THE **IDDAADS JOURNAL** VOLUME 6 : NO 5 : DECEMBER 2015 : ISSN 0976-3104

Institute of Integrative Omics and Applied Biotechnology Journal Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.



Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief Integrative Omics and Applied Biotechnology (IIOAB) Journal



Prof. Vasco Azevedo Federal University of Minas Gerais Brazil

Editor-in-Chief

Integrative Omics and Applied Biotechnology (IIOAB) Journal Editorial Board:



Nina Yiannakopoulou Technological Educational Institute of Athens Greece



Rajneesh K. Gaur Department of Biotechnology, Ministry of Science and Technology India



Vinay Aroskar Sterling Biotech Limited Mumbai, India



Arun Kumar Sangalah VIT University Vellore, India



Bui Huy Khoi Industrial University of Ho Chi Minh City Vietnam



Moustafa Mohamed Sabry Bakry Plant Protection Research Institute Giza, Egypt



Atun RoyChoudhury Ramky Advanced Centre for Environmental Research India



Bui Phu Nam Anh Ho Chi Minh Open University Vietnam



Sanjay Kumar Gupta Indian Institute of Technology New Delhi, India



Sumathi Suresh Indian Institute of Technology Bombay, India



Rohan Rajapakse University of Ruhuna Sri Lanka

Tetsuji Yamada Rutgers University New Jersey, USA



N. Arun Kumar SASTRA University Thanjavur, India

Steven Fernandes Sahyadri College of Engineering & Management India RESEARCH ARTICLE OPEN ACCESS



EVALUATION OF CLEANING OF ISTHMUSES USING DIFFERENT IRRIGATION TECHNIQUES - AN IN VITRO STUDY

Ashwini Ghogare¹, Shail Jaggi ¹, Nitin Shah ¹, Jyoti Mandlik^{1*} and Nikhil Sharma²

1Department of Conservative Dentistry and Endodontics, Dental College and Hospital, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, INDIA 2Department of Conservative Dentistry and Endodontics, Modern Dental College, Indore, Madhya Pradesh, INDIA

ABSTRACT

Purpose: The aim of this in vitro study was to evaluate the efficacy of different irrigation systems in cleaning the isthmuses and lateral canals in the apical and middle thirds of the root canal. **Methodology:** Thirty extracted human mandibular first molar teeth were selected for the study. The distal roots of teeth were removed and mesial roots were used for the study. Radiographs of the teeth were taken in bucco-lingual and mesio-distal directions to check the presence of any lateral canals. The samples were randomly divided into three experimental groups (n=10); Group1: Manual agitation irrigation technique, i.e. 30 gauges Navi-tip. Group2: Passive ultrasonic irrigation (PUI) with Ultrasonic tip i.e. Irrisafe. Group3: EndoVac. Canals were prepared using HyFlex files and obturated with gutta flow-2 sealer and gutta purcha point. All the samples were cleared by using the method of tooth clearing adopted from Robertson et al. **Method of data analysis**: Morphological analysis was performed using a stereomicroscope to reveal details of accessory canal filling. **Results:** The samples treated with endoVac irrigation technique showed maximum number of cleaned lateral canals and isthmuses in the apical as well as in the middle third of the root canals. The PUI technique was better than manual agitation irrigation technique.

 Received on: 19th-Aug-2015

 Revised on: 28th-Sept-2015

 Accepted on: 2nd-Oct-2015

 Published on: 8th-Oct-2015

KEY WORDS

Isthumuses; Manual Irrigation techniques; Passive Ultrasonic Irrigation; EndoVac Irrigation Technique; Chemo-mechanical Preparatio,; HyFlex files; Tooth Clearing Technique; Gutta flow-2 Sealer and gutta percha

*Corresponding author: Email: drjyoti8@gmail.com; Tel: +91- 9421385056

INTRODUCTION

The success of endodontic treatment depends on the eradication of microbes from the root-canal system and prevention of reinfection. The root canal is shaped with hand and rotary instruments under constant irrigation to remove the inflamed and necrotic tissue, microbes/biofilms, and other debris from the root-canal space. The main goal of root canal instrumentation is to facilitate effective irrigation, disinfection, and filling. Several studies using advanced techniques such as microcomputed tomography (CT) scanning have demonstrated that proportionally large areas of the main root-canal wall remain untouched by the instruments, emphasizing the importance of chemical means of cleaning and disinfecting all areas of the root canal [1]. Irregularities in canal systems such as narrow isthmuses and apical deltas prevent complete debridement by mechanical instrumentation alone. An isthmus is often poorly accessible to root canal instruments, irrigants, and medications and acts as tissue and bacterial reservoir, that might account for the failure of nonsurgical root canal treatment [2]. Hence chemomechanical preparation is recommended wherein the irrigant serves as a physical flush to remove debris as well as serving as a bactericidal agent, tissue-solvent and lubricant [3]. The irrigation technique has influence in cleaning the complex root canal anatomy including the isthmuses. Various irrigation systems have been advocated by the researchers. The purpose of this in vitro study was to evaluate the cleaning of the isthmuses using different irrigation techniques.

MATERIALS AND METHODS

Data collection

The study protocol was approved by the institutional research committee. Thirty extracted human mandibular first molar teeth were selected for the study. The teeth were extracted for either periodontal reasons or poor restorability. The criteria to exclude teeth from

DENTAL SCIENCE



the study were; teeth with external resorptive defect seen with the naked eye, Hypercementosis, evident root fracture checked by transillumination, incompletely formed apex, extreme root curvature and previous root canal treatment

Preparation of samples

After extraction, the teeth were washed under tap water. The distal roots of teeth were removed and mesial roots were used for the study. All the samples were stored in normal saline for further use. Radiographs of the teeth were taken in bucco-lingual and mesiodistal directions to check the presence of any lateral canals. The samples were randomly divided into three experimental groups (n=10).

- Group1- Manual agitation irrigation technique, i.e. 30 gauge Navi-tip.
- Group2- Passive ultrasonic irrigation with Ultrasonic tip i.e. Irrisafe.
- Group3- EndoVac.

Endo-access cavity was done for all samples. Working lengths were established by introducing a size 06 K-files until the tip could be seen at the foramen. Root Canal instrumentation was performed with hand K-files 8, 10, 15, 20 till full working length and then rotary Hyflex file (0.60) system was used. During instrumentation, the chamber was flooded with 5% NaOCI replenished after each instrument.

Preparation for group 1

Manual agitation irrigation technique was performed with 30 gauge Navi-Tip. For irrigation 3 ml of 5% NaOCl was used. The irrigant was delivered after use of each instrument using a 30-gauge side vented needle for 15sec, then canal was rinsed with normal saline and after completion of instrumentation, the canal was agitated with 17% EDTA for 15 Sec.

Preparation for group 2

Passive ultrasonic activation was performed with a Irrisafe ultrasonic tip ISO 10 (Satelec Acteon Group, Merignac Cedex, France) mounted on a Suprasson P5 Booster ultrasonic unit (Satelec Acteon Group). The file was inserted 2 mm short of the working length and passively activated using a power setting of 3, according to manufacturer's recommendations. The file was passively inserted into the canal without any filing motion. This procedure was performed in three cycles of 20 seconds each for a total activation time of 1 minute.

Preparation for group 3

Root canal procedure was same as for group 1and 2. Irrigation was done by Endo Vac and 30 gauge Navitip was used as microcannula. For this irrigation protocol, first the macro-irrigation was accomplished over a 30 second period this was done by using the macro-cannula which was constantly moved up and down in the canal from a point where it started to bind to a point just below the orifice the canal space was then left undisturbed, full of irrigant for 30 seconds. Three cycles of micro irrigation followed. During a cycle of micro-irrigation the pulp chamber was filled with irrigant while the Navi-tip was placed at the working length for 6 seconds, then it was positioned 2 mm from working length for 6 second and then moved back to working length for 6 seconds. This up and down motion continued until 30 seconds had elapsed. This completed one micro irrigation cycle. The first cycle used 5% NaOCI, as an irrigant, the second cycle normal saline and the third cycle 17% EDTA. The final irrigation was done with normal saline.

The samples from three groups were then obturated with gutta flow-2 sealer and gutta purcha point and cleared using the method of tooth clearing recommended by Robertson et al [4]. Morphological analysis was performed using a stereomicroscope to reveal details of accessory canal filling. Observations were performed by counting the number of visible lateral canals as well as isthmuses within the middle and apical third of the roots. All accessory canals and spaces detected between the canals were observed from all the four surfaces (mesial, buccal, distal and palatal). The complete root was studied under 17X magnification and the areas of interest, the apical 1/3 and the middle 1/3, were studied under 25X magnification. The scoring was done in the following manner:

- 0: No filling of lateral canals and isthmuses
- 1: Partial filling of lateral canals and isthmuses
- 2: Complete filling of lateral canals and isthmuses

The scores were tabulated and analyzed statistically using Kruskal Wallis ANOVA test for 'within group' comparison.



