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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.

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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

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RESEARCH ARTICLE OPEN ACCESS

DESIGN AND CONSTRUCTION OF 250 LITERS PLASTIC BIO-DIGESTER AND EVALUATION OF BIOGAS PRODUCTION USING 4 CO-SUBSTRATES

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ABSTRACT

Biogas is a gas produced by the breakdown of organic matter in the absence of oxygen. This work involves the construction of (250L) plastic biodgiester using plastic 'Storex' tank as reactor vessel and non-corroding metal as the agitating device. The substrate used in this work includes rice husk, paper waste, maize husk and cow dung were perpetrated using hydrothermal process (except the latter) before been prepared in slurry form to achieve 8% solid content. A total 170 liters slurry was used to fed the digester. In this study, batch fermentation system was used. During fermentation, agitation of the reaction medium was carried out (6-8 times daily) with the aid of agitating device. Biogas production was measured by the downward water displacement method. Measurable gas was observed at the 13th day of the study (600ml), while highest gas production was observed at 47th day of the work (5500ml). Fluctuation in environmental condition such as temperature and changing in pH value leads to inconsistence daily gas production. The pH of the medium ranged from 4.45 – 6.85. The use of plastic material for the construction of biodigester and a non-corroding metal as agitating device aids biogas production. This is because the plastic is cheap, durable, consistent, and gas leakage problem can be easily controlled. Also a mixture of animal waste and plant waste materials with proper pretreatment of these wastes prior to discharge to biodigester has a positive effect on biogas yield.

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KEY WORDS

Biogas; Biodigester; Substrates; Pretreatment; Construction; Fermentation

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[I] INTRODUCTION

Rotting vegetable matter and some plant waste as well as detoxification of toxic compound present in waste waters are known to give up flammable gas. During the course of treatment, it was observed that the gas generated can be used as a source of heat and energy to power industrial plant. This same idea was brought to the United Kingdom in 1895, when the gas produced was used to light street lamps. In the early 1900s this system was developed in some other parts of the United Kingdom and in Germany for the treatment of sewage. Centralized drainage systems were being installed in many towns in Europe and anaerobic digestion was seen as a means to reduce the volume of solid matter in sewage. The gas produced was occasionally used as a source of energy, especially during the Second World War [1].

In Bombay 1930s the use of farm manure in a floating steel gas drum to generate methane was developed. This program provides villagers with cooking fuel. The first allusion to animal manure comes from Humphrey DAVY; who reported early in the 19th century the presence of this combustible gas in fermenting farmyard manure. This was used for the inversion of the safety lamp [2].

In the year 1940, many municipal sewage treatment plants in the United States and elsewhere were already employing anaerobic digestion as part of the treatment of municipal waste and thus generating methane which was used to generate electricity for the plant [3]. This indicates that in pollution control, the anaerobic digestion process is proven effective with additional benefit in the form of a supply of useful gas [3].

Biogas typically refers to a gas produced by the biological break down of organic matter in the absence of oxygen [4]. According to Uzodinma (2008) [5] define biogas as a renewable natural gas containing approximately 70% methane (CH4) and roughly 30% carbon dioxide and trace amount of other gases. Biogas can also be defined as a gas resulting from anaerobic degradation of waste materials, or industrial waste materials. It is viable alternative source of energy [6]. Biogas from renewable raw materials contains methane, carbon dioxide, water vapor and other gases in trace form which include hydrogen sulphide, hydrogen ammonia etc. [7].

Biogas is mixture of gas produced by methanogenic bacteria while acting upon biodegradable materials in an anaerobic condition. Biogas is about 20 percent lighter than air and has an

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ignition temperature in the range of 650 degrees to 750 degree Celsius. It is an odorless and colorless gas that burns with clear blue flame similar to that of LPG gas [8]. Biogas is lighter than air and highly explosive!

Biogas can be generated from a wide range of energy crops such as maize, wheat, sunflower, grass, animal manures and by-products from industrial processes. After anaerobic digestion, the digestion residue (spent medium) can be used as a valuable fertilizer for agricultural crops [9].

Millions of cubic meters of methane in the form of swamp gas or biogas are produced every year by the decomposition of organic matter, both animal and plant vegetables [10]. It is almost identical to the natural gas pumped out of the ground by oil companies and used by many people for heating houses and cooking meals [10].

Apart from the anaerobic digestion process, methane can also be produced artificially; for example, production of methane from wood products or the use of biomass in a process called thermal gasification. This is also a renewable source of methane [11].

Biogas can be generated from a wide range of energy crops such as maize, wheat, sunflower, grass, animal manures and by-products from industrial processes. After anaerobic digestion, the digestion residue (spent medium) can be used as a valuable fertilizer for agricultural crops [9].
Economical biogas production requires high biogas yields and guidelines on optimum energy production, optimum harvesting time, optimum nutrient composition, optimum conservation and pre-treatment technology must be development [12].
There are two basic types of organic decomposition that can occur during biogas production: Aerobic and Anaerobic.

All organic materials, both animal and vegetables can be broken down by these two process, but the products of decomposition are quite different in the two cases. Aerobic decomposition fermentation will produce carbon dioxide, ammonia and some other gases in small quantities, heat in large quantities and a final product that can be used as a fertilizer. Anaerobic decomposition will produce methane, carbon dioxide, some hydrogen and other gases in traces, very little heat and final product with a higher nitrogen content than is produce by aerobic fermentation [13].

The increasing global industrialization, urbanization population explosion in major metropolitan cities have significant affected the amount of wastes generated from municipal solid wastes. Beukering et al. (1999) [14] accounted on urbanization as an important factor that increases waste generation. Anand (1999) [15] focused on urbanization as an important variable in waste generation, while Halla and Majani (1999) [16] emphasized on population explosion as an important determining factor in waste generation. Thus, if these wastes are not properly managed, they may pose a severe threat to public health [17].

With over 80% of the industries in Nigeria located in the industrial cities of Lagos, Kano and Port Harcourt [17], the need to strongly pursuer the conversion of wastes generated into fuel (biogas) to reduce its nuisance value to the environment is important. It is known that potentially, all organic waste materials contain adequate quantities of the nutrients essentials for the growth and metabolism of the anaerobic bacteria in biogas production [18]. This biogas is a renewable high quality energy source that should be explored, particularly in developing countries where energy is costly, and is much needed for developmental activities. In the anaerobic process, a complex mixture of interacting microorganisms, mainly bacterial, carries out the complete degradation of organic materials of biogas. The breakdown of the complex organic compounds occurs in a 3-stage process, involving 3 main groups of independent microbes. They are fermentative, proton reducing acetogenic and methanogenic bacteria [19, 20].
On a global scale, investigations on biogas production have been performed from olive-mill waste water [11], plant biomass used for phyto-remediation of industrial wastes [21], when diluted with poultry manure [18], cotton wastes and food/vegetable residue [22]. However, in Nigeria, most studies used animal dung as substrate [17]. A few investigations have been made on other substrate such as water hyacinth [18] and aquatic weeds [22].

There are several physical and chemical parameters that are known to influence biogas production. Those parameters are listed below [23]

Temperature: Mesophilic temperature which is between 20^0C – 40^0C is the best temperature of which the methanogens can metabolize complex organic wastes.

pH: Anaerobic digestion will occur best within a pH range of 6.8 – 8.0. More acidic or basic mixtures will ferment at a lower speed.

Carbon Nitrogen Ratio: Anaerobic process requires both element, but when other conditions are favorable, a carbon – nitrogen ratio of about 30 - 1 is ideal for the raw material fed into a biogas plant.

Total Solid Content: For proper biogas production, the waste material should be prepared in slurry form with a solid content of about 10% so that the slurry can move freely inside the bioreactor.

Retention time: This is based on the temperature of the fermentation and the type of substrate used.
Agitation: This is highly required in some fermentation processes in other to avoid slurry being settled out and form a hard scum on the surface, preventing the release of biogas.

Other factors that aid biogas production are: feeding rate, acid accumulation, initial inoculum, types of substrate etc.

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Biogas production in Nigeria and other African developing countries has not been put into proper consideration in energy generating sectors thus, the use of biogas in houses for cooking and at industrial level to generate electricity which can be used to power industrial plants has not been put in proper position. This is so due to the fact that the government and people depend on the energy derive from fossil fuel. This result into various form of pollution which includes oil spillage, oil contamination of water source, heavy metals from automobile exhaust and effluent from oil refineries which are known to rendered the ecosystem unpleasant for man, animal, plants, and microorganisms. This research work aimed at (i) Design and construction of 250 liters household biodigester and (ii) Evaluation of the performance of the digester using mixture of substrates.

[II] MATERIALS AND METHODS

2.1. Biodigester design

The The 250L plastic biodigester was designed and constructed. It has 2 openings; one at the top of the biodigester in which PVC pipe (8cm in diameter) was inserted which serves as feeder and carries 15cm in diameter and 20cm in length plastic rubber (capped), thus serve as the feeding place for the biodigester. The second opening was located at the bottom. At the second opening a 4cm in diameter PVC pipe was inserted and also carries the same size of rubber plastic as in the feeder, thus allows easy removal of the spent medium. The two openings are in opposite direction to each other. The gas chamber is located inside the reactor vessel at the upper part of the digester. At the top of the gas chamber, a rubber hose with 0.5cm in diameter is inserted for gas collection. This is allowed to pass through the lid of the 5L rubber plastic of water displacement.

