) and peroxide of extracted olive oil samples were determined according to the A.O.A.C. (1990) methods. K232 and K270 values were determined accourding to the methods of A.O.C.S., (1991). Polyphenols were determined according to the method of Gutfinger (1981). TBA value was determined using method of Sidwell *et al.,* (1954).

Listing the prerequisite programs:

The prerequisite programs (PRPs) represent the conditions and/or the necessary basic activities to develop the seven principles of HACCP system during olive oil extraction in the olive oil extraction plant under investigation were evaluated according to Egyptian Standard (ES): 3393/2005.

Application of HACCP system:

According to the NACMCF (1988 and 1992), HACCP system was implemented during extraction steps of olive oil using hydraulic pressing method based on the following seven principles: 1) Conduct a hazard analyses. 2) Identify the critical control points (CCPs). 3) Establish critical limits for preventive measures associated with each identified CCP. 4) Establish CCP monitoring

requirements.5) Establish corrective actions to be taken when monitoring indicates then a deviation from an established critical limit. 6) Establish verification procedures. 7) Establish record-keeping and documentation procedures. The results were summarized with reference to CCPs and their monitoring on the HACCP worksheet.

RESULT AND DISCUSSION

Assessment the prerequisite programs (PRPs):

The results of assessment of PRPs represent the conditions and/or the necessary basic activities to maintain a hygienic practices for different extraction steps of olive oil plant under investigation. In which, must be established in processing area before application HACCP system using recommended chick list by ES 3393/2005. The obtained data revealed that, location of extraction plant, walls, devices machines and disposals tanks were satisfactory according to the criteria listed in ES: 3393/2005. On the other hand, for more controlling of microbial contamination during extraction the following areas in the extraction plant should be physically isolated: olive receiving, washing, extraction and separation, storage and packaging, (Petrakis, 1994). In the same time, quality control practices and its device were not applicable where the temperature of the olive oil extraction plant was not recorded and the microbiological characteristics of water used were not followed periodically this could be one of the major contamination sources during extraction steps. At the same time, control measures were not applicable, so, we suggestion of simple HACCP system to establish some of control measures could be used for improving the safety of extracted oil. Also, maintenance and sanitation programs were not applicable where program of pest control was not applicable and there is no program for waste management of produced wastes during extraction process. According to the results of observation of personal hygiene of the workers it could be observed that, hygiene and sanitation practices of workers in the extraction plant were not enough and workers should trained more about personal hygiene especially hand washing. The aforementioned notices suggested as corrective actions for prerequisite programs should be established in the extraction plant under investigation. Verification of prerequisite programs of HACCP system could be evaluated by assessment of microbiological criteria of air and water samples and swab samples from workers hands and equipments used.

The results of microbiological loads of air samples of different locations of the olive oil extraction plant under investigation, (A) olive fruit receiving and cleaning, (B) crushing, (C) malaxation, (D) pressing and (E) separation are shown in Fig. (1). It could be noticed that the total aerobic count of collected air samples was ranged from 1.81 to 1.94 log cfu/plate for hydraulic pressing plant. Where there are no microbial criteria of air in the food establishment, these results could be reflect the microbiological quality of extraction plant air where, the aerobic bacterial count of air samples of different locations are satisfactory.

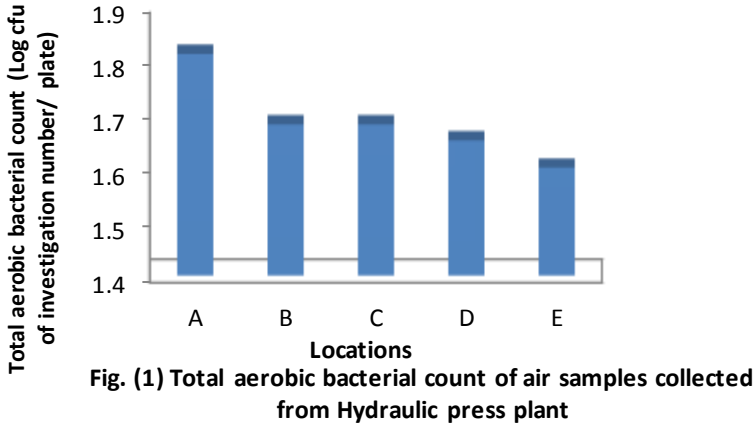


Fig. (1) Total aerobic bacterial count of air samples collected from Hydraulic press plant

Water is an important factor for the olive oil extraction it was used for washing of olive fruits, cleaning equipments and utensils and cleaning hands workers. As well as, water takes place as raw material in the oil separation process. Results of microbiological analysis of water sample collected were presented in Fig. (2). It could be noticed that all collected water samples contained aerobic and coliform counts higher than those recommended limits of ES 1589/2005. In which aerobic bacterial count of water used directly in food processing did not exceed 20 cell, beside it must be coliform free. This may be due to the water line of Hydraulic press is old and may have many disorders caused this contamination. This point was listed as major contamination sources and should be corrected by maintains the water line supply of extraction plant and periodic microbiological analysis of water used in extraction plant.

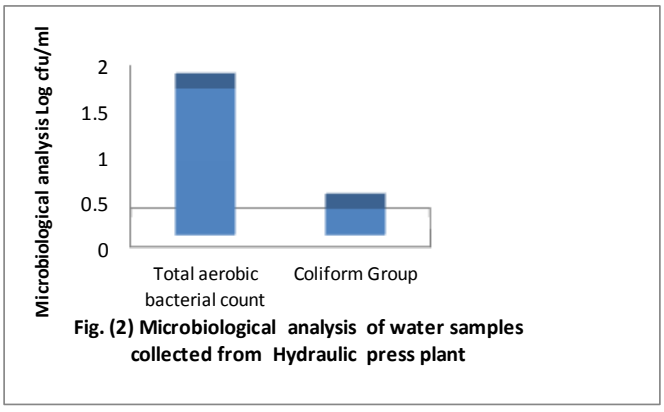


Fig. (2) Microbiological analysis of water samples collected from Hydraulic press plant

The microbiological analysis data of the swabs taken from workers in the plant are shown in Table (2). Swabs of worker's hands were tested for total aerobic bacterial count and coliform group as microbiological criteria for evaluating of personal hygiene. Result in Table (2), revealed that, total aerobic bacterial count of swab from worker's hands was ranged between 0.85 to 2.28 log cfu/cm^2. On the other hand, coliform group did not detect in the swabs of worker's hands of feeding and washing steps, crushing and malaxation and pressing steps values of 0.32 and 0.95 cfu/cm^2, respectively. The presence of coliform group in the hands of workers are reflect a poor personal hygiene of workers thus could be solved by training program for workers about good personal hygiene during the extraction steps of olive oil. At the same time, the personal hygiene one of the prerequisite program should be established in the plant for application HACCP system.

Table (2). Microbiological analysis of different swabs from hands of workers in Hydraulic press plant

Workers	Microbiological analysis Log cfu/cm^2	
	Total aerobic bacterial count	Coliform Group
Worker in feeding step	0.85	<1
Worker in washing step	1.93	<1
Worker in crushing and malaxation step	1.88	0.32
Worker in pressing step	2.28	0.95

<1: viable colony was not detected at detection limit < 10^1 cfu/ cm^2

Total aerobic bacterial and coliform group counts of different equipments used was determined, the results are presented in Table (3). It could be noticed that, total aerobic bacterial count of washing tank, crusher, malaxation 1, malaxation 2, hydraulic press and pressing stainless disk was 1.65, 1.70, 1.66, 1.88, 1.94 and 2.40 log cfu/cm^2, respectively. About coliform group which used as sanitation criteria, it could be noticed that, coliform group was not detected (detection limit < 10^1 cfu/cm^2) in washing tank, crusher, malaxation tank 1. On the other hand, malaxation tank 2, hydraulic pressure and disk contained 0.85, 2.00 and 1.74 log cfu/cm^2, coliform group respectively. This microbiological load may be due to the absence of detailed plan for cleaning and disinfection processes for equipments and tools used, therefore a planned cleaning and disinfection program of equipment must be corrected and verification its performance periodically.

Table (3). Microbiological analysis of different swabs from equipments used in Hydraulic pressing plant

Microbiological analysis Log cfu/cm^2		Equipment
Coliform Group	Total aerobic bacterial count	
<1	1.65	Washing tank
<1	1.70	Olive crusher
<1	1.66	Malaxation 1
0.85	1.88	Malaxation 2
2.00	1.94	Hydraulic pressure
1.74	2.40	Disk

<1: viable colony was not detected at detection limit < 10^1 cfu/cm^2

Application of HACCP system:

Hazard analysis:

Typical preparation, associated hazards, and critical control point of different extraction steps of olive oil extracted by Hydraulic press method are illustrated in Fig (3). The possibilities of contamination, survival of contaminants, and growth of microorganisms are analyzed in process reviews. Sources of contamination are worker's hand, utensils, equipments and raw materials.

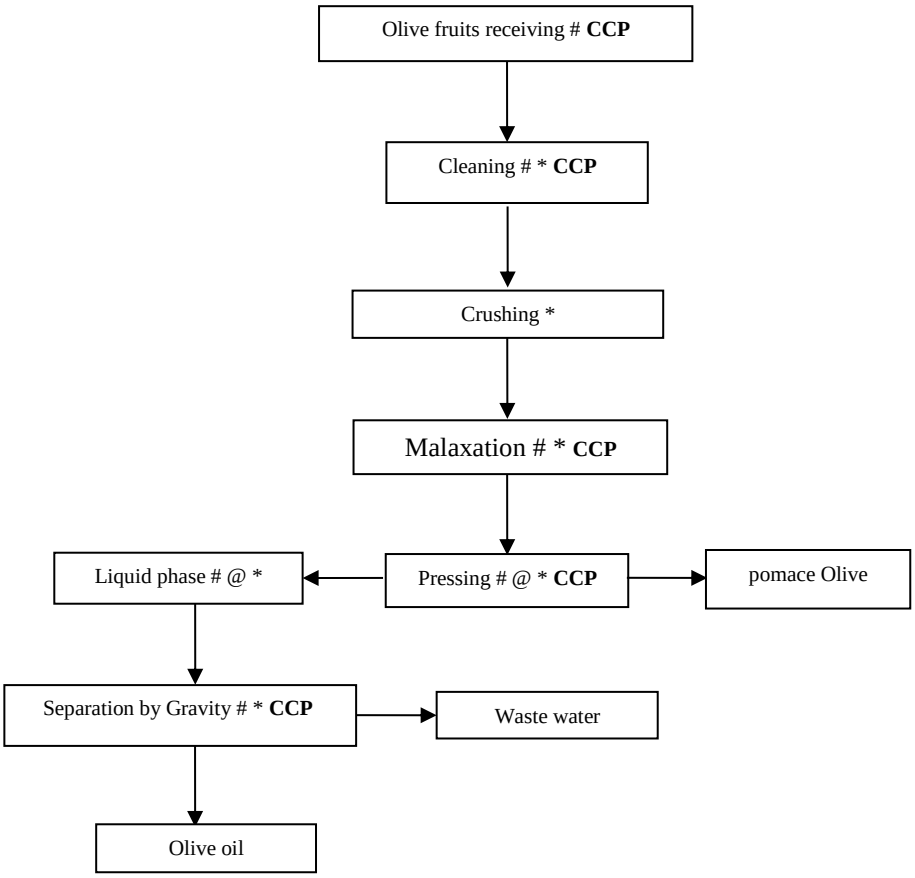


Fig. (3): Flow diagram of the olive oil extraction by Pressing method

CCP: Critical Control Point, #: Possible contamination, *: Contamination from equipment and utensil surfaces and @: Contamination by persons handling product.

The microbiological analysis of different steps during olive oil extraction by Hydraulic press was determined, the results are presented in Table (4). The main extraction steps were examined for their contents of total aerobic bacterial, coliform group, yeast & mold and aerobic spore forming bacterial counts. From the data recorded in Table (4) it could be observed that, total aerobic bacterial count reduced from 4.76 log cfu/g for fresh olive fruits before washing to 3.89 log cfu/g after washing then it was reduced to 2.60 log cfu/g after crushing, which may be due to the events of mechanical damage of microbial cell walls during crunching process. Meanwhile, total bacterial counts intended to increase sharply during two malaxation

steps 1 and 2, whereas, it was 4.4 and 4 73 log cfu/g, respectively. The same trend was also observed for yeast & mold count, which decreased from 4.26 log cfu/g for olive fruits before washing to 2.95 log cfu/g for washed olive fruits, then decreased to 2.60 log cfu/g after crushing. On the other hand, yeast & mold count was increased during malaxation steps 1 and 2 comparing to olive paste sample after crushing step where it was 2.85 log cfu/g during malaxation 1 step and 2.95 log cfu/g during malaxation 2. According to the results of coliform group, which was increased from 2.18 log cfu/g for olive fruits before washing to 2.30 log cfu/g for olive fruits after washing, and it was slow decreased to 2.26 log cfu/g during crushing process, furthermore, coliform group was increased during malaxation 1 and 2 steps where it was 3.00 log cfu/g during malaxation 1 and 2 steps.

Table (4). Microbiological analysis of different extraction steps of olive oil by Hydraulic pressing method

Extraction steps	Microbiological analysis Log cfu/g			
	Total aerobic bacterial count	Coilform group	Yeast & Mold count	Aerobic spore forming bacterial count
Olive fruit before washing	4.76	2.18	4.26	2.43
Olive fruit after washing	3.89	2.30	2.95	2.19
Olive past during crushing	2.60	2.26	2.60	2.04
Olive paste during malaxation 1	4.40	3.00	2.85	2.08
Olive paste during malaxation 2	4.73	3.00	2.95	2.38

The same table, revaled that, aerobic spore forming bacterial count tended to decreased from 2.43 log cfu/g for fresh olive fruits to 2.19 log cfu/g for olive fruits after washing, and decreased to 2.04 log cfu/g during crushing process. Also, aerobic spore forming bacterial counts was increased during malaxation 1 and 2 steps comparing to olive paste sample after crushing step where it was 2.08 log cfu/g during malaxation 1 and 2.38 log cfu/g during malaxation 2, respectively. According to the aforementioned results it could be concluded that, washing step was very important in reducing the microbial load of olive fruits and could be determined as one of the critical control points during the processing steps of olive oil extraction using a hydraulic press method, beside the characteristics of water used in this step was a control measures should be established in this step. At the same time, malaxation step must be controlled as the microbial load was increased especially

during malaxation step where it may be one of the reasons of increasing the microbial load of olive past, therefore, established a good sanitation program of the aforementioned equipments could be used as control measures during application of HACCP system during extraction process.

Identifying critical control points:

A Critical Control Point (CCP) refers to a processing steps, or procedure in a food process at which an essential control measure can be applied to eliminate, prevent or reduce an identified food hazard to an acceptable limit, (ISO 22000-2005). Each CCP has one or more critical limits to ensure that hazards are prevented, eliminated and reduced to acceptable levels. During a flow diagram's checking procedure using the decision tree, (Codex 2003a), several critical control points of extraction flow diagram of olive oil have been set up and tabulate in Table (5). From the aforementioned data, it could be noted that, olive fruits receiving, cleaning olive fruits, malaxation, extraction of olive oil and separation of olive oil are detected as a CCPs.

Table (5). CCP of olive oil extraction processing line

Processing step	Category and identified hazard	Q1	Q2	Q3	Q4	CCP
1- Receiving of olive fruits	Microbiological infection (B)	No	No			
	Suitability of olive (P&B)	Yes	Yes			
	Mechanical damage (P)	No	Yes	*		
	Insect infection (P)	No	Yes	**		CCP
	Foreign materials(P)	No	No			
	Pesticides residues (C)	No	Yes	***		
	Enzymatic reactions (C)	Yes	Yes			
2- Cleaning (leaves removal, washing)	Water quality (B)	Yes	Yes			
	Reuse of excessive wash water (B)	Yes	Yes			
	Washing time (B)	Yes	Yes			CCP
	Foreign materials (P)	Yes	Yes			
3- Crushing	Microbiological infection (B)	No	No			
	Foreign materials (P)	No	No			

Continue Table (5).

Processing step	Category and identified hazard	Q1	Q2	Q3	Q4	CCP
4- Malaxation	Microbiological infection (B)	Yes	Yes			CCP
	Emulsions formation (C)	Yes	Yes			
	Enzymatic reactions (C)	Yes	Yes			
5-Extraction of olive oil by hydraulic pressing	Microbiological infection (B)	Yes	No	No	No	CCP
	personal hygiene (B)	Yes	No	Yes	No	
	Water quality (M)	Yes	Yes			
	Enzymatic reactions (C)	Yes	Yes			
	Emulsions formation (C)	Yes	Yes			
6- Separation by Gravity	Impurities (P)	Yes	Yes			CCP
	Enzymatic reactions (C)	Yes	Yes			
	Oil degradation (C)	Yes	Yes			

B=Biological; C=Chemical; P=Physical Hazards

*= Modifying (Sorting), **=Modifying (Insect control program), ***=Modifying (Safety period)

Setting the critical limits for identified critical control points:

The critical limits must be established to guarantee that; the acceptable level for each hazard at each CCP is not exceeded. During CCP identification, establish critical limits should be measurable where it could be reasonably demonstrated that the threshold level has not been exceeded. Critical limits might be based upon factors such as: olive quality, water quality, adequate renew frequently wash water, good hygiene, good performance of equipment, temperature and time, good personal hygiene and some physicochemical properties of olive oil such as (FFA, PV, TBA, K_{232}, K_{270} and Polyphenols).