RESULTS

"Within group" Comparison, using Kruskal Wallis ANOVA

The analysis of scores obtained by using Kruskal Wallis ANOVA test for the within group comparison at apical and middle third showed that the p-value was more than 0.05.hence the mean values of the number of filled canals were not statistically significant. However, in the EndoVac group, all the samples showed filling either partial or complete, no sample has '0' score and more no of samples (eight) with complete filling **[Table-1 and 2]**; The values in the bracket are the percentage and the number indicates the number of samples showing filling).

Table: 1. Comparison of root canal filling in the apical third:

Irrigation techniqe	Type of filling, Number (%)			P value (Kruskal Wallis ANOVA)
	No filling	Partial filling	Complete filling	
Group 1	4 (40)	2 (20)	4 (40)	
Group 2	3 (30)	2 (20)	5 (50)	0.103
Group 3	0 (0)	2 (20)	8 (80)	

Table-1 shows the filling in the apical third of the samples for all three groups. In Group 1(Navi-tip irrigation group), four samples showed no filling, two samples showed partial filling and four samples showed complete filling in the lateral canals and isthmuses. In Group 2(Passive Ultrasonic irrigation group), three samples showed no filling, two samples showed partial filling and five samples showed complete filling in the lateral canals and isthmuses. In Group 3(EndoVac irrigation group), zero samples showed no filling, two samples showed complete filling in the lateral canals and eight samples showed complete filling in the lateral canals and isthmuses.

Table: 2.Comparison of root canal filling in the middle third

Irrigation technique	Type of filling, Number (%)			P value (Kruskal Wallis ANOVA)
	No filling	Partial filling	Complete filling	
Group 1	2 (20)	3 (30)	5 (50)	
Group 2	2 (20)	2 (20)	6 (60)	
Group 3	0 (0)	3 (30)	7 (70)	0.532

Table-2 shows the filling in the middle third of the samples for all three groups. In Group 1(Navi-tip irrigation group), two samples showed no filling, three samples showed partial filling and five samples showed complete filling in the lateral canals and isthmuses. In Group 2(Passive Ultrasonic irrigation group), two samples showed no filling, two samples showed partial filling and six samples showed complete filling in the lateral canals and isthmuses. In Group 3(EndoVac irrigation group), zero samples showed no filling, three samples showed complete filling and seven samples showed complete filling in the lateral canals and isthmuses.

DISCUSSION

For this in-vitro study, thirty freshly extracted human mandibular first molars were used. We selected only mesial root because of their high incidence of canal isthmuses [5]. All the samples of our experiment were obturated with gutta-flow with an assumption that it would flow in all the intricacies of the canals including the isthmuses. But it will flow effectively only if these isthmuses are cleared off debris with the irrigation technique. The main purpose of the present study was to evaluate the potential of different irrigation techniques in clearing the debris from isthmuses and lateral canals and the method we opted for this evaluation was based on this assumption. To observe whether the lateral canals and isthmuses were filled with gutta-flow or not, we used the tooth clearing technique. After clearing, stereomicroscopic examination of the entire length of the sample was done at 17X magnification. The accessory and lateral canals in the apical 1/3 and the middle 1/3, were studied at 25X magnification.

In the present study the group 1 was treated with manual agitation irrigation technique. We used 30 gauge, side vented Navi-tip needle as it could reach the apex, ensuring that the irrigating solution too reaches close to the apex and be mechanically effective. Moser and Heuer from observations of their in vitro study determined that smaller diameter size needle can be placed closer to the apex and can be more efficient in flushing the debris; however the smaller diameter needles require more pressure for activation of the plunger than larger diameter needles [6].





Fig: 1. Stereomicroscopic images of the group 1 (Navi-Tip, manual irrigation) samples



Fig: 2. Stereomicroscopic images of the group 2 (Irrisef, Passive Ultrasonic Irrigation) samples

Ya Shen et al conducted a study to investigate the effect of irrigation needle tip design on irrigant flow pattern by using a 3-dimensional computational fluid dynamics (3D CFD) model. The results showed that needle tip design influences flow pattern, flow velocity, and apical wall pressure, all important parameters for the effectiveness and safety of irrigation. They observed that side vented needle require low apical pressure and hence were safer [7]. In our study the stereomicroscopic examination of the needle irrigation group revealed that in 40% of the samples apical isthmuses were filled with gutta-flow where as isthmuses in middle third were filled with gutta flow in 50% of the samples [Figure- 1]. This group was poorest in performance as far as cleaning of isthmuses is concerned.

In the present study the second group recived treatment with Passive ultrasonic irrigation technique. We used Irrisafe instruments. Satelec recommends the IrriSafe instruments for Passive Ultrasonic Irrigation (PUI), the safe



removal of the smear layer, dentine debris and bacteria from the root canal. The instrument's shape improves microstreaming and microcavitation in fluids. Non-cutting rounded end prevents damage to the apical constriction [8]. In our study, the stereomicroscopic examination of this group revealed that in 50% of the samples apical isthmuses were filled with gutta-flow where as isthmuses in middle third were filled with gutta flow in 60% of the samples [Figure- 2]. The Satelec system used in this study is a piezoelectric unit that does not require an external cooling source and is more powerful than magnetostrictive units. It functions on principal of acoustic streaming and cavitation [9]. The delivery of fresh irrigating solution within the root canal may have contributed to the improved cleanliness values.



Fig: 3. Stereomicroscopic images of the group 3 (IEndoVac Irrigation) samples

S-J Lee, M-K Wu & P R Wesselink compared the ability of syringe irrigation and ultrasonic irrigation to remove artificially placed dentine debris from simulated canal irregularities within prepared root canals. They concluded that both forms of irrigation reduced the debris score significantly. The debris score was statistically significantly lower after ultrasonic irrigation than after syringe irrigation [10]. T. Rödig, M. Sedghi, F. Konietschke, K. Lange, D. Ziebolz & M. Hülsmann compared the efficacy of syringe irrigation, RinsEndo and passive ultrasonic irrigation (PUI) in the removal of dentinal debris from simulated irregularities in root canals with different apical sizes. They found that passive ultrasonic irrigation removed debris significantly better from the artificial canal irregularities than RinsEndo and syringe irrigation [11]. Tina Rödig, Meral Bozkurt, Frank Konietschke, and Michael Hülsmann, conducted an in vitro study to compare the efficiency of conventional syringe irrigation, the Vibringe System, and passive ultrasonic irrigation (PUI) in the removal of debris from simulated root canal irregularities. Results showed that ultrasonic irrigation removed debris significantly better from the artificial canal irregularities than the Vibringe System and syringe irrigation [12]. The results of our study are in agreement with the above mentioned studies. In our study also the PUI technique was more effective than manual needle irrigation technique. The needle irrigation technique (group-1) could not clean the isthmuses effectively. This could be attributed to the formation of apical vapor lock during canal irrigation. Tay et al showed that the presence of the apical vapor lock adversely affected the debridement efficacy of needle irrigation technique [13]. The PUI technique (group-2), in this study, showed better efficiency in cleaning isthmuses. This finding suggests that ultrasonic technique activated the irrigant with sufficient force to overcome the apical vapor lock. Another reason for better performance of PUI technique may be that during ultra sonic irrigation much higher velocity and volume of irrigant flow was used in the canal.

In the third experimental group of our study, we used the Endo-Vac system. This system works on the principle of the Apical Negative Pressure (ANP) technique of irrigation [14]. The stereomicroscopic examination of Endo-Vac group revealed that in 80% of the samples apical isthmuses were filled with gutta-flow, where as isthmuses in



middle third were filled with gutta flow in 70% of the samples [Figure- 3]. This was the best performance observed amongst all the experimental groups.

Benjamin A. Nielsen, J. Craig Baumgartner compared the efficacy of Endo-Vac irrigation system and irrigation needles to debride root canals at 1 mm and 3mm from working length. The result showed significantly better debridement using EndoVac compared with needle irrigation [15]. Chris Siu and J. Craig Baumgartner conducted an in vivo study to compare the debridement efficacy of EndoVac irrigation versus conventional needle irrigation. EndoVac irrigation resulted in significantly less debris at 1mm from working length compared to needle irrigation [16]. Anchu Rachel Thomas, Natanasabapathy et al. evaluated the canal isthmus debridement efficacy of new modified EndoVac irrigation in mesial roots of mandibular molars. Intragroup analysis revealed a statistically significant difference in the percentage reduction of debris after cleaning and shaping and after final irrigation protocol in all the groups (P < .001). The final irrigation protocol produced significantly cleaner canal isthmuses in all the groups (P < .001). On intergroup analysis, the modified EVI group performed significantly better than the other groups. The EVI and PUI groups performed better than the needle irrigation group. There was no statistical significance between the EVI and PUI groups [17]. Results of our study are somewhat similar to this study. In our study for EndoVac group we used NaviTip for micro-irrigation like the modified Endovac irrigation protocol of this study.