The water displacement unit is made up of 2 identical 5L transparent rubber plastic container labeled “A” and “B”. About 4 liters of water was discharged into “A” which is then transferred into “B” through a 1cm rubber hose connected to both containers. The water moved due to the pressure as a result of gas production. This can be seen in **Figure– 2**.

The agitating device is inserted from the top of the digester in form of a rod or shaft which carries the mixer i.e. blade (3 in number attached to the shaft at 1200 to each other) of 10cm in diameter. The shaft carries a roller or motor driven device with a diameter of 20cm, which aids agitation of reaction medium, facilitate and also makes stirring easy. The stirrer is also equipped with 3 metal bars (with a diameter of 1/10 of the digester diameter). Thus serves as baffle unit, prevent dislocation of the spagar and prevent vortexing during agitation. **Figure– 1** shows biodigester diagram.

2.2 Substrate collection and pretreatment

2.2.1. Collection

The substrates used in this study were collected in 4 different locations in Ogbomoso town. Rice bran was obtained from one feed mill industries along “Apake” area, corn husk from “Odo Oba” market, Cow dung from “Kara” and paper waste (Old newspaper) from “Sabo” area. The substrates are in equal proportion of 3.4kg each.

2.2.2 .Pretreatment

For ease of digestion as well as to have maximum biogas yield, substrate for biogas production must undergo one or more treatment before been charged into the biodigester. Three out of four substrates are known to contain cellulosic and ligninosic materials, therefore to remove these macromolecules, hydrothermal decomposition was carried out. This is done by milling the substrates into powdered form, followed by boiling at 100^0C for one hour or more. The substrates that involve in hydrothermal decomposition include rice bran, maize husk and paper waste.

2.2.3. Slurry preparation

After hydrothermal decomposition of the cellulose and lignin compound present in the 3 substrates mentioned earlier, pretreatment process continued by making the substrate in slurry form. This was done by mixing each substrate with water to achieve 8% solid content [24]. After successful slurry preparation the substrates are now ready to be feed into digester.

2.3. Digester feeding and inoculation

2.3.1. Feeding

The pretreated substrates were charged into the digester through the feeding port of the digester (feeder). This is aided with the use of big plastic funnel placed at the mouth of the feeder plastic thus ensuring easy movement of the substrate through the 8cm in diameter feeder PVC pipe. A total volume of 170L of slurry prepared substrate was used to feed the digester.

2.3.2. Inoculation

In this study, fresh rumen content of cow was use to inoculate the digester. The rumen content was collected and transported under anaerobic condition to maintain the viability of the microorganism. 20% inoculum density was used. This is to ensure that appropriate numbers of microorganism cells were used to initiate the fermentation. Inoculation is done through the feeder [25].

2.4. Fermentation

In this study, batch fermentation system was used. During fermentation, the digester was stirred at least 5 times (5 - 8 times) daily for about 20 - 30mins each time with the aid of agitating device. Also pH of the digester content was measured every week by opening of effluent tap to allow about 20 - 30ml of the digester mixture to flow into a beaker (this is done after vigorous agitation) and then read with a pH meter. The digester was run under mesophilic temperature.

2.5. Gas quantification

The gas generated was measured by down ward displacement of water. The volume of water displaced was measured daily. **Figure– 2** show water displacement unit.

2.6. Data collection

The volume of biogas produce was recorded on a daily basis. The mean weekly gas yield and other parameters were recorded. This was monitored for 10 weeks and 4days. The daily ambient temperature over these periods varied from 28^0C – 32^0C while the average was 29^0C.

[III] RESULTS AND DISCUSSION

3.1. Changes in pH value during fermentation

Figure– 3 shows the variations in pH value of the reaction mixture. This is taken at an interval of seven days. At the

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beginning of the work, the initial pH is 6.42, moreover after 7 days, change in pH value was observe to be 4.89 (acidic) which may be due to metabolic activities of the fermenting microbes. The pH values continue to fall till it reaches 4.45 at 28th day then rose to 5.48 at 35th day of the fermentation. The pH continues to rise till 56th day which is nearly close to neutral at 6.85. Fluctuation in pH may be attributed to the interactions and metabolic activities of microorganisms in the fermenting medium.

3.2. *Gas production*

A measurable gas production started at 13th day of the work after feeding the digester with the treated waste and the inoculum. Although this is against the work Chidi et al. (2008) [26] in which they reported to have a measurable gas production at 4th day of their work, this may be due to variation in temperature, inoculum density, viabilities of microbes and interactions, substrate composition as well as digester size and amount of substrate been feed into the digester.

At the 14th day of the work the volume of water displaced dropped from 600ml to 85ml, 3days after there is no water displacement which may be due to drop in environmental temperature. There after daily water displacement fluctuation continue between 55ml to 400ml, then rose back at 34th – 48th day with a displacement ranging from 1020ml – 5,500ml. The highest volume of water displaced was observed at 47th day of the work (5,500ml) which is correlated to the work of Uzodinma (2008) [5] in which they have gas production of 150 liters at 47th day of fermentation. Gas production was obstructed by heavy rainfall for about 8 consecutive days (49th – 56th) while it reawakes at 57th day but at lower rate, this may be due to cool weather and or reduction in nutrients content of the medium. This is represented in **Figure– 4**.

Gas production rate depends on the ability of microbes to utilize available nutrient under a favorable condition. These conditions includes physical parameters such as pH, temperature, agitation e.t.c. pH of the medium is one of the most important parameters that affect biogas production. When all other conditions are favorable, optimum biogas production occurs at pH value ranging from 6.5 – 78.5. Uzodinma and Ofoefuhe (2008) [27] reported that pH value of their work ranged from 5.93 – 7.73 which is higher than the pH recorded in this work, with a pH value ranging from 4.45 – 6.85. This may be due to differences in substrates composition.

Fig: 1. Plastic bio-digester

Fig: 2. Water displacement unit

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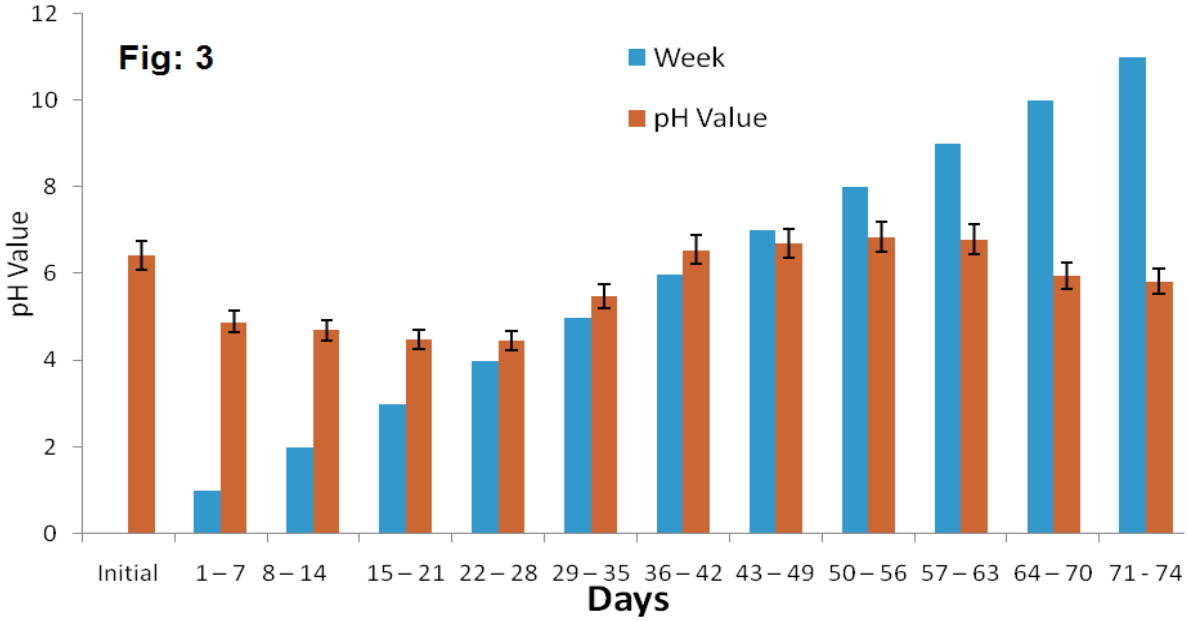