Establishing monitoring procedures for the critical control points:

Planned observations and measurements are conducted to assess whether a CCP is under control and produce an accurate record for a future verification process. Monitoring procedures during olive oil extraction plant were included, visual inspection of each items, microbiological and physicochemical analysis of water, physicochemical properties of olive oil (FFA, PV, TBA, K_{232}, K_{270} and Polyphenols), Chemical analysis of samples, Visual inspection of wash water dirt, temperature and time, periodic visual inspection of equipment, correct application of the programs preventive cleaning and disinfection of equipment and good hygiene practices.

Chemical parameters of extracted olive oil:

The results of chemical parameters of olive oil extracted by hydraulic pressing method are in Table (6).

Table (6). Chemical properties of olive oil extracted by Hydraulic press method

Parameters	Extracted olive oil	Egyptian standard *	Codex standard **
Acidity (% as oleic)	1.46 ± 0.025	3.3	3.3
Peroxide value (meq peroxide / kg oil)	10.26 ± 0.157	20≤	20≤
TBA (mg Malonaldhid / kg oil)	1.86 ± 0.025	-	-
K232	2.17 ± 0.064	-	3.5
K270	0.45 ± 0.015	0.25	0.3
Polyphenols (as ppm gallic acid)	300 ± 5	-	-

* E S 49 (2000)

** Codex (2003b)

± SD

The obtained results in Table (6) was showed that, acidity (free fatty acids as % Oleic acid) of the produced oil by hydraulic press (1.46% as oleic acid) was in the allowed limits of olive oils to be categorized as virgin olive oil according to the codex standards (Codex 2003b) in relation to acidity. The commonly used methods for measuring the auto-oxidation statues of oil are the formation of peroxides and Thiobarbituric acid (TBA) test. The first parameter gives a whole picture on the course of oil oxidation, whilst, the second presents the production of secondary oxidation products, which are responsible for off flavors of rancid oil (Basuny, 1996). In relation to the peroxide value of the produced oil by hydraulic press system (only olive oil was allowed to have peroxide value reaches to 20 because it contains desirable bio-active materials which interfere in determination of peroxide value). It could be noticed that the produced oil has high peroxide value (10.26) this means that, oil oxidation takes place in this oil sample. From the results shown in the table, we found the TBA (as mg malonaldhid / kg oil) recorded high value reached to 1.86, this means that secondary oxidation of the oil had been occurred. From the aforementioned results of K232 and K270 [K232 value measures the oxidation whereas the secondary oxidation could be measured by K270 (Ranalli *et al.,* 2000), at the same time it could be noticed that K232 was 2.17 and K270 was 0.45. K232 did not reach to the allowed values by codex

standards (Codex 2003b) which stated the max value of K232 is 3.5 for olive oil to be categorized as virgin olive oil. But K270 was higher than the max value of standards (0.3). The results of peroxide value and TBA are in agreement of those of K232 and K270. These results indicated that oil oxidation had happened not only primary oxidation but also, secondary oxidation. The aforementioned data showed obviously that, total poly phenols (as ppm gallic acid) were 300 ppm. This may be due to the used extraction system (Hydraulic press) which required high amount of water to separate the olive oil.

Establish a corrective action:

During investigation of olive oil extraction plant some specific corrective actions were established for each identified CCP. These corrective actions are performed when the monitoring procedures had been indicated that, the critical limits are exceeded. The corrective actions were designed to rapidly regain control over the CCP and prevent recurrence. Corrective action during extraction olive oil should include: reject unsuitable items, poor quality fruits must processed separately, switch point water supply, conduct training for operators, Increase changes wash water, visual inspection of temperature and time and correct preventive programs for maintenance, equipment cleaning and disinfection.

Establish verification procedures:

The verification procedures were established in order to check if the HACCP system is working as suggested HACCP plan established in the extraction olive oil under investigation; this could be done by establishing accurate records of the previous and ongoing measurements as well as the tracking of those products that have exceeded the critical limits. These verification procedures were including: develop particular specifications for the product, recording raw materials, frequency of changes water, preventive programs for maintenance, equipment cleaning and disinfection and Corrective measures.

The different aforementioned principles of designed HACCP plan during the investigation of olive oil extraction plant were tabulated in Table (7).

CONCLUSION

The main sources of hazards during olive oil extraction come from environment handling and processing techniques. Olives might be contaminated biologically by microorganisms, pesticides and heavy metal. Hygiene handling and Good Manufacturing Practice during receiving of fresh olive fruits, washing and different extraction steps are very important, because many microorganisms and enzymatic reactions may be accrued and affected the safety and quality of extracted olive oil. The establishment of HACCP system as Food Safety tools in the extraction plants of olive oil allows controlling of different hazards affecting the safety of consumers. Application of HACCP system could be used for harmonizing the practices of different HACCP principles for controlling the different identified hazards during

different steps of olive oil extraction. Various microbiological methods of analysis and procedures have been developed to monitor the safety of olive oil at an early stage and during extraction steps. The PRPs implementation allowed us to master the likelihood of incidence of physical, chemical and microbiological hazards. The HACCP plan principles were established and have been enabled to monitor and control of identified microbiological hazards.

Table (7) HACCP work sheet for Hydraulic pressing system

Critical control point (CCP)	**Hazard**	**Control measures**	**Critical limits**	**Monitoring action**	**Corrective action**	**Verification procedures**
Receiving of olive fruits	*Microbiological infection *Insect infection *Suitability of olive * Mechanical damage *Pesticides residues *Heavy metal *Enzymatic reactions *Foreign materials	*Cleaning efficiency *Separate ground of flight and health olives *High quality drinking water *Good transportation handling	*Separation of ground of flight and health olives *Meeting the specifications of drinking water *Adequate sanitary conditions *Good transportation	*Visual inspection of each items *Microbiological and physicochemical analysis of water *Chemical analysis of samples	*Reject unsuitable items *Poor quality fruits must processed separately *Switch point water supply *Conduct training for operators	*Develop particular specifications for the product *Corrective measures *Recording raw materials
Cleaning (leaves removal, washing)	*Water quality *Foreign materials *Washing time *Reuse of excessive wash water	*Program effectiveness cleaning and disinfection *Renew frequently wash water *Oil loss *Equipment	*Adequate renewal of the washing water *Increase wash time *Good hygiene *Good performance of equipment	*Visual inspection of wash water dirt *Correct application of the programs preventive cleaning and disinfection of equipment	*Increase changes wash water *Correct programs preventive cleaning and disinfection of equipment	*Frequency of changes water * Programs preventive cleaning and disinfection of equipment *Corrective measures

Continue Table (7) .

Critical control point (CCP)	Hazard	Control measures	Critical limits	Monitoring action	Corrective action	Verification procedures
Malaxation process	*Microbiological infection *Enzymatic reactions *Emulsions formation	*Equipment (state, cleaning) *Olive past temperature and malaxation time (<30ºC, 45min) *oil loss	*Good performance of equipment *Suitable conditions (25-30 ºC, 45-60 min)	* Malaxation temperature and time	*Visual inspection of malaxation temperature and time *Correct programs preventive cleaning and disinfection of equipment	* Cleaning and disinfection programs of equipment *Visual inspection of malaxation temperature and time
Extraction of olive oil by hydraulic pressing (liquid phase)	*Microbiological infection *Personal hygiene *Enzymatic reactions *Emulsions formation *Water quality	*Personal hygiene *Operations (time, efficiency, oil loss) *Water temperature and water count *Equipment (state, cleaning) *Cleaning program and adequate disinfections *Oil loss	*Good personal hygiene *Time processing * Water count (1/2 L/Kg past) *Water temperature (25-28 ºC) *Minimum oil loss	*Visual inspection of time processing, water count and water temperature	*Correct programs maintenance preventive cleaning and disinfection of equipment * Adjusting water count and temperature	* Preventive programs for cleaning and disinfection of equipment * Adjusting water count and temperature
Oil separation	*Impurities *Oil degradation *Microbiological infection *Enzymatic reactions	*FFA, PV, TBA, K_{232}, K_{270}, Polyphenols *Filtration suitable amount of water *Water temperature	*FFA, PV, TBA, K_{232}, K_{270} , Polyphenols *Olive oil specification *Good performance of equipment *Good hygiene *Good handling practices	*Visual inspection of FFA, PV, TBA, K_{232}, K_{270}, Polyphenols *Good hygiene practices *Periodic visual inspection of equipment	*Correct preventive maintenance programs, cleaning and disinfection of equipment *Conduct training to operators	* Preventive programs maintenance, equipment cleaning and disinfection *Training courses *Visual inspection of FFA, PV, TBA, K_{232}, K_{270}, Polyphenols

REFERENCES

A.O.A.C. (1990). Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists. Washington, DC.

A.O.A.C. (2007). Official Methods of Analysis. 18th ed., Association of Official Analytical Chemists. Washington, DC.

A.O.C.S. (1991). Official and Tentative Methods of the American Oil Chemists Society 14th ed. Published by the American Oil Chemist's Society. 35, East Wacker Drive, Chicago, IIIinois, USA.

Alarcón de la Lastra, C., M.D. Barranco, V. Motilva and J.M. Herrerías (2001). Mediterranean Diet and Health: Biological Importance of Olive Oil. ***Current Pharmaceutical Design***, *7*, 933-950.

Basuny, A. M. M. (1996). Biochemical studies on olive oil. M. Sc. Thesis, Agriculture Biochemistry, Fac. of Agric. Cairo University.

Codex Alimentarius Commission (2003a). Hazard Analysis and Critical Control Point (HACCP) system and Guidelines for its Application. Formerly CAC/RCP 1-1969; (Rev. 4 - 2003)**.**

Codex Alimentarius Commission (2003b). Standard for olive oils and olive pomace oils. Formerly CAC/RS 33-1970; Revised in 1989, Rev. 2-2003.

Egyptian Organization for Standardization and quality control (ES) 1589 (2005). Botteled /packaged drinking waters (other than natural mineral water).

Egyptian Organization for Standardization and quality control (ES) 49 (2000). The Egyptian standards of vegetables edible oils, part II-Edible olive oil. Cairo, Egypt.

Egyptian Organization for Standardization and quality control (ES): 3393 (2005). General principles of food hygiene for establishment of food products.

Gandhi, A. P. (2008). Development of HACCP Procedure for the Production of Full Fat Soy Flour. International Food Research Journal 15(2): 141-154.

Gutfinger, T. (1981). Polyphenols in olive oil. J.A.O.C.S., 58: 966- 968.

ISO 21527 (2008). Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 2: Colony count technique in products with water activity less than or equal to 0,95

ISO 22000 (2005). Food safety management systems Requirements for any organization the food chain

ISO 4832 (2006). Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms -- Colony-count technique

ISO 4833 (2003). Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 degrees C.

José, G.M. and K., Yousfi (2006). The postharvest of mill olives. GRASAS Y ACEITES, 57 (1), 16-24.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (1992). Hazard analysis critical control point System. Journal of Food Microbial. 16:1-23.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (1998). Hazard analysis critical control point principles and application guidelines. Journal of Food Protection. 61: 1246-1259.

Petrakis, C. (1994). Good Manufacturing Practice (GMP) guidelines for virgin olive oil production. Grasas y Aceites. 45(1-2): 53-54.

Ranalli, A., A. Malfatti and P. Cabras (2001). Composition and quality of pressed virgin olive oils extracted with a new enzyme processing aid. J.Food Sci., 66 (4): 592-603.

Ranalli, A., L. Pollastri, S. Contento and E. Iannucci (2003). The glyceridic and nonglyceridic constituents of virgin olive oil after use of a novel method of enzyme extraction. International J. Food Sci., Tech., 38:17-27.

Ranalli, A., N. Costantini, G. De Mattia and M.L. Ferrante (2000). Evaluating two kinds of centrifuged virgin oils arising from continuous olive processing. J. Sci. Food Agric. 80: 673-683.

Sidwell, C.G.; S. Harold; B. Milado and J. H. Mitchell (1954). The use of thiobarbituric acid as a measure of fat oxidation. J.A.O.C.S., 31: 503-606.

Stinson, C. G. and N. P. Tiwari (1978). Evaluation of quick bacterial count methods for assessment of food plant sanitation. Journal of Food Protection. 41:269-271.

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 161-174 (July 2015/Ramadan 1436H)

Immunomodulatory And Antioxidant Activity Of Pomegranate Juice Incorporated With Spirulina And Echinacea Extracts Sweetened By Stevioside

Soha H. Mahmoud[1]; Mahmoud R.M.[2]; I.S. Ashoush[2, 3] and M.Y. Attia[1]

[1]*Obstetrics and Gynecology Dept., Fac. Medicine, Ain shams Univ. Hospital, Cairo, Egypt*

[2]*Food Science Dept., Fac. Agric., Ain Shams Univ., Shoubra El-kheima Cairo, Egypt*

[3] *Food Science and Human Nutrition Dept., Agric. and Vet. Med. College, Qassim Univ., Qassim, KSA.*

(Received 21 /2/2015; accepted 27/6/2015)

ABSTRACT. Pomegranate juice (PJ), *Spirulina platensis* extract (SP) and *Echinacea purpurea* Extract (EP) was chromatographically analyzed by High performance liquid chromatography (HPLC) for phenolic and flavonoid compound. Also, this extracts was determined for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activates in terms of scavenging DPPH free radicals. The TPC of (PJ) was 26.15mg gallic acid/g of extract, and TFC was 17.6 mg catechin/g of extract, while total phenolic content for (SP) and (EP) extracts were 0.153, 0.773mg gallic acid/ml, respectively. The total flavonoids content for each of them was 2.599, 8.063mg catechin/ml, respectively. Identification of phenolic compounds showed high amount of ellagic acid, pyrogallol, catechein, benzoic, cinnamic, syringic, gallic, aminobenzoic, protocatechuic, catechol, epicatechein, caffeine, P.OH.benzoic, caffeic, vanillic, p-Coumaric, Ferulic, α-Coumaricand Salycilic. Also, identification of the flavonoids compounds appeared high amount of rutin, quercitrin, Hisperidin, Quercetrin, luteolin, kampferol, narengin and rosmarinic. Formulated pomegranates beverage (FPB) was also investigated for antioxidant activities and total phenolics content. Furthermore, phagocytosis inhibition test was carried out to measure immunomodulatory activity of pomegranate juice, stevioside, Spirulina, echinacea and formulated pomegranates beverage. Immunological tests showed highly significant increase in phagocytosis of macrophages for Formulated pomegranate beverage (FPB). Depending on sensory evaluation, the formulated pomegranates beverage showed overall acceptance compared to normal pomegranate juice. So, it could be concluded the suitability of pomegranate juice, spirulina, echinacea and stevioside as a supplementation in the production of health promoting beverage.

Keywords: Pomegranate juice, Echinacea, Spirulina, Stevioside, Antioxidant status, Immunomodulatory, Sensory evaluation.

INTRODUCTION

Pomegranate fruit (*Punica granatum* L.) has gained commercial importance in recent years in the food and health industries due to better health outcomes (Fawole and Opara, 2013). Pomegranates have high levels of polyphenols and may be a rich source of compounds that had antiviral activity (Sundararajan *et al*., 2010). The antioxidant capacity of pomegranate juice is greater than other fruit juices and beverages (Seeram *et al.,* 2008). This antioxidant activity has been attributed to the high content of phenolic compounds (Gil *et al*., 2000). Pomegranate is known to contain considerable of phenolic compounds, including anthocyanin and ellagic acid (Gonzalez-Molina *et al*., 2009).

Pomegranate juice has important clinical implications, and it has even been recommended in the treatment of acquired immune deficiency syndrome (AIDS) due to rich concentration of diverse bioflavonoids and to their known free radical scavenging activity and inhibition of lipoxygenase. Furthermore, pomegranate is one of nine herbs included in a Japanese-patented formula for treating AIDS (Schubert, 1999; Lee and Watson 1998).

Echinacea purpurea was a popular herbal immunomodulator. Recent reports indicate that echinacea products inhibit nitric oxide production in activated macrophages (Zhai *et al.*, 2009). *Echinacea purpurea* is a plant originally used by native Americans to treat respiratory infections and have long been used to aid in wound healing and to enhance the immune system (Lee *et al.*, 2010).The active ingredients of a medicinal plant are phenolic compound that is also an important antioxidant (Khanavi *et al*., 2009; Huda-Faujan *et al*., 2009).

Spirulina, filamentous blue-green microalgae have received increasing attention due to most promising sources of compounds with biological activity that could be used as functional ingredients (Pulzand Gross, 2004). Their balanced chemical composition (good quality proteins, balanced fatty acid profiles, vitamins, antioxidants and minerals) and their interesting attributes can beapplied in the formulation of novel food products (Spolaore *et al*., 2006).

Furthermore, spirulina contains β-carotene and xanthophyll pigments, ɣ-tocopherol and phenolic compounds, which are responsible for the antioxidant activities of these microalgae, as shown by several authors' for in vitro and in vivo experiments (Miranda *et al*., 1998; Patel *et al*., 2006). Moreover, the benefits of Spirulina in building immunity and improving resistance to viral infections and several pre-clinical animal studies have shown good immune stimulatory effects in a variety of species. A hot water extract of spirulina has shown very promising effects on the human immune system as reported by (Blinkova *et al.*, 2001).

Stevioside, an abundant component of *Stevia rebaudiana* leaf, has become well-known for its intense sweetness (250–300 times sweeter than sucrose) and is used as a non-caloric sweetener in several countries. Based on direct observations on human and animals, it has been proven that stevioside had to be non-mutagenic, non-teratogenic and non-carcinogenic effects. Stevia has been consumed by human

beings for centuries without any negative effects and had approved for use as a sweetener by the Joint Food and Agriculture Organization / World Health Organization Expert Committee on Food Additives (JECFA). Also, Stevioside offers therapeutic benefits, having anti-hyperglycaemic, anti-hypertensive, anti-inflammatory, anti-tumour, anti-diarrhoea, diuretic, and immunomodulatory actions, (Chatsudthipong & Muanprasat 2009, Anton *et al.* 2010 and Roberto *et al.* 2012).