In the present study maximum numbers of lateral canals and isthmuses were cleaned off in the EndoVac group. This could probably be attributed to the design of EndoVac irrigation system in which the micro irrigation is done upto the working length. Hence the observations of this study show that the canal isthmus debridement efficacy of EndoVac group was found to be superior to PUI and Needle irrigation. EndoVac could have performed better because of the concomitant delivery of the irrigant till the working length and effect of micro and macro-irrigation and evacuation of the irrigant creating a negative apical pressure, eliminating the apical vapour lock effect and enabling the irrigant to be pulled across the isthmus region and flushing out the debris.

CONCLUSION

Knowing the anatomic complexities of the root canal system it is important to use a suitable irrigation system while cleaning and shaping of the root canal, that will enable the irrigant delivered to flush of the debris from canal aberrations. From the observations of present study we conclude that EndoVac system fulfills the requirements of an ideal irrigation technique. However further research is needed to develop an irrigation system that will give 100% results in cleaning the root canal system.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENT

We thank the department of Oral and Maxillofacial Sugery for giving permission to collect the extracted teeth, Officials at IIESER Research Center, Pune for extending helping hand in stereomicroscopic examination of the samples to be tested.

FINANCIAL DISCLOSURE

This research was carried out in our institution, the stereomicroscopic examination was conducted at IIESER Research Center, Pune, and we were not supported by any financial assistance.

REFERENCES

- [1] Markus Haapasalo, Ya Shen, et al.[2010] Irrigation in Endodontics. *Dent Clin N Am* 54:291–312.
- [2] Bing Fan, Yihuai Pan, Yali Gao, et al. [2010] Three-dimensional Morphologic Analysis of Isthmuses in the Mesial Roots of Mandibular Molars. *Journal of Endodontics*, 36, 1866–1869.
- [3] Stephen Cohen, Richard Burns pathways of the pulp 8th edition, cleaning and shaping of the root canal system. Page no-231.
- [4] MVenturi, C Prati, G Capelli, M. Falconi L. [2003]Breschi, A preliminary analysis of the morphology of lateral canals after

root canal filling using a tooth-clearing technique. *International Endodontic Journal*, 36, 54-63.

- [5] T von Arx. [2005] Frequency and type of canal isthmuses in first molars detected by endoscopic inspection during periradicular surgery. *International Endodontic Journal*, 38: 160–168.
- [6] Kenneth Hargreaves, Stephen Cohen, pathways of the pulp 10th edition, tooh morphology and access cavity preparation, 139



- [7] Ya Shen, Yuan Gao, et al.[2010] Three-dimensional Numeric Simulation of Root Canal Irrigant Flow with Different Irrigation Needles. *Journal of Endodontics*, 36:884–889.
- [8] LWM van der Sluis, M Versluis, MK Wu, PR.[2007] Wesselink Passive ultrasonic irrigation of the root canal: a review of the literature. *International Endodontic Journal*, 40:415–426
- [9] Rubin Gutarts, John Nusstein, Al Reader, and Mike Beck. [2007] In Vivo Debridement Efficacy of Ultrasonic Irrigation Following Hand-Rotary Instrumentation in Human Mandibular Molars. *Journal of Endodontics*, x 31, 166–170.
- [10] SJ Lee, MK. Wu, PR Wesselink.[2004] The effectiveness of syringe irrigation and ultrasonics to remove debris from simulated irregularities within prepared root canal walls. *International Endodontic Journal*, 40: 415–426.
- [11] T Rodig, M Sedghi, F Konietschke, et al. [2010]evaluated the efficacy of syringe irrigation, RinsEndo and passive ultrasonic irrigation (PUI) in the removal of dentinal debris from simulated irregularities in root canals with different apical sizes. *International Endodontic Journal* 43: 581–589.
- [12] Tina Rödig, Meral Bozkurt, et al.[2010] Comparison of the Vibringe System with Syringe and Passive Ultrasonic Irrigation in Removing Debris from Simulated Root Canal Irregularities. *Journal of Endodontics*, 36:1410–1413.

- [13] Franklin R Tay, Li-sha Gu, G. et al.[2010] Effect of Vapor Lock on Root Canal Debridement by Using a Side-vented Needle for Positive-pressure Irrigant Delivery. *Journal of Endodontics*, 36: 745–750.
- [14] Gregori M. Kurtzman, Positive versus negative pressure irrigation.roots1_2012,16-22 (internet):http://www.dentaltribune.com/htdocs/uploads/printarchive/editions/13b0e64af31d 4be843db2cf752b783a7_16-22.pdf
- [15] Benjamin A. Nielsen, and J Craig.[2007]Baumgartner Comparison of the EndoVac system to needle irrigation to debride root canals. *Journal of Endodontics* 33:611–615.
- [16] Chris Siu, and J. Craig Baumgartner.[2010] comparison of the debridement Efficacy of the EndoVac irrigation system and conventional needle root canal irrigation in vitro. *Journal of endodontics*, 11:1782–1785.
- [17] Anchu Rachel Thomas, Natanasabapathy et al.[2014] comparative evaluation of canal isthmus debridement efficacy of a modified EndoVac technique with different irrigation system. *Journal of* endodontics, 1–5.

ABOUT AUTHORS

Dr. Ashwini Ghogare, the post graduate student in the Department of Conservative Dentistry and Endodontics at Bharati Vidyapeeth Deemed University's Dental College and Hospital Pune, India.

Prof. Dr. Shail Jaggi is Professor in the Department of Conservative Dentistry and Endodontics at Bharati Vidyapeeth Deemed University's Dental College and Hospital Pune, India.

Prof. Dr. Nitin Shah is the Head of Department, Conservative Dentistry and Endodontics at Bharati Vidyapeeth Deemed University's Dental College and Hospital Pune, India.

Dr. Jyoti Mandlik is an Associate Professor in the Department of Conservative Dentistry and Endodontics at Bharati Vidyapeeth Deemed University's Dental College and Hospital Pune, India.

Dr. Nikhil Sharma is an Assistant Professor in the Department of Conservative Dentistry and Endodontics at Modern Dental College, Indore, Madhya Pradesh, India.

RESEARCH ARTICLE OPEN ACCESS



APPLICATION OF FAT FRACTIONATION IN TRADITIONAL EGYPTIAN RAS CHEESE

Nadia A. Abou-zeid^{1*} and Awad S²

¹Dairy Science and Technology Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, EGYPT ²Dairy Science and Technology Department, Faculty of Agriculture El -Shatby, Alexandria University, EGYPT

ABSTRACT

Ras cheese was manufactured from a mixture of skim cows and buffaloes milk of equal amounts, standardized at 5 % fat by anhydrous milk fat (A cheese), anhydrous milk fat fraction of a low or a high melting point (H cheese) and (L cheese), respectively. Cheeses were analyzed for sensory evaluation, texture profile, yield, chemical composition, ripening indices and fatty acid profile. Samples varied in hardness between hard (L cheese), less hard (A cheese), firm /typical (H cheese). H cheese received the highest sensory scores, yield, as well as the best texture profile and nutritional properties.

Received on: 27th-July2015 Revised on: 27th-Sep-2015 Accepted on: 7th-October-2015 Published on: 2nd-Nov-2015

KEY WORDS

Ras cheese; 0iling off defect: Fat fractionation

*Corresponding author: Email: nadia_abou_zeid@yahoo.com

INTRODUCTION

Ras cheese is known as the most popular Egyptian hard cheese. It is similar to the Greek variety "Kefalotyri" [1]. Cheese is considered a soft solid material consisting of a network composed mainly of protein, water, and lipid [2]. The mechanical properties of cheese are related to network composition, structure, and interactions among molecules within the network [3-5]. Although the utilization of milk fat fractions provides potential benefits in a variety of products; and unique properties are imparted by fractions optimized for their use [6-7] no work has been done yet in this field in Ras cheese. The oiling-off at room temperature in summer, excess hardness, dryness and the lake of springy, flexible firm body and smooth texture are the most common defects that may be found in Ras cheese and may be contributed to fat. Fat functionally modification of the properties of milk fat fractions was the main purpose of this investigation. Fractionation of milk fat can be done by several methods that separate the triglycerides of milk fat [8].

MATERIALS AND METHODS

Butter was melted at 60° c and filtered through Whatman number 1 filter paper under vacuum to remove protein and other materials .The obtained anhydrous milk fat (AMF) was separated and then fractionated using a dry fractionation process using the method described by Grall and Hartel [9].This method included a two-step process : during the first step, the anhydrous milk fat was fractionated at 25.5 °c to produce a high melting point (solid) fraction, during the second step, the solid fraction obtained from the 25.5°c fractionation was refractionated at 35° c to produce a higher melting point (more solid) fraction which is referred as high melting point fraction. Liquid fractions obtained at 25.5° c and 35° c were combined together to be referred as low melting point fraction. Part of the same lot of anhydrous milk fat (AMF) was used as it is.

