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## Bio-Tech & Genetics

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Discovering Thoughts, Inventing Future

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# Proteomix and Enzyme Kinetics in Normal and Cataractous Human Lens

By Dr. Ajit V. Pandya
*C. U. Shah Science College, India*

*Abstract-* Opacity of lens is called cataract. Blindness due to cataract increases to great extent globally, more than 50% people are experiencing profound or total loss of vision due to cataract. Oxidative damage plays major role in cataract development and defects are recorded in the antioxidant and related enzymes in lens during this disease. The mean value of GLUTATHION REDUCTASE activity is 1.463 ± 0.079 and 0.730 ± 0.062 n moles/min/mg, GLUTATHION-S-TRANSFERASE activity is 1.780 ± 0.069 and 0.545 ± 0.342 n moles/min/mg and Y-GLUTAMYL TRANSPEPTIRASE activity is 9.595 ± 0.094 and 3.7 ± 0.216 n moles/min/mg respectively for normal and cataractous lenses. An explanation for fall in GLUTATHION REDUCTASE activity would be the inhibitory effect of oxidants on the activity of reducing enzymes. The activity of GLUTATHION-S-TRANSFERASE is very low compared to GLUTATHION REDUCTASE and Y-GLUTAMYL TRANSPEPTIRASE. The turn over of GSH by Y-GLUTAMYL TRANSPEPTIRASE and GLUTATHION-S-TRANSFERASE is thought to be groups led with several factors including GSH level. Altered activity of the enzymes associated with the synthesis, catabolism and utilization of glutathione in the lens have been reported in mixed type of cataract.

*Keywords: glutathion reductase (GR), glutathione s- transferase (GST), glutamyl trans peptidase (GTP), human lens.*

*GJSFR-G Classification : FOR Code: 060107*


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*Keywords: glutathion reductase (GR), glutathione s-transferase (GST), glutamyl trans peptidase (GTP), human lens.*

## I. Introduction

Cataract is an opacification of the ocular lens that results in diminished vision and eventual blindness (Thylefors et al., 1995). Out of 41 million people blind globally, about 42% people are experiencing profound or total loss of vision due to cataract. The number of blinds in India is estimated to 18.7 million, out of which 9.5 million, the blindness is due to cataract. A single primary cause of cataract most likely does not exist.

Epidemiological literature indicates that the prevalence of cataract is related to geographical location, climate and sun hours (Hiller et al., 1977, Zigman et al., 1979). Oxidative stress plays major role in cataractogenesis and defects are recorded in the antioxidant and related enzymes in lens and peripheral blood during this disease.

The protecting glutathione system of the lens including glutathione redox cycle and its enzymes, glutathione reductase, glutathione-s-transterase, g-glutamyl transpetidase, GSH, GSSG, NADPH, etc. have Been reported in the lens. Thus, GSH metabolism can be expected to be a significant factor in the defense of lens against cataractogenesis. Altered activity of the enzymes associated with the synthesis, catabolism and utilization of glutathione in the lens have been reported with the progression of cataract (Rathbun et al., 1983).

Quantitatively, at least, the most significant protective system in the lens is that involving the reversible oxidation of glutathione. Normal lenses maintain a steady state of concentration of GSH; however this begins to drops in lenses undergoing cataract formation. This has been found to be true in almost all experimental cataracts and also in human senile cataracts.

One of the functions of this high level of reduced Glutathione is probably to maintain protein sulfhdryls in the reduced form (Kinoshita et al., 1964). Being small and mobile molecules, glutathione (GSH) reacts with potential oxidants before they could interact with the lens proteins. Thus, it would react with oxygen and also act as a scavenger for any free radicals generated by ionizing radiation, UV and visible light, or univalent reduction of oxygen.

Lens glutathione related enzymes are also capable of clearing mixed disulphide of glutathione and lens proteins. This provides the lens with a possible additional route for the regeneration of protein sulphydryl. Thus the study related with such protective enzymes help in identifying their key roles in the cataractogenesis and oxidative insult in the lens.

## II. Materials and Methods

### a) Collection of Material

The lenses of the patients who were undergoing cataract surgery at Nagari Eye Hospital, Ahmedabad, by medical Dr. involved in surgery, were collected. The cataract type in these patients was diagnosed with the help of slit-lamp biomicroscope. Several eyes with clear lens were obtained from C. H. Shamaria eye bank, Red Cross society, Ahmedabad, India.

1) The Y-GLUTAMYL TRANSPEPTIRASE activity was assayed by the method of Tate and Meister [7].

The activity was expressed as units/hr/g fresh lens, where a unit of Y-GLUTAMYL TRANSPEPTIRASE is equal to the u moles of p-nitroaniline released per minute.

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2) The GLUTATHION-S-TRANSFERASE activity was assayed by Worholm et al., method (1985).

The enzyme activity was expressed as units/hr/g fresh lens. One unit of GLUTATHION-S-TRANSFERASE is the amount of the enzyme that catalyses the formation of 1 u mol of 1 chloro-2, 4 dinitrophenyl glutathione per minute at 30 ° C.

3) Glutathion Reductase activity was estimated by the method of Carlberg and Mannervij (1985).

The activity of GLUTATHION REDUCTASE was expressed as units/hr/g fresh lens, where a unit of GLUTATHION REDUCTASE activity is defined as the amount of enzyme that catalyzes the reduction of l u mole of NADPH per minute.

4) Determination of Proteins: The soluble, insoluble, total proteins of the lens and total proteins of the AQH were determined by the standard method of Lowry et al., (1951).

*b) Statistical analysis*

All results were expressed in mean ± SD. One way analysis of variance (ANOVA) was used to test the significance of difference and Bonferroni test to test the significance of difference between control and different cataract types. The p value less than 0.05 is considered as significant. The results are expressed Glutathion Reductase by considering values of control lens as control as 100%.

## III. Results

*a) Glutathion-S-Transferase*

As shown in Table-1, the enzyme GLUTATHION-S-TRANSFERASE activities in both normal and cataractous lenses. The GLUTATHION-S-TRANSFERASE activity is 1.780 ± 0.069 and 0.545 ± 0.342 n moles/min/mg (mean ± S.E.) for normal and cataractous lenses. The decreases in activity during cataractous condition are by 69.38% for GLUTATHION-S-TRANSFERASE. This major change in activity of crucial enzymes is highly significant and p-value is less than 0.01.

One of the key enzymes associated with GSH metabolism is GLUTATHION-S-TRANSFERASE. GLUTATHION-S-TRANSFERASE, a family of proteins having multiple detoxification effects have been observed which is responsible for this reason . It was also observed that the activity of GLUTATHION-S-TRANSFERASE reduces with increase age and severely reduces under cataractous condition i.e. 70% compared to normal human lens. One of them has reported 73% reduced activity of GLUTATHION-S-TRANSFERASE in the brown dense cataracts (Rao et. al., 1983).