In this context the aim of the present work was to design a functional beverage combining the health-promoting properties of pomegranate juice incorporated with bioactive compounds from Spirulina and Echinacea extract sweetened with stevioside as a natural sweetener. The immunomodulatory and antioxidant activity effect were also studied.

MATERIAL AND METHODS

Material

Pomegranate fruits were obtained from local market. Spirulina (*Spirulina platensis*) biomass was obtained from Algae Biotechnology Unit in National research center, Giza, Egypt. *Echinacea purpurea* leaves and flower in dried form were purchased from local market in Cairo, while stevioside crystalline, white powder, with sweetness power of 280 times as sucrose, procured from Stevia International Company for Agro-industry Product, in Cairo, Egypt (SICAP). Ethanol, Sodium carbonate, Glycerin and methanol were obtained from El-Gomhoreya Co., Cairo, Egypt. 2, 2-diphenyl-2-picrylhydrazyl radical (DPPH) and Folin-Ciocalteus phenol reagent were purchased from Sigma–Aldrich Inc. (St Louis, MO, USA).

Preparation of fresh pomegranate juice (PJ)

Pomegranate fruits were washed in cold tap water and drained. They were manually cut-up and the outer leathery skin which encloses hundreds of fleshy sacs was removed. The juice that is located in the sacs was manually pressed and extracted. The juice obtained had a deep-red color. It was stored at -18 ^{o}C until using (Maskan, 2006).

Preparation of echinacea extract (EP)

Echinacea **(**EP) powder was extract with 55% ethanol to distilled water (1:10, w/v) under 55°C for 3 h, after filtering the extracts was collected and stored under -20℃ until analyzed or the filtrate evaporated to dryness under vacuum and lyophilized (Ivana *et al.*, 2009).

Preparation of spirulina extract (SP)

The spirulina were extracted by the method of Kajimoto and Murakami (1999). That is, the matter was treated with 10 volume of distilled water and boiled for 10min. The extracts were filtered through cheesecloth and the filtrate was used for the following tests.

Preparation of stevioside Extract (SE).

Stevioside was prepared as freshly homogenized suspension in 1:100 (w/v) soluble in distilled water. The filtration by using suction filtration through filter papers (Toyo No. 5B), according to the method described by Sytar *et al*., (2012).

Preparation of the formulated pomegranate beverage (FPB)

formulated pomegranate beverage (FPB) prepared from pomegranate juice (PJ) mainly incorporated with Spirulina (SP): Echinacea (EP) extracts with ratio and sweetened by Stevioside (SE) according to the most appropriate concentration for each extract based on phagocytosis inhibition assay.

Method of analysis

Determination of total phenols content:

Spectrophotometric determination of total phenols content was determined with the Folin-Ciocalteau method, adapted by the method of Arnous *et al*., (2001) as follow: In Eppendorf microtube, 790 µg of Milli-Q water, 10µl of sample appropriately diluted with MeOH, and 50µl of Folin-Ciocalteau reagent were added and vortexed for 1min, then 150 µl of aqueous 20% sodium carbonate were added, then vortexed and allowed to stand in the dark at room temperature, for 120 min. The absorbance was recorded at 750nm, and quantified using gallic acid as a standard. Results were expressed as mg per ml of gallic acid equivalents (GAE).

Determination of total flavonoids:

Total flavonoids (TF) content was determined by Aluminum Chloride Method (Chang *et al*., 2002) as follows; reaction mixture include 1.0 ml of extract, 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120mm) was incubated at room temperature for 30 min and absorbance was measured at 415nm. Catechin can be used as positive control. Results were expressed as milligram catechin equivalents /ml fresh matter from Total content of flavonoids was expressed as equivalent of catechin in one gram of dry plant material.

Evaluation of the free-radical scavenging capacity:

In this method, the 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical was used to measure the antioxidant activity of extracts and beverage (Lim *et al*.,2001) The percentage of antioxidant activity (AA %) of each substance was assessed by DPPH free radical assay. The reaction mixture consisted of adding 0.5mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of ethanol (3.3mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA %) was determined according to Mensor *et al*., (2001) as follow.

$$\mathbf{AA\% = 100 - [(Abs_{sample} - Abs_{blank}) \times 100 / Abs_{control}]}$$

Identification of phenolic acids and flavonoid compounds by HPLC

Total phenols content were determined by using HPLC (Hewlett Packard series 1050) according to Goupy *et al*., (1999).While, flavonoids content were determined by using HPLC system employed consisted of an HP 1090M Series II High-performance liquid chromatography equipped with an HP 1090 Series II diode array and an eight-channel electrochemical colometric array detector (EC; Esa Inc., USA).Method according to Mattila *et al*., (2000).

Sensory evaluation of formulated pomegranate beverage (FPB)

Ten members semi trained panelists from the staff of Food Science Department Ain Shams University were asked to score the formulated pomegranate beverage (FPB) as a functional beverage compared to a normal Fresh pomegranates juice for their appearance, color, odor, taste, consistency and overall acceptability using the individual products were scored for the intensity of the different attributes on a scale of 0 to 10, where; 0 = non perceptible intensity and; 10 = extremely high intensity according to Calin-Sanchez *et al*., (2012).

Phagocytosis inhibition assay:

As mentioned before, the pomegranate juice, green alga, Echinacea extract and stevioside were used to formulate pomegranate beverage, the immunonodulatory effects of this material and their mixture (formulated beverage) were studied, in vitro. The technique adapted by soliman and attia (2007) was followed. Briefly, Preparation of 0.1% nitroblue tetrazolium (NBT) solution labeled by flurescence material (if flurescence microscope will be using): 5mg NBT was dissolved in 2.5 ml 0.9% NaCl protected from light by wrapping tube. Then the tube was placed in a conical plastic centrifuge tube, shacked until dissolved (30 to 60 unites). Then, 2.5 ml of 0.15M phosphate buffered saline was added and tube labeled and stored at 2^{o} to 8^{o}C, this may be used up to 1 week from time of preparation.

Procedure:

(1) For each culture and control, 0.1 ml of 0.1% NBT solution was placed into each well, in a plastic microtiter plate; 7 wells were used, these represent 5 types of examined material, control positive (Histamine) and Control Negative (Saline).

(2) Buffy coat cell was collected after centrifugation of heparinized tubes with removal of plasma, and 0.1 ml of this buffy coat was then pipetted into each well. This procedure would increase the concentration of phagocytic cells and was strongly recommended, better than using whole heparinized blood.

(3) Approximately 0.100 ml of examined materials was added to each culture and control wells, labeled as stimulated assay.

(4) With a plastic pipette mixing was recommended. Then incubation done for 60 minutes, then blood smear was prepared by putting a drop on a microscopic slide.

(5) For each well of all patients a blood smear repeated and control.

(6) One slide selected from each group and flooded with freshly filtered Wright's stain for 3.5 minutes, then in equal volume of Sorenson buffer for 10 minutes more, then washed by distilled water and dried.

(7) Each slide scored under low power for the greater concentration of leucocytes then, using Flurescence Microscope, and at least 50 neutrophils were counted, by recording both total neutrophils and the number which contain deposits of black formazone (reduced NBT dye). Only cells containing black material larger than granule, normally appearing in neutrophils, were counted.

The results are expressed as the percent of cells found having phagocytosed, the normal control should have > 25% of the cells exhibiting reduction of NBT dye, the percentage of neutrophils abnormal in NBT if less than 25%.

Statistical analysis:

Data were analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test with $P \leq 0.05$ being to determine the significant differences in results using SAS software (SAS, 1996).

RESULTS AND DISCUSSION

Phenolic compounds content

The phenolic components of the Spirulina (SP) and Echinacea (EP) extract was calculated based on calibration curves of external standards built for each of the analyzed compounds. The content of major phenolics detected in the formulated pomegranate beverage (FPB), spirulina (SP) and echinacea (EP) extracts ranged from the highest concentration pyrogallol, 3-OH-Tyrosol, chlorogenic, e-vanillic, 4-aminobenzoic, catechol, epicatechein, benzoic, protocatechuic and caffeicwere the concentration values as follows(122828.95, 83902.91, 54662.78, 24627.05, 24621.19, 21037, 20984.01, 9119.59, 7022.28 and 5314.69µg/100mg)respectively, as shown in table (1). These results are in harmony with those obtained by (Elfalleh *et al*., 2011 & Fischer *et al*., 2011), (Gil *et al*., 2000; Seeram *et al*., 2005; He *et al*., 2011).

Table (1). Phenolic compounds composition of Spirulina (SP) Echinacea (EP) extracts and formulated pomegranate beverage (FPB) extract mixtures

Phenolic compounds	Concentration compounds (µg/100mg)		
	Echinacea	Spirulina	FPB*
Syringic	6609.03	50373.13	2411.47
Gallic	863.39	367.65	2557.01
Pyrogallol	18897.24	42647.31	122828.95
4- Aminobenzoic	90.51	731.16	24621.19
3-OH-Tyrosol	ND	ND	83902.91
Protocatechuic	662.55	3524.11	7022.28
Catechein	810.17	3445.16	4976
Chlorogenic	821.81	5629.91	54662.78
Catechol	6053.86	5726.55	21037
Epicatechein	1701.56	7978.49	20984.01
Caffeine	1122.30	1892.20	4272.42
P.OH.benzoic	1667.73	6144.07	8767.03
Caffeic	218.95	7614.26	5314.69
Vanillic	716.11	1788.39	2478.19
p-Coumaric	5953.45	2820.65	470.04
Ferulic	2119.76	1186.66	552.57
Iso-ferulic	749.63	1574.00	902.61
Reversetrol	ND	ND	648.13
Ellagic	1882.25	1686.68	5045.64
e-vanillic	ND	ND	24627.05
α-Coumaric	187.92	368.51	267.33
Benzoic	3494.72	7563.84	9119.59
Salycilic	1754.42	2565.67	3598.64
p-coumaric	ND	ND	60.01
Coumarin	235.65	269.71	96.92
3,4,5Methoxy Cinnamic	98.65	305.15	295.49
Cinnamic	15.65	39.59	2756.10

*formulated pomegranates beverage (FPB): pomegranate juice (PJ) 85%, stevioside (SE) 5%, Spirulina (SP) 4% & echinacea (EP) 6%)

ND: Not Detect

Flavonoids compounds content

The data in table (2) showed flavonoids contents which were relatively high inpomegranate, spirulina and echinacea extract. it contained relatively high amount of narengin, quercetrin, hisperidin, rutin, quercetin and rosmarinic were the concentration values as follows (472721.50, 319744.32, 288382.98, 191591.81, 136421.11 and 119626.87 µg/100mg) respectively.These results are in parallel with those of (Gonzalez-Molina *et al*., 2009).

Table (2). Flavonoids compounds composition of Spirulina (SP), Echinacea (EC) extracts and formulated pomegranate beverage (FPB) extract mixtures.

Concentration (µg/100mg)			Flavonoids compounds
FPB	**Spirulina**	**Echinacea**	
472721.50	2188.65	951.14	Narengin
191591.81	2455.66	2651.50	Rutin
288382.98	13288.09	4671.89	Hisperidin
119626.87	673.05	700.10	Rosmarinic
319744.32	821.20	2875.07	Quercetrin
136421.11	213.59	83.33	Quercetin
112.18	1910.86	595.75	Luteolin
895.89	95.49	99.01	Narenginin
2520.79	248.73	243.97	Kampferol
2512.94	487.67	60.11	Hispertin
275.12	116.57	5.56	Apegnin
1514.65	135.92	2.05	7-Hydroxy flavon

*formulated pomegranates beverage (FPB): pomegranate juice (PJ) 85%: stevioside (SE) 5%: spirulina (SP) 4%: echinacea (EP) 6%

Phagocytosis inhibition assay:

Pomegranate juice, spirulina, echinacea, stevioside extracts and formulated pomegranate beverage (FPB) was tested against phagocytic function Table (3). Phagocytosis is the first step in the response of macrophages to invading microorganisms. The results showed that each tested material could enhance the ability of uptake of neutral read by macrophages compared with positive control (histamine). Furthermore, pomegranate juice, stevioside, spirulina, Echinacea and formulated pomegranate beverage (FPB). These results indicated that the

incorporated pomegranate with spirulina, Echinacea and stevioside could activate innate immune response and may be considered as a supplementary therapy. A highly significant difference was observed for the FPB.

Table (3). Immunomodulatory effect of pomegranate extracts, Stevioside (ST) Spirulina (SP), Echinacea (EC) and formulated Pomegranate beverage (FPB) extract mixtures on phagocytic response of macrophages

Treatment groups	Phagocytosis Percentage
Negative control (saline)	44± 1.26[a]
Positive control (Histamine)	16± 0.23[f]
Stevioside	40± 0.12[b]
Echinacea	40± 0.51[b]
Spirulina	41± 0.16[c]
Pomegranate juice	38± 0.12[d]
Formulated pomegranate beverage(FPB)	43± 0.34[a]

Means with the same letter are not significantly different P<0.05.

Total phenolic content, total flavonoids and scavenging activity of stevioside (ST) Spirulina (SP), Echinacea (EC) and formulated pomegranate beverage (FPB):

The data presented in table (4) show that pomegranate extract was characterized by high content of total phenols 2.15 mg/ml and had a great free radical scavenging activity 92.95%. These results are in agreement with those obtained by (Panichayupakaranant *et al*., 2010; Amani *et al*., 2014). Also, it could be noticed that the formulated pomegranate beverage (FPB) is a good source of total phenolics content 2.294 mg/ml and had a high free radical scavenging activity 94.54% due to incorporation of formulated pomegranate beverage (FPB). Also, (SP) extract of and (EP) Total phenolic compounds for both of them (0.153, 0.773 mg / ml) mg of gallic acid equivalents (GAE)/ml of extract respectively. The total flavonoid each of them (2.599, 8.063 mg / ml) mg of catechin equivalent to the extract respectively. These results are in parallel with those of Davey *et al*. (2000) and Elena *et al*. (2009). Therefore, this beverage increases health benefits by increasing antioxidant properties as reported by Reza *et al*. (2014).The same trend was found in antioxidant activity no significant between pomegranate extracts (PJ) and formulated pomegranate beverage (FPB) extract mixtures. While found to significant between the other stevioside (ST), spirulina (SP) and echinacea (EC) extract compared to pomegranate extracts (PJ) and formulated pomegranate beverage (FPB) extract mixtures.

Pomegranate fruit has taken a great attention for its health benefits in the last years, numerous studies on the antioxidant activity have shown that pomegranate juice contains a higher level of antioxidant that most of the other fruit juices and beverages Seeram *et al*., (2008).

Table (4). The total phenolic, total flavonoids and antioxidant activity of pomegranate extracts (PJ), stevioside (ST), spirulina (SP), echinacea (EC) and formulated pomegranate beverage (FPB) extract mixtures

Extract and Beverage	T. Phenolics mg gallic acid/ml	T. Flavonoid mg catechin/ml	Antioxidant activity %
PJ	2.15 ± 0.15^{b}	1.76 ± 0.45^{d}	92.95 ± 0.44^{a}
EP	0.773 ± 0.22^{c}	8.063 ± 0.25^{a}	69.02 ± 0.24^{c}
SP	0.153 ± 0.34^{d}	2.599 ± 0.09^{b}	71.09 ± 0.28^{b}
SE	ND	ND	67.14 ± 0.32^{d}
FPB	2.294 ± 0.64^{a}	2.084 ± 0.55^{c}	94.54 ± 0.18^{a}

Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$), ND: Not Detect.

Sensory evaluation of formulate pomegranate beverage

Data presented in Table (5) revealed that no significant differences were noticed between the pomegranate juices and formulate pomegranate beverage in appearance, color, odors and consistency, while the other parameters including taste and overall acceptability showed a significant decrease in the mean values of pomegranate juice (PJ) compared to fresh formulate pomegranate beverage. The differences in the sensory parameters could be attributed to the different raw materials and components of the two juices.

Table (5). Sensory evaluation of formulated pomegranate beverage

	Appearance	Color	Taste	Odors	Consistency	Overall Acceptability
PJ	8.1 ± 0.05^{a}	8.0 ± 0.43^{a}	7.7 ± 0.45^{c}	8.3 ± 0.18^{a}	8.5 ± 0.15^{a}	41.0 ± 0.26^{d}
FPB	8.7 ± 0.11^{a}	8.5 ± 0.21^{a}	9.9 ± 0.41^{a}	8.6 ± 0.52^{a}	8.7 ± 0.19^{a}	45.8 ± 0.11^{a}

n=10, Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

PJ: pomegranate juice (100 %)

FPB: pomegranate juice (PJ) 85%: stevioside (SE) 5%: spirulina (SP) 4%: echinacea (EP) 6%

CONCLUSION

Based on the above results, it could be concluded that pomegranate beverage incorporated with spirulina and Echinacea extract sweetened by stevioside exhibited a potential source for antioxidant and phytochemical act as immunomodulation effect. They can be incorporated in pomegranate to enhance their flavor and introduced health promoting beverage.

REFERENCES

Amani S. Al-Rawahi, G. Edwards, Mo. Al-Sibani, G. Al-Thani2, A. S. Al-Harrasi, and M. S. Rahman (2014). Phenolic Constituents of Pomegranate Peels (*Punica granatum L.*) Cultivated in Oman. European Journal of Medicinal Plants, 4(3): 315-331.

Anton S.D., C.K. Martin, H. Han, S. Coulon, W.T. Cefalu and P. Geiselman (2010).Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite, 55:37–43.

Arnous A, D. P. Makris and P.Kefulas (2001). Effect of principal polyphenol components in relation to antioxidant characteristics of aged red wines. J. Agric. Food Chem. 49(12):5736-5742.