Cheese making

Ras cheese was manufactured from a mixture of fresh cows and buffaloes milk of equal amounts obtained from the herds of the Faculty of Agriculture, Menofiya University. Four batches of milk were prepared for cheese making. The first batch of milk without any addition standardized at 5% fat C milk, the other three batches were skimmed and standardized at 5% fat (A, H and L milk) using one of the following:



1) Anhydrous milk fat, (A milk)

2) High melting point (solid) anhydrous milk fat fraction, (H milk)

3) Low melting point (soft) anhydrous milk fat fraction, (L milk).

Cheese making was carried out by the method described by Nadia [10] using the previously prepared milk to manufacture control cheese(C), anhydrous milk fat cheese (A), high melting point fat fraction cheese (H) and low melting point fat cheese (L) with the same respect mentioned above in milk preparing.

Methods of cheese analysis

Cheese was analyzed after 5 months of storage as following:

Sensory evaluation: Taste panel of 20 persons was chosen from the staff and assistants at the department of Dairy Science, Faculty of Agriculture, Menofiyia University .The panelists scored the cheese according to the description of Foegeding and Drake [11] .The texture was classified into soft, firm/typical, hard and very hard.

Reological analysi: The texture of Ras cheese was qualified by texture analyzer CNS – (The Farnell, England). The probe was TA17 (with angle 30° and 25 mm in diameter) using a speed of 1 mm/ second and a distance 10 mm in cheese. Cheese samples were cut into cubes 3cm3 and kept at 12° c for 1 hour before analysis.

Cheese yield: The cheese yield was calculated as kg cheese /kg milk. Cheese that obtained the highest organoleptic scores was subjected to the rest of analysis

Chemical composition and ripening indices determination: The cheese samples were analyzed for moisture, acidity, fat, total nitrogen and soluble nitrogen according to Ling [12], total free amino acids nitrogen was assessed according to the method of Chebotarev and Veltsova [13] with slight modification as adopted by Nasr [14].Soluble tyrosine and soluble tryptophane were determined as outlined by Vakaleris and Price [15]. Total volatile fatty acids were determined by the method described by Kosikowski [16]

Fatty acids profile analysis: Fat was extracted from cheese samples according to the method of Bligh and Dyer [17]. Fatty acid methyl esters were injected by an auto sampler into a Hewlett-Packard 5890A gas chromatograph with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA) Loor [18].

Statistical analysis: Factorial design of three replicates and the completely randomized design were used to analyze all the data. Newman Keuls test was then used to make the multiple comparisons using Costat program. Evaluation was based on a 1% and 5 % significance levels.

RESULTS AND DISCUSSION

Sensory evaluation

Supplementary Table –1 shows the sensory evaluation. The texture of the samples was described by panelists and classified into hard cheese (samples C and L), semi hard cheese (sample A) and semi soft & typical /firm cheese (sample H). The data obtained demonstrated that cheese of 5 months age gained equal or higher scores compared with cheese of 6 months age , therefore we shall discuss only the organolepic data for cheese at 5 months age only. Samples (C and L) were characterized by the highest hardness and crumbling under compression forces. When they were cut with a knife into layers and left at summer room temperature ($35^{\circ}c$) for 12 hours ,they exhibited oiling–off defects. These samples also showed the least flavor piquancy .They received total organoleptic score of 75 and 73, respectively.

Sample (A) also showed oiling-off defects when left for 12 hours at summer room temperature (35°c) after being cut into layers .It showed moderate flavor piquancy. It received total organoleptic score of 81. Sample (H) exhibited spring flexible firm body, smooth texture and piquant flavor. It was pliable with no fractures observed when compressed in hand. It possessed high degree of elasticity. It did not exhibit oiling –off defects when left at summer room temperature 35°c for 12 hours after being cut into layers. It received the highest total organoleptic score 98.

Organoleptic scores significantly increased by the replacing milk fat with anhydrous milk fat (A cheese) or high melting point anhydrous milk fat fraction (H cheese), while it significantly decreased by the replacing milk fat with low melting point anhydrous milk fat fraction (L cheese).



Cheese yield

Supplementary Table -2 shows the yield of cheese at both fresh and ripe states. Cheese yield significantly increased by replacing milk fat with anhydrous milk fat (A cheese) or high melting point anhydrous milk fat fraction (H cheese), while it significantly decreased by the replacing milk fat with low melting point anhydrous milk fat fraction (L cheese). This may be due to the applied of higher melting point fraction in Ras cheese making could be a tool to modulate the oiling -off which causing an increase in cheese yield.

Reological characteristics

Supplementary Table –3 shows the reological characteristics of the Ras cheese samples as affected by modified milk fat. The average of hardness for (C) and (L) Ras cheese samples was 982 and 932 g respectively. Sample (A) was hard but less than (C) and (L) samples, its average hardness value was 812. Sample (H) recorded the lowest hardness value with average of 621 g. It is clear from **Supplementary Table–4** that there is a great difference between C and H cheese in consistency. H cheese had the lowest consistency. Therefore, there is a positive relationship between hardness and consistency. The difference in springiness between C and L cheese was insignificant, but it was significant between C, A and H cheese ($P \le 0.01$). The H cheese had the highest springiness value because the higher melting point fraction yielded a higher viscosity which causing an increase in springiness. Significant differences were noticed between control and treatment samples in chewiness. H cheese had the lowest value .C cheese had chewiness value about one and a half times more than H cheese. A positive relationship was found between hardness and chewiness, these results agree with that reviewed by Katsiari et al., Bryant et al. and Fox et al.[7, 22, 23]. There is a positive relationship between either fracturability or Cohesiveness and hardness of Ras cheese. H cheese had the lowest fracturability value.

Very significant differences were noticed in Adhesiveness between H cheese and all other cheese samples including C cheese. There were also significant differences between C cheese and other treatments. L cheese had the highest value, so there is a negative relationship between hardness and adhesiveness.

Chemical composition

Moisture, acidity, salt/water, fat/D.M. values were higher for H cheese ($P \le 0.01$) than for the corresponding control cheese (Table 4). This may be attributed to the ability of the cheese curd to maintain the high melting point fraction of anhydrous milk fat to a greater extend than milk fat, this is because milk fat contains low melting point fat fraction which was completely melted during the process of cheese manufacturing and thus allowing alot of it to be lost in whey. Results also showed that pH and T.N./ D.M. had opposite trend to moisture, acidity, salt/water, fat/D.M. values. Lower N/D.M. % in H cheese is attributed to its higher fat content compared with C cheese.

Ripening indices

The ripening indices of the cheese at both fresh and 5 months age are shown in **Supplementary Table** -5 for S.N./T.N.%, T.F.A.A.N %, T.V.F.A, S.tyr and,S.trp . H cheese had higher values than C cheese .This may be due to higher moisture content in H cheese which stimulates microbial and enzymatic activities leading to more protein decomposition and fat hydrolysis [24].

Fatty acid profiles

Supplementary Table –6 shows the fatty acid profiles for H and C cheese .Distinct differences were found between them : C cheese contained more saturated fatty acids than H cheese .C cheese contained one and a half times more short chain (C4:0 to C8:0) and medium-chain (C10:0 to C12:0) fatty acids than H cheese. The proportion of lauric acid (C12:0) in H cheese is 63.96% of its proportion in C cheese. Ulbricht and Southgate [25] proposed an atherogenic index (AI) for lipids as a dietary risk indicator for cardiovascular disease. The AI is the sum of the proportion in the fat of lauric and palmitic acids and four times myristic acid divided by the proportion of the total unsaturated fatty acids. H cheese had more long-chain saturated fatty acids (C16:0 to C18:0). The most distinct difference between the two cheese samples in the long chain fatty acid content was the amount of



C18:0, which increased from 13.24 in C cheese to 16.39 in H cheese. Cholesterol showed great decrease in H cheese compared with C cheese. Shukla et al. [19] reported that HMT (high melting triglyceride) butter offers the advantages of lower cholesterol and saturated fat contents. However, the contents of C8 and C10 fatty acids were also reduced.

CONCLUSION

Modified cheese (H) exhibited modifications in the nutritional and functional properties compared with the control one (C). Lauric acid (C12:0) content in H cheese is 63.96% of its content in the control cheese. H cheese had more unsaturated fatty acids than C cheese. The high solid fat content of H cheese has led to greater emulsion stability at elevated temperatures. This increased stability was reflected in higher texture scores for H cheese. The improved texture and emulsion stability eliminated the oiling-off to cheese surface at 35°C.

Therefore, cheese made from high melting point fat fraction shows good potential for use at ambient and higher temperatures at which the control cheese normally exhibits problems such as oiling- off, hardness and crumbling. Additionally, storage cost of this modified cheese would be lower because the product is stable at room temperature and does not require refrigeration to overcome the problem of oiling-off at the cheese surface. A lower cholesterol and saturated fat content of the product offer added advantages that consumer's desire.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENT

.