*b) ϒ-Y-Glutamyl Transpeptirase*

Similarly Table-1 shows Y-GLUTAMYL TRANSPEPTIRASE activities in normal and cataractous human lenses. The Y-GLUTAMYL TRANSPEPTIRASE activity is 9.595 ± 0.094 and 3.7 ± 0.216 n moles/min/mg (mean ± S.E.) respectively for normal and cataractous lenses. This reduce activity under cataractous condition is by 61.43% for Y-GLUTAMYL TRANSPEPTIRASE. The difference in activity of this crucial enzyme is highly significant and p-value is less than 0.01.

Since GSH is entirely degraded within the lens (Sippel, 1983), the r-glutamyl cycle seems to play an important role in the lens. Y-GLUTAMYL TRANSPEPTIRASE reacts very effectively with GLUTATHION-S-TRANSFERASE and all the enzymes involved in the GSH cycle. The activity of Y-GLUTAMYL TRANSPEPTIRASE is very low compared to GLUTATHION REDUCTASE and GLUTATHION-S-TRANSFERASE. The oxidation of GSH by Y-GLUTAMYL TRANSPEPTIRASE is thought to be groups led with transport of amino acids across the membrane by the same enzyme. This mechanism is highly effective in the lens, since it has a rapid turnover of GSH and is able to transport amino acids in to the tissue (Reddy, 1979). Any change in such mechanism may alter the Y-GLUTAMYL TRANSPEPTIRASE activity in the lens. The rapid turnover of GSH here would indicate rapid detoxification (oxidation) mechanisms coming into being.

Several epidemiological studies have claimed that antioxidants such as GSH and vitamin C have prevention role against the development of cataract (Bates, et.al, 1999, Bleau, et.al, 1998, Kupfer C, et.al, 1994, Carr, et.al,1999, Diplock, et.al,1998, Bunce,et.al, 1996, Malik A, et.al ,1995, Ringvold A, et.al, 1996). Eventually the cumulative action of oxidative activities on GSH bringing about its oxidation could hamper the detoxifying mechanism causing reduction in the GSH levels. Due to fall in r – glutamyl cysteine synthetase (r-GCS) activity, the level of GSH decline rapidly in lenses with increase in age. Since GSH is a substrate for Y-GLUTAMYL TRANSPEPTIRASE, its decrease would inhibit the feedback mechanism thus lowering the activity. All together GSH and its metabolizing enzyme activity affect one another which are key factor for cataract development.

*c) Glutathion Reductase*

Also Table-1 shows GLUTATHION REDUCTASE activities in normal and cataractous human lenses. The age Glutathion Reductase groups are matched and are compared for both normal and cataractous eye. The mean value of Glutathion Reductase activity is 1.463 ± 0.079 and 0.730 ± 0.062 n moles/min/mg (mean ± S.E.) in normal and cataractous lenses respectively. It decreases under cataractous condition by 50.1%.

Reported results show activity of GLUTATHION REDUCTASE decreases during aging and cataractogenesis. However, cataractous lenses contain

substantial amount of GLUTATHION REDUCTASE and mixed disulphide. Other Glutathion Reductase groups of scientist has also reported decrease activity of GLUTATHION REDUCTASE (Rao, et. al, 1983). The fall in Glutathion Reductase activity would result in decrease in GSH concentration, since, the system loses its capacity to regenerate GSH from G-S-S-G.

The decrease in Glutathion Reductase activity will also affect the proteins, since the fall in GSH levels would result in disturbances in the maintenance of protein-SH Glutathion Reductase groups in reduced form giving rise to protein – protein disulfide bonds or protein – GSH disulfide bonding leading to Glutathion Reductase aggregation of these proteins.

An explanation for fall in Glutathion Reductase activity would be the inhibitory effect of oxidants on the activity of reducing enzymes (Zigman, 1980). For the same reason GLUTATHION REDUCTASE is an – SH dependent enzyme. Since photo or chemical oxidation of specific amino acids (i.e. Tryptophane) can react with proteins and GSH – SH Glutathion Reductase groups, it may be postulated that the oxidative loss of Glutathion Reductase activity is due to tying – up of essential –SH Glutathion Reductase groups in the enzyme (Zigman, 1980). Nevertheless protein disulphide bonds still accumulate slowly. Perhaps, this indicates that the GLUTATHION REDUCTASE system cannot quite cope with the rate of oxidation in the cataractous lens. Decreases activity of GLUTATHION REDUCTASE could affect two major constituent or the lens – proteins and GSH, leading to the accumulation of GSSG, and $H_2O_2$ which is toxic to the lens as they are strong oxidative substance.

## IV. Protein Profiles of Lens

The average values for total protein (TP), soluble protein (SP) and insoluble protein (ISP) in normal and cataractous human lenses ate shown in table-2. It shows significant differences between normal and cataractous lens. There is significant increase in the level of ISP in cataractous condition where as significant decrease in the level of SP in cataractous lens compared to normal lens.

The change in amount of TP is negligible. With reference to TP there is insignificant difference between normal and cataractous lenses. The percentage of SP in normal and cataractous lenses ate 79.19% and 35.56% respectively, whereas that of ISP are 20.8 and 64.30 respectively compared to total protein. If shows increase in the level of ISP during cataractous condition. Significant difference exists between these two parameters in normal and cataractous lenses. All values are expressed as mean ± s.e. and p-value is less than 0.01. The changes leading to the production of these modified proteins isolated from the cataractous and normal human lens and the relationship between these is discussed here.

Many of the changes observed during all types of nuclear cataract. Opecification and aggregate formation of proteins during cataractous condition could be due to oxidation. Examples of such changes would be methionine sulphoxide and disulphide bond formation in the proteins. His had lead young scientist to consider are due to the oxidative modification of proteins in the nucleus of the lens. It has long been believed that oxidation is involved in many types of cataract. This had led to the development of a most of their levels in the lens.