Blinkova LP, Gorobets OB and Baturo AP (2001).Biological activity of Spirulina: Review. Mikro. bial. Epidemiol. Immunobiol. 2:114-118.

Calin-Sanchez A., A. Figiel,.Hernandez, P. Melgarejo and A. A. Carbonell-Barrachina (2012). Convective and microwave drying influence on the chemical composition, functional properties and sensory quality of pomegranate arils and rind. II International Symposium on the in pomegranate, Options Mèditerranèe. A (103): 165-167.

Chang C., M. Yang, H. Wen and J. Chern (2002). Estimate of total flavonoid content in propolis by two complementary colorimetric methods. J.Food Drug. Anal., 10:178-182.

Chatsudthipong V. and C. Muanprasat (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacology & Therapeutics, 121:41–54.

Davey M. W, Van Montagu, M. Inze´, D. Sanmartı´n, M. Kanellis, A. Smirnoff. N., Benzie, I. J. J. Srain, J. J. Favell and D., Fletcher (2000). J. Plant L-ascorbic: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric.80:825-860.

Elfalleh, W., N Tlili, N Nasri, Y Yahia, H Hannachi, N Chaira, M Ying, A Ferchichi (2011). Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum* L) fruits. Journal of Food Science 76:707–713.

Fawole O.A; and U.L. Opara (2013). Effects of maturity status on biochemical content, polyphenol composition and antioxidant capacity of pomegranate fruit arils (CV. Bhagwa). South African Journal of Botany, 85:23-31.

Fischer U, R Carle and D Kammerer (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MS. Food Chem. 127:807–821.

Gil M.I.; F.A. Tomas-Barberan; B. Hess-Pierce; D. M. Holcroft; A. A. Kader (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem.48: 4581-4589.

Gonzalez-Molina.E; D.A. Moreno and C. Garcia-Viguera (2009). A new drink rich in healthy bioactive combining lemon and pomegranate juices. Food Chem, 115: 1364-1372.

Goupy P., **M. Hugues., P. Boivin and M. J. Amiot (1999).** Antioxidant composition and activity of barley (HordeumVulgare) and malt extracts and of isolated phenolic compounds. J. Sci. Food. Agric. 79: 1625-1634.

He, L., H. Xu, X. Liu, W. He, F. Yuan., Z. Hou and Y. Gao (2011). Identification of phenolic compounds from pomegranate (*Punica granatum* L.) seed residues and investigation into their antioxidant capacities by HPLC–ABTS+ assay. Food Research International 44: 1161–1167.

Huda-Faujan.N; A. Noriham; and A. S. Norrakiah (2009). Babjia Antioxidant of plants methanolic extracts containing phenolic compounds. Afr. J. Biotechnol. 8: 484-489.

Ivana Stanisavljević, SašaStojičević, Dragan Velićkovič, VladaVeljković and MiodragLazić (2009).Antioxidant and Antimicrobial Activities of Echinacea (*Echinacea purpurea L.*) Extracts Obtained by Classical and Ultrasound Extraction. Chinese Journal of Chemical Engineering, 17(3):478-483.

Kajimoto, G. and C. Murakami (1999).Antioxidant activity of several commercial teas and their components (in Japanese). Journal of Japanese Society of Nutrition and Food Science, 52:209–218.

Khanavi .M; M. Hajimahmoodi; M. Cheraghi-Niroomand; Z. Kargor; Y. Ajani; A. Hadjiakhoondi and M. R. Oveisi.(2009). Comparison of the antioxidant activity and total phenolic contents in some stachys species. Afr. J. Biotechnol. 8:1143-1147.

Lee .J; and R.R. Watson. (1998). Pomegranate: a role in health promotion and AIDS? Nutrition Food and AIDS; Ed.; CRC. Press: Bo Ca. Raton. FL; R.R. Wastson; 179-192.

Lee .Tzu-Tai; Ching-Chiang.Huang; Xiao-Hua.Shieh; Chung-Li. Chen; Liang –Jwu.Chen and Bi.Yu.(2010). Flavonoid, phenol and polysaccharide

contents of *Echinacea purpurea* L. and its immuno stimulant Capacity In vitro. International Journal of Environmental Science and Development. 1(1):5-9.

Lim K. **T., C. Hu and D. D. Kitts (2001).**Antioxidant activity of a Rhusverniciflua stokes ethanol extract. Food Chemical Toxicology, 39: 229-237.

Maskan, M. (2006). Production of pomegranate (*Punica granatum* L.) juice concentrate by various heating methods: color degradation and kinetics. Journal of Food Engineering, 72: 218–224.

Mattila P., **J. Astola and J. Kumpulainen (2000).** Determination of Flavonoids in Plant material by HPLC with Diode-Array and Electro-Array Detections. J. Agric. Food Chem. 48: 5834-5841.

Mensor L.L, F. S. Menezes, G.G. Leitao, A. S. Reis, T. C. Dos Santos and C. S. Coube (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15: 127-130.

Miranda, M., Cintra, R., Barros, S., and Mancini-Filho, J. (1998). Antioxidant activity of the microalga Spirulina maxima. Brazilian Journal of Medical and Biological Research, 31:1075-1079.

Ordonez A. A. L., J. D. Gomez., M. A. Vattuone and M. I. Isla (2006). Antioxidant activities of sechiumedule (Jacq) swartz extracts. Food Chem., 97:452-458.

Panichayupakaranant P, Tewtrakul S. and Yuenyongsawad S. (2010). Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract. Food Chemistry, 123:400-403.

Patel, A., Mishra, S. and Glosh, P. (2006). Antioxidant potential of C-phycocyaninisolated from cyanobacterial species Lyngbyaphormidium and Spirulina sp. Indian Journal of Biochemistry and Biophysics, 43:25-31.

Pulz, O., and Gross, W. (2004).Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology, 65:635-648.

Reza MalekiBaladi, S. Moghaddaszadeh-Ahrabi and Mohammad Afrouziyeh (2014). Extraction of phenolic composition and quantification of the total phenolic of pomegranate pomace. European Journal of Zoological Research.3 (1):133-135.

Roberto L.M., V.G. Antonio, Z.B. Liliana, K. Ah-Hen (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chem. 132:1121–1132.

SAS (1996). **SAS/ Stat Users Guide**: **Statistics, System for Windows**, version 4.10 (release 8.01 TS level 01M0), SAS Inst., Inc. Cary, North Carolina, USA

Schubert S. Y.; E. P. Lansky and I. Neeman (1999). Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. J. Ethnopharmacol., 66: 11-17.

Seeram N, R. Lee, M. Hardy and D. Heber (2005). Rapid large scale purification of ellagitannins from pomegranate husk, a by-product of the commercial juice industry. Sep. Purif. Technol. 41:49–55.

Seeram, N. P.; M. Aviram; Y. Zhang; S. M. Henning; L. Feng; M. Dreher; and D. Heber (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J. Agric. Food Chem., 56: 1415-1422.

Soliman M.D.E. and M.Y. Attia (2007). Specific phagocytic inhibition test (A new assay) in diagnosis of foods, aspirin and inhalents allergy. Sc. J. Az. Med. Fac. (Girls), 28(3):559-575.

Spolaore, P., Joannis-Cassan, C., Duran, E., andIsambert, A. (2006). Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 101:87-96.

Sundararajan A; Ganapathy R.; L. Huan; J.R. Dunlap; Webby R.J.; Kotwal G.J.; and Sangster, M. Y. (2010). Influenza virus variation in susceptibility to inactivation by pomegranate polyphenols is determined by envelope glycoproteins. Antiviral Research, 88: 1-9.

Sytar O; Brestic M.; Rai M. and Shao, H.B. (2012). Phenolic compounds for food, pharmaceutical and cosmetics production. Journal of Medicinal Plants Research, 6(13): 2526-2539.

Zhai. Z; Solco A.; Wu L.; Wurtele E.S.; Kohut M.L.; Murphy P.A. and Cunnick, J.E. (2009). Echinacea increases arginase activity and has anti-inflammatory properties in RAW 264.7 macrophage cells, in dicative of alternative macrophage activation. Of Ethnopharmacology. 122: 76-85.

Plant production and protection

Journal of Agricultural and Veterinary Sciences
QassimUniversity, Vol. 8, No. 2, pp. 177-186 (July 2015/Ramadan 1436H)

A Checklist to the Family *Chenopodiaceae* in Qassim Region, Saudi Arabia

[(1)] Wail E. Abdalla, [(1)] Gamal E.B. El Ghazali and [(2)] Abdul Rahman A. Al-Soqeer

[(1)]Faculty of Science and Arts at Al Rass, [(2)]Faculty of Agriculture and Veterinary Medicine, University of Qassim, Saudi Arabia.

(Received 24/3/2015; accepted 3/5/2015)

ABSTRACT. The aim of the present study is to provide an updated checklist of members of the family *Chenopodiaceae* in Qassim region, Saudi Arabia, a family that has been observed to be well adapted and successfully dominate many parts of this region. A total number of 38 species belonging to 15 genera were encountered in the area. This checklist comprises 28 wild species, 9 weeds of cultivation and one introduced species. This list includes species recently collected from the area in addition to specimens previously deposited at Regional Herbaria. The genus *Salsola* with 9 species was found to be the most dominant genus in the family in the area, followed by the genus *Chenopodium* with 5 species. It is hoped that this checklist will be updated continuously in an attempt to contribute to biodiversity assessment and setting strategic plans for land management, especially in arid and semiarid regions.

Keywords: Floristic studies, Checklist, Wild plants, Weeds, Introduced species, Xerophytes, Biodiversity.

Corresponding author: Wail E. Abdalla, Faculty of Science and Arts at Al Rass, E-mail address: wailelsadig@gmail.com

INTRODUCTION

Qassim region is considered as the main agricultural region in Saudi Arabia, and is located in the central part of the Saharo-Arabian floristic region (Al-Nafie, 2008). It is situated between latitudes 24° 25' – 27° 15' North and longitudes 41° 30' – 45° 41' East, and is bounded by Hail region in the North and Northwest, Al Madina region in the West, and Riyadh region in the South and East. The climate of the Qassim region is characterized by very hot dry summers and mild to cool winters. (Prince Naïf bin Abdul-Aziz International Airport, Qassim, Saudi Arabia-meteorological Airport Station number 40405) indicated that the climate in Qassim region has a mean monthly rainfall of 11.4 mm. Rain may also occur in the spring and its rainfall is very rare, and normally non-existent. Mean monthly air temperature 25.1°C, but winters are much cooler than summer. Mean monthly air temperature is 34 °C and 15 °C, in summer and winter seasons, respectively (Alharbi & Gomaa, 2014).

Floristic explorations and taxonomic studies can provide efficient and convenient information about the nomenclature, distribution, ecology, utility of various plant species, and thus about an ecosystem (Subramanyam & Nayar, 1974). Studies on the flora and fauna of any region are considered prerequisite for biodiversity assessment and setting strategic plans for land management, especially in arid and semi-arid regions.

Family *Chenopodiaceae* comprises about 102 genera and 1400 species of worldwide distribution, but commonly in xerophytic and saline habitats. To adapt to such environments, plants of *Chenopodiaceae* are mostly succulents, halophytes or xerophytes, with well developed or much reduced leaves (Perveen & Qaiser, 2012). Family *Chenopodiaceae* is represented in the main Flora of the kingdom of Saudi Arabia by 73 species belonging to 23 genera (Chaudhary, 1999). While Al- Turki (1997) documented 29 species belonging to 14 genera in the Qassim region. *Chenopodiaceae* plants, commonly known as 'Goosefoot' family, are mostly grow as weed and some are food plants like spinach, chard, beets, sugar beet and quinoa. The family was placed in the order *Caryophyllales* by Cronquist (1981), Takhtajan (1969) and Dahlgren (1975). Hutchinson (1959) and Thorne (1968, 1992) included the family in the order *Chenopodiales*, Ulbrich (1934) in the order *Centrospermae* and Bentham & Hooker (1880) in the series *Curvembryeae*. The APG (Angiosperm phylogeny group) system (1998), the APG II (2003) and APG III (2009) have included the chenopods in the family *Amaranthaceae* on the basis of evidence from molecular phylogenies (Paul, 2012).

The present study is designed to document the members of the family *Chenopodiaceae* in the Qassim region, Saudi Arabia, a family that has been observed to be well adapted and successfully dominate many parts of this region - especially at the prominent sebkhas - in order to provide pre-requisite data for biodiversity assessment and setting plans for land management in this semi-arid region.

MATERIAL AND METHODS

The present checklist constitute plant material recently collected via several field surveys conducted by the authors within the study area, voucher specimens deposited at King Suad University Herbarium (KSU), and chenopds recorded from the literature to inhabit Qassim region (Al Turki 1997; Chaudhary 1999).

Several field surveys were conducted in Qassim region, Saudi Arabia, in the period from September 2012 to January 2015, covering different seasons and various ecological habitats within the study area to collect members of the family *Chenopodiaceae*. The visited sites include: Buraydah, Uqlat As Suqur, Al Awshazayah, Unayzah, Nabhaniyah, Badaiai, Qiba, Melaida, Al Maznab, Shehiya and Rahiaya.

The collected plant material was pressed, dried using blotting papers at room temperature, and was identified with the use of available literature, and further compared with authenticated specimens at the herbarium of Botany and Microbiology department, King Saud University, Riyadh (KSU). Voucher specimens were deposited at the Faculty of Science and Arts at Al-Rass, Faculty of Agriculture and Veterinary Medicine (University of Qassim), and partly at the herbarium of Botany and Microbiology department, King Saud University, Riyadh (KSU).

The collected plant specimens were identified according to Chaudhary (1999) and Boulos (1999), and were confirmed by comparison with authenticated specimens at the herbarium of Botany and Microbiology department, King Saud University, Riyadh (KSU).

RESULTS

A total of 38 species belonging to 15 genera of the family *Chenopodiaceae* were reported to exist in Qassim region. The present checklist comprises about 28 wild species, 9 weeds of cultivation, and one species (introduced) an escaped weed in alfalfa fields (*Atriplex suberecta*). A list of these plants is presented in Table 1. The genera and the representative species in each genus were arranged alphabetically. Vernacular names (in brackets), source of the voucher specimen and/or number of herbarium specimen for each species were also included.

Table (1).

Species list	Reference and/ or Voucher Specimen No.
1- Agathophora	
A. alopecuroides (Del.) Fenzl ex Bunge (*Humaidal*)	Al- Turki (1997)
2- ***Agriophyllum***	
A. minus Fischer & C. Meyer ex Ledeb.	J. Thomas 1190/ 1993, Qassim 160 km for Riyadh (KSU Herbarium).
3- ***Anabasis***	
A. lachnantha Aellen & Rech. (*Ujrum;Ujayriman; Hurdh; Ghaslah*)	Al- Turki (1997); El Ghazali *et al.* 2/36, Awashajiya (Fac. of Sc. & Arts at AlRass Herbarium)
A. setifera Moq. (*Sha'aran; Sha'ar*)	Al- Turki (1997); El Ghazali *et al.* 18/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
4- ***Atriplex***	
A. dimorphostegia Kar. & Kir (*Rughaylah*)	Al- Turki (1997); El Ghazali *et al.* 108/ 32, Badai (Fac. of Sc. & Arts at AlRass Herbarium)
A. leucoclada Boiss. (*Rughal*)	Al- Turki (1997); El Ghazali *et al.* 8/36, widespread (Fac. of Sc. & Arts- AlRass)
A. suberecta Verd.	El Ghazali *et al.* 104/32, Badai (Fac. of Sc. & Arts at AlRass Herbarium); M Al-Saweed, 22195/2013, Al-Ghat, Qassim (KSU Herbarium).
5- ***Bassia***	
B. arabica (Boiss.) Maire & Weiller**.**	Al- Turki (1997).
B. eriophora (Schrader) Asch. in Schweinf.	Al- Turki (1997); El Ghazali *et al.* 113/32, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
B. muricata (L.) Asch. in Schweinf. (*Dhinnaban; Hartabeel; Haytham*)	Al- Turki (1997); El Ghazali *et al.* 109/32, widespread (Fac. of Sc. & Arts at AlRass Herbarium)

Continue Table (1).

6- *Beta*	
B. vulgaris L. subsp. ***maritima*** (L.) Arcang. (*Salq Barri*).	Al- Turki (1997); El Ghazali *et al.* 33/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
7- *Chenopodium*	
C. acerifolium Andrz. (*Zorbeih*).	Chaudhary (1999), Al- Turki (1997)
C. album L. (*Zorbaih*)	Al- Turki (1997); El Ghazali *et al.* widespread (Fac. of Sc. & Arts at AlRass Herbarium)
C. ambrosioides L.	El Ghazali *et al.* 30/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
C. glaucum L. (*Ghubaira; Zorbaih*)	El Ghazali *et al.* widespread (Fac. of Sc. & Arts at AlRass Herbarium)
C. murale L. (*Zorbaih*)	Al- Turki (1997); El Ghazali *et al.* 31/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
8- *Cornulaca*	
C. aucheri Moq.	Al- Turki (1997)
C. monacantha Del. (*Huwaydhan; Ha'adh*) Fig. 1B	Al- Turki (1997); El Ghazali *et al.* 11/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
9- *Halothamnus*	
H. bottae Jaub. & Spach. (*Tahama; Hamadal Arnab; Tihyan*).	Al- Turki (1997); El Ghazali *et al.* 7/36, Melaida (Fac. of Sc. & Arts at AlRass Herbarium)
H. iraqensis Botsch. (*Tahama; Qadqad*) Fig. 1A	Al- Turki (1997); El Ghazali *et al.* 1/36, Awashajiya (Fac. of Sc. & Arts at AlRass Herbarium)
H. lancifolius (Boiss.) Kothe-Heinrich	Migahid A., 12814 /1976 (KSU Herbarium), Buraidah, (as *Aellenia lancifolia* (Boiss.) Ulbr.)