None.

FINANCIAL DISCLOSURE

No financial support was received to carry out this project.

REFERENCES

- Phelan JA, Renaud L and Fox PF. [1993] Some non European cheese varieties .In Cheese: Chemistry, Physics and Microbiology.2.Major cheese groups.Chapman and Hall ,London, p 421-465.
- [2] Walstra P. [2003] Physical Chemistry of Foods. Marcel Dekker, Inc., New York, NY.
- [3] Lucey JA, ME Johnson, aDS Horne. [2003] Perspectives on the basis of the rheology and texture properties of cheese. *J Dairy Sci* 86:2725–2743.
- [4] Siew Kim Lee, Henning Klostermeyer, Skelte G Anema. [2015] Effect of fat and protein-in-water concentrations on the properties of model processed cheese *International Dairy Journal* 50: 15–23.
- [5] Stocco G, Cipolat-Gotet C, Cecchinato A, Calamari L, Bittante G. [2015] Milk skimming, heating, acidification, lysozyme, and rennet affect the pattern, repeatability, and predictability of milk coagulation properties and of curd-firming model parameters: A case study of Grana Padano. *J Dairy Sci* 98:5052–5067.
- [6] Hartel RW. [1996] Applications of milk fat fractions in confections. J Am Oil Chem Soc 73:945.
- [7] Katsiari, MC, Voutsinas LP, Kondyli E. [2002]. Improvement of sensory quality of low-fat Kefalogra-viera-type cheesewith commercial adjunct culltues. *Inter dairy J* 12:757.
- [8] Fox PF and PLH Mcsweeney. [2006] Advanced dairy chemistry. Volume 2 Lipids.Third Edition.Springer.com. U. S. A.
- [9] Grall D, and RW Hartel. [1992] Kinetics of butterfat crystallization. J Am Oil Chem Soc 69:741.

- [10] Nadia A Abou Zeid. [2008] Cheese and its fractions. first Edition Alzahraa Publishers, Inc., El reyad, Sodia Arabic kingdam pages 102–106.
- [11] Foegeding EA and MA Drake. [2006] Invited Review: Sensory and Mechanical Properties of Cheese *Texturel J Dairy Sci* 90:1611-1624.
- [12] Ling,ER.[1963] A Text Book of Dairy chemistry .Vol 2 Third edition.,Champan and Hall ,London.
- [13] Chebotarev A Land Veltsova, S Maxia. [1959] Composition and changes in the amino acid complex in the process of cheese ripening *Inter Dairy Congr* Xv: 691.
- [14] Nasr WI.[2001] .Studies on acceleration manufacture steps of hard cheese .M. Sci. Thesis, Moshtohor, Zagazig University.
- [15] Vakaleris DG.and Price WV. [1959]. A rapid spectrophotometric method for measuring cheese ripening *J Dairy Sci* 47: 264-276.
- [16] Kosikowski,FV.[1978]Cheese and fermented milk foods 2 nd Ed .Cornell Univ.Ithaca,New York.
- [17] Bligh EG, and WJ Dyer. [1959] A rapid method for total lipid extraction and purification. *Can J Biochem. Physiol* 37:911– 917.
- [18] Loor JJ. [2001] Alterations in mammary gland synthesis and secretion of fatty acids in response to trans isomers of octadecenoic acid or conjugated linoleic acid isomers. Ph.D. Diss., Virginia Tech, Blacksburg, Virginia.
- [19] Shukla A, Bhaskar AR, Rizvi SSH, Mulvaney SJ. [1994] Physicochemical and Rheological Properties of Butter Made from Supercritically Fractionated Milk Fat . *Journal of Dairy Science*,77: 1.

FOOD TECHNOLOGY



- [20] Awad SEI Atter, A, Ahmed, N. and El-Soda, M. [2002]. Proteolysis and texture changes during the ripening of white soft cheese (Talaga) .1st Arab Mansoura Conference for Food and Dairy Science & Technology, 1-3 October 2002.305–316.
- [21] Awad SEI –Atter,A, Ayad EHE and El-Soda M.[2003] Characteristic of Egyptian market Ras cheese .,sensory evaluation ,rheological,physico-chemical properties and microbiological analysis .*Egyptian J Dairy Science* 31:289–303.
- [22] Bryant A, Z Ustunol, and J Steffe. [1995] Texture of Cheddar cheese as influenced by fat reduction. *J Food Sci* 60:1216–1219, 1236.
- [23] Fox PF, TP Guinee, TM Cogan, and PLH McSweeney. [2000] Cheese Rheology and texture. Pages 305–333 in Fundamentals of Cheese Science. Aspen Publishers, Inc., Gaithersburg, MD.
- [24] Law BA.[1997]Microbiological and Biochemistry of cheese and fermented milk 2ndEd.T.J.Podstow.Cornwall,Great Britain.
- [25] Ulbricht TLV, Southgate DAT.[1991] Coronary heart disease: seven dietary factors. *Lancet* 338:985–992.

SUPPLEMENTARY TABLES (as provided by authors)

Supplementary Table: 1. Effect of modified milk fat on sensory evaluation of Ras cheese after ripening for five and six months

	Ras cheese age(months)					
Scoring points	Five			Six		
	Taste & Flavour 60	Body& Texture 40	Total 100	Flavor 60	Body & texture 40	Total 100
С	51.4ª	31.1ª	84.5ª	52.2ª	33.8ª	85.0ª
А	50.8ª	31.2ª	82.0ª	51.3ª	32.3ª	83.6ª
L	47.4 ^b	26.3 ^b	73.7 ^b	48.2 ^b	26.5 ^b	74.7 ^b
Н	59.3°	39.5°	98.8	58.9°	39.6°	98.5°

a–c Values for each parameter with the same letter in the same column do not differ (α = 0.01)

Supplementary Table: 2. Effect of modified milk fat on yield % of fresh and ripe Ras cheese

Cheese samples	Ripening periods			
	Fresh	5 months		
С	14.8ª	13.7ª		
А	13.5ª	12.5ª		
L	11.6 ^b	10.5 ^b		
Н	18.2°	17.1°		

a–c Values for each parameter with the same letter in the same column do not differ ($\alpha = 0.01$)

Supplementary Table: 3. Effect of modified milk fat on rheological properties of Ras cheese after ripening for five months.

Samples	Hardness (g)	Consistency g/sec	Springiness Mm	Factorability (g)	ShewinessG mm	Cohesiveness ratio	Adhesiveness g/sec
С	812.3a	4062.3a	4.9a	787.4a	3952.3a	0.86a	42.6a
A	832.4b	4342.5b	4.7a	812.3b	4192.4b	0.99a	40.3a
L	982.7c	4522.6c	4.0b	853.3c	4316.8c	1.3b	46.4b
Н	621.6d	3663.2d	5.9c	675.9d	2756.1d	0.67c	33.5C

a–d Values for each parameter with the same letter in the same column do not differ ($\alpha = 0.01$)



Supplementary Table: 4. The chemical composition of H and C cheese at fresh state and after ripening for five months

Chemical composition%	Ras cheese samples					
	Fre	esn	5 month age	•		
	С	Н	С	Н		
Moisture	40.2 ^a	43.7 ^b	29.8ª	33.5 [⊳]		
Acidity	1.3ª	1.5ª	2.3ª	2.6ª		
pН	5.7ª	5.5ª	5.4ª	5.2ª		
Salt/water	6.9 ^a	6.7 ^a	13.0ª	11.5⁵		
Fat/D.M.	51.4ª	60.5 ^b	42.2ª	51.9 ^b		
T.N./D.M.	7.5 ^a	6.1 ^b	6.3ª	5.2 ^b		

a–b Values for each parameter with the same letter in the same row do not differ ($\alpha = 0.01$)

Supplementary Table: 5. The ripening indices for H and C cheese at fresh state and after ripening for five months

Ripening indices	I	Ras cheese samples					
indices		Fresh	1	5 months age			
		С	Н	С	Н		
S.N./T.N.	%	7.4 ^a	6.9 ^a	15.5 ^a	18.3 ^b		
T.F.A.A.N	1%	0.06 ^a	0.04 ^a	0.18 ^a	0.64 ^b		
T.V.F.F.A	\ *	6.8 ^a	7.9 ^a	13.6ª	21.6 ^b		
S.tyr.**		60.2 ^a	74.6 ^a	178.5 ^a	223.9 ^b		
S. trp ***		47.2 ^a	50.7 ^a	143.1 ^a	175.5 ^b		

a-cValues for each parameter with the same letter in the same row of the first and the second columns or the third and the fourth columns do not differ (α = 0.01); * ml 0.1 N NaOH /100g of cheese; ** mg/100g of cheese; **mg/100g of cheese