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| | | |
|---|---|---|
| **ϒ-GTP** (n moles/min/mg) | 9.595 ± 0.094 (n = 22) | 3.70 ± 0.216 (n = 39) |
| **GR (n moles/min/mg)** | 1.463 ± 0.079 (n = 22) | 0.730 ± 0.062 (n = 42) |

➢ *All values are expressed as mean ± S.E.*

- - - - - - - ▶ *Numbers in the parenthesis are sample sizes (n).*
- - - - - - - ▶ *For all p-value < 0.01*

Total Protein, Soluble Protein and Insoluble Protein Content in Normal and Cataractous Human Lenses

| Type | TotaL Protein (ug/mg) | Soluble Protein (ug/mg) | Insoluble Protein (ug/mg) |
|---|---|---|---|
| Normal (n = 28) 60 ± 10 Yrs. | 382.09 ± 29 | 302.60 ± 26 | 79.48 ± 7 |
| Catractous (n = 48) 58 ± 12 Yrs. | 402.15 ± 31 | 143.03 ± 14 | 258.59 ± 21 |

- - - - - - - ▶ *All values are expressed as mean ± S.E.*
- - - - - - - ▶ n = sample sizes.
- - - - - - - ▶ For all p-value < 0.01

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# Extensive Study of Antioxidant Activity in *Agaricus Bisporus, Calocybe Indica* and *Pleurotus Ostreatus* Under Varying Cooking Conditions

By Kirthikaa B, Sathish Kumar T & Shanmugam S
*Kumaraguru College of Technology, India*

*Abstract-* The aqueous extracts of three edible mushroom species *viz. Agaricus bisporus, Calocybe indica and Pleurotus ostreatus* were analyzed for antioxidant activity using *in vitro* biochemical assays. In this study, the effect of widely used three cooking conditions, boiling, steaming and microwaving on the antioxidant content of the mushroom species were explored by comparatively analyzing the raw and cooked mushrooms. Cooking the food affects the total antioxidant content due to release of antioxidant, destruction or creation of redox-active metabolites. The radical scavenging ability and reducing power were observed to increase in cooked mushrooms while the ion chelating ability decreased than the fresh one. The DPPH radical scavenging ability for fresh *P.ostreatus, A.bisporus and C.indica* were 44.7, 42.7 and 40.3% respectively at the highest concentration. The scavenging ability for steam cooked P.ostreatus, A.bisporus and C.indica were 94.4, 91.8 and 89.3% respectively. The overall results suggest that the total antioxidant activity of the mushroom species were in the order of *P.ostreatus> A.bisporus >C.indica.* Among the cooking methods adopted in this study, the antioxidant content was highest in steam cooked mushrooms followed by boiled and microwaved. Thus, steaming is considered as the best cooking method of choice in order to release/ conserve antioxidants in mushrooms.

*Keywords: antioxidant; radical scavenging ability; redox-active metabolites; agaricus bisporus; calocybe indica and pleurotus ostreatus.*

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Kirthikaa B [α], Sathish Kumar T [σ] & Shanmugam S [ρ]

*Abstract-* The aqueous extracts of three edible mushroom species *viz. Agaricus bisporus, Calocybe indica* and *Pleurotus ostreatus* were analyzed for antioxidant activity using *in vitro* biochemical assays. In this study, the effect of widely used three cooking conditions, boiling, steaming and microwaving on the antioxidant content of the mushroom species were explored by comparatively analyzing the raw and cooked mushrooms. Cooking the food affects the total antioxidant content due to release of antioxidant, destruction or creation of redox-active metabolites. The radical scavenging ability and reducing power were observed to increase in cooked mushrooms while the ion chelating ability decreased than the fresh one. The DPPH radical scavenging ability for fresh *P.ostreatus, A.bisporus* and *C.indica* were 44.7, 42.7 and 40.3% respectively at the highest concentration. The scavenging ability for steam cooked *P.ostreatus*, *A.bisporus* and *C.indica* were 94.4, 91.8 and 89.3% respectively. The overall results suggest that the total antioxidant activity of the mushroom species were in the order of *P.ostreatus*> *A.bisporus* > *C.indica.* Among the cooking methods adopted in this study, the antioxidant content was highest in steam cooked mushrooms followed by boiled and microwaved. Thus, steaming is considered as the best cooking method of choice in order to release/ conserve antioxidants in mushrooms.

*Keywords: antioxidant; radical scavenging ability; redox-active metabolites; agaricus bisporus; calocybe indica and pleurotus ostreatus.*

## I. Introduction

Oxidation is the key factor for the living organisms in the production of energy for biological processes. Although oxidation is necessary, excess formation of oxygen - derived free radicals initiates many disorders such as tumor development, autoimmune diseases and also in degenerative processes associated with ageing [1]. These highly reactive oxygen derived free radicals which are capable of oxidizing biomolecules leading to cell death and tissue damage are produced by chemical and metabolic processes of the human body [2]. Almost all organisms are well protected against free radicals induced damage by oxidative enzymes such as super oxide dismutase (SOD), catalase (CAT), antioxidants such as α- tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione [3].The antioxidants present in the plant foods serves as a protection by scavenging free radicals. In addition to naturally occurring antioxidants the most commonly used synthetic antioxidants are butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and tert-butylated hydroxyquione (TBHQ). [4]. Some synthetic antioxidants have been reported to be carcinogenic, hence research on the potential applications of natural antioxidants, have received much attention [5-8].

Mushrooms are readily available source for water soluble vitamins. Edible mushrooms in cooked or other processed forms are rich in nutrients and antioxidants [9] and are suitable for diabetic and heart patients. Some recently isolated and identified compounds in mushrooms show significant medical properties, such as immuno-modulatory, cardiovascular, liver protective, anti-inflammatory and anti-diabetic [10-13].

Many plant products are studied for antioxidant property, but, till date, the effects of cooking and comparison of cooking methods on antioxidant capacity of foods have not been well studied. [14].Though there are studies on antioxidant activity of mushrooms, only little information is available about the effect of cooking on antioxidant activity of the mushrooms. In this study, we explored the effects of three cooking methods (boiling, steaming and microwaving) on antioxidant capacity of edible mushroom species *Agaricus bisporus, Calocybe indica* and *Pleurotus ostreatus* .

## II. Materials and Methods

### a) Chemicals

All chemicals used were of analytical grade; 1,1- Diphenyl – 2 – picryl –hydrazil (DPPH), ferrous chloride, 3- (2-pyridyl ) -5,6-bis (4- phenyl – sulfonic acid- 1,2,4-triazine) (Ferrozine), trichloroacetic acid (TCA), potassium ferric cyanide, ferric chloride, cerium

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sulphate and ethanol were obtained from Sigma chemicals, USA and Himedia chemicals, Mumbai, India.