Continue Table (1).

10- *Haloxylon*	
H. salicornicum (moq.) Bunge ex Boiss. (*Rimth*) Fig. 1C	Al- Turki (1997); El Ghazali *et al.* 12/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
H. persicum Bunge ex Boiss. (*Ghada*) Fig. 1D	El Ghazali *et al.* 13/36, Badai (Fac. of Sc. & Arts at AlRass Herbarium)
11- *Salicornia*	
S. europaea L.	Al- Turki (1997); El Ghazali *et al.* 4/36, Awashajiya (Fac. of Sc. & Arts at AlRass Herbarium)
12- *Salsola*	
S. arabica Botsch.	El Ghazali *et al.* 24/36, Melaida (Fac. of Sc. & Arts at AlRass Herbarium)
S. cyclophylla Baker (*Ara'ad*) Fig. 2C	Al- Turki (1997); El Ghazali *et al.* 21/36, Al rass (Fac. of Sc. & Arts at AlRass Herbarium)
S. drummondii Ulb.	El Ghazali *et al.* 27/36, Melaida (Fac. of Sc. & Arts at AlRass Herbarium)
S. imbricata Forssk. (*Khareet; Harm*) Fig. 2B	Al- Turki (1997); El Ghazali *et al.* 19/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
S. incanescens C. Meyer in Eichw.	Al- Turki (1997) (as *S. volkensii* Schweinf. & Asch.)
S. jordanicola Eig.	Al- Turki (1997)
S. schwenifurthii Solm-Laub. (*Hamth*) Fig. 3B	Al- Turki (1997); El Ghazali *et al*. 26/36, Melaida (Fac. of Sc. & Arts at AlRass Herbarium)
S. spinescens Moq. (*Ara'ad; Jerram*).	Al- Turki (1997)
S. villosa Schults in Roem & Schultes (*Rautha*) Fig. 2D	Al- Turki (1997); El Ghazali *et al.* 9/36, Melaida (Fac. of Sc. & Arts at AlRass Herbarium)

Continue Table (1).

13- *Seidlitzia*	
S. rosmarinus Bunge ex Boiss. (*Shinan; Ushnan; Duwwayd*) Fig. 2A	Al- Turki (1997); El Ghazali *et al.* 14/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
14- *Suaeda*	
S. aegyptiaca (Hasselq.) Zoh. (*Mulleih; Suwwad; Hatlas; Qulman; Khareiza*) Fig. 3A	Al- Turki (1997); El Ghazali *et al.* 28/36, Bekaria (Fac. of Sc. & Arts at AlRass Herbarium)
S. fruticosa Forssk. ex J.F. Gmelin**.**	El Ghazali *et al.* 25/36, Melaida (Fac. of Sc. & Arts at AlRass Herbarium)
S. vermiculata Forssk. ex J.F. Gmelin in L. (*Swwad; Tahma*) Fig. 3C	Al- Turki (1997); El Ghazali *et al.* 16/36, widespread (Fac. of Sc. & Arts at AlRass Herbarium)
15- *Traganum*	
T. nudatum Del. (*Dhumran*)	Al- Turki (1997)

DISCUSSION

A total number of 38 species belonging to 15 genera of the family *Chenopodiaceae* were encountered in the Qassim region, Saudi Arabia.

Al-Turki (1997) reported 29 species belonging to 14 genera, 25 species belonging to 13 genera were already deposited at the Herbarium of Botany and Microbiology Department, King Saud University, Riyadh (KSU), while 28 species belonging to 12 genera were collected by the authors of this study. Nine species were reported by El Ghazali & El Soqeer (2013) as weeds of cultivation in the study area.

One species, *Salsola volkensii* Schweinf. & Asch., which is reported by both Chaudhary (1999) and Al- Turki (1997), is now considered as a synonym for *S. incanescens* C. Meyer in Eichw., a species which is reported by Chaudhary (1999) but not mentioned by Al- Turki (1997).

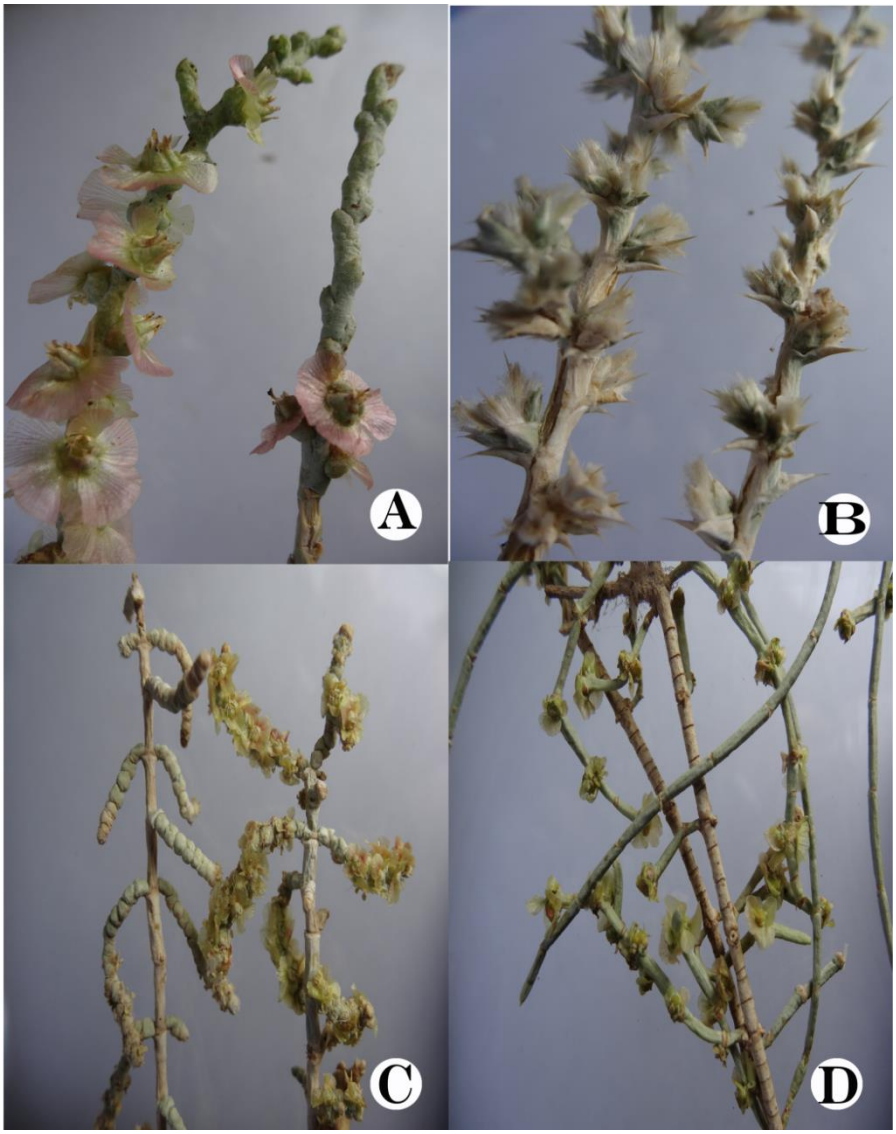


Fig.(1). A: ***Halothamnus iraqensis*****, B:** ***Cornulaca monacantha*****, C:** ***Haloxylon salicornicum*****, D:** ***Haloxylon persicum*****.**

Floristic studies usually provide useful data for setting strategic plans for land management, especially in arid and semi-arid regions. Such regions are characterized by low and erratic rainfall, high evapo-transpiration, shallow soils with

low water holding capacity and low soil fertility (Mwamburi & Musyoki, 2010). Thus, the ecosystem can easily be affected by mismanagement. These plants play a vital role in providing fodder to livestock, fuel wood for the inhabitants, and maintaining the land from degradation.

The realized dominance of members of the family *Chenopodiaceae* in such semi-arid region might be attributed to the ecological adaptations of these species. As mentioned by Perveen & Qaiser (2012), members of this family are mostly succulents, halophytes or xerophytes, with well developed or much reduced leaves.

It is recommended that more eco-taxonomical studies to be conducted in the area, to nominate the dominant communities and species and to investigate the characters and adaptations that helped them to succeed in such an ecosystem.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the generous financial support provided by the Deanship of Scientific Research, Qassim University. Thanks are also due to the staff of the herbarium of Botany and Microbiology department, King Saud University, Riyadh (KSU), for their cooperation.

REFERENCES

Alharbi, A.B. and Gomaa, F.A. (2014). Physio-chemical properties of airborne particles collected from some locations in Qassim region. *Life Science Journal*, **11**(9): 1031.

Al-Nafie, A.H. (2008). Phytogeography of Saudi Arabia. *Saudi Journal of Biological Sciences*, **15**(1): 159- 176.

Al-Turki, T.A. (1997). A preliminary checklist of the flora of Qassim, Saudi Arabia. *Fedd. Rep.* **108**(3-4): 259-280.

APG; Angiosperm Phylogeny Group (1998). An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* **85**:514-533.

APG II (2003). An update of the Angiosperm phylogeny group classification for the orders and families of flowering plants. *Bot. J. Linn. Soc.* **141**: 399 – 436.

APG III (2009). An update of the Angiosperm phylogeny Group classification for the orders and families of flowering plants. *Bot. J. Linn. Soc.* **161**(2): 105 – 121.

Bentham, G. and Hooker, J.D. (1880). Genera Plantarum. **3**: 43 – 78. L. *Reeve & Co.,* Williams & Norgate, London.

Boulos, L. (1999). Flora of Egypt, *Al Hadara Publishing*, Cairo, **1**: 92-129.

Chaudhary S.A. (1999). Flora of the Kingdom of Saudi Arabia. *Ministry of Agriculture and Water*, Riyadh, Saudi Arabia, **1**: 157-233.

Cronquist, A. (1981). An integrated system of classification of flowering plants. *Columbia University Press*, New York.

Dahlgren, R.A. (1975). A system of classification of the Angiosperms to be used to demonstrate the distribution of characters. *Bot. Notiser,* **128**: 119 – 147.

El Ghazali, G.E.B. and Al-Soqeer, A.A. (2013). A Checklist of the weed Flora of Qassim Region, Saudi Arabia. *Australian Journal of Basic and Applied Sciences*, **7**(2): 900-905.

Hutchinson, J. (1959). The families of flowering plants. ed. 2, vol. I & II. *Oxford University Press*, UK.

Mwamburi, A. and Musyoki, J. (2010). Improving tree survival in the dry lands of Kenya: A guide for farmers and tree growers in the dry lands. *Kenya Forestry Research Institute* (KEFRI) Information Bulletin, **2:** 11.

Paul, T.K. (2012). A synopsis of the family *Chenopodiaceae* in India. *Pleione,* **6**(2): 273- 297.

Perveen, A. and Qasier, M. (2012). Pollen flora of Pakistan- LXX: *Chenopodiaceae*. *Pak. J. Bot,* **44**(4): 1325- 1333.

Subramanyam, K. and Nayar, M.P. (1974). Plant taxonomy – its past role and future lines of action in India. *Bull. Bot. Surv. India* **13**:147.

Takhtajan, A. (1969). Flowering Plants – Origin & Dispersal. Oliver & Boyd. *Edinburge*.

Thorne, R. (1968). Synopsis of a putatively phylogenetic classification of the flowering plants. *Aliso,* **6**(4): 57 – 66.

Thorne, R. (1992). Classification and geography of the flowering plants. *Botanical Rev.*, **66**: 441 – 647.

Ulbrich, E. (1934). *Chenopodiaceae*. In: Engler and Prantl., *Die. Nat. Pflanzenfam*., **16c**: 377 – 584. Duncker & Humbolt, Berlin.

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 187-200 (July 2015/Ramadan 1436H)

Effect of Mother Bulb Size and Planting Space on Seed Production of Onion (*Allium cepa*, L.) Cultivar Giza 6 Mohassan

El-Damarany, A.M.[1], El-Shaikh, K.A.A.[1], Obiadalla-Ali, H.A.[1,2], and Abdel-Kader, M.M.[1]

[1]*Horticulture Department, Faculty of Agriculture, Sohag University, Sohag 82786, Egypt.*

[2]*Plant Production and Protection Department, College of Agriculture and Veterinary Medicine, Qassim University, P. O. 6622, 51452. Buryadh, Kingdom of Saudi Arabia*

(Received 22 /4/2015; accepted 22/6/2015)

ABSTRACT. This study was carried out at the experimental farm of Agriculture Faculty, Sohag University during two successive seasons, 2010/2011 and 2011/2012, to investigate the effect of plant spacing and bulb size on seed production of onion cultivar Giza 6 Mohassan. Three different plant spacing (30cm, 40cm and 50 cm) and three bulb size (3:4.5cm, 4.5 >: 6cm and 6 >: 7.5cm) were used in this study. The planting distance between rows was 50 cm. The new Duncan's multiple range tests showed that the mother bulb size factor had significant effect on all traits. Moreover, planting space significantly affected seed yield parameters except days to sprout. The maximum seed yield per feddanwas recorded withthe large bulb size and the medium planting space.Seed yield per plant and per feddanwereinfluenced by days to sprout, plant height, number of leaves per plant, number of stalks per plant, days to flowering, flower stalks height and weight of seeds per umbel. This study is useful for plant breeders and all those interested in the field of onion seed production and application of such investigations may be encourage other researchers to produce seeds in other cultivars.

***Keywords*:** Bulb Size, Plant Spacing, Onion Seed Production, Coloration.

Corresponding author:Hazem A. Obiadalla-Ali, Department of Horticulture, Faculty of Agriculture, Sohag University, Sohag, Egypt. E-mail: drhazemaoa@yahoo.com

INTRODUCTION

Onion (*Allium cepa*, L.) is one of the most important vegetable crops in the world for the various aspects of economic, health and food. The earliest record comes from Egypt where onion appear as carvings on pyramid walls and in tombs from the third and fourth dynasties (2700 BC) (**Tackholm and Drar, 1954**). Onion's name derived from "Onia" which probably ranked to a city built in 1703 B.C. near the Gulf of Suez (**Dawar *et al.*, 2007**). It is widely used to increase taste of different type of food and considered as a rich source of carbohydrates, proteins and vitamin C besides minerals like phosphorus and calcium (**Jaggi, 2005**) and prevents stomach carcinoma (**Dorant *et al.*, 1996**). These pharmacological effects of onion can be ascribed both organosulfur compounds which are responsible for the typical odor and flavor and flavonoids (**Kato *et al.*, 1983; Deschner *et al.*, 1991**).

In Egypt, onion is considered one of the major sources for hard currency, due to the early availability for foreign markets as well as its higher quality compared to other onion due to high nutritional value and pungency. The annual onion production in Egypt was 2304 thousand (MT) represent 484 $ million as income in 2011 compared to 1486 thousand (MT) represent income of $ 312 million in 2007 according to **FAO (2011)**. Sohag Governorate produced 331 thousand tons represented 15% from the total production as mentioned by the yearly book of economics and statistics of the **Ministry of Agriculture (2011).**

Agricultural production and productivity are influenced by several factors. Seed is one of the vital input factors which significantly helps in enhancing the productivity of crops. The seed production of onion is very difficult phenomena as it is produced in two phases. In first phase, the bulb production is required, while under second phase the seed production takes place from the bulbs.

Giza 6 Mohassan onion cultivar is a high yielding and popular in Sohag Governorate. It is yellow skinned having flat bulbs. It has got excellent keeping quality, early exportation and dehydration process. It is consider the best cultivar inUpper Egypt according to **Ministry of Agriculture (2011)**.

Bulb-to-seed method is appropriate for onion seed production **(Rahim *et al.*, 1982)**. Bulb size is one of the most important factors affectingonion seed production **(Nehra *et al.*, 1988; Abedin, *et al.*, 1999; Karim *et al.*, 1999; Mirshekari and Mobasher 2006; Khodadadi, 2009; Asaduzzaman *et al.*, 2012 and Khodadadi, 2012).** As well as, plant spacing isthe key factor in producing qualitativeonion seeds **(Barakauskienzi *et al.*, 2002; Mirshekari and Mobasher, 2006; Elhag and Osman 2013).** The interaction between bulb size and plant spacing was studied by many investigators **(Lial *et al.*, 1987; Ali *et al.*, 1998; Baloch*et al.*, 1998; Navab *et al.*, 1998; Singh and Sachan, 1999a, b)**

The target of onion production can not be achieved due to theacute crisis of onion seeds. Improved seed contributes substantially to enhance crop yield as high as 30% **(Shaikh *et al.*, 2002).**

Due to the lack of adequate information on different aspects of seed production, such as proper size of mother bulbs, the growers are reluctant to seed production. Bulb size and plant spacing are the key factors in producing onion seeds of high quality (**Mirshekari and Mobasher, 2006**). However, the information on bulb size and plant spacing of the cultivar Giza 6 Mohassan is limitedin respect of seed production. Therefore, the present study was conducted to investigate the effects of bulb size and plant spacing on seed production of onion cultivar Giza 6 Mohassan under Sohag conditions.

MATERIALAND METHODS

Location:

The present study was conducted during the during two successive seasons of2010/2011 and 2011/2012 at the experimental farm of Agriculture Faculty, Sohag University, Sohag, Egypt where the soil is newly reclaimed. Physical and chemical properties of used soil are shown in Table (1).