Supplementary Table: 6. Fatty acid profiles for H and C cheese ripened for five months

Fatty acids	С	heese			
	С	Н			
	Fatty acids concentration %				
C 4:0	3.00ª	1.25 ^b			
C 6:0	0.38ª	0.25ª			
C 8:0	1.28ª	0.43 ^b			
C 10:0	1.49ª	0.31ª			
C 12:0	3.08ª	1.97 ^b			
C 13:0	0.19ª	0.14ª			
C 14:0	10.88ª	9.49 ^b			
C 14:1 n9c	1.51ª	1.53ª			
C 15:0	1.59ª	1.96 ^b			
C 16:0	29.38ª	29.14ª			
C 16:1 n9c	2.03ª	0.95 ^b			
C 17:0	0.88ª	1.49 ^b			
C _{18:0}	13.24ª	16.39 ^b			
C 18 : 1 n9t	26.72ª	29.34 ^b			
C 18:1 n9c	0.00	0.00			
C 18 : 2 n6c	1.63ª	1.34 ^b			
C _{20 : 1}	0.25ª	1.44ª			
C 18 : 3 n3	0.72ª	0.45 ^b			
C 20:0	1.40ª	1.03 ^b			
C 22:0	0.15ª	0.23 ^b			
C 22:1 n9	0.20ª	0.37ª			



Cholesterol, mg/10	00 g	112.92ª	53.69 ^b
Short –chain C ₄ -C ₈		4.66 ^a	1.93 ^b
Medium chain	C ₁₀ -C ₁₂	4.57 ^a	2.28 ^b
Long-chain	C14-C22	90.58ª	95.75 ^b
Unsaturated		\ 33.06	35.42

a-b Values for each parameter with the same letter in the same row do not differ ($\alpha = 0.01$)

RESEARCH ARTICLE OPEN ACCESS

ENVIRONMENTAL ASSESSMENT OF CUCUMBER FARMING USING ENERGY AND GREENHOUSE GAS EMISSION INDEXES

Moslem Sami¹*and Habib Reyhani²

¹Bank Keshavarzi Iran, Isfahan, IŘAN ²Independent Researcher, IRAN

ABSTRACT

Energy and GHG (greenhouse gas) emissions evaluation is from the most common methods for assessment of environmental status of production activities. In this study energy and GHG balance in the greenhouse cucumber production in Yazd province of Iran were assessed. Data of this study were collected using a face to face questionnaire method from the farmers growing cucumber crops. Study results showed that the average annual crop yield of the greenhouses was 89868.54 kg/ha which demanded an average energy input of 699217.04 MJ/ha. Diesel fuel and electricity were the biggest energy consumers in the farms with shares of 59.31 and 25.58% of total input energy. These two inputs also were the biggest air pollutant with the emissions of 33128.65 and 62309.23 kg CO2-eq per hectare respectively. The results also showed that a quadratic model was the best for modeling the relations between the crop yield and total energy input, GHG per yield and energy intensity; and linear model was the best for modeling relation between yield and energy productivity.

Received on: 4th-Sept-2015 **Revised on:** 24th-Oct-2015 **Accepted on:** 25th-Oct-2015 **Published on:** 5th-Dec-2015

KEY WORDS

Cucumber farming, Energy indexes, GHG emission, Yield sensitivity

*Corresponding author: Email: sami.moslem@gmail.com Tel: +98 913 222 8623

INTRODUCTION

When we discuss about agriculture, its unwanted effects on the environmental cannot be ignored. Agriculture and energy have a close relationship since agriculture is both consumer and producer of energy in the form of bioenergy [1]. Efficient use of energy is one of the principal requirements of sustainable agriculture. To evaluate the sustainability of agriculture, its energy efficiency must be considered, and major sources of energy waste must be identified and assessed [2]. Direct fossil energy use by agriculture is about 3.0-4.5% of the total energy consumption in the developed countries of the world [3]. On the other hand the indirect energy used for production, formulation, storage and distribution of agricultural inputs and their application with tractorized equipment involves a greet share of total consumed energy in world. This trend in consumption of energy is along with the emissions of CO₂ and other Greenhouse Gases (GHGs) into the atmosphere [4]. Agriculture accounts for one-fifth of the annual increase in anthropogenic greenhouse warming. Agriculture is the main source of non-carbon dioxide GHGs, emitting nearly 60% of nitrous oxide (N₂O) and nearly 50% of methane (CH₄) [5]. If agricultural production is going to significantly increase while also minimizing its impact on future climate change, it is important to understand the current status of energy and GHG budgets and their link with farms outputs. Energy and GHG emission analysis in agricultural production operations results in determining overuse sectors and may act as a platform to improve production processes.

Considering the energy balance of crop production was much debated in the early 1970s when the world energy crisis made people aware from limitation of fossil supply [31]. After that considerable studies have been conducted in energy efficiency of various agricultural productions such as grains [7-9], greenhouse crops [10,11], hay crops [12], fruits [13,14], vegetables [15] etc. While, there are few studies on the topic of gas emissions as result of agricultural in-farm and off-farm activities. Shortages of information about gas emissions in the production processes of agricultural inputs may be one of the most important reasons of this ignorance. However, there are some studies that tried to evaluate and analyze the gas emissions of agricultural activities [16,17,4]. In this study we analyzed the energy and GHG balance in cucumber production and evaluated the sensitivity of energy and GHG criteria to the output yield.

ENV. SCIENCE



The cucumber (Cucumis sativus) is a widely cultivated plant in the gourd family Cucurbitaceous which include squash and the same genus as the muskmelon. It is a warm-season plant and grows rapidly at 24–29°C temperatures [35]. China is biggest producer of cucumbers on the planet. Iran is the next big producer of cucumber. In Iran, it was cultivated on 77,951 ha (in field and in greenhouse) and the production was 1715024 tons in 2010 [25]. In 2012 Iran exported more than 188000 tons of cucumber which brought more than 141 million dollars income for Iran. Iran was the fourth largest cucumber exporter after Spain, Mexico and Netherlands. Yazd province with cultivated area of 1003 ha was one of the major Iranian cucumber greenhouse producers with the total production of 300,165 t in 2010 [25].

MATERIALS AND METHODS

Data collection

Data used in this study were obtained by using a face-to-face questionnaire method from 32 farmers growing single crop of cucumber in greenhouses in Taft county of Yazd province, Iran during 2011-2012. These farmers were selected randomized according simple random sampling method. Yazd province, with area of 7215 ha, is located in the center of Iran within 29°48 to 33°30 north latitude and 52°45 to 56°30 east longitude. The study region climate is hot and relatively dry and the average annual rainfall is reported to be 50- 350 mm. In the studied province the greenhouse cucumber is raised only in the cold seasons of year. The supplementary data on the farms were obtained from Ministry of Jihad-e-Agriculture of Iran. The sample size was calculated according equation 1 as described by Ghasemi Mobtaker et al., [18]:

$$n = \frac{N(s \times t)^2}{(N-1)d^2 + (s \times t)^2}$$
⁽¹⁾

where n is required sample size. N is the number of holdings in target population (102 in this study), s is standard deviation (calculated as 0.173), d is acceptable error (permissible error was chosen as 5%) and t is confidence limit (1.96 in the case of 95% reliability).

Energy calculation

Farm inputs and outputs can be expressed in terms of energy equivalents. The total energy use per unit of activity can be expressed in terms of MJ/ha, indicating overall energy consumption. In this study energy budget was calculated based on a mix of actual data from farms and energy coefficients. The energy equivalents for different inputs and outputs used in energy budget calculation are shown in column 3 of Table-1. The energy cost of inputs and practices were adapted from different sources of estimations that best fit Iran conditions. We calculated energy intensity and energy productivity as indexes of energy use efficiency using Equations 1 and 2 [19]:

Energy productivity =
$$\frac{\text{Output yield (kg ha^{-1})}}{\text{Energy input (kg ha^{-1})}}$$
 (2)

Energy intencity =
$$\frac{\text{Energy input (Mj ha^{-1})}}{\text{Cucumber output (kg ha^{-1})}}$$
 (3)

The input energy is also classified into direct and indirect; and renewable and non-renewable forms. The indirect energy included energy embodied in chemicals, manure, machine and equipment; while the direct energy includes human power, fuel and electricity in the production process. On the other hand, non-renewable energy includes diesel, electricity, pesticides and fertilizers; while renewable energy consists of human and manure fertilizer [20].