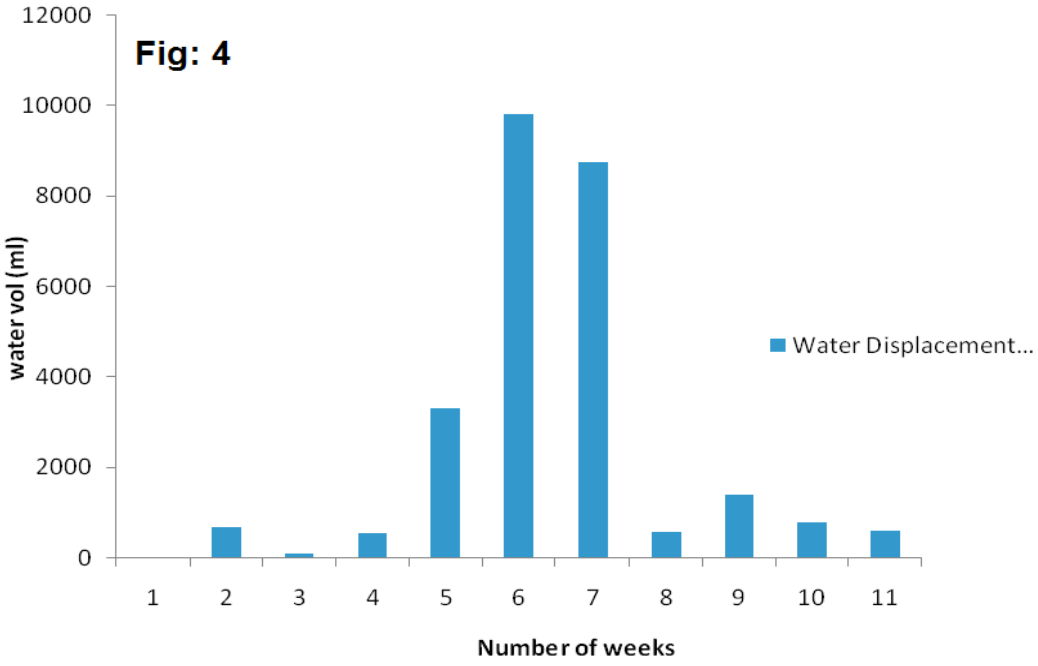

Fig: 3. pH Value and number of weeks; Fig: 4. Weekly water Displacement (ml)

[IV] CONCLUSION

From the results obtained and analysis of charts, graphs and tables, the following conclusions are made:
The use of plastic material for the construction of biodigester and non-corroding metal as agitating device should be employed during design and construction of biodigester for biogas production. This is because the plastic is cheap, durable, consistence, and gas leakage problem can be easily controlled.

Also mixture of animal waste and plant waste materials has positive effect on biogas yield [27], and pretreatment of these waste prior to discharge to biodigester also aid biogas yield [18].

Batch system of fermentation makes the work simpler and economical because it does not required daily pretreatment of waste, feeding as well as daily remover of spent medium. Also the pH of the medium was found to range from 4.45 (acidic) –

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6.85 (neutral), this is within the ranges that support biogas production [28]. The work was carried out under mesophilic temperature that ranged from 25 – 27^{0}C during which gas production occurs, but gas production continue to ceased due to temperature reduction being a result of cool weather and heavy rainfall.

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CONFLICT OF INTEREST

There is no any form of conflict of interest

FINANCIAL DISCLOSURE

No financial sponsor in the form of person, institution or organization is involved in the present work

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GLYCAEMIC AND LIPID PROFILES IN HIV POSITIVE PATIENTS ON ANTIRETROVIRAL THERAPY IN SOKOTO STATE, NIGERIA

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ABSTRACT

Background: *This study assessed the effects of Human Immunodeficiency Virus (HIV) and Antiretroviral Drugs (ARVs) on glycaemic and lipid profiles, and to determine the pattern of glucose and lipid abnormalities at different periods of the treatment.* ***Method***: *Two hundred (200) participants were enrolled in the study, which comprised fifty (50) HIV negative, fifty (50) HIV positive not on therapy (HAART naïve), fifty (50) on therapy for 1-6 months and fifty (50) on HAART for 7-12 months. Glycaemic and lipid profiles were analyzed using enzyme based methods while CD_4 cell counts were enumerated using flow cytometry.* ***Results:*** *Total Cholesterol, TAG, VLDL-C and FBS of control group did not differ significantly ($P>0.05$) in the entire three groups. The mean HDL-C was significantly lower ($p<0.05$) in the HAART naïve group, and those on treatment for 1-6 months compared to control subjects. HDL-C increased with the increase in the level of CD4 cell counts. The LDL-C in the control was observed to be significantly lower ($p<0.05$) than those on treatment for 1-6 months. A significant increase ($P<0.05$) in LDL-C/HDL-C ratio was observed in the entire groups, except those on therapy for 7-12 months compared to control group.* ***Conclusions***: *From our study we suggest that glycaemic and lipid profile should be part of routine test for all HIV patients as part of monitoring their management.*

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KEY WORDS

HIV; ARVs; T-Cholesterol; TAG; HDL-C

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[I] BACKGROUND

Human immunodeficiency virus (HIV) belongs to the genus lentivirus, a family of lentivirus [1]. Acquired immunodeficiency syndrome (AIDS), is incurable illness caused by human immunodeficiency virus (HIV), which are known by long period of replication before manifestation of the disease [2]. Losses of cellular immunity due to metabolic syndrome are one of the effects of AIDS [3].

The acquired immunodeficiency syndrome (AIDS) was initially identified in 1981 [4]. In Nigeria, the first two cases of AIDS were identified in 1985 and reported in 1986 during an International AIDS conference [5].

The HIV has thus far infected between 21.6 and 24.1 million people in Sub-Saharan Africa with South Africa having the highest figure followed by Nigeria [6]. Another report released by WHO [7] showed that 9.1 million of HIV infected people in Sub Saharan Africa are receiving antiretroviral therapy in 2013, which make the region to has the highest HIV positive patient on HAART worldwide.

In Nigeria, a total of 3.1 million people are living with HIV as at the end of 2011 and about 300, 000 new infections are occurring annually [6]. The national prevalence rate is 3.6% and 3.1% in 2011 and 2012 respectively [8]. Furthermore, about 1.5 million of people living with HIV in Nigeria have access to antiretroviral therapy [6].

Sokoto State, with a total population of 3,702,676 (2006 National Census Figures) has a HIV prevalence rate of 6.0% in 2008 [9], which dropped to 3.3% in 2010, below the National prevalence rate of 4.6% [10]. A total of 15,095 and 18,504 people were tested for HIV in 2013 and 2014 respectively, out of which 1,238 and 1,204 individuals were positive [11]. On the total of newly patient enrolled on ART, the figure showed that 714 and 525 patients are newly enrolled in 2013 and 2014 respectively [11].

Based on the WHO recommended guidelines for the treatment of HIV positive patients, a total of 1.5 million (30%) people infected with HIV infection in Nigerian are on therapy [6]. HAART has dramatically decreased the morbidity and mortality

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associated with HIV infection and rebuilds the immune system [12].

A number of side effects such as dyslipidaemia and lipodystrophy have been reported to be induced by HAART [13, 14]. Cardiovascular disease account for more than 20% of death [15] and diabetes [16,17]. Previous studies has associated ARVs particularly protease inhibitors with lipid and glucose abnormalities such as hyperglyceridaemia, hyper cholesterolaemia, hypo HDL- Cholesterolaemia, hyper insulinaemia [18, 19], high levels of LDL-C [18], insulin resistance, impaired glucose tolerance and diabetes mellitus, which leads to ischaemic heart disease [20, 21].

In Sokoto State antiretroviral drugs have been used for the management of HIV infection since 1998. Although, glycaemic and lipid profiles in HIV patients on ART has been widely studied in many places across the globe, none has been documented from Sokoto State. Thus, this study is set out to examine if some factors like viral virulence, genetic, race and environmental difference can modify the outcome of management of AIDS patients and whether some measures need to be taken to ensure that better therapies are received by the people living with HIV in the state.

[II] EXPERIMENTAL DESIGN

2.1. Research location

The research was carried out in Sokoto State, Nigeria. The sample was collected from HIV Clinics of Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital, Sokoto. An estimated 70 to 80% of the HIV positive patients in the state receive treatment from the two HIV clinics, hence the two centres are the major ART centres and enrolled about three fourth of the patients in the state. Ethics and Research Committee's approval of the two hospitals were obtained. Patients enrolled were informed using a standard informed consent form and written interview to subjects that gave their consent to participate in the study.

2.2. Sample Size

A total of two hundred (200) samples comprising fifty (50) apparently healthy HIV negative volunteers, fifty (50) sample from HIV positive pre-HAART (HAART naïve), fifty (50) HIV positive patients on treatment for 1 to 6 months, and fifty (50) HIV positive patients on HAART for 7 to 12 months.

2.3. Sample Collection and Sample treatments

On enrollment, 5mls of blood samples were collected using multiple sample needles with sterile vacutainers blood specimen bottle and centrifuged for five minutes at 3000g. The serum was removed and transferred into serum container by means of disposal transfer pipette for the assay of biochemical parameters.

[III] MATERIALS AND METHODS

3.1. Reagents

All reagents used were of analytical grade. For quantitative determination of total cholesterol, HDL-Cholesterol, triglyceride and fasting blood glucose in serum, enzymatic calorimetric kits were procured from Randox Laboratories Limited, United Kingdom. Kit for CD_4 count was procured from Partee Munster, Germany.