Table (1): Soil characterization of the experimental site

Sampling depth	E.C. (1:5) dSm^{-1}	pH (H_2O) (1:2.5)	O.M %	$CaCO_3$ %	Clay %	Silt %	Sand %	Soil Texture	Total N (%)	$NaHCO_3$-P ppm	Available K ppm
0 - 25	0.21	7.35	2.51	11.27	29.70	23.12	47.18	SCL	0.199	8.3	374
25 - 45	0.15	7.73	0.09	52.15	3.19	6.00	90.81	S	0.053	19.5	178
45 - 65	0.19	7.90	0.40	55.49	2.90	7.18	89.92	S	0.004	19.9	144
65 - 80	0.20	7.85	0.31	22.50	2.60	7.22	90.18	S	0.004	6.5	102

SCL= Sandy Clay Loam, S= Sand, $NaHCO_3$-P= $NaHCO_3$-P extractable-P.

Experimental details:

Giza 6 Mohassan Onion cultivar was used in this study. Three onion bulb size (S_1= 3:4.5cm, S_2= 4.5 >: 6cm and S_3= 6>: 7.5cm) and three planting spaces (P_1=

30cm, P_2= 40cm and P_3= 50cm) were used in this study. The treatments were arranged as a split-plot in randomized complete-blocks (RCB) design with three replicates. The three onion bulb size were arranged in the main plots and three planting space were assigned in the sub-plots.

Each experimental unit was 6.3 m^2 consisted of three ridges 70 cm apart and 3 m length. Bulbs were planted on 10 November in both seasons. Recommended cultural procedures other than the applied treatments were followed. The mature umbels were harvested in the morning with a small portion of flowering stalk between 15 and 25 April in both seasons, when 15-20% of the fruits exposed black seeds.

Data collection:

Days to sprouting: measured by registeringthe number of days from planting until 50 % of bulbs having sprout. Plant height (cm) and number of leaves per plant wererecorded from 5 randomly sampled plants per plot at 90 days from planting. Number of stalks per plantand flower stalks height (cm) wererecorded from 5 randomly sampled plants per plot at 140 days from planting. Days to flowering wasmeasured by registeringthe number of days from planting until 50% of the plants havingthe first opened flower. Umbels diameter (cm) was measured using Vernier Caliper after 140 days from planting. Weight of seeds per umbel(g) was recorded from 5 randomly selected plants after the completion of flowering. One thousand seeds were counted from each plot then weighed with electric balance in gram up to two decimal units. Seed yield per plant (g) was recorded after harvest from 5 randomly sampled plants per plot. Seed yield per feddan (kg, feddan is equal 4200 square meters, which is approximately equal to 0.42 of a hectare) was measured after harvest by converting the respective seed yield per plot. Weight of 1000 seeds (g)was recorededafter harvestby anelectric balance. For the determination of germination (%) percentage three dishes of 50 seeds were cultivated per treatment. The dishes were closed and placed in the germinator at the temperature 20◦C. Germination percentage was calculated according to the following equation:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of cultivatedseeds}} \times 100$$

Statistical analysis

Data obtained during the two seasons of the study were statistically analyzed and treatments means were compared using the Duncan's multiple range tests **(Gomez and Gomez, 1984)**.

RESULTS AND DISCUSSION

Effect of mother bulb Size

Different sizes of onion mother bulb showed significant effect on growth and yield components (Table 2, 3 and 4). The maximum days to sprouting (9.92), the highest plant height (87.83cm), number of leaves per plant (81.72) and number of stalks per plant (7.44) were produced by large mother bulb, which was significantly higher than the medium and small sized bulb (Table 2). The maximum days to flowering (122.8A), flower stalk height (97.51cm), umbel diameter (8.28cm) and weight of seeds per umbel (3.65g)were obtained from the large mother bulb, which was significantly higher than the medium and small sized bulb (Table 3). Similar trend was found in Seed yield per plant (27.38 g), seed yield per feddan (409.0 kg), weight of 1000 seed (4.31 g)and germination percentage (87.45%) were obtained from large mother bulb, which wassignificantly different from the two other sizes of mother bulb (Table 4). The superiority of large bulbs may be related to itshigher food reserves. These results are in harmony with those reported by **Nanendra and Ahmed (2005), Khan *et al.*, (2005)** and **Maria and Roman (2009).**

Whereas, the minimum effect of mother bulb size on days to sprouting, plant height, number of leaves per plant, number of stalks per plant, days to flowering, flower stalk height, umbel diameter, weight of seeds per umbel, seed yield per plant,seed yield per feddan, weight of 1000 seed andgermination percentage (6.92, 74.26cm, 59.79, 4.67, 112.4, 80.36cm, 4.90cm, 2.69g, 12.84g, 197.9kg, 3.96g, 85.11%, respectively) were found withsmall mother bulbs.

Effect of planting space

No significant effect was found for planting spaces on days to sprouting (Table 2). While using different planting spaces had significant affect on plant height, number of leaves per plant, number of stalks per plant, days to flowering, flower stalk height, umbel diameter, weight of seeds per umbel, seed yield per plant,seed yield per feddan,weight of 1000 seed andgermination percentage (Table 2, 3 and 4). The significant variation in plant height (83.91cm) was obtained from the widest space, and the lowest plant height (78.42cm) was observed from the bulbs spaced with the closest space.Also, the maximum number of leaves per plant (77.98) was recorded by planting bulbs at 50cm (P_3), while the lowest value (68.48) produced by the closest space. The highest values for number of stalks per plant, days to flowering, flower stalk height, umbel diameter, weight of seeds per umbel, seed yield/plant,weight of 1000 seed andgermination percentage (6.73, 118.7, 92.96cm,7.52cm, 3.56g, 24.4g, 4.26g and 87.33% respectively) were obtained from mother bulbs planted at the widest space, which were significantly different from the two other planting spaces (Table 2, 3 and 4).

On the contrary, the closest space gave the maximum value for seed yield per feddan (322.8 kg) trait whereas the minimum seed yield per feddan (292.1 kg) obtained from planting the bulbs at the widest space. Plants of the widest spacing produced more green leaf probably due to the less competition onnutrients, light, space, and moisture. Later on, these leaves accumulated photosynthate and ultimately encouraged producing more number of flowering stalk,**Singh and Sachan (1999a, b).**

On the other hand, planting onion bulbs at 30cm (P_1) significantly decreased all growth and yield parameters including plant height, number of leaves per plant, number of stalks per plant, days to flowering, flower stalk height, umbel diameter, weight of seeds per umbel, seed yield per plant,weight of 1000 seed and germinationpercentage. Except seed yield per feddan, which was the opposite of that, as well as daysto sprouting parameter was not affected by using different planting spaces.

Interaction effect of bulb size and spacing

Significant variations from the interaction effect of bulb size and planting spacing was observed on both vegetative growth and yield component parameters(Table 2, 3 and 4). Interaction between (S_3× P_3) gave the maximum days to sprouting (10.03), whereas the earliest sprouting (6.76) was registered when the smallest bulbs wereplanted at widest space.The interaction between the largest bulb size (S_3) and thewidest planting distance (P_3) gave the tallest plants (92.61cm) which were significantly higher than the other interactions. On the contrary, the shortest plants (74.11cm) were obtained from the interaction between the smallest bulbs andthe closest planting space (Table 2). These results could be attributed to the effect ofnarrow spacing on the reduction of light intensity which encouraged IAA synthesis (**El-Khayat and Zaghloul, 1999**). The increase in IAA concentration in stem tissues caused cell enlargement and hence plant height**(Uggla *et al.*, 1998)**. These results are in harmony with those reported by **Dharmendra *et al.*, (2001) and Dawar *et al.*, (2007).**The highest number of leaves per plant (92.67) and number of stalks per plant (8.42) were obtainedfromthe largest bulb size (S_3) widest planting distance (P_3), which were significantly higher than the other interactions, while, the lowest values (59.52 and 4.65 respectively) were recorded from the interaction between the smallest bulbs with the closest planting space (Table 2).

The maximum period for flowering (123.0 days) was recorded from the interaction (S_3 × P_3);the largest bulbs with the widest planting space which wassignificantly higher than the other interactions. The longest flower stalks (102.5cm) was observed on plants developed from the interaction (S_3 × P_3), while (S_1 × P_1) gave the shortest (79.50cm) flower stalks (Table 3). The present results are in agreement with those found by **Thapa *et al.*, (2004).**The maximum umbel diameter (9.73 cm) was obtained from plants emerged from the large size bulbs and thewidestspace (Table 3), while, the lowest values were recorded from the interaction (S_1×P_1). The same result was reported by **Asaduzzaman *et al.*, (2012).**The combination between the two studied factors indicated that the interactions significantly affected yield components.The highest weight of seeds perumbel, seed yield per plant, weight of 1000 seeds and the germination percentage (4.14g, 34.38g, 4.52g and 88.90%, respectively) were obtain from the largest bulb size with the widest planting distance (Table 3 and 4) which were significantly higher than the other interactions. The heaviest weight of seed yield per feddan (427.2 kg) was obtained from the interaction (S_3 × P_2);the largest bulb size with the medium planting space (Table 4 and Figure 1).

Table (2). Effect of bulb size, planting space and their interaction on days to sprouting, plant height, number of leaves per plant and number of stalks per plant of onion cultivar Giza 6 Mohassan in both growing seasons

Treatments		Days to Sprouting		Plant height (cm)		Number of leaves/plant		Number of stalks /plant	
		2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012
Bulb sizes	S_1	6.96 c	6.92 c	74.97 c	74.26 c	61.30 c	59.79 c	4.67 c	4.86 c
	S_2	8.41 b	8.55 b	83.10 b	83.20 b	75.88 b	74.19 b	6.41 b	6.20 b
	S_3	9.86 a	9.92 a	87.13 a	87.83 a	81.72 a	80.87 a	7.44 a	7.42 a
Planting paces	P_1	8.41 a	8.56 a	78.97 c	78.42 c	68.48 c	67.02 c	5.49 c	5.47 c
	P_2	8.41 a	8.37 a	82.53 b	82.96 b	72.43 b	71.56 b	6.39 b	6.33 b
	P_3	8.42 a	8.47 a	83.88 a	83.91 a	77.98 a	76.27 a	6.73 a	6.67 a
Bulb sizes *Planting spaces	S_1*P_1	7.00 c	7.12 c	74.94 e	74.11 e	60.72 e	59.52 f	4.65 g	4.65 g
	S_1*P_2	7.12 c	6.88 c	74.52 e	74.16 e	61.47 e	60.05 f	4.71 fg	5.03 f
	S_1*P_3	6.76 c	6.76 c	75.44 e	74.53 e	61.72 e	59.80 f	4.92 f	4.90 fg
	S_2*P_1	8.45 b	8.55 b	80.45 d	80.48 d	72.13 d	71.12 e	5.74 e	5.64 e
	S_2*P_2	8.33 b	8.45 b	83.92 c	84.51 c	75.93 c	73.50 d	6.65 c	6.14 d
	S_2*P_3	8.45 b	8.65 b	84.92 c	84.59 c	79.57 b	77.95 c	6.85 c	6.82 c
	S_3*P_1	9.77 a	10.00 a	80.97 d	80.67 d	72.60 d	70.42 e	6.08 d	6.14 d
	S_3*P_2	9.75 a	9.78 a	89.17 b	90.22 b	79.90 b	81.12 b	7.82 b	7.82 b
	S_3*P_3	10.03 a	9.98 a	91.27 a	92.61 a	92.67 a	91.07 a	8.42 a	8.30 a

Means followed by different letter are significantly different at 5% level of significance.

Table (3). Effect of bulb size, planting space and their interaction on days to flowering, flower stalk height, umbel diameter and weight of seeds per umbel of onion cultivar Giza 6 Mohassan in both growing seasons

Treatments		Days to Flowering		Flower stalk height (cm)		Umbel diameter (cm)		Weight of seeds/umbel (g)	
		2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012
Bulb sizes	S_1	113.9 c	112.4 c	80.36 c	4.92 c	4.92 c	4.90 c	2.69 c	2.70 c
	S_2	118.3 b	115.8 b	89.66 b	7.20 b	7.20 b	7.17 b	3.31 b	3.44 b
	S_3	122.8 a	121.3 a	97.09 a	8.28 a	8.28 a	8.14 a	3.63 a	3.65 a
Planting spaces	P_1	117.9 b	116.2 a	85.57 c	6.08 c	6.08 c	6.08 c	2.91 c	2.99 c
	P_2	118.4 ab	116.5 a	89.14 b	6.80 b	6.80 b	6.64 b	3.21 b	3.23 b
	P_3	118.7 a	116.8 a	92.39 a	7.52 a	7.52 a	7.48 a	3.52 a	3.56 a
Bulb sizes *Planting spaces	S_1*P_1	113.3 d	112.3 c	79.50 f	4.90 f	4.90 f	4.86 e	2.54 f	2.56 f
	S_1*P_2	114.3 d	112.3 c	80.97 f	4.93 f	4.93 f	4.90 e	2.63 f	2.64 f
	S_1*P_3	114.0 d	112.7 c	80.60 f	4.93 f	4.93 f	4.93 e	2.91 e	2.90 e
	S_2*P_1	117.7 c	115.7 b	84.33 e	6.23 e	6.23 e	6.33 d	3.02 de	3.25 d
	S_2*P_2	118.3 bc	116.0 b	90.57 d	7.47 c	7.47 c	7.23 c	3.35 c	3.43 c
	S_2*P_3	119.0 b	115.7 b	94.07 bc	7.90 b	7.90 b	7.93 b	3.56 b	3.64 b
	S_3*P_1	122.7 a	120.7 a	92.87 c	7.13 d	7.13 d	7.06 c	3.16 d	3.18 d
	S_3*P_2	122.7 a	121.3 a	95.90 b	8.00 b	8.00 b	7.80 b	3.64 b	3.61 b
	S_3*P_3	123.0a	122.0 a	102.50 a	9.73 a	9.73 a	9.57 a	4.07 a	4.14 a

Means followed by different letter are significantly different at 5% level of significance.

Table (4). Effect of bulb size, planting space and their interaction on seed yield per plant, seed yield per feddan, weight of 1000 seeds and germination percentage of onion cultivar Giza 6 Mohassan in both growing seasons

Treatments		Seed yield/plant (g)		Seed yield/feddan* (kg)		Weight of 1000 seed (g)		Germination (%)	
		2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012	2010/ 2011	2011/ 2012
Bulb sizes	S_1	12.84 c	13.13 c	197.9 c	202.5 c	3.96 b	3.99 c	85.11 c	85.56 c
	S_2	21.35 b	21.42 b	324.8 b	327.0 b	4.26 a	4.27 b	86.44 b	86.48 b
	S_3	27.35 a	27.38 a	408.1 a	409.0 a	4.29 a	4.31 a	87.37 a	87.45 a
Planting spaces	P_1	16.14 c	16.59 c	322.8 a	331.8 a	4.07 c	4.09 c	84.85 b	85.15 b
	P_2	21.07 b	20.89 b	315.9 a	313.3 b	4.18 b	4.22 b	86.96 a	87.00 a
	P_3	24.34 a	24.45 a	292.1 b	293.4 c	4.25 a	4.26 a	87.11 a	87.33 a
Bulb sizes *Planting spaces	S_1*P_1	11.81 h	11.90 i	236.3 e	237.9 f	3.97 d	3.99 gh	84.67 de	85.33 c
	S_1*P_2	12.39 h	13.29 h	185.8 f	199.3 g	3.92 e	4.01 g	85.33 cd	85.33 c
	S_1*P_3	14.32 g	14.20 g	171.8 f	170.4 h	3.99 d	3.98 h	85.33 cd	86.00 c
	S_2*P_1	17.34 f	18.33 f	346.8 c	366.6 c	4.23 c	4.24 e	85.56 c	85.56 c
	S_2*P_2	22.33 d	21.08 d	334.8 c	316.2 d	4.26 c	4.27 d	86.78 b	86.78 b
	S_2*P_3	24.39 c	24.84 c	292.7 d	298.1 e	4.29 c	4.30 c	87.00 b	87.11 b
	S_3*P_1	19.26 e	19.54 e	385.3 b	390.8 b	4.01 d	4.03 f	84.33 e	84.56 d
	S_3*P_2	28.48 b	28.29 b	427.2 a	424.3 a	4.37 b	4.39 b	88.78 a	88.89 a
	S_3*P_3	34.31 a	34.38 a	411.7 a	411.8 a	4.50 a	4.52 a	89.00 a	88.90 a

*Feddan is 4200 m^2, approximately 0.42 ha.

Means followed by different letter are significantly different at 5% level of significance.

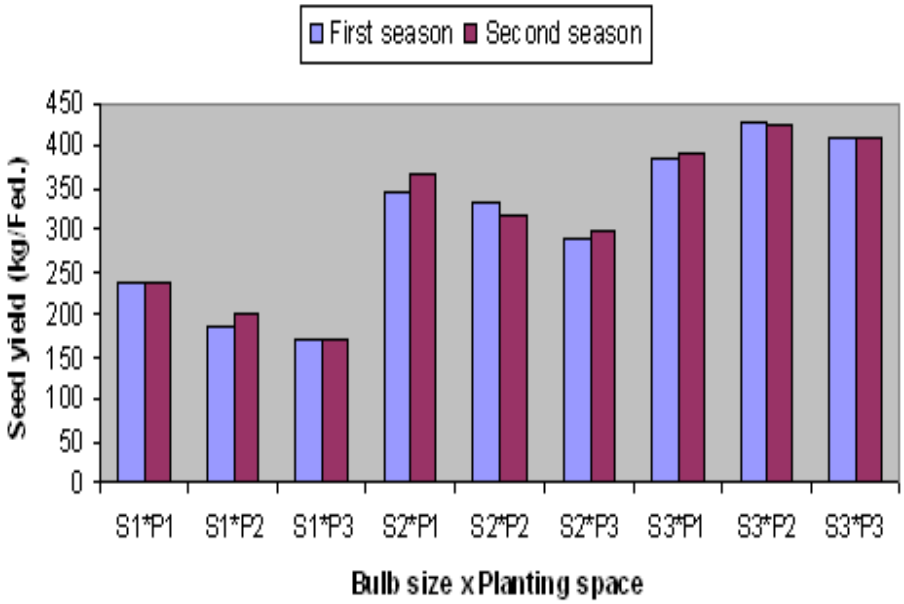


Figure (1):Interaction effect of bulb size and plant spacing on seed yield per feddan (kg) of onion cultivar Giza 6 Mohassan in both growing seasons

Correlation between traits

Relationships between 10 characters related to seed production of onion cultivar Giza 6 Mohassan are presented in Table (5). Seed yield per plant and per feddanwereinfluenced by days to sprout, plant height , number of leaves/plant, number of stalks per plant, days to flowering, flower stalks height and weight of seeds per umbel. Correlation between each of these two traits with days to Sprout, plant height, number of leaves per plant, number of stalks per plant, days to flowering, flower stalks height and weight of seeds per umbel were (0.804 and 0.935), (0.973 and 0.830), (0.986 and 0.822), (0.986 and 0.807), (0.833 and 0.904), (0.964 and 0.804) and(0.967 and 0.752), respectively. They were highly significant ($P< 0.01$).