*b) Standards*

The three standards used in this study are reported to remove the oxygen free radicals and acts as antioxidants. The standards were obtained from Sigma chemicals, USA.

a. Quercetin – Flavonoid – $C_{15}H_{10}O_7$
b. Rutin - glycoside between the quercetin and the disaccharide rutinose - $C_{27}H_{30}O_{16}$
c. Ascorbic acid - Form of Vitamin C - $C_6H_8O_6$

*c) Plant Material and Extraction*

Three mushroom species *A. bisporus, C. indica* and *P. ostreatus* were commercial samples. Fresh mushrooms from each species were divided into small pieces; 20g was weighed and grounded by using homogenizer, then mixed with aqueous solvent, water in room temperature at 150rpm for overnight. The extract was filtered over Whatman No.1 filter paper and the filtrate was collected, solvent was removed by evaporating at 50°C to obtain the dry extract. The extracts were placed in a plastic bottle and then stored at 4°C to prevent oxidative damage until analysis of the extracts.

*d) Cooking Methods*

The antioxidant activity of cooked mushrooms is analyzed by adapting three common cooking methods *viz.,* microwaving, steaming and boiling.The mushrooms were washed under running tap water and remaining water is blotted before weighing.

i. *Microwaving*

Mushrooms of 50g and 200ml of distilled water were added to a glass beaker and micro waved for 5 minutes. The mushrooms were then cooled for few minutes to room temperature and homogenized then the extract was prepared as stated above.

ii. *Steaming*

50g of mushroom was placed over 95°C water in a closed water bath for 5 minutes, after which the mushrooms were removed, cooled and processed as above.

iii. *Boiling*

200ml of boiled distilled water were added to a 50g portion of mushroom and placed in water maintained at 95°C for 5 minutes. After cooking the mushrooms were cooled, homogenized and extracted as above.

*e) Determination of total Antioxidant Activity*

The total antioxidant activities of the extracts were determined by the cerium (IV) sulphate method [15]. Cerium (IV) sulphate solution of 2mM concentration was added to extracts and standards of varying concentrations (100-1000$\mu$g/ml). Each concentration is done in triplicates. The solution is mixed well and incubated for 30minutes at room temperature. Absorbance of each solution was measured at 320nm using UV/ VIS spectrophotometer (Beckman DU-530).

*f) Determination of Reducing Power*

The reducing power of the aqueous extracts was determined by ferricyanide method [16]. Varying concentrations of standards and aqueous extracts (100-1000$\mu$g/ml) were mixed with phosphate buffer (0.2M, pH 6.6) and 1% potassium ferricyanide. The reaction was incubated at 50°C for 20 minutes and rapidly cooled followed by addition of 10% trichloroaceticacid. The contents are centrifuged at 1000g for 10 minutes. The supernatant obtained was mixed with 0.1% ferric chloride and allowed to stand for 10 minutes at room temperature. The absorbance was measured at 700nm using UV/ VIS spectrophotometer (Beckman DU-530).

*g) DPPH radical scavenging assay*

The free radical scavenging ability of the extracts was determined by using $DPPH^+$ [17]. DPPH, (1, 1-Diphenyl-2-picryl-hydrazil) radical solution of 1mM was added to varying concentration (100-1000$\mu$g/ml) of test solution and standards. The reaction mixture was mixed and incubated at room temperature for 30 minutes. The absorbance was recorded at 517 nm using UV/ VIS spectrophotometer (Beckman DU-530). $DPPH^+$ scavenging activity was determined as:

$DPPH^+$ scavenging activity (%) = [1-(test sample absorbance/ blank sample absorbance] × 100.

*h) Ferrous ion Chelating Ability*

The ferrous ion chelating ability [18] was used to determine the metal chelation ability of the mushroom extract. The varying concentrations (100 – 1000$\mu$g/ml) of extracts and standards were mixed with 2mM $FeCl_2$ and 5mM ferrozine solutions. The reaction was incubated for 10 minutes at room temperature. The absorbance at 562nm of the resulting solutions was measured and recorded using UV/ VIS spectrophotometer (Beckman DU-530). The ion chelating ability is calculated by using the formulae:

Ferrous ion chelating ability (%) = [1-(test sample absorbance/ blank sample absorbance] × 100

*i) Statistical Analysis*

The experimental data were evaluated and graphs were plotted by using Microsoft Excel. The statistical analysis such as one way ANOVA was performed using Graphpad prism 5.0 software

## III. Results and Discussion

*a) Determination of Cerium (IV) sulphate antioxidant capacity*

The Cerium (IV) sulphate assay was based on the oxidation of antioxidant by Ce (IV) ions. The absorbance indicates the concentration of unreacted Ce (IV) ions. The antioxidant capacity of samples increases

with increasing concentration and this was observed from the decrease in absorbance.

The total antioxidant activity of *P.ostreatus* was found to be higher than *A.bisporus* and *C.indica*. The antioxidant activity of cooked mushrooms was observed to be higher than the raw ones [Figure 1a-e]. Among the cooking processes performed the antioxidant activity is higher in the steam cooked mushrooms. The antioxidant activity of all three steam cooked mushrooms were plotted separately for detailed explanation. These differences were statistically significant at 5% level ($P<0.05$) using one way ANOVA.

*b) Determination of Reducing Power*

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [19]. $Fe^{3+}$ - $Fe^{2+}$ transformation was investigated in the presence of sample for the measurements of the reductive ability. In this method, the antioxidants reduced the oxidant probe $Fe^{3+}$ to $Fe^{2+}$. This ion then conjugated with the ferricyanide ion to form a Prussian blue coloured product, which was spectrophotometrically measured at 700nm. An increase in the absorbance was due to an increase in the extract concentration, which indicates a significant reducing power [Figure 2a-e]. The reducing power of the fresh mushrooms were in the order of *P.ostreatus*> *A.bisporus* >*C.indica*. The reducing power of the cooked ones was higher than the raw mushrooms. The reducing power was also higher in the steam cooked mushrooms than the other ones which are shown in Figure 2. These differences were statistically significant at 5% level ($P<0.05$) in one way ANOVA.

*c) DPPH Radical Scavenging Assay*

The DPPH reacts with methanol or absolute ethanol to give a purple colour DPPH radical ($DPPH^{+}$). The presence of antioxidants in the sample will scavenge the formed DPPH radical and there by a decreasing the formed colour. Scavenging effects of extracts from mushroom species on DPPH radicals increased with increasing concentrations. [20, 21]

The DPPH radical scavenging ability for fresh *P.ostreatus*, *A.bisporus* and *C.indica* were 44.7, 42.7 and 40.3% respectively at the concentration of 1000μg/ml. The scavenging ability for steam cooked *P.ostreatus*, *A.bisporus* and *C.indica* were 94.4, 91.8 and 89.3% respectively. The free radical scavenging ability for methanolic extracts of *A.bisporus* and *P.ostreatus* were reported to be 77.5 and 81.3% at the concentration of 180 μg/ml [1]. The scavenging ability was found to be lower for aqueous extract than methanol extract yet methanol was not preferred in this study because of its toxic nature. The radical scavenging ability for three mushrooms under varying cooking conditions along with fresh ones and standards were depicted in [Figure 3a-e].