3.2. Statistical Analysis

Statistical analysis was performed using Graph pad Instat version 3.02 (Graph pad Corp., San Diego, USA). The data was described using descriptive statistics and analysis of variance (Benferroni compare all columns) to test for the level of significance between the mean. A P value < 0.05 was taken as statistically significant.

[IV] RESULTS

The fasting total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), Atherogenic index (AI), Triglyceride (TAG), fasting blood glucose, and CD_4 count were evaluated.

The fasting total cholesterol and TAG of the three groups (HIV positive HAART naïve, those on HAART for 1-6 months and 7-12 months on HAART) did not differ significantly ($P>0.05$) in comparison to control **[Supplementary Figures-1 and -2].**

The serum HDL-C levels of the three HIV positive groups were significantly lower ($P<0.05$) than the control group **[Supplementary Figure– 3]**. The level of HDL-Choleterol increases with the increase in CD_4 cell count, though no positive or negative significant correlation exists between the two parameters in the entire groups as shown in **Supplementary Figure– 4**. LDL-C level of the three HIV positive groups are higher compared to control, though not statistically significant ($P>0.05$) except those on treatment for 1 to 6 months **[Supplementary Figure– 5]**.

The VLDL-C levels of all the three HIV positive groups were not statistically significant ($P<0.05$) when compared with control subjects **[Supplementary Figure–6]**. A significant increase ($P<0.05$) in LDL-C/HDL-C ratio was observed in the two HIV positive groups (treatment naïve and those on treatment for 1-6 months) when compared with control **[Supplementary Figure–7]**.

Moreover, HIV positive subjects have higher level of fasting blood sugar when compared with the control, though the increase in not statistically significant ($P>0.05$) as shown in **Supplementary Figure-8** below. The glucose level of HIV positive subjects have been shown to increase with the increase in the level of CD_4 T-cell count, even though the two indices are not significantly correlated throughout the groups **[Supplementary Figure– 9]**.

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[III] DISCUSSION

No significant difference (P>0.05) in total cholesterol, triglyceride and very low density lipoprotein cholesterol of the HAART naïve groups, those on treatment for 1 to 6 months and 7 to 12 months on treatment compared to control, though there is a slight increment in the level of the three parameters, but is statistically insignificant.
Our findings on the lipid and glycaemic profiles are consistent with a report by Iffen *et al.* [22], in which no significant difference in the levels of fasting total cholesterol of control group when compared with HIV positive subjects was reported. Likewise, Kumar and Sathian [23] reported no significant change in TAG level of HIV positive patient when compared with the control. A study by Kumar *et al.* [24] revealed that very low density lipoprotein cholesterol were markedly elevated in HIV/AIDS patients compared to normal subject, which are in conformity with our finding, though the elevation is not significant.

Francis and Onyinye [25] reported significant increase in the serum total cholesterol, triglycerides and very low density lipoprotein cholesterol of HIV positive patients when compared with the control group. This study contrasts the finding of the present study.

The VLDL-C consists mainly of triglycerides, which may substitute the reason for no significant change in the VLDL-C level was observed when no significant change in TAG was also noticed among the three HIV positive groups.

The present study corroborates the finding by Khiangte *et al.* [26] who reported the mean HDL-C to be significantly higher in HAART group compared with HAART naïve group. The finding of this study is also consistent with the a study by Ducobu and Payen [27], where he reported that HIV infection induces early decrease of HDL-C, which is proportional to the lowering of CD_4 count, which revealed the severity of infections. Chandrasekaran *et al.* [28] suggested that use of non-nucleoside reverse transcriptase inhibitors based therapy (which was mostly used by the patients of this study) result in an elevation in HDL- levels and therefore may be less atherogenic than protease inhibitors.

Khiangte *et al.* [26] reported a decrease in the level of HDL-C as progression of HIV disease continues by concomitant decline in the levels of CD_4 cells. Progression of HIV infection was fully known with depletion of CD_4 cells count which is also accompanied by decrease in HDL-Cholesterol as reported by Obirikorang *et al.* [29]. As the virus (HIV) weaken the immune system various co-infections may likely to occur, which may lead to fever, malnutrition, diarrhea, loss of appetite etc. Fat from the food is the major source of HDL-C, which will reduce as a result of malabsorption of fat from the food caused by diarrhea [26].

The study by Francis and Onyinye [25] on the effects of HAART on lipid profile in HIV infected people contradict the finding of the present study, where they observed no significant difference (P>0.005) in the mean serum HDL-C levels of the HIV infected subjects on HAART compared to those without HAART and HIV seronegative individuals.
Additionally the study revealed that level of LDL-C in the treatment naive subject, those on HAART for 1-6 months and patient on HAART for 7 – 12 months are lower (thought not significantly) compared to control subject.

Ducobu and Payen [27] also reported that patient with AIDS had higher levels of LDL-C when compared with seronegative group, which conform to the finding of this current study. Other studies [24, 22] reported higher LDL-C levels in HIV positive patients when compared with control group.

Miserez *et al.* [30] reported that HIV positive patients taking ARVs commonly have high levels of LDL-Cholesterol, which conform to the present finding in this study as it was observed in 1-6 month of treatment group. The mechanisms by which ARVs result in metabolic disorders are not fully understood, but lipid abnormalities in HIV patients receiving protease inhibitors (PIs) treatment are more evident [31].

The LDL/HDL-C ratio (Atherogenic Index) of HAART naïve and those on HAART for 1-6 months was significantly higher (P<0.05) compared to control, but at subjects on HAART for 7-12 month the difference was insignificant.

Furthermore, the result of the study revealed that, the fasting blood glucose in HIV positive subject is higher, though not statistically significant compared to control subjects. Muthumani *et al.* [32] showed no significant change in the level of fasting blood sugar of HIV positive subjects compared to the control group, which corroborate to the finding of this study. Hadigan *et al.* [33] reported that fasting blood sugar levels remain in normal range in most of the patients receiving potent antiretroviral therapy. Gadd [34] reported that low CD_4 count in HIV positive subjects is associated with high glucose level, which may be serve as the reason for the higher (not statistically significant) glucose level in all the three groups when compared with control (with highest CD_4 cell count).

[IV] CONCLUSION

In conclusion, from the finding of our study the total cholesterol, Triglyceride, very low density lipoprotein and fasting blood sugar remain significantly unaltered in all the three HIV positive groups in comparison with the control. HDL-C was found to be significantly altered in the three HIV positive groups compared with HIV negative group, which was also observed to increase as the CD_4 T-cell count increase. A slight higher (not statistically significant) level of LDL-C was found in all the three HIV positive groups except those on HAART for

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1-6 months, where LDL-C level was significantly increased in comparison with control.

Our study was limited to one year (12 month) duration of treatment which may not have been enough to assess long term changes in lipids and glucose profiles. Data on individual dietary history are unavailable; therefore role of dietary intake cannot be commented on. The study did not include hypertensive and diabetic individuals throughout the study

CONFLICT OF INTEREST

The authors have declared no conflict of interest. The authors further declared that no other relationships or activities that could appear to have influenced this study