Many researchers found one or more correlations between onion traits**(Sidhu et al. 1996; Bolandnazar 1998; Mohanty, 2000; Aklilu et al., 2001; Sultana et al., 2007 and Rafieipour et al., 2011)**.

Table (5). Phenotypic correlation coefficients among 10 traits of Giza 6 Mohassan onion cultivars sown during 2010/2011 and 2011/2012 seasons

Characters*	1	2	3	4	5	6	7	8	9	10
1	-	0.829 **	0.830 **	0.822 **	0.922 **	0.857 **	0.218 **	0.786 **	0.804 **	0.935 **
2		-	0.977 **	0.971 **	0.852 **	0.953 **	0.380 **	0.946 **	0.973 **	0.830 **
3			-	0.968 **	0.831 **	0.976 **	0.387 **	0.969 **	0.986 **	0.822 **
4				-	0.864 **	0.941 **	0.359 **	0.925 **	0.986 **	0.807 **
5					-	0.838 **	0.370 **	0.775 **	0.833 **	0.904 **
6						-	0.399 **	0.962 **	0.964 **	0.804 **
7							-	0.413	0.416	0.249
8								-	0.967 **	0.752 **
9									-	0.780 **
10										-

* 1= Days to Sprout, 2=Plant Height, 3= Number of leaves/plant, 4= Number of stalks/plant, 5= Days to flowering, 6= Flower stalks height (cm), 7= Umbels diameter(cm), 8= Weight of seeds umbel (g), 9= Seed yield/plant (g), and 10= Seedyield/feddan (kg).

* = Correlation is significant at the 0.05 level, ** = Correlation is significant at the 0.01 level

CONCLUSION

From the data presented in this study, it could be concluded that the combination between medium planting space (40 cm) and the large mother bulb size (6 >: 7.5 cm) give the highest seed yield perfeddan fromGiza 6 Mohassan onion cultivar under such conditions.Seed yield per plant and per feddan were significantly correlated with days to sprout, plant height, number of leaves per plant, number of stalks per plant, days to flowering, flower stalks height and weight of seeds per umbel.

REFERENCES

Abedin, M.J.;Rahim,M.A.;Islam,K.S. andHaider, M.A. (1999). Effect of planting date and bulb size on the yield and quality of onion seed. Bangladesh. *J. Seed Sci. Tech.*,**3**(1&2): 25-28.

Aklilu, S.;Dessalegne, L. and Currah, L. (2001). Association of characters and path coefficient analysis of seed yield and yield components in onion (*Allum cepa*L.). *Acta Agronomica Hungarica.*,**49**(2):175-181.

Ali, N.;Baloch,M. A.;Hussain,S. A. and Ali,N. (1998). Study on the effects of planting space and bulb size on seed production in onion crop. *Sharhad J. Agric.*,**14**(6): 563-568

Asduzzaman, M.D.;Hasan,M. D.;Hasan, M .D. andMoniruzzaman, M. D.(2012). Effect of bulb size and plant spacing on seed production of onion (*Allium cepa*L.). *Bangladesh J. Agric. Res.*,**37**(3): 405-414.

Baloch, M. A.;Hussain, S. A. and Ali,N. (1998). Study on the effects of planting space and bulb size on seed production in onion crop. *Sarhad J. Agric.*,**14**: 563-568.

Barakauskienzi, Z.;Dris,R. and Oladele,O.I. (2002). Effect of plant spacing and seed rate on onion quality and seed yield. *Nigerian Journal of Horticultural Sciences*,**7**(1): 22-25.

Bolandnazar, S.A.(1998). Evaluation of Effect of Onion Cutting on Yield and Quality of Onion (*Allium cepa*L.) Seed. *MSc Thesis, Faculty of Agriculture, Tabriz University,* Iran,126p

Dawar, N. M.;Wazir,F. K.;Dawar, M. and Dawar,S. H.(2007). Effect of planting density on growth and yield of onion varieties under climatic conditions of Peshawar. *Sarhad Journal of Agriculture*,**23**(4): 912-917.

Deschner, E. E.;Ruperto,J.;Wong,G. and Newmark,H. L. (1991).Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis*,**12**(7): 1193-1196.

Dharmendra K.;Singh,P.V. and Kumar,A.(2001). Effect of different levels of spacing on growth and yield of onion (*Allium cepa* L.). *India, Agric. Sci. Digest*,**21**(2): 139 – 140.

Dorant, E.;Brandt,P. A. V. D.;Goldbohm,R. A. andSturmans, F.(1996). Consumption of onions and a reduced risk of stomach carcinoma. *Gastroenterology*,**110**(1): 12- 20.

Elhag, A. Z. and Osman,H. M.(2013). Effect of Plant Spacing on Onion (*Allium cepa* L.) Seeds Quality. *Universal Journal of Applied Science*,**1**(2): 52-55.

El-Khayat, A.S. and Zaghloul, R.A. (1999). Biofertilization and Organic Manuring Efficency on Growth and Yield of Caraway Plants (*Carum carvi* L.). *Annals of Agric., Sc., Moshtohor*,**37**(2): 1379-1397.

FAO. (2011). *FAO website (faostat.fao.org)* accessed on 24th February.

Gomez, K.A. and A.A. Gomez.(1984). Statistical Procedures for Agricultural Research. *John Wiley and Sons, New York*, 680p.

Jaggi, R. C. (2005). Sulphur as production and protection agent in onion (*Allium cepa* L.). *Indian J. Agric. Sci.*,**75**(12):805 – 808.

Karim, R. M.;Ahmed,F. and Hoque,M. M.(1999). Effect of bulb size and spacing on the bulb yield of onion. *Bangladesh J. Agnil. Review*,**24**(4): 609-6 13.

Kato, R.;Nakadate,T.;Yamamoto,S. and Sugimura,T.(1983). Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion and ornithine decarboxylase activity by quercetin: possible involvement of lipoxygenase inhibition. *Carcinogenesis*,**4**(10): 1301- 1305.

Khan, M.A.;Hassan,M.K.;Ara,R.;Alam,M.M. and Brahma,S.(2005). Effect of bulb size and harvesting time on the growth and yield of onion. *Prog. Agric.*,**16**: 25–29.

Khodadadi, M.(2009). Effects of motheral bulb size, plant density and planting date on the yield and yield components of onion Ghole Ghese variety. *Abhar Islamic Azad University Pub.*, Iran, 28 pp.

Khodadadi, M.(2012). The Effects of planting date and motheral bulb size on quantitative and qualitative seed traits of onion Red Rey variety. *International Journal of Agriculture: Research and Review*,**2**(4): 324-327.

Lial, S.; Y.S Malik and U.C. Pandey. (1987). Effect of bulb size and spacing on seed production of onion. *Haryana, J. of Hort. Sci.*,**16**:164-168

Mengistu T. and Yamoah,C. (2010). Effect of sowing date and planting density on seed production of carrot (*Daucus carota* var. *sativa*) in Ethiopia. *African Journal of Plant Science*,**4**(8): 270-279.

Ministry of Agriculture(2011). The yearly book of Economics and Statistics, Egypt, winter crops section, Onion.

Mirshekari, B. and Mobasher,M.(2006). Effect of sowing date, plant density and onion size on seed yield of Azashahr Red onion variety in Tabriz. *J. Agri. Sci. Islamic Azad Univ.*,**12**(2),397-405.

Mohanty, B. K. (2000). Genetic variability, correlation and path analysis in onion seed crop. *Indian J. Hort.*,**57**: 329-333.

Morozowska M., and Hołubowicz,R.(2009). Effect of bulb size on selected morphological characteristics of seed stalks, seed yield and quality of onion (*Allium cepa* L.) seeds. *Flora Hort.*,**21**(1): 27-38.

Narendra, S. and Ahmed,Z.(2005). Influence of bulb size and spacing on onion seed production in Leh (Ladakh). *Vegetable science*,**32**(1): 101-102.

Navab, A.; BalochM.A.; Hussain S.A. andAli, N. (1998). Study on the effects of planting space and bulb size on seed production in onion crop. *Sarhad Journal of Agriculture*,**14**(6): 563-568

Nehra, B.K.;Pandita,M. L. and Singh,K. I. (1988). Cultural and nutritional studies in relation to seed production in onion (*Allium cepa* L.). II. Effect of bulb size, spacing and nitrogen on plant growth and seed yield. *Haryana J. Hort. Sci.*,**17**: 1-2.

Rafieipour M.;Azar, A. M.;Mahna, N.;Kazemnia, H. D.;Kazemiani, S. and Yarmohamadi, F. (2011). Evaluation of Genetic Variability of Six Iranian Landraces of Onion (*Allium cepa* L.) for Seed Yield and Yield Components. ISSN 1068_3674, *Russian Agricultural Sciences*,**37**(5): 385–391.

Rahim, M.A.;Hussain, A. and Siddique,M.A. (1982). Seed production ability of three onion cultivars. *Bangladesh Hort.*,**10**(1):31-38.

Shaikh, A. M.;Vyakaranahal,B.S.;Shekhargouda, M. and Dharmatti,P. R.(2002). Influence of bulb size and growth regulators on growth, seed yield and quality of onion cv. *Nasik Red. Seed Res.*,**30**(2):223-229.

Sidhu, A.S.;Kanwar,J.S. and ChadhaM.L. (1996). Seed Production Potential of Different Genotypes of Onion. *Onion Newsletter for the Tropics*,**7**:38–41.

Singh, S.R. and Sachan, B.P.(1999a). Interaction of bulb size and spacing on seed yield and yield attributing trait of onion (*Allim cepa* L.) cv. Kalyanpur Round Red. *Sci. Hort.*,**6**:125–128.

Singh, S.R. and Sachan, B.P. (1999b). Evaluation of different bulb size, spacing and varieties for higher seed yield and attributing traits on onion (*Allium cepa* L.). *Crop Res. Hissar*,**17**(3):351-355.

Sultana, M.;Islam, A. K. M.;Rasul,M. G.;Mian,M. A. K. and Hossain,T.(2007). Estimation of Correlation and Path Coefficients of Seed Yield Contributing Traits in Onion (*Allium cepa* L.). *Bangladesh J. Life Sci.*,**19**(2):133-139.

Tackholm, V. and Drar,M.(1954). Flora of Egypt. *Bulletin of the Faculty of Science Cairo University*,Vol. 3, Cairo,

Thapa, U.;Das,R.;Mandal,A. R. and Maity,T. K.(2004). Growth and seed yield of onion (*Allium cepa* L.) as influenced by spacing and bulb size. *Seed ResearchNew Delhi*,**32**(2):126-129.

Uggla, C.; Mellerowicz, E. J. and Sundberg, B. (1998). Indole-3-Acetic Acid Controls Cambial Growth in Scots Pine by Positional Signaling. *Plant Physiol.*,**117**: 113–121.

Journal of Agricultural and Veterinary Sciences
Qassim University, Vol. 8, No. 2, pp. 201-212 (July 2015/Ramadan 1436H)

Comparison of Plant Propagation Efficiency Under Hydroponic/Soilless Culture and Traditional/Soil Culture Systems

Anber M. A. Hassanein

Present address: Plant Production and Protection Dept., College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia.

Permanent address: Horticulture Dept.(Ornamental Plants), Faculty of Agriculture, Sohag University, Egypt.

(Received 16/3/2015,accepted 28/5/2015)

ABSTRACT. The work aimed to investigate plant propagation efficiency of hydroponic/soilless culture system compared to traditional/soil culture system, and the possibility of its application in the production of different ornamental plants. For the comparison, cuttings of the most important ficus species, *F. penjamina* and *F. Hawaii*, were cultivated under the two systems in two separated experiments. Three effective factors including plant species, cutting type and growth regulators type were studied under the two culture systems. Terminal and intermediate cuttings of both ficus species were cultivated hydroponically in water or traditionally in an equal mixture of sand and peat moss. Both media were supplemented with 50 ppm of (IAA), 3-indole acetic acid, (IBA), indole-3-butyric acid or (NAA), 1-naphthyl acetic acid solutions besides control treatment without any growth regulator. To investigate the application of hydroponic system in the production of different plant genera, cuttings of Schefflera and Ficus were cultivated under hydroponic system feeding by 50 ppm of IAA, IBA, NAA or water for control treatment. The studied factors had important roles in the propagation of plants via cuttings under the two culture systems. *F. penjamina* responded better than *F. hawaii*. Terminal cuttings were better under traditional system but intermediate cuttings were better under hydroponic system. Treatment of cuttings by growth regulators was essential under both systems, and IAA was the best one. A difference was also found between the two studied culture systems where traditional system was better for survival of cuttings however, hydroponic system was better for rooting and growth of cuttings. The application of hydroponic system on scheffelera proved the importance of plant type factor. The reported work showed the differences between the two culture systems, and the possibility of their application for the production of different plants. The obtained results could be of great importance for the production of plants at commercial scale by any culture system.

Keywords: Ficus, Schefflera, soilless, Cuttings, Propagation, Growth regulators, Rooting

Corresponding author: Anber M. A. Hassanein, Dept. of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, KSA, E-mail: anber100@yahoo.com

INTRODUCTION

Soil is not a proper medium for pot plants because of drainage and aeration problems. Hydroponic systems include all systems that deliver the nutrients in a liquid form. Depending on the growth media of roots, the hydroponic system may be liquid where roots are hanging into nutrient solution, or aggregate where roots grow into an inert medium such as sand, gravel or other media then irrigated with a complete nutrient solution. The hydroponic system may be open where nutrient solution is distributed from a reservoir then drained, or closed where the nutrient solution is collected and reused. Production of plants under hydroponic system has many advantages including the possibility of production on non-arable land, the isolation of plants from pests and diseases, the control of nutrients and pH, the efficient use of water and nutrients and the ease of transplanting. Hydroponic systems can also produce herbs of high quality and roots free of environmental toxins such as heavy metals in soils **(Hayden, 2006)**.

Hydroponic production of medicinal crops provided opportunities for improving quality, purity, consistency, bioactivity, and biomass production on a commercial scale. The comparison of biomass data of aeroponic vs. soilless culture or field grown production of various medicinal crops including *Arctium lappa*, *Urtica dioica* and *Anemopsis californica* proved that hydroponic systems can be used to produce very clean, high-quality herb and root crops for the natural products industry **(Hayden, 2006)**. The potential of soilless growing media for raising roselle (*Hibiscus sabdariffa*) seedlings was studied and found to be possible **(Navindra *et al.*, 2011)**. To reduce peat consumption, the effect of different soilless growing media including perlite, composted tree bark, composted tea wastes and rice husks were studied on *Ficus benjamina* as commercially grown ornamental plant **(Abouzari *et al.*, 2012)**. The water culture was suggested as a simple and efficient rooting method worthy of practical application for rooting woody cuttings. The water culture of 30 plant species showed that 20 gave almost equal percentages of rooting in water and in sand. Some species also rooted more rapidly in water and developed more vigorous roots than in sand **(Komissarov, 1968)**. Cuttings of *Ficus benjamina* as woody plant and *Chrysanthemum x morifolium* as herbaceous plant were rooted and responded similarly under aero-hydroponic systems. Cuttings of stirred water constantly rooted and formed more roots than of unstirred water. Maximum rooting occurred in misted sections suspended in the aero-hydroponics chambers (**Soffer and Burger, 1988)**.

Ornamental plants are usually produced in soil or in pots which is costly and cause many soil related problems as above described. Stem cutting is the most frequent method used for vegetative propagation of many plant species from herbaceous to woody plants. Ficus is one of the most economically important ornamental trees mainly propagated by cuttings. *F. benjamina* and *F. hawaii* are the most important species of Ficus genus for their multiple uses as ornamental, indoor, garden and roadside trees **(Wanger *et al.*, 1999; Hadia *et al.*, 2008).** It was found that cuttings of micropropagated stock plants of *Ficus benjamina* are more efficient

than cuttings of traditionally propagated stock plants **(Kristiansen, 1991)**. Success of propagation via stem cuttings is usually affected by many factors including the status of mother plant or cutting source, type of culture medium, type of cutting, rooting hormones and environmental conditions. Growth hormones and rooting media were found to be effective factors for rooting of many plant species **(Akinyele, 2010)**. In our previous work, we studied the effect of factors influencing production of plants via stem cuttings under traditional system. Our study included culture media, cutting type, growth regulators, plant type and concentration of growth regulator (**Hassanein, 2013**). However, no reference was available on the comparison of propagation systems or the effect of those factors under hydroponic system. The present work aimed to compare efficiency of traditional and hydroponic systems in the production of plant species via stem cuttings. Effective factors as plant genus, plant species, cutting type, growth regulators were studied under the two systems.