The one way ANOVA analysis shows that fresh and cooked mushrooms were found to be significantly different.

*d) Ferrous ion chelating ability*

In this assay the mushroom extracts and standard antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator.

Ferrous ion chelating ability for *P.ostreatus*, *A.bisporus* and *C.indica* were 93.0, 91.3 and 89.6% respectively at the concentration of 1000μg/ml. The metal chelating ability for steam *P.ostreatus*, *A.bisporus* and *C.indica* were 91.3, 89.8 and 87.2% respectively. The metal chelating ability for methanol extracts of *A.bisporus* and *P.ostreatus* were 58.5 and 62.5% at the concentration of 100μg/ml [1].

These differences were statistically measured at 5% level ($P<0.05$) using one way ANOVA. The difference is not statistically significant. This shows that ion chelating ability though reduced to certain extent in cooked mushrooms than the raw ones it is not significant reduction. The ferrous ion chelating ability for fresh mushrooms along with the cooked ones were shown in [Figure 4a-e].

## IV. Conclusions

Based on the results of this study, it is clearly indicated that the aqueous extracts of fresh and cooked mushroom species *A.bisporus, C.indica* and *P.ostreatus* has significant antioxidant activity against various antioxidant systems *in vitro.* The chelating ability of *P.ostreatus* was found to be similar to the potent antioxidant standards, rutin, quercetin and vitamin C. The results presented here clearly demonstrate that process of cooking can make the antioxidant capacity of cooked food quite different from that of uncooked ones. This is most probably caused by a variety of effects, including destruction, release and transformation of food components. In this study, steaming was shown to be best method to retain and/or enhance the antioxidant capacity, followed by boiling and then by microwaving. To conclude, steaming is the preferred method to enhance the potential to obtain antioxidants from mushrooms. Hence these mushroom species can be used as an easily accessible source of natural antioxidants in fresh and cooked forms and also as a possible food supplement or in pharmaceutical industry.

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## Contribution by Authors

K.B – Performed experiment, data analysis and paper work.
R.P – Acted as study director and provided trouble shooting support.
S.T – Co-ordinated and designed the study.

## Figures Legends

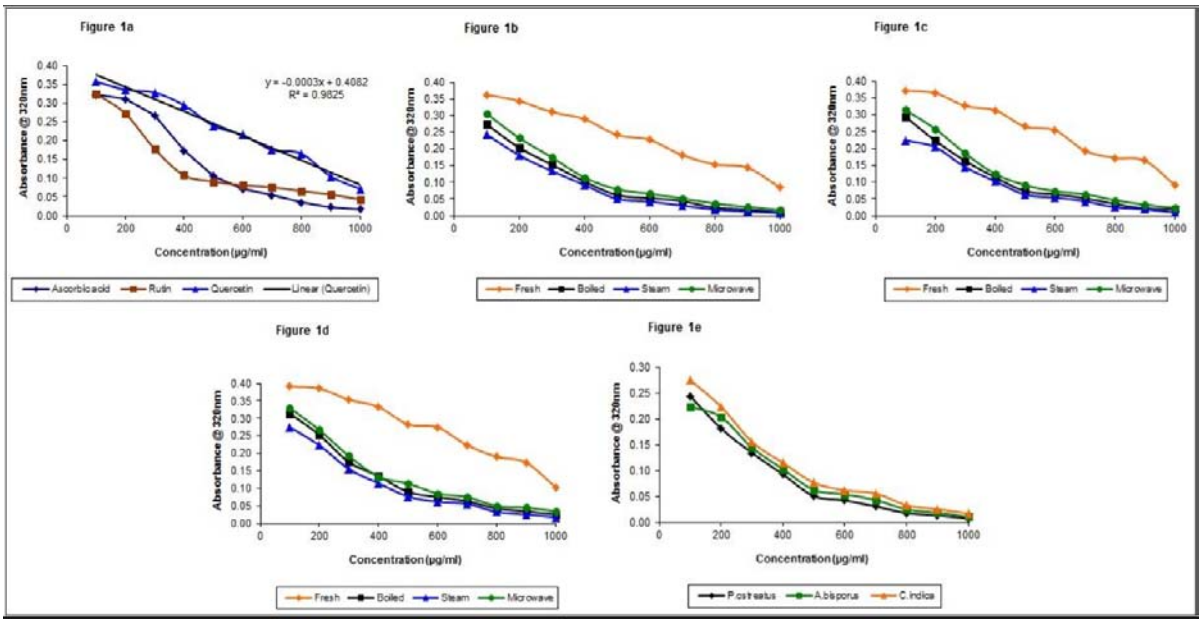


*Figure 1a :* Standard graph for total antioxidant activity.
*b :* Total antioxidant activity of P.ostreatus in cooking conditions.
*c :* Total antioxidant activity of A.bisporus in cooking conditions.
*d :* Total antioxidant activity of C.indica in cooking conditions.
*e :* Total antioxidant activity of steam cooked mushrooms.

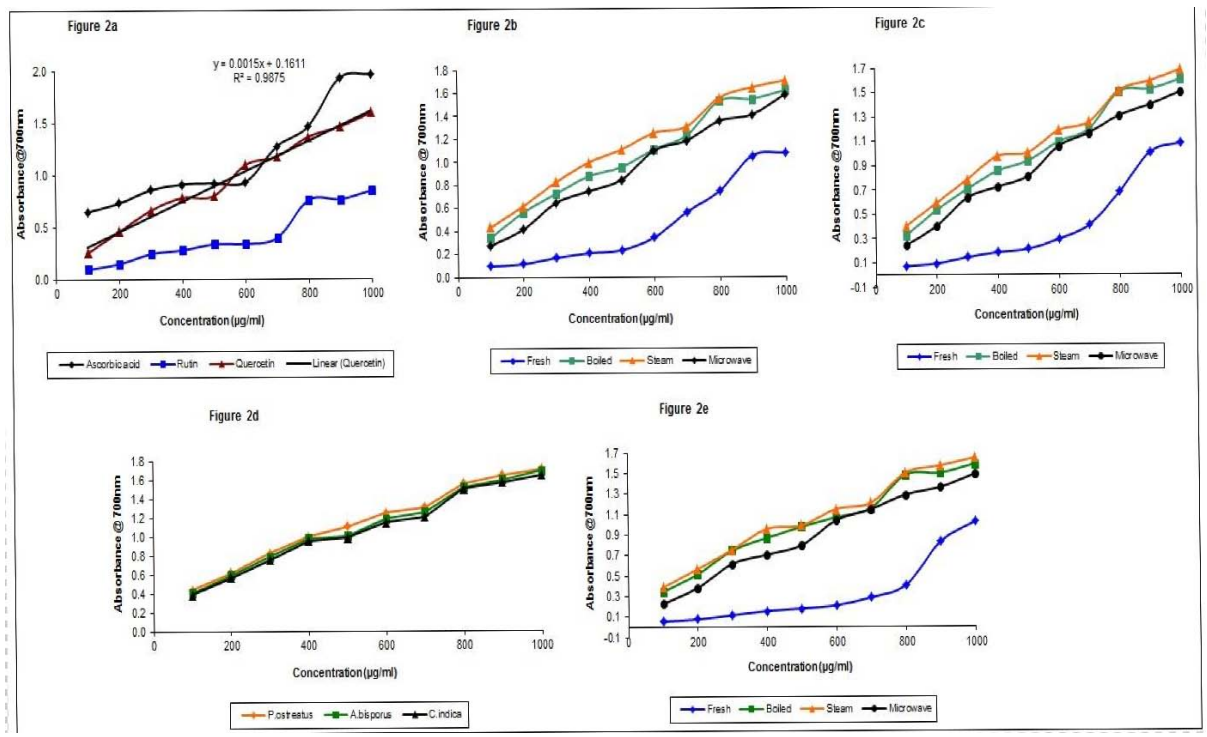


*Figure 2a :* Standard graph for determination of reducing power.
*b :* Reducing power of P.ostreatus in cooking conditions.
*c :* Reducing power of A.bisporus in cooking conditions.
*d :* Reducing power of C.indica in cooking conditions.
*e :* Reducing power of steam cooked mushrooms.

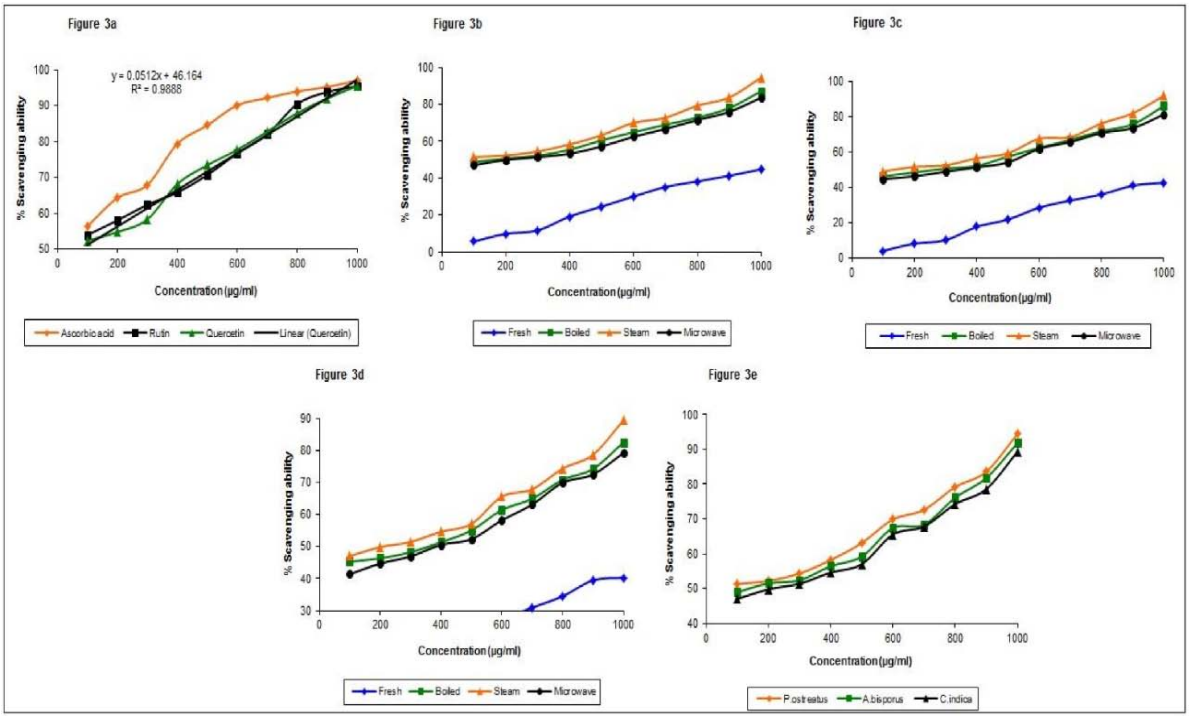


*Figure 3 a :* Standard graph for DPPH radical scavenging ability.
*b :* Radical scavenging ability of P.ostreatus in cooking conditions.
*c :* Radical scavenging ability of A.bisporus in cooking conditions.
*d :* Radical scavenging ability of C.indica in cooking conditions.
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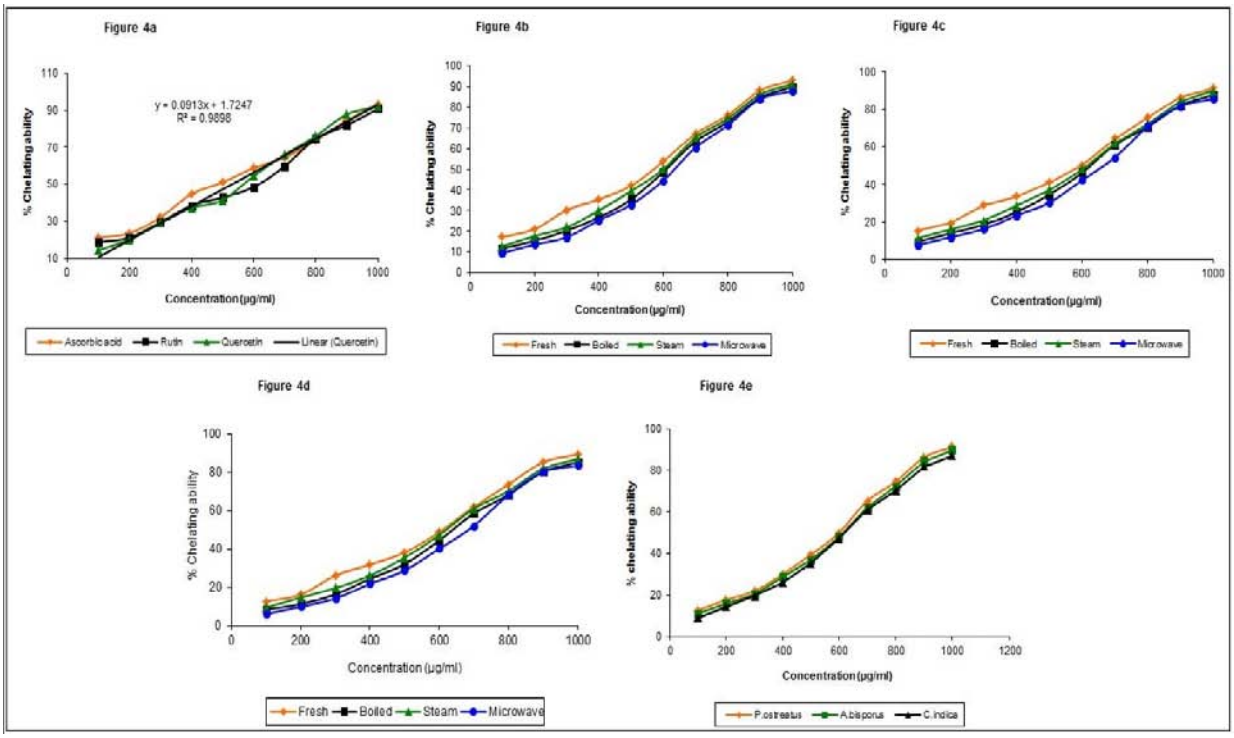


*Figure 4 a :* Standard graph for ion chelating ability.
*b :* Chelating ability of P.ostreatus in cooking conditions.
*c :* Chelating ability of A.bisporus in cooking conditions.
*d :* Chelating ability of C.indica in cooking conditions.
*e :* Chelating ability of steam cooked mushrooms.

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# A Fast Multiple Attractor Cellular Automata with Modified Clonal Classifier Promoter Region Prediction in Eukaryotes

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*Abstract-* DNA is a very important component in a cell, which is located in the nucleus. DNA contains lot of information. For DNA sequence to transcript and form RNA which copies the required information, we need a promoter. So promoter plays a vital role in DNA transcription. It is defined as "the sequence in the region of the upstream of the transcriptional start site (TSS)". If we identify the promoter region we can extract information regarding gene expression patterns, cell specificity and development. So we propose a novel fast multiple attractor cellular automata (MACA) with modified Clonal classifier for promoter prediction in eukaryotes. We have used three important features like TATA box, GC box and CAAT box for developing this classifier. The proposed classifier is tested with datasets from Eukaryotic Promoter Database, EPDnew which is a collection of promoters of human, mouse, zebrafish and D.melanogaster. In training phase of the classifier 100% specificity was obtained. In testing phase 84.5% sensitivity and 92.7% specificity was achieved in an average. The time taken to predict the promoter region of length 252 in an average is .7 nano seconds.

*Keywords: cellular automata (CA), multiple attractor cellular automata (MACA), clonal classifier (CC), promoter.*

*GJSFR-G Classification : FOR Code: 270199p, 270899p*


*Strictly as per the compliance and regulations of :*

# A Fast Multiple Attractor Cellular Automata with Modified Clonal Classifier Promoter Region Prediction in Eukaryotes

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*Abstract-* DNA is a very important component in a cell, which is located in the nucleus. DNA contains lot of information. For DNA sequence to transcript and form RNA which copies the required information, we need a promoter. So promoter plays a vital role in DNA transcription. It is defined as "the sequence in the region of the upstream of the transcriptional start site (TSS)". If we identify the promoter region we can extract information regarding gene expression patterns, cell specificity and development. So we propose a novel fast multiple attractor cellular automata (MACA) with modified Clonal classifier for promoter prediction in eukaryotes. We have used three important features like TATA box, GC box and CAAT box for developing this classifier. The proposed classifier is tested with datasets from Eukaryotic Promoter Database, EPDnew which is a collection of promoters of human, mouse, zebrafish and D.melanogaster. In training phase of the classifier 100% specificity was obtained. In testing phase 84.5% sensitivity and 92.7% specificity was achieved in an average. The time taken to predict the promoter region of length 252 in an average is .7 nano seconds.

*Keywords: cellular automata (CA), multiple attractor cellular automata (MACA), clonal classifier (CC), promoter.*

## I. Introduction

Most of the problems in bioinformatics can be address through bioinformatics. Promoter prediction plays a vital role in protein formulation and DNA transcription. Some of the genetic diseases which are associated with variations in promoters are asthma, beta thalassemia and rubinstein-taybi syndrome. Promoter sequence [1] can be used to control the speed of translation from DNA into protein. It is also used in genetically modified foods.

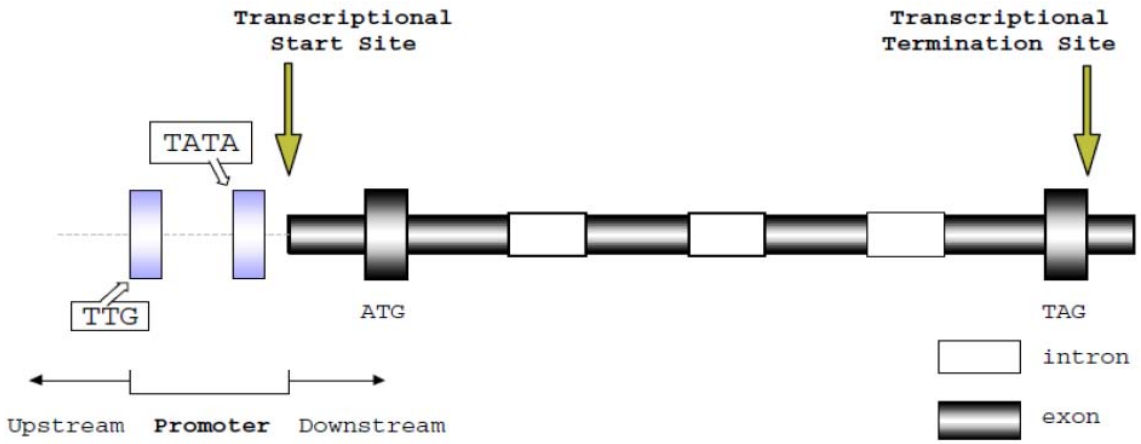


*Figure 1:* Promoter Region in a DNA sequence

Fig: 1 shows the location of promoter and protein coding region in untranslated region (UTR). Promoter is located towards the upstream (5') of the DNA sequence. Promoter initiates the Transcription. The start codon (ATG) of the protein coding region and stop codon (TAG) were also indicated in the fig 1.

Cellular Automata (CA) is a basic model of a spatially developed decentralized system, made up of various unique components called Cells. It is a computing model which can provide a good platform for performing complex computations with the available local information. Each cell in the system has a specific state which changes with over time depending on the neighboring states.

Von Neumann[2] and Stanislaw Ulam initially proposed the model of Cellular Automata in 1940. Stephen Wolfram[3] did a detailed study on one-dimensional CA (Elementary CA). He later published a book on "A New Kind of Science" in 2002 which dealt with basic and neighborhood structure of CA has pulled in scientists from different disciplines. It has been

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subjected to thorough numerical and physical dissection for most recent fifty years and its requisition has been proposed in diverse extensions of science - both social and physics.

So we apply a special class of CA [4] termed as multiple attractor cellular automata which uses fuzzy logic strengthened with modified Clonal classifier to predict the promoters efficiently and fastly.

## II. Literature Survey

Vladimir B. Bajic [1] et al. have developed ANN (Artificial Neural Networks) based program for finding promoters using micro-structural promoter component recognition. Authors have considered features like TATA box, CCAAT box, Inr and GC box for promoter prediction. All these features are cascaded and every feature has a corresponding ANN developed. The output of all features will be given to the integration layer ANN to give the final output. Authors have compared their work with Audic, Autogene, Promoter 2.0, NNPP, Promter Find, Promoter Scan, TATA, TSSG, TSSW, IMC, SPANN, SPANN2 for True Positives and False Positives.

Jih-Wei Hung [5] has developed an effective forecast calculation that can expand the recognition (power =1 - false negative) of promoter. Authors introduce two strategies that utilize the machine force to ascertain all conceivable examples which are the conceivable characteristics of promoters. The primary strategy we exhibit FTSS (Fixed Transcriptional Start Site) utilizes the known TSS positions of promoter arrangements to prepare the score record that helps us in promoter forecast. The other strategy is NTSS (Nonfixed TSS). The TSS positions of promoter arrangements utilized as a part of NTSS are thought to be obscure, and NTSS won't take irrefutably the positions of Tsss into attention. By the exploratory effects, our expectation has higher right rate than different past systems

Marshall S.Z. Horwitz [6] et al. have chosen an assembly of Escherichia coli promoters from irregular DNA groupings by swapping 19 base sets at the -35 promoter area of the etracycline safety gene te" of the plasmid pbr322. Substitution of 19 base sets with artificially blended irregular groupings brings about a greatest of 419 (something like 3 x 1011) conceivable swap groupings. From a populace of in the ballpark of 1000 microscopic organisms harboring plasmids with these irregular substitutions, tetracycline choice has uncovered numerous practical -35 promoter successions. These promoters have held just halfway. homology to the -35 promoter accord grouping. In three of these promoters, the agreement operator moves 10 nucleotides downstream, permitting the RNA polymerase to distinguish an alternate Pribnow box from inside the definitive pbr322 succession. Two of the successions advertise translation more determinedly than the local promoter.

## III. Design of maca Based Modified Clonal Classifier

A Cellular Automata which uses fuzzy logic is an array of cells arranged in linear fashion evolving with time. Every cell of this array assumes a rational value in the interval of zero and one. All this cells changes their states according to the local evaluation function which is a function of its state and its neighboring states.

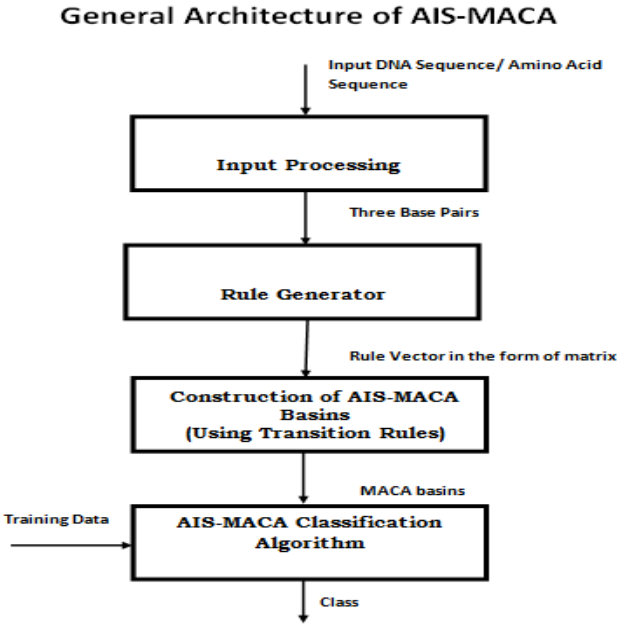


*Figure 2 :* General Design of MACA

The general design of MACA [7], [8], [9] based Modified Clonal Classifier is indicated in the figure 2. Input to this algorithm and its variations will be DNA sequence and Amino Acid sequences. Input processing unit will process sequences three at a time as three neighborhood cellular automata is considered for processing DNA sequences. The rule generator will transform the complemented and non complemented rules in the form of matrix, so that we can apply the rules to the corresponding sequence positions very easily. MACA basins are calculated as per the instructions of proposed algorithm and an inverter tree as in Fig 3,4 named as AIS multiple attractor cellular automata is formed which can predict the class of the input after all iterations.

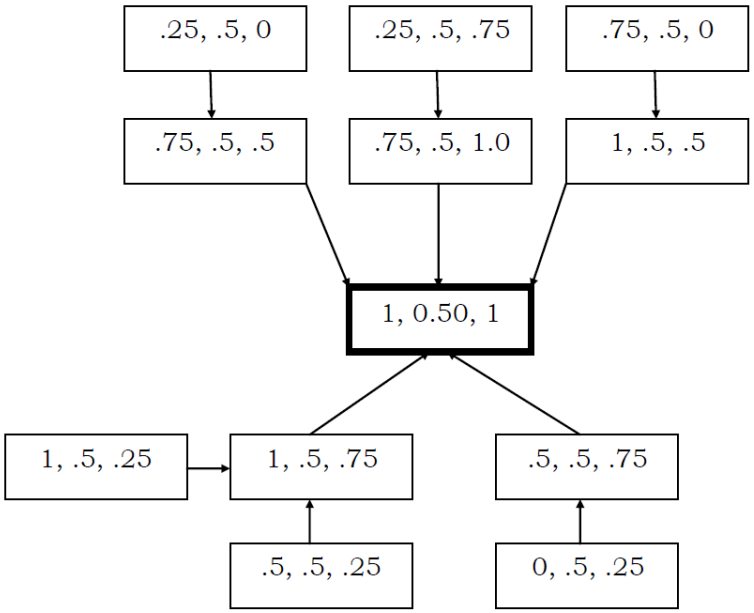


*Figure 3 :* MACA-Modified Clonal Classifier Tree with basin 1, .50, 1