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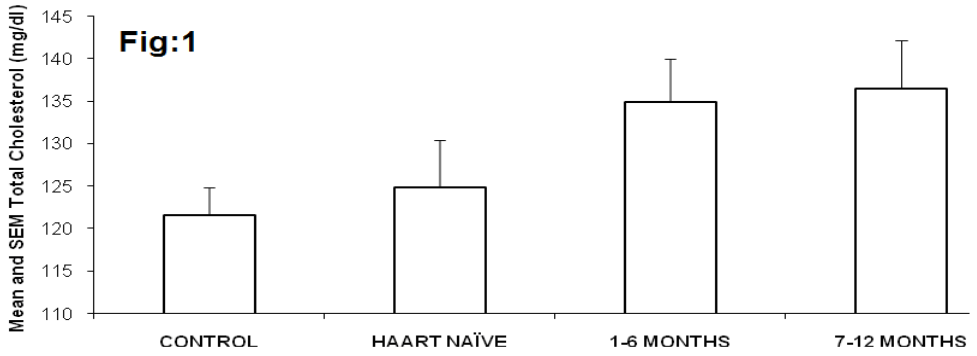
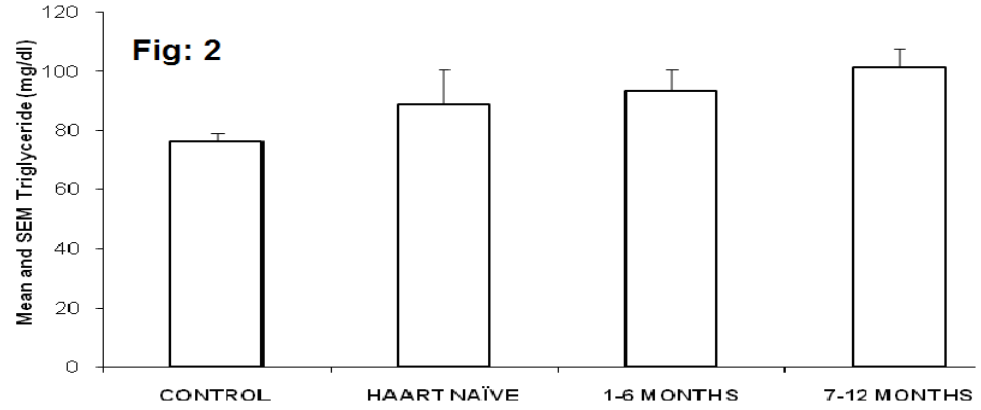
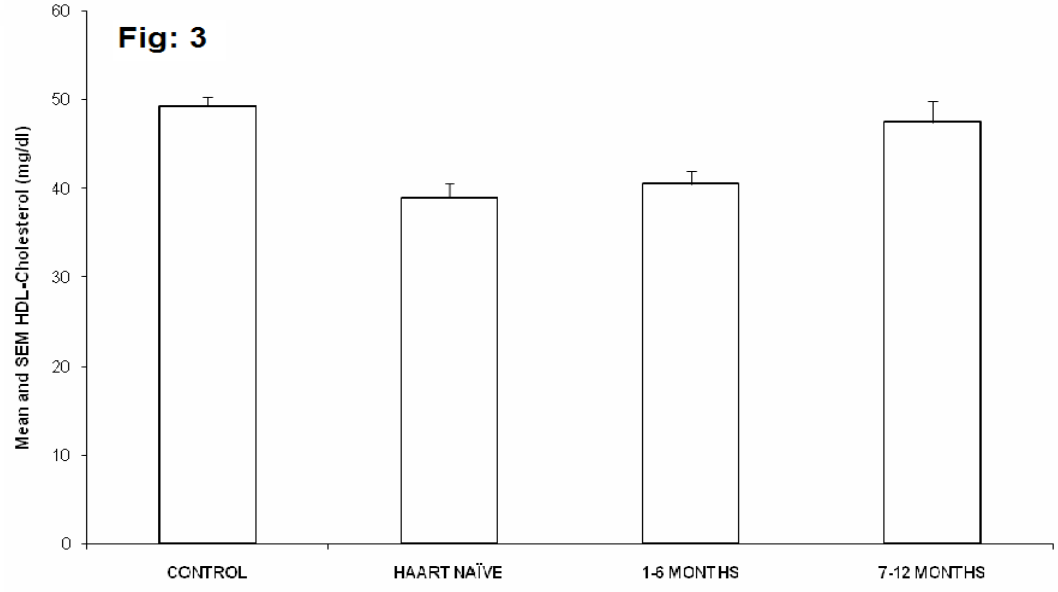
Supplementary Information (As provided by author)







Supplementary Figure 1: Total-Cholesterol level of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)
Supplementary Figure 2: Triglyceride level of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)
Supplementary Figure 3: HDL-Cholesterol level of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)

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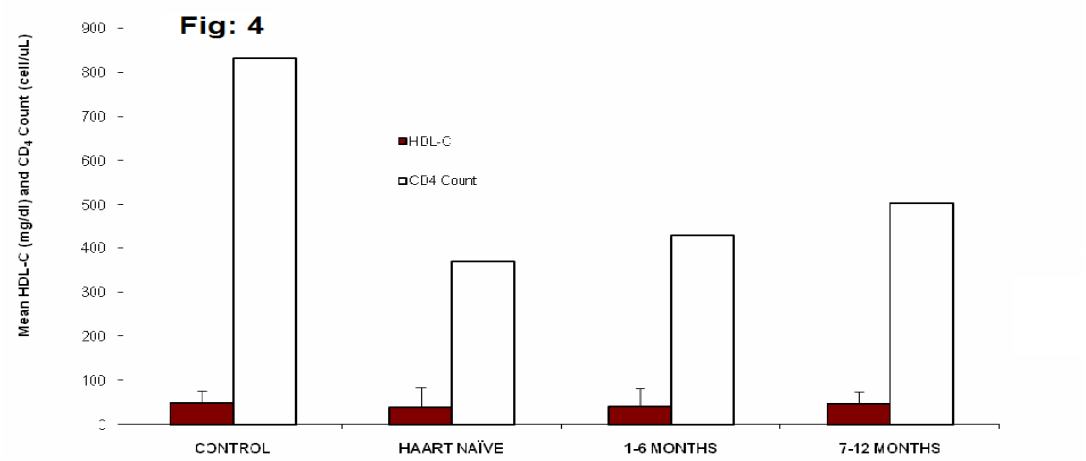

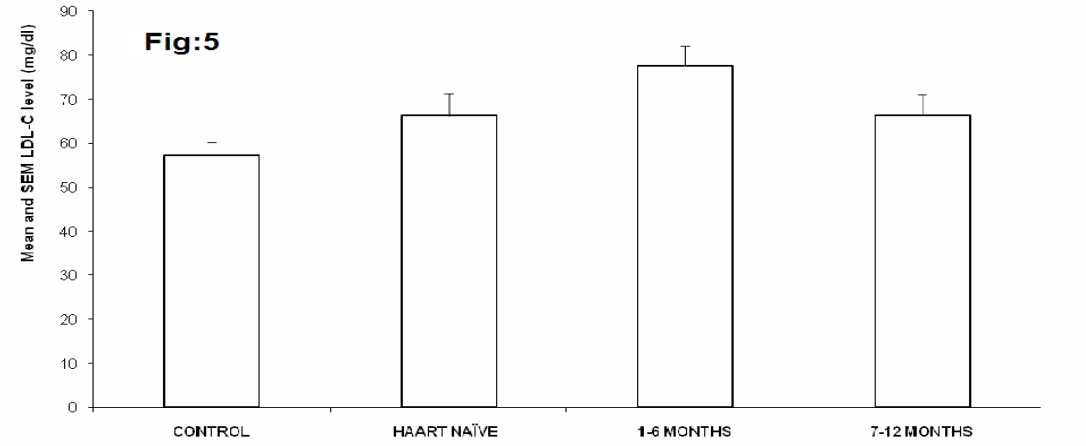

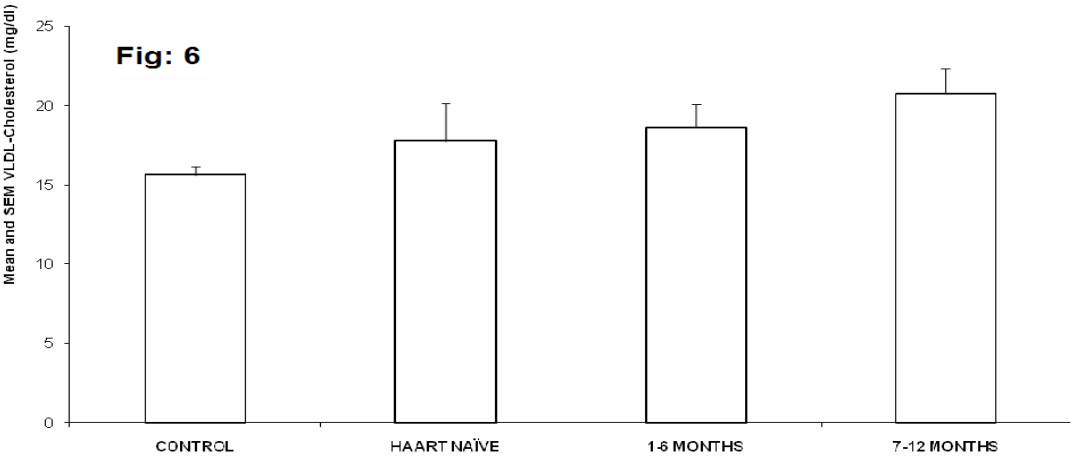

Supplementary Figure 4: HDL-Cholesterol and CD_4 T-cell Count of HIV negative subject, HIV positive, HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)
Supplementary Figure 5: LDL-Cholesterol level of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)
Supplementary Figure 6: VLDL-Cholesterol level of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)

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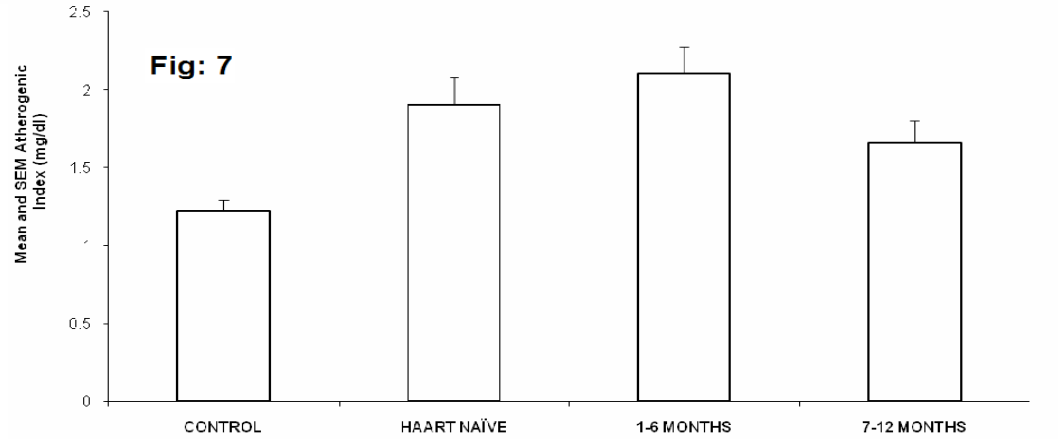