MATERIALS AND METHODS

Establishment of study

This study was conducted under plastic house conditions at the Floriculture Experimental Farm, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia during 2013 and 2014. Three separated experiments were carried out to achieve the aims of the study. Two experiments were performed on two *Ficus* species at the same time on the same factors to compare between the propagation systems; traditional and hydroponic systems. The third experiment was achieved to investigate the application of hydroponic system on other plant genera.

Comparison of propagation systems on *Ficus*

Two separated experiments were carried out at the same time to compare between hydroponic/soilless culture and traditional/soil culture systems. To study the propagation efficiency of the studied systems on the production of plants via cuttings, three factors were studied. The first factor was plant species where experiments were effectuated on two of the most economically important ficus species, *Ficus penjamina* and *Ficus hawaii*. The second factor was cutting type where, cuttings were taken from shoot tips (terminal) or shoot center (intermediate) of mother plants grown under plastic house conditions. Cuttings were of 10 cm long and contain 4-5 buds for both cutting types and both species. All leaves, except the terminal leaf, were separated from both cutting types before planting. Cuttings were planted traditionally in pots of 30 cm diameter filled with an equal mixture of sand and peat moss for traditional/soil culture system or in bored water tubes for hydroponic system **(Fig.1)**. Traditionally cultivated cuttings were irrigated similarly when needed. The third factor was growth regulator where cuttings were dipped in 50 ppm of (IAA), 3-indole acetic acid (FULKA), (IBA), indole-3-butyric acid (WINLAB) or (NAA), 1-naphthyl acetic acid (Riedel-de Haen) solutions for 30 min

or water for control treatment before traditional cultivation. For cuttings cultivated under hydroponic system, the system was fed by 50 ppm of IAA, IBA or NAA solutions or water for control treatment.

Application of hydroponic system on different genera

A separated experiment was carried out to investigate the application of hydroponic system on different plant genera. The system was applied on *Ficus penjamina* and *Schefflera arboricola* as previously described. Terminal cuttings of both species were prepared and planted in bored water tubes as above mentioned. Cuttings were fed by water (control) or 50 ppm of IAA, IBA or NAA solutions **(Fig. 2)**.

Experimental design and data analysis

The first two experiments were arranged in a split-split plot design with three factors and the third experiment were arranged in a split plot design with two factors. Fifty cuttings were cultivated in four replicates per treatment for all experiments. By the end of each experiment, one month after cultivation, viability percentage recorded as the percent of cuttings still alive or green of cultivated cuttings, rooting percentage calculated as the percent of rooted cuttings of cultivated cuttings or of viable cuttings, and growth percentage recorded as the percent of cuttings developing new leaves of the cultivated cuttings were collected from all studied treatments in all experiments. All data were subjected to analysis of variance (ANOVA) to determine significant differences followed by the comparison of means at significant level of 5% using Excel 2010.

RESULTS AND DISCUSSION

Effect of plant species

Data presented in **Table (1)** show results on the production of two ficus species, *F. penjamina* and *F. hawaii*, via cuttings under traditional/soil and hydroponic/soilless culture systems. Generally, plant species significantly affected viability, rooting and growth percentages of cuttings under both culture systems. *Ficus penjamina* showed significantly better results compared to *F. hawaii* under the two studied systems. The interaction between ficus species and cutting type also showed significant differences. Under traditional culture system, the studied species gave similar rooting and growth percentages but cuttings of *F. penjamina* showed significantly higher viability percentages. Under hydroponic culture system, intermediate cuttings of both species responded similarly but terminal cuttings of *F. penjamina* showed significantly better survival, rooting and growth percentages compared to that of *F. Hawaii*. Significant differences were also found in the response of ficus species to growth regulators under the two culture systems **(Table 1)**. The favorable effect of plant species factor on germination and growth was previously proved **(Hassanein, 2010)**. Plant species was also found to be an effective factor significantly influencing traditional production and growth of ficus species via stem cuttings **(Hassanein, 2013)**.

Effect of cutting type

Results on the effect of cutting type on the production of ficus under traditional and hydroponic system are shown in **Table (1)**. Generally, terminal cuttings showed significantly higher rooting and growth percentages under traditional culture system. However, intermediate cuttings showed higher rooting percentage under hydroponic culture system. This result can be related to the higher tolerance of intermediate cutting to water. The interaction between cutting type and plant species was mentioned above. Terminal cuttings gave higher rooting and growth percentages for both ficus species under traditional culture system. Under hydroponic culture system, terminal cuttings were better with *F. penjamina* but intermediate cuttings were better with *F. Hawaii*. The interaction of cutting type with growth regulators also showed significant effect **(Table 1)**. The higher rooting capacity of intermediate cuttings may be resulted from their higher nutrition and humidity content. Intermediate cutting is the type widely used for the propagation of woody plants **(Kristiansen, 1991)**. However, the growth speed caused by the presence of apical buds in terminal cuttings may explain their better growth. These results were also in harmony with our findings with traditionally cultivated cuttings of ficus and chrysanthemum **(Hassanein, 2013)**.

Effect of growth regulators type

Effects of growth regulators type on the propagation efficiency of two ficus species under traditional and hydroponic culture systems are illustrated in **Table (1)**. The general effect of growth regulator was significant. The treatment of cuttings with growth regulators, regardless their type, gave significantly higher viability percentage under traditional culture system and significantly higher rooting and growth percentage under hydroponic system when compared to control. Indole acetic acid (IAA) was the best growth regulator under both culture systems. Similar favorable effects were obtained (IBA). However, the lowest results were recorded on cuttings treated by naphthyl acetic acid (NAA). The interaction of growth regulators with cutting type or plant species was also significant **(Table 1)**. The role of growth regulators on production of plants via cuttings was previously reported on many plant species **(Akinyele, 2010)**. The obtained results are also in harmony with those previously obtained in our previous work effectuated in pots **(Hassanein, 2013)**. The superior effect of IAA may relate to its type as natural growth hormone produced by plants.

Comparison of propagation systems

The production of two ficus species via cuttings under traditional/soil and hydroponic/soilless culture systems as affected by cutting type and growth regulator is shown in **Table (1) and Figure (1)**. Results proved the possibility of production such species under any system. However, many differences were found between the two culture systems. Viability of cuttings under traditional system was higher (meanly 92.2 %) than that of hydroponic system (meanly 43.1 %). However, rooting percentage was higher under hydroponic system, 33.8 % as average, compared to

that of traditional system, 6.6 % as average. Cuttings also grew better under hydroponic culture system. Under traditional system, the best results were obtained when terminal cuttings were treated by IAA for both species where 20-25% of cuttings rooted and developed new leaves. Under hydroponic system, the best results were obtained when intermediate cuttings of both species were treated IAA where 80 – 100 % of cuttings rooted and 40 – 50 % developed new leaves. Similar good results could be obtained from the terminal cuttings of *F. penjamina*. These results are in harmony with those obtained by **Komissarov (1968)** where some species rooted more rapidly and developed more vigorous roots in water compared to sand. Results showed a difference between the two culture systems. A difference was previously found between aeroponic, soilless and field production of various medicinal crops **(Hayden, 2006)**.

Application of hydroponic system

Application of hydroponic system for the production of ficus and scheffelera via cuttings is shown in **Table (2) and Figure (2)**. Response of ficus cuttings was better than the response of scheffelera. The maximum rooting percentage was obtained with IAA. Under the best conditions, 50 % of ficus cuttings rooted versus only 10 % of schefflera cuttings. The comparison between the response of ficus and schefflera to similar factors showed the effectiveness of plant type factor. This result can be explained by the nature of ficus as woody tree and schefflera as soft shrub. The higher tolerance of ficus cuttings may also be another cause of this difference. We also previously reported on the difference between herbaceous and woody plants under traditional culture system **(Hassanein, 2013)**.

CONCLUSION

It can be concluded that several factors have important roles in the propagation of plants via cuttings under traditional/soil and hydroponic/soilless culture systems. Concerning plant species, *F. penjamina* responded better than *F. hawaii*. Terminal cuttings were better under traditional system but intermediate cuttings were better under hydroponic system. Treatment of cuttings by growth regulators was found to be essential under both systems, and IAA was the best one. A great difference was found between the two studied culture systems. Traditional system was better for survival of cuttings where hydroponic system was better for rooting and growth of cuttings. The application of hydroponic system on scheffelera showed different responses which proved the importance of plant type factor. The reported work showed the differences between the two culture systems and proved the possibility of their application for the production of different plants. The obtained results could be of great importance for the production of plants at commercial scale by any culture system depending on available facilities.

REFERENCES

Abouzari, A.; Rouhi, S.; Eslami, A. and Kaviani, B. (2012). Comparison of the effect of different soilless growing media on some growth characteristics of benjamin tree (*Ficus benjamina*). *Int. J. oF Agric. & Biol.*, **14**(6): 985–988.

Akinyele, A.O. (2010). Effects of growth hormones, rooting media and leaf size on juvenile stem cuttings of *Buchholzia coriacea*, Engler. *Ann. For. Res*., **53**(2): 127-133.

Hadia, H.A.; El-Mokadem, H.E. and El-Tayeb, H.F. (2008). Phylogenetic relationship of four *Ficus* species using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. *Journal of Applied Sciences Research*, **4**(5): 507-514.

Hassanein, A.M.A. (2010). Improving seed germination and seedling growth of some economically important trees by seed treatments and growing media. *Journal of Horticultural Sciences & Ornamental Plants*, **2**(1): 24-31.

Hassanein, A.M.A. (2013). Factors influencing plant propagation efficiency via stem cuttings. *Journal of Horticultural Sciences & Ornamental Plants*, **5**(3): 171-176.

Hayden, A. L. (2006). Aeroponic and hydroponic systems for medicinal herb, rhizome, and root crops. *Hortscience*, **41**(3) 16-18.

Navindra, B.; Gianjeet, D. and Joyce, G-S. (2011). Influence of soilless growing media, pot size and sieved media on the production of *Hibiscus Sabdariffa* L. seedlings. *AJAE*, **2**(5):147-154.

Komissarov, D.A. (1968). Biological basis for the propagation of woody plants by cutting. *Israel Program for Scientific Translations*. Jerusalem, 34-38.

Kristiansen, K. (1991). Post-propagation growth of cuttings from *in vitro* and *in vivo* propagated stock plants of *Ficus benjamina*. *Scientia Horticulturae*, **46**: 315-322.

Soffer, H. and Burger, D.W. (1988). Effects of dissolved oxygen concentrations in aero-hydroponics on the formation and growth of adventitious roots. *J. Amer. Soc. Hort. Sci*., **113**(2):218-221.

Wagner, W.L.; Herbst, D.R. and Sohmer, S.H. (1999). Manual of the flowering plants of Hawaii. *Bishop Museum Special Publication 83*, University of Hawaii and Bishop Museum Press, Honolulu, HI.

TABLES

Table (1) Production of ficus species from cuttings as affected by cutting and growth regulators types under two culture systems one month after cultivation

Ficus specie	Cutting type	Growth regulator	Traditional system			Hydroponic system		
			Viability %	Rooting %	Growth %	Viability %	Rooting %	Growth %
F. penjamina	Terminal	Control	100.0 a	10.0 cd	10.0 cd	60.0 c	0.0 e	0.0 e
		NAA	100.0 a	15.0 bc	15.0 bc	60.0 c	20.0 d	20.0 d
		IAA	100.0 a	25.0 a	25.0 a	60.0 c	60.0 c	40.0 bc
		IBA	100.0 a	15.0 b	15.0 b	80.0 b	80.0 b	80.0 a
	Intermediate	Control	100.0 a	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
		NAA	100.0 a	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
		IAA	100.0 a	0.0 e	0.0 e	80.0 b	80.0 b	0.0 e
		IBA	100.0 a	0.0 e	0.0 e	100.0 a	100.0 a	40.0 bc
F. Hawaii	Terminal	Control	65.0 e	5.0 de	5.0 de	50.0 c	0.0 e	20.0 d
		NAA	90.0 bc	10.0 cd	10.0 cd	0.0 e	0.0 e	0.0 e
		IAA	100.0 a	20.0 ab	20.0 ab	0.0 e	0.0 e	0.0 e
		IBA	95.0 ab	5.0 de	5.0 de	0.0 e	0.0 e	0.0 e
	Intermediate	Control	65.0 e	0.0 e	0.0 e	10.0 d	10.0 de	0.0 e
		NAA	75.0 d	0.0 e	0.0 e	80.0 b	80.0 b	30.0 cd
		IAA	100.0 a	0.0 e	0.0 e	100.0 a	100.0 a	50.0 b
		IBA	85.0 c	0.0 e	0.0 e	10.0 d	10.0 de	0.0 e
F. penjamina	---	---	100.0 a	8.1 a	8.1 a	55.0 a	42.5 a	25.0 a
F. Hawaii	---	---	84.4 b	5.0 b	5.0 b	31.3 b	25.0 b	12.5 b
---	Terminal	---	93.8 a	13.1 a	13.1 a	38.8 a	20.0 b	22.5 a
---	Intermediate	---	90.6 a	0.0 b	0.0 b	47.5 a	47.5 a	15.0 a
---	---	Control	82.5 c	3.8 b	3.8 b	30.0 c	2.5 c	5.0 c
---	---	NAA	91.3 b	6.3 ab	6.3 ab	35.0 bc	25.0 b	12.5 b
---	---	IAA	100.0 a	11.3 a	11.3 a	60.0 a	60.0 a	27.5 a
---	---	IBA	95.0 ab	5.0 ab	5.0 ab	47.5 ab	47.5 a	30.0 a

Continue Table (1)

Ficus specie	Cutting type	Growth regulator	Traditional system			Hydroponic system		
			Viability %	Rooting %	Growth %	Viability %	Rooting %	Growth %
F. penjamina	Terminal	---	100.0 a	16.3 a	16.3 a	65.0 a	40.0 a	40.0 a
F. penjamina	Intermediate	---	100.0 a	0.0 b	0.0 b	45.0 b	45.0 a	10.0 bc
F. Hawaii	Terminal	---	87.5 b	10.0 a	10.0 a	12.5 c	0.0 b	5.0 c
F. Hawaii	Intermediate	---	81.3 b	0.0 b	0.0 b	50.0 b	50.0 a	20.0 b
F. penjamina	---	Control	100.0 a	5.0 ab	5.0 ab	30.0 d	0.0 d	0.0 c
F. penjamina	---	NAA	100.0 a	7.5 ab	7.5 ab	30.0 d	10.0 d	10.0 de
F. penjamina	---	IAA	100.0 a	12.5 a	12.5 a	70.0 b	70.0 b	30.0 b
F. penjamina	---	IBA	100.0 a	7.5 ab	7.5 ab	90.0 a	90.0 a	60.0 a
F. Hawaii	---	Control	65.0 c	2.5 b	2.5 b	30.0 d	5.0 d	10.0 de
F. Hawaii	---	NAA	82.5 b	5.0 ab	5.0 ab	40.0 cd	40.0 c	15.0 cd
F. Hawaii	---	IAA	100.0 a	10.0 ab	10.0 ab	50.0 c	50.0 c	25.0 bc
F. Hawaii	---	IBA	90.0 ab	2.5 b	2.5 b	5.0 e	5.0 d	0.0 e
---	Terminal	Control	82.5 bc	7.5 bc	7.5 bc	55.0 b	0.0 e	10.0 e
---	Terminal	NAA	95.0 ab	12.5 b	12.5 b	30.0 d	10.0 e	10.0 e
---	Terminal	IAA	100.0 a	22.5 a	22.5 a	30.0 d	30.0 d	30.0 b
---	Terminal	IBA	97.5 a	10.0 b	10.0 b	40.0 c	40.0 c	40.0 a
---	Intermediate	Control	82.5 c	0.0 c	0.0 c	5.0 e	5.0 ef	0.0 c
---	Intermediate	NAA	87.5 bc	0.0 c	0.0 c	40.0 c	40.0 c	15.0 de
---	Intermediate	IAA	100.0 a	0.0 c	0.0 c	90.0 a	90.0 a	25.0 bc
---	Intermediate	IBA	92.5 ab	0.0 c	0.0 c	55.0 b	55.0 b	20.0 cd

Percentages were calculated from the cultivated cuttings (50 cuttings per treatment). Means with similar letter at the same column and partition are not significantly different at $\alpha = 0.05$.

Table (2). Production of ficus and schefflera under hydroponic system using terminal cuttings one month after cultivation

Plant genus	Growth regulator	Viability of cultivated cuttings %	Rooting of cultivated cuttings %	Rooting of viable cuttings %
Ficus	---	50.0 a	27.5 a	53.8 a
Schefflera	---	51.3 a	6.9 b	13.4 b
---	Control	60.0 a	10.0 b	30.0 a
---	NAA	37.5 b	15.0 b	37.5 a
---	IAA	65.0 a	25.0 a	30.0 a
---	IBA	65.0 a	10.0 b	15.0 b
Ficus	Control	20.0 f	10.0 cd	50.0 b
	NAA	40.0 e	30.0 b	75.0 a
	IAA	80.0 b	50.0 a	60.0 ab
	IBA	60.0 cd	20.0 bc	30.0 c
Schefflera	Control	100.0 a	10.0 cd	10.0 d
	NAA	35.0 ef	0.0 d	0.0 d
	IAA	50.0 de	0.0 d	0.0 d
	IBA	70.0 bc	0.0 d	0.0 d

Percentages were calculated from the cultivated cuttings (50 cuttings per treatment). Means with similar letter at the same column and partition are not significantly different at $\alpha = 0.05$.

FIGURES

Fig. (1). Production of two ficus species under traditional (A) and hydroponic (B) culture systems.

Fig. (2).: Production of shefflera and ficus under hydroponic culture system using terminal cuttings.