GHGs emission

Production, storage and application of inputs in agricultural farms invoke combustion of fuels, which results in CO₂ and other GHGs emission. Global Warming Potential (GWP) is an index presenting the impact of gaseous gases on the atmosphere's capacity of absorbing infrared radiation, which contributes to the global greenhouse gas effect. The GWP is expressed in kg CO2 equivalent (CO2-eq), which is taken to be 1 for CO2, 296 for N2O and 23 for CH4 (IPCC 2006). Conversion coefficients CO2-eq is calculated for each farm input based on its GHGs emissions during its production or/and consumption and can be expressed in kg CO₂-eq per weigh of input. Total CO₂-eq index is calculated by the sum of CO₂-eq of all farms inputs in terms of kg/ha [21]. Used conversion coefficients in this study are presented in column 5 of Table-1. We used conversion coefficients for different fuels and electricity for Iran calculated by Sami et al., [21]. N fertilizer has two sources of GHGs emission; off-farm emissions which involve GHGs emissions from production, packaging and transporting of fertilizers and on-farm emissions which involve



emissions from soil denitrification and nitrification processes in the field after distribution of fertilizers. Precise measurement of N_2O emissions from soil denitrification and nitrification processes is difficult since it depends on many complex interactions taking place in the soil, and can considerably vary depending on temperature, moisture, available N, organic matter, soil aeration, pH and so on. Nevertheless, direct N_2O emissions have been shown to relate to N inputs. Therefore, amounts of N_2O emissions are often calculated using an emission factor that represents the percentage of any N applied that emits in the form of N_2O [22]. According to IPCC [23], the amount of C lost via harvested crops is considered to be replaced by C uptake in the following crop and there is no significant long-term accumulation of C in crops products. Therefore, we did not take into account this carbon cycle [21]. We used two indexes including GHG per yield and GHG per hectare to present the GHG emissions status of farms

Item	Unit/ha	Energy equivalent (MJ/unit)	References	CO ₂ -eq coefficient (kg/unit)	References
N fertilizer	kg	78.10	[32]	3.97 (off-farm) + 2.96 (on-farm)	[21]
P fertilizer	kg	17.40	[32]	1.30	[34]
K fertilizer	kg	13.70	[32]	0.71	[34]
Micro fertilizer	kg	8.80	[32]	0.66	[34]
Manure	ton	303.00	[32]	27.50	[4]
Diesel fuel	lit	41.06	[21]	3.28	[21]
Electricity*	kWh	12.00	[32]	4.18	[21]
Fungicides	kg or l	210.00	[33]	14.49	[21]
Insecticides	kg or l	101.20	[32]	29.00	[34]
Human labour	h	2.20	[6]	-	-
Cucumber seed	kg	1.00	[25]	-	-

Table:1. Coefficients of CO₂-eq and energy of inputs in farms

*the data for electricity is for Iran distribution network electricity which is combined of thermal energy sources and hydroelectric energy sources

RESULTS

The amount of inputs used in the production of cucumber was specified in order to calculate energy and CO_2 equivalences in the study. Inputs in cucumber production were: human power, diesel fuel, fertilizers, pesticides, electricity and seed. The output was considered cucumber yield. The related energies of different inputs used in the studied greenhouses are shown in Table-2 (column 3). As it can be seen, the highest energy input belonged to diesel fuel with a share of 59.31% of total energy input (414,714.13 MJ/ha). The diesel was mostly used as the fuel for greenhouse heaters. This high rate of diesel consumption in the greenhouses of the studied region could be attributed to cold weather conditions of growing seasons on the one hand and low thermal efficiency of greenhouses buildings on the other. Reducing heat exchange between outside and inside of greenhouses by using double layer plastic film plus internal thermal blanket can decrease the amount of diesel fuel consumption in greenhouses [25]. Fuel for heating was reported as the biggest energy consumer in the greenhouses by many researchers. Taki et al., [26], Heidari and Omid [24], and Pishgar-Komleh et al., [25] reported the fuel as the most important input energy in their studies with proportions of 40, 54 and 68% of total input energy respectively. The second most demanding energy input for cucumber production in the studied region was electricity (25.58%). Electricity was mostly used for air conditioners to circulate and exchange air and also for water pumping and spraying. Use of more efficient fans and water pumps may considerably decrease the consumed electricity in the greenhouses. this result was in agreement with the results of Pishgar-Komleh et al., [25] who reported the electricity as second most important energy input in greenhouse cucumber farming. Shares of other inputs in the total energy input were insignificant. Least energy demanding inputs were micro fertilizers and seed (with shares of 0.02 and 0.001 respectively).

The share and amount of GHG emitted by each input in cucumber cultivation are shown in columns 5 and 6 of **Table-2**. Electricity in the farms was the dominant source of GHG emissions with a share of 61.60% of total CO_2 -eq emissions (62309.23 kg/ha). After electricity the diesel fuel had the highest share (32.75%). Other than electricity and fuel other inputs had ignorable shares of total GHG emissions in the studied farms. Fertilizers with a share of 5.31% of total CO2-eq emissions were ranked as the third air pollutant in terms of GHG emission. Nitrogen and manure were dominant sources of GHG emissions among fertilizers and almost 43.31% of total CO_2 -eq emissions from fertilizer use and 2.30% of total CO_2 -eq emissions from farming systems belonged to each of them.



ltem	Quantity of input used per hectare (unit/ha)	Input energy (MJ/ha)	%	CO2 equivalent (kg/ha)	%
Labor	17063.00	37538.60	5.37	0.00	0.00
Diesel fuel	10100.20	414714.13	59.31	33128.65	32.75
Pesticides	-	3813.50	0.55	346.40	0.34
Insecticide	4.13	417.47	0.06	119.63	0.12
Fungicide	16.17	3396.03	0.49	226.77	0.22
Fertilizers	-	64271.96	9.19	5369.10	5.31
Manure	84621.48	25640.31	3.67	2327.09	2.30
Nitrogen	335.18	26177.61	3.74	2322.80	2.30
Potassium	673.87	9232.08	1.32	478.45	0.47
Phosphor	177.79	3093.54	0.44	231.13	0.23
Others	15.08	128.42	0.02	9.63	0.01
Electricity	14906.52	178878.18	25.58	62309.23	61.60
Seed	0.07	0.67	0.00	0.00	0.00

Table: 2. Input and outputs of farms and their related indexes in terms of energy

Calculated farm indexes are reflected in **Table-3**. The average annual crop yield of the greenhouses was estimated as 89868.54 kg/ha which demanded an average energy input of 699,217.04 MJ/ha. Pashaee et al., [27] estimated the total energy input for greenhouse tomato production in Kermanshah Province of Iran at 123,130 MJ/ha. In another study conducted by Ozkan et al., [28], the total energy inputs for greenhouses produced cucumber in any one period of plant cultivation were reported to be 134,771.3 MJ/ha. Energy productivity of farms was 0.13 kg/MJ. This means that 0.13 kg of output was obtained per unit of input energy. This energy productivity rate is in ranges of other similar reports for greenhouse crops (e.g. 0.11, 0.25 and 0.12 kg/MJ by Pahlavan et al., [15], Salami et al., [29] and Pishgar-Komleh et al., [25]). The average energy intensity of the studied farms was 9.66 MJ/kg. This index shows that 9.66 MJ of energy was used for production of one kilogram of cucumber. The total energy input of studied farms could be classified as direct (90.26%), indirect (9.74%) or renewable energy (9.04%) and non-renewable energy (90.96%). In the several past studies the ratio of direct energy was reported higher than that of indirect energy, and the ratio of non-renewable energy greater than that for renewable energy [e.g. 12,14,1]. In the process of cucumber cultivation, as it can be seen in the **Table-2**, 101,153.38 kg CO₂-eq per hectare and 1.42 kg per weight of crop was emitted.

Table: 3. Calculated indexes of the farms

Calculated indexes	Unit	Quantity	%
Total output yield	kg/ha	89868.54	-
Energy intensity	MJ/kg	9.66	-
Energy productivity	kg/MJ	0.13	-
Indirect energy	MJ/ha	68086.12	9.74
Direct energy	MJ/ha	631130.92	90.26
Renewable energy	MJ/ha	63179.58	9.04
Nonrenewable energy	MJ/ha	636037.46	90.96
Total energy input	MJ/ha	699217.04	100.00
GHG per output	kg/ kg	1.42	
GHG per hectare	kg /ha	101153.38	

In this study we also evaluated the relations between the output yield and total input energy, GHG per yield, energy intensity and energy productivity. The plots of observed values of the total crop yield versus calculated indexes are presented in **Figures-1 and -4**. The regression coefficients in relationship between parameters and the corresponding R^2 values are given in these Figures. The coefficient of determination (R^2) between yield and total input energy, GHG per yield, energy intensity and energy productivity were 0.29, 0.75, 0.92 and 0.83, respectively. The best relationship between crop yield and total energy input, GHG per yield and energy intensity

ENV. SCIENCE



were expressed in the form of second degree polynomial regression. Quadratic model for prediction of crop yield using total input energy was also suggested by Canakci and Akinci [30] or cucumber in Turkey. Figure-1 shows that the crop yield in greenhouses increased in response to the total input energy at first, but with more increase in the input energy, yield showed a decrease. This shows that the highest energy efficiency provided with the use of special rate of input energy and more increase in the input energy is along with the waste of energy. However obtained coefficient of determination in this figure is low and therefore the presented polynomial regression cannot be suggested as a reliable model for estimation but the plot provides a total overview on the relationship of crop yield and total input energy. Figures-2 and -3 show that the least energy consumption and GHG emissions per weight of crop yield and energy productivity was expressed as linear regression [Figure-4]. This shows that the energy productivity in greenhouses increased by increasing in the crop yield.



Fig:3. GHG per yield versus crop yield

Fig:4. Energy productivity versus crop yield

CONCLUSION

The present study evaluated the energy and GHG balance of greenhouse cucumber in Yazd province of Iran. The results indicated that fuel and electricity were the most important environmental pollutant in terms of energy consumption and GHG emission. The cucumber production was very dependent on direct and non renewable energies so that the share of direct energy from total input energy was very greater than indirect energy and the share of non renewable energy was also very greater than renewable energy. Assessing relations between crop



yield and calculated environmental indexes showed that the highest efficiency of energy and the lowest GHG emissions were achieved in the specific rate of yield production per hectare.

CONFLICT OF INTEREST

The authors declare having no competing interests

ACKNOWLEDGEMENT

None.

FINANCIAL DISCLOSURE

No financial support was received for this study.

REFERENCES

- Ozkan B, Akcaoz H, Fert C. [2004] Energy input-output analysis in Turkish agriculture. *Renewable Energy* 29: 39–51.
- [2] Pervanchona F, Bockstallerb C, Girardinc P. [2002] Assessment of energy use in arable farming systems by means of an agroecological indicator: the energy indicator. *Agricultural Systems* 72:149–172.
- [3] Enquete Commissio. [1995] Protecting Our Green Earth. How to manage global warming through environmentally sound farming and preservation of the world's forests. 683 pp. Bonn Germany Economia Verlag.
- [4] Lal R. [2004] Carbon emissions from farm operations. *Environment International* 30: 981–990.
- [5] Smith P, Martino D, Cai Z, et al. [2007] Agriculture. Chapter 8 of Climate change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (B. Metz O R. Davidson P R. Bosch R. Dave and LA. Meyer (Eds)) Cambridge University Press Cambridge United Kingdom and New York NY USA.
- [6] Pimentel D, Pimentel M. [1979] Food, Energy and Society. In: Resource and Environmental Science Series. Edward Arnold Publication, London.
- [7] Asakereh A, Safaienejad M, Sami M. [2011] Energy and economic analysis of dry farming chickpea in Iran a case study: Lorestan province. *Journal of Agricultural Technology* 7(3): 547–555.
- [8] Mousavi Avval S H, Rafiee S, Jafari A, Mohammadi A. [2011]. Investigating the energy consumption in different operations of oilseed productions in Iran. *Journal of Agricultural Technology* 7(3):557–565.
- [9] Raei Jadid S, Homayounifar M, Sabuhi Sabuni M, Mohammadi A. [2012] Energy analysis and optimization of inputs for wheat production in Marand region. *Indian Journal of Agricultural Sciences* 82(2): 21–24.
- [10] Taki M, Haddad M. [2012] A novel method with multilayer feedforward neural network for modeling output yield in agriculture. *International Journal of Modern Agriculture* 1(1):13–23.
- [11] Firoozi S, Sheikhdavoodi M, Sami M. [2014] Evaluation the ability of different artificial intelligence-based modeling techniques in prediction of yield using energy inputs data of farms. *Journal of Life Science and Biomedicine* 4(3): 214–220.
- [12] Hassanzadeh Ghorttapeh H, Taherifard E, Gerami F. [2012]. Energy efficiency in alfalfa (Medicago sativa L.) production system in north west of Iran (Case Study: Mahabad City). *Annals of Biological Research* 3(5): 2469-2473.

- [13] Sami M, Shiekhdavoodi MJ, Asakereh A. [2011] Energy use in apple production in the Esfahan province of Iran. *African Crop Science Journal* 19:125–130.
- [14] [14] Loghmanpour Zarini R, Yaghoubi H, Akram A. [2013]. Energy use in citrus production of Mazandaran province in Iran. *African Crop Science Journal* 21(1): 61–65.
- [15] Pahlavan R, Omid M, Akram A.[2012] The relationship between energy inputs and crop yield in greenhouse basil production. Journal of Agricultural Science and Technology 14: 1243–1253.
- [16] Eshun J F, Apori S O, Wereko E. [2013] Greenhouse gaseous emissions and energy analysis in rice production systems in Ghana. African Crop Science Journal 21(2):119 – 125.
- [17] Sami M, Shiekhdavoodi M J, Almassi M, Marzban A. [2013]. Assessing the sustainability of agricultural production systems using fuzzy logic. *Journal of Central European Agriculture* 14(3): 318–330.
- [18] Ghasemi Mobtaker H, Akram A, Keyhani A. [2010]. Economic modeling and sensitivity analysis of the costs of inputs for alfalfa production in Iran: A case study from Hamedan province. *Ozean Journal of Applied Sciences* 3(3): 313–319.
- [19] Singh JM. [2002]. On farm energy use pattern in different cropping systems in Haryana India. MA. Thesis International Institute of Management University of Flensburg Germany Stout B.A. 1990. Handbook of Energy for World Agriculture. New York: Elsevier; 504 pp.
- [20] Demircan V, Ekinci K, Keener H M, Akbolat D, Ekinci C. [2006]. Energy and economic analysis of sweet cherry production in Turkey: a case study from Isparta province. *Energy Conversation Management* 47(13–14):1761–1769.
- [21] Sami M, Shiekhdavoodi MJ, Pazhohanniya M, Pazhohanniya F. [2014] Environmental comprehensive assessment of agricultural systems at the farm level using fuzzy logic: A case study in cane farms in Iran. *Environmental Modelling and Software* 58: 95– 108.
- [22] Flynn H C, Smith P. [2010] Greenhouse gas budgets of crop production – current and likely future trends. First edition, International Fertilizer Industry Association Paris France.
- [23] IPCC.[2006] IPCC Guidelines for National Greenhouse Gas Inventories Prepared by the National Greenhouse Gas Inventories Programme H.S. Eggleston L. Buendia K. Miwa T. Ngara and Tanabe K. (Eds). Institute for Global Environmental Strategies Tokyo Japan.
- [24] Heidari MD, Omid M. [2011] Energy use patterns and econometric models of major greenhouse vegetable productions in Iran. *Energy* 36: 220–225.
- [25] Pishgar-Komleh SH, Omid M, Heidari MD. [2013]. On the study of energy use and GHG (greenhouse gas) emissions in



greenhouse cucumber production in Yazd province. *Energy* 59: 63–71.

- [26] Taki M, Ajabshirchi Y, Ghasemi Mobtaker H. [2012] Energy consumption input–output relationship and cost analysis for greenhouse productions in Esfahan province of Iran. *American Journal of Experimental Agriculture* 2(3): 485–450.
- [27] Pashaee F, Rahmati MH, Pashaee P. [2008] Study and determination of energy consumption to produce tomato in the greenhouse. In: The 5th National Conference on Agricultural Machinery Engineering and Mechanization 27–28 August Mashhad Iran. PP. 1–12.
- [28] Ozkan B, Akcaoz H, Karadeniz F. [2004] Energy requirement and economic analysis of citrus production in Turkey. Energy Conversion and Management 45, 1821–1830.
- [29] Salami P, Ahmadi H, Keyhani A. [2010]. Estimating the energy indices and profitability of strawberry production in Kamyaran zone of iran. *Energy Research Journal* 1(1): 32–35.
- [30] Canakci M, Akinci I. [2006]. Energy use pattern analyses of greenhouse vegetable production. Energy 31: 1243–1256.
- [31] Pimentel D, Hurd LE, Bellotti AC, et al. [1973]. Food production and the energy crisis. Science 182: 443–449.
- [32] Kitani O. [1998] Energy and environment in agricultural engineering research. International Engineering Conference Bangkok. Bangkok: *Thai Society of Agricultural Engineering*.
- [33] Barber A. [2004] Seven Case Study Farms: Total Energy & Carbon Indicators for New Zealand Arable & Outdoor Vegetable Production. AgriLINK New Zealand Ltd.
- [34] Macedo IC, Seabra JEA, Silva jEA R. [2008] Green house gases emissions in the production and use of ethanol from sugarcane in Brazil: the 2005/2006 averages and a prediction for 2020. Biomass Bioenergy 32: 582–595.
- [35] Mohammadi A, Omid M [2010] Economical analysis and relation between energy inputs and yield of greenhouse cucumber production in Iran. *Applied Energy* 87: 191–196.

Moslem Sami Is currently associated with Bank Keshavarzi Iran, Isfahan, Iran. He is interested and work in Biosystem analysis, environmental protection, modeling and fuzzy inference.

Habib Reyhani is Independent researcher. He is interested and work in energy analysis, environmental protection, agricultural mechanization.