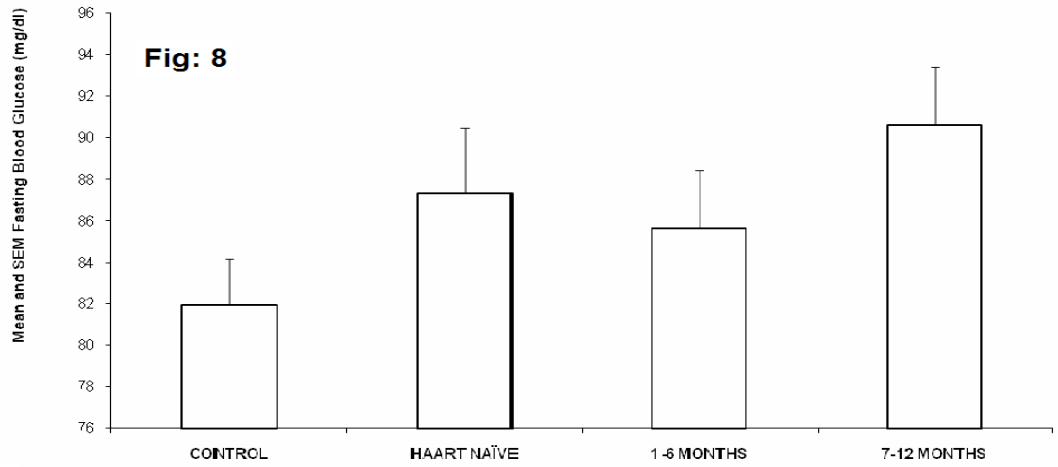

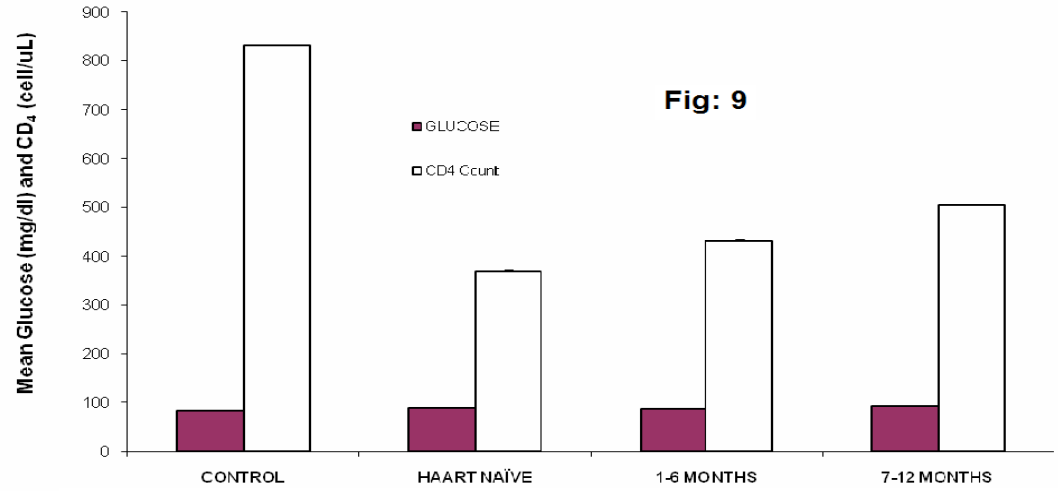

Supplementary Figure 7: Atherogenic Index of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)
Supplementary Figure 8: Fasting Blood Glucose level of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 month and 7- 12 months on HAART (n=50)
Supplementary Figure 9: Fasting Blood Glucose and CD_4 T-cell Count of HIV negative subject, HIV positive HAART naïve, Positive on HAART for 1-6 months and 7- 12 months on HAART (n=50)

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RESEARCH ARTICLE OPEN ACCESS

THE USE OF PLANT ENZYMES FOR RIPENING ACCELERATION OF RAS CHEESE

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ABSTRACT

Plant enzymes could be the cheap replacement of the costs microbial enzymes used in acceleration cheese ripening .Purified enzymes extract was prepared from the composite weed Cichorium pumilum. Cichorium enzymes purified extract (CEPE) was added at concentrations of 0.1, 0.2, or 0.3% (V /V) to milk (M treatments) or to cheese curd (P treatments). Proteolytic and lipolytic parameters were higher in treatments than control and in P treatments than in corresponding M ones. Rheological characteristics illustrated that cheese with CEPE had lower hardness, springiness, chewiness, gumminess, and higher adhesiveness and cohesiveness than control. Sensory evaluation proved that both M2 and P1cheese acquired after ripened for 60 days only the typical full flavor and body & texture of control cheese ripened for 150 days.

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KEY WORDS

Ripening acceleration, Ras cheese, Cichorium pumilum enzymes purified extract

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[I] INTRODUCTION

Ras cheese requires a long period of time to develop the full flavor and texture of ripened cheese. Accelerated ripening of cheese was achieved by addition bacterial strains or by addition of microbial proteases [1-3]. Both bacterial strains and microbial proteases are expensive, also bitterness was reported as the most common flavor defect in cheeses from milk or curd treated with microbial proteases [4-12,2,13].Although plant enzymes could be the cheap replacement of the costs microbial enzymes used in acceleration of cheese ripening, little work has been done in this field. Sadeq et al reported the possibility of using partially purified Calotropis procure leaves protease (Calotropin) to accelerate ripening of Monterey cheese [14]. Nadia and El Sisi used Cichorium enzymes crude extract for acceleration ripening of Domiati cheese [15].

Cichorium is a safe weed; US Food and Drug Administration Poisonous Plant Database, Cornell University Poisonous Plants Informational Database and Provet Poisonous Plants Database do not list any toxic effects of Cichorium. Rasheeduz and Mujahid reported that Cichorium had anti- hepatoxic effects in Wistar strain of Albino rats against carbon tetrachloride induced hepatic damage [16]. Vohra reported that Chicory has been listed as one of the 38 plants that are used to prepare Bach flower remedies, a kind of alternative medicine [17].Thus, in view of fermentation, the objectives of this study are to evaluate the possibility of utilization CEPE to accelerate Ras cheese ripening.

[II] MATERIALS AND METHODS

2. 1. Cichorium enzymes Crude extraction

Plant stems were cut into small pieces, dried, ground then acidic Cichorium enzymes crude extract was prepared as described by Vadde and Ramakrishna [18] with some modifications by stirring 10 grams of stem powder in 100 ml chilled 0.05 M tris-HCl buffer, pH 7.2 in blendor for 1 to 2 minutes The extract was filtered through cheese cloth and centrifuged at 20000 rpm for 15 min. The residue is discarded. The supernatant was referred as Cichorium enzymes crude extract (CECE).

2. 2. Purification of crude extract

Purification was done using the procedure described by Vadde and Ramakrishna [18].

2. 3. Estimation of proteins

Protein content in the Cichorium extract was estimated by the method of Lowry [19].

2. 4. Assay of proteolytic enzymes

Endopeptidase activity was measured using the method described by Sarath et al [20] and modified by Vadde and Ramakrishna [18].

2. 5. Assay of lipoletic enzymes

Lipase activity was measured using the method described by Qixin and Charles [21].

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2. 6. Ras cheese manufacture

Ras cheese was made by the conventional method described by Nadia [22]. Fresh mixture of buffaloes' and cows' milk (1:1) (obtained from the herds of the Faculty of Agriculture Menoufia University) was divided into seven portions. The first portion was left without treatment and served as a control (C). Cichorium enzymes Purified extract CEPE was added at levels of 0.1, 0.2 or 0.3 % to both of milk prior to renting (M treatments) and cheese curd particles before hoping (P treatments). Cheese was ripened at 14 ± 1°C for 5 months.

2. 6. Methods of cheese analysis

2. 6. 1. Chemical analysis

Moisture, acidity, total & soluble nitrogen and non protein nitrogen contents were determined according to Ling [23]. Schilovish index was estimated as described by Tawab and Hofi [24].

Determination of free amino acid was carried out as described by Bertacco et al [25].Total and individual free fatty acids (FFA) were determined by the method of Deeth et al [26].

2. 6. 2. Rheological analysis

Texture analyzer CNS – (The Farnell, England) was used. The probe was TA17 (30 angle and 25 mm diameter) at speed of 1 mm/ second and 10 mm distance in cheese. Cheese samples were cut into cubs 3cm3 and kept at 12 c for 1 hour before analysis.

2. 6. 3. Electrophoresis

Samples for electrophoresis were prepared as described by Sood et al [27].

2. 6. 4. Organoleptic evaluation

The organoleptic properties of the cheese samples were evaluated by regular score panels chosen from the staff members of Department of Dairy Science , Faculty of Agriculture ,Menoufia University according to the method of Nadia and Wafaa [28].

2. 6. 5. Statistical analysis

Factorial design 1 and 2 factors , 3 replicates was used to analyze all the data Steel and Torrie [29].

[III] RESULTS AND DISCUSSION

3. 1. Purification of protease

The results of the purification are summarized in **Table –1**. The crude extract had specific activity of 32.62 units/mg. The precipitate obtained between 40 – 60% NH2SO4 saturation recovered 1.68 fold by this step. The purified enzyme was about 2.56 fold for application of CG cellulose. In the next step, The purified enzyme was about 4.53 fold by DEAE-cellulose. The final efficient step in the purification procedure is the fractionation on Sephadex G75. The enzyme was highly purified by Sephadex G75 gel filtration chromatography. The specific activity of the protease enzyme is very high (286.48 units/mg). The purified enzyme was about 8.78 fold by this step

.

3. 2. Purification of lipase

The crude extract had lipase specific activity of 48.41units/mg **[Table– 1]**. The precipitate obtained between 40 – 60% NH2SO4 saturation recovered 1.12 fold by this step. In the next step, the purified enzyme was about 2.19 fold. Specific activity was 185.50 of application of DEAE cellulose chromatography for the purification of lipase. The enzyme was highly purified by Sephadex G 75gel filtration chromatography. The specific activity of the lipase enzyme was very high (418.2 units/mg). The purified enzyme was about 8.64 fold by this step.

Table: 1. Summary of purification

TEPS	PROTEIN(MG/ML)	SPECIFIC ACTIVITY Of protease U/mg	SPECIFIC ACTIVITY of lipase U/mg
Crude extract	3.2	32. 62	48.4
NH_2SO_4 fractionation	2.5	54. 70	53.6
CM-cellulose	1.8	83.42	106.12
DEAE-celellulose	0 .9	147.73	185.5
Sephadex G 75	0.3	286.48	418.2

3. 4. Chemical analysis of cheese

3. 4. 1. Ripening parameters

Data in **Table– 2** illustrate that the level of soluble nitrogen (SN/TN), non protein nitrogen (NPN/TN), Schilovish index and TVFA in the control cheese were significantly lower than those of the corresponding treated cheeses.

3. 4. 2. Free amino acid:

Data in **Table–3** shows the amount of free amino acids after 60 days in control, P1 and M1 cheese. Acids generally increased during ripening and were significantly higher in the CEPE treated cheeses than in the control cheeses, and they were higher in P1 than in M1 cheese. From these results, it seems that the

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CEPE increased liberation of FAA significantly and it was more effective when added to cheese curd than cheese milk.

Table: 2. Ripening properties of Ras cheese during ripening as affected by CEPE

Chemical properties	Ripening period	Treatments							
		C	M1	M2	M3	P1	P2	P3	LSD
Acidity	0	0.85^{a}	0.87^{a}	0.83^{a}	0.86^{a}	0.87^{a}	0.84^{a}	0.83^{a}	±0.05
	30	1.36^{a}	1.42^{b}	1.56^{c}	1.63^{d}	1.56^{c}	1.74^{e}	1.94^{f}	
	60	1.57^{a}	1.77^{b}	1.85^{c}	1.92^{d}	1.89^{c}	1.98^{d}	2.12^{e}	
	120	1.49^{a}	1.37^{b}	1.29^{c}	1.09^{d}	1.34^{b}	1.08^{d}	1.05^{d}	
	150	1.26^{a}	1.13^{b}	1.07^{c}	1.06^{c}	1.15^{b}	1.08^{c}	1.06^{c}	
SN/TN %	0	6.18^{a}	6.22^{a}	6.15^{a}	6.16^{a}	6.21^{a}	6.20^{a}	6.11^{a}	±0.15
	30	8.05^{a}	8.85^{b}	9.95^{c}	10.96^{d}	9.84^{c}	10.73^{d}	12.81^{e}	
	60	11.51^{a}	12.23^{b}	15.01^{c}	17.51^{d}	15.12^{c}	17.67^{d}	18.92^{e}	
	120	14.63^{a}	16.84^{b}	19.63^{c}	21.92^{d}	19.72^{c}	21.73^{d}	22.03^{e}	
	150	17.13^{a}	19.14^{b}	22.41^{c}	25.17^{d}	22.22^{c}	25.24^{d}	27.24^{e}	
N.P.N/TN%	0	4.13^{a}	4.15^{a}	4.18^{a}	4.19^{a}	4.20^{a}	4.21^{a}	4.15^{a}	±0.32
	30	4.32^{a}	4.73^{b}	5.97^{c}	7.96^{d}	5.92^{c}	7.74^{d}	9.17^{e}	
	60	5.65^{a}	6.88^{b}	8.21^{c}	10.67^{d}	8.19^{c}	10.43^{d}	14.16^{e}	
	120	8.87^{a}	9.54^{b}	10.88^{c}	12.08^{e}	11.41^{d}	12.33^{e}	15.12^{f}	
	150	10.43^{a}	10.97^{b}	11.51^{c}	13.44^{e}	12.97^{d}	13.18^{e}	15.71^{f}	
Schilovich index	0	15.92^{a}	16.24^{a}	16.23^{a}	16.31^{a}	15.82^{a}	15.98^{b}	15.94^{a}	±0.78
	30	28.16^{a}	32.92^{b}	38.97^{c}	45.93^{d}	38.77^{c}	45.65^{d}	56.15^{e}	
	60	45.56^{a}	55.82^{b}	67.16^{c}	71.86^{d}	67.22^{c}	73.12^{e}	80.77^{f}	
	120	58.11^{a}	69.33^{b}	82.16^{c}	92.55^{d}	85.52^{e}	95.61^{f}	108.52^{g}	
	150	71.92^{a}	80.66^{b}	96.22^{c}	109.29^{d}	99.13^{e}	113.39^{f}	125.18^{g}	
T.V.F.A	0	18.41^{a}	18.27^{a}	18.14^{a}	18.28^{a}	18.14^{a}	18.38^{a}	18.12^{a}	±0.84
	30	32.19^{a}	37.12^{b}	45.92^{c}	61.74^{d}	45.84^{c}	61.33^{d}	73.11^{e}	
	60	41.83^{a}	46.64^{b}	53.16^{c}	72.92^{d}	53.36^{c}	72.87^{d}	89.62^{e}	
	120	52.11^{a}	57.68^{b}	68.41^{c}	96.49^{e}	69.13^{d}	96.72^{e}	109.52^{f}	
	150	59.82^{a}	67.88^{b}	84.68^{c}	118.6^{f}	86.75^{d}	114.32^{e}	133.67^{g}	

See **Table– 1** for details, a–g Means within a row with different superscript letters are significantly different (P < 0.05)

Lysine, proline, phenylalanine and tyrosine, were in large quantities in all cheeses whereas glycine, Cysteic acid, Iso-leucine and therionine existed only in small amount. Ivana et al reported that in twenty five cheeses commercially available in the Czech Republic glutamic acid, proline, aspartic acid, leucine, valine, and phenylalanine are present in higher concentrations [30]. Other amino acids are present in lower concentrations. Sood et al mentioned that asparagine, glutamine, valine, leucine, phenylalanine, and arginine were in large quantities in all Cheddar cheeses whereas proline and histidine existed initially only in traces but increased later [27].

The determination of free amino acids plays an important role in assessing the nutritional quality of foods [31-33].

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3. 4. 3. Free fatty acids

The percentage concentration of free fatty acids (FFA) in Ras cheeses are shown in **Table– 4**. In terms of short (C4-C8) and long (C16, C18, C18:1) chain fatty acids, a significant interaction ($P<0.05$) was found between ripening period and cheese samples.

Amounts of butyric, caproic and caprylic acids were higher in samples with added CEPE than in the control cheese sample and in P1 than M1. Amount of free fatty acids were approximately equal in m2 and p1 cheese . From these results, it seems that the CEPE increased liberation of FFA significantly and it was more effective when added to cheese curd than cheese milk.

In control cheese sample the level of butyric, caproic, caprylic acids increased until 60 days, but then did not increase anymore. Adding *Cichorium* enzymes increased the level of butyric, caproic, caprylic acids until 150 days. Concentrations of capric, lauric and myristic acids increased more obviously in the cheese with added CEPE ($P<0.05$). Katsiari *et al* and Collins *et al* reported that the short and medium chain fatty acids contribute more to the flavor formation than the long chain ones [1],[34].

Palmitic, stearic and oleic acids were the most abundant FFA in all the cheeses throughout the ripening especially oleic acid. These results are in accordance with the findings of Guller and Kondyli *et al* [2, 35].

Table: 4. Free fatty acids of total FFAs in Ras cheese during ripening as affected by CEPE

Free fatty acids %	Storage Periods	cheese samples* C	M1	M2	P1
Butyric $C_{4:0}$	0	0.09	0.12	0.15	0.13
	60	0.14	0.17	0.34	0.37
	150	0.18	0.18	0.53	0.56
Caproic $C_{6:0}$	0	0.04	0.06	0.08	0.09
	60	0.06	0.08	0.16	0.18
	150	0.05	0.08	0.37	0.36
Caprylic $C_{8:0}$	0	0. 04	0.05	0.06	0.05
	60	0.05	0.07	0.15	0.18
	150	0.06	0.06	0.32	0.35
Capric $C_{10:0}$	0	0.15	0.18	0.21	0.19
	60	0.19	0.25	0.35	0.38
	150	0.18	0.26	0.52	0.56
Lauric $C_{12:0}$	0	0.43	0.50	0.58	0.52
	60	0.51	0.67	0.75	0.79
	150	0.62	0.78	1.88	1.95
Maristic $C_{14:0}$	0	1.5	1.6	1.8	1.8
	60	2.1	2.3	4.3	4.2
	150	3.7	4.1	6.3	6.5
Palmitic $C_{16:0}$	0	19.1	18.5	18.5	18.5
	60	17.6	16.2	12.0	11.8

*See **Table– 1** for details, Values are means of 3 replicates

3. 4. 4. Textural characteristics of cheese

Rheological characteristics **[Table-5]** illustrated that cheese with CEPE had lower hardness, springiness, chewiness, gumminess, and higher adhesiveness and cohesiveness than control. Data also illustrate that there was sharp decrease in gumminess, and chewiness values of all cheese as ripening period advanced. A negative relationship was found between hardness and the values found for gumminess, springiness and chewiness. These results agree with that reviewed by Bryant et al , Fox et al and Katsiari et al [1, 36, 37].

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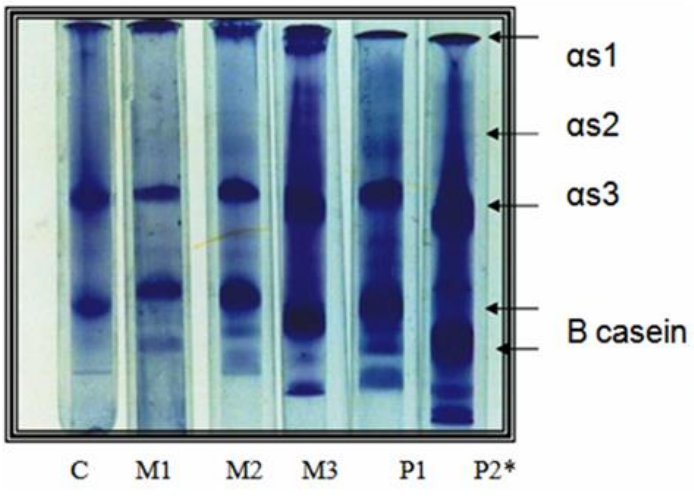
Table: 5. Rheological properties of Ras cheese during ripening. as affected by CEPE

Rheological properties	Ripening periods	cheese samples*							
		C	M1	M2	M3	P1	P2	P3	
Hardness (g) C	0	722.2^{a}	718,9^{a}	724.8^{a}	721.3^{a}	720.2^{a}	722.3^{a}	719.4^{a}	±9.2
	60	968.4^{a}	899.5^{b}	855.9^{c}	803.1^{e}	845.3^{d}	804.2^{e}	658.7^{f}	
	150	8112.1^{a}	751.7^{b}	534.8^{c}	411.5^{d}	528.9^{c}	415.9^{d}	332.9^{e}	
Adhesiveness gs^{-1}	0	9.27^{a}	9.18^{a}	9.34^{a}	9.25^{a}	9.32^{a}	9.23^{a}	9.24^{a}	±0.72
	60	15.48^{a}	18.12^{b}	32.25^{c}	22.88^{d}	31.85^{c}	21.65^{e}	14.35^{f}	
	150	39.30^{a}	35.15^{b}	14.67^{c}	5.08^{d}	14.38^{c}	4.88^{d}	4.05^{e}	
Springiness m m	0	8.91^{a}	8.72^{a}	8.73^{a}	8.82^{a}	8.99^{a}	8.88^{a}	8.84^{a}	±0.21
	60	6.16^{a}	6.56^{b}	5.45^{c}	4.67^{d}	5.62^{c}	4.51^{d}	3.22^{e}	
	150	5.85^{a}	4.98^{b}	4.08^{c}	2.88^{d}	4.05^{c}	2.76^{d}	2.05^{e}	
Cohesiveness ratio	0	1.38^{a}	1.32^{a}	1.36^{a}	1.35^{a}	1.30^{a}	1.31^{a}	1.36^{a}	±0.14
	60	2.92^{a}	2.51^{b}	1.58^{d}	0.89^{e}	1.72^{c}	0.92^{e}	0.72^{f}	
	150	0.74^{a}	0.71^{a}	0.62^{b}	0.60^{b}	0.74^{a}	0.66^{b}	0.51^{c}	
Gumminess gs^{-1}	0	1011.2^{a}	1014.5^{a}	1012.1^{a}	1017 .4^{a}	1013.3^{a}	1018.6^{a}	1015.7^{a}	±11.3
	60	943.3^{a}	953.8^{a}	756.7^{b}	542.3^{c}	747.8^{b}	550 .8^{c}	445.4 ^{d}	
	150	740.8^{a}	680.5^{b}	558.6^{c}	465.2^{d}	548.3^{c}	426.1^{e}	278.7^{f}	
Chewiness gs^{-1}	0	11304^{a}	11293^{a}	11298^{a}	11292^{a}	11303^{a}	11292^{a}	11297^{a}	±13.1
	60	3456^{a}	3124^{b}	2541^{c}	1342^{d}	2533^{c}	1326^{d}	819^{e}	
	150	1934^{a}	1740^{b}	1265^{c}	856^{d}	1254^{c}	846^{d}	563^{e}	

*See **Table 1**



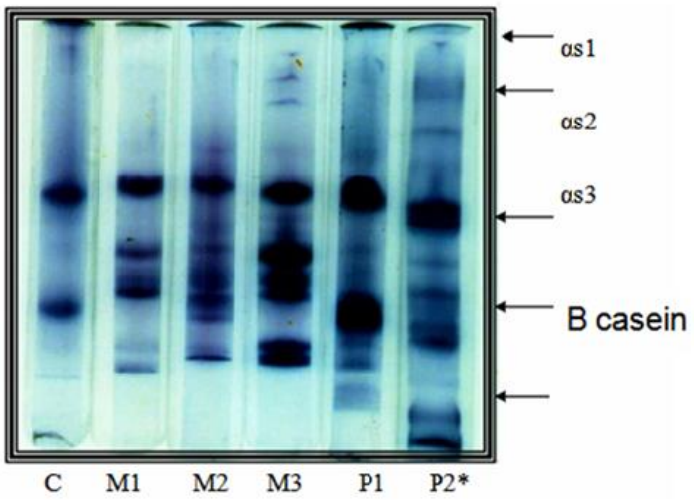


Fig: 1. Gel electrophoretic pattern of 60 days old Ras cheese. Fig: 2. Gel electrophoretic pattern of 150 days old Ras cheese

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3. 4. 5. Electrophoresis of cheese casein

Hydrolysis of αs1 casein was significantly greater and faster in cheeses treated with CECE than in control cheeses. The αs2 and αs3 caseins also were hydrolyzed but less than αs 1. The proteolysis of B-casein was greater in the Cichorium enzyme treated cheeses than in the control as well as was greater and faster in P treatments than M ones **[Figures– 1 and -2]** . Addition of CECE to Ras cheese milk or curds accelerated casein proteolysis. The B-casein, which remains essentially intact in conventional Ras cheeses was considerably degraded in the enzyme-treated cheeses. The α1- casein rose in all cheeses during ripening with more increase in CECE -treated cheeses. This suggests that added CECE either facilitate the action of rennin on the phenylalanine (24)-valine (25) bond as of casein or that the Cichorium enzymes attacks that particular bond. These results was in agreement with what found by sood *et al* [27].

3. 4. 6. Organoleptic properties of cheese

M1and P2 gained the highest total score after 60 days of storage .After ripening for 60 days only, they acquired the typical full flavor, body and texture of control cheese ripened for 150 days **[Table– 6]**.

[IV] CONCLUTION

Generally the results obtained lead to the conclusion that Cichorium could be used as a cheap source of plant enzymes; used to accelerate Ras cheese ripening. This reduces manufacturing costs for both producers and consumers. The most successful method of CEPE addition is adding 0.1% to cheese curd. It reduces the period required for Ras cheese ripening to 60 days instead of 150 days without any defects in cheese properties

Table: 6. Statistical analysis of total scores of organoleptic properties

		cheese samples*							
	Ripening Period	C	M1	M2	M3	P1	P2	P3	LSD
Total Criticisms (100Point	0	46.1[a]	45.2[a]	45.3[a]	46.3[a]	43.2[a]	44.4[a]	44.4[a]	± 3.8
	60	70.3[a]	83.1[b]	98.3[c]	85.4[b]	99.3[c]	85.2[b]	71.6[a]	
	150	98.5c	97.3[c]	67.1[b]	64.2[b]	67.2[b]	59.5[a]	56.2[a]	

*See **Table– 1**

ABBREVIATIONS: (CEPE) Cichorium pumilum enzymes Purified extract, C control cheese, M1,M2 and M3 cheese treated with 0.1 ,0.2 or 0.3% CEPE added to cheese milk – P1,P2or P3 cheese treated with 0.1 ,0.2 or 0.3% CEPE added added to cheese curd.

CONFLICT OF INTEREST

The Author assures that there is no conflict of interest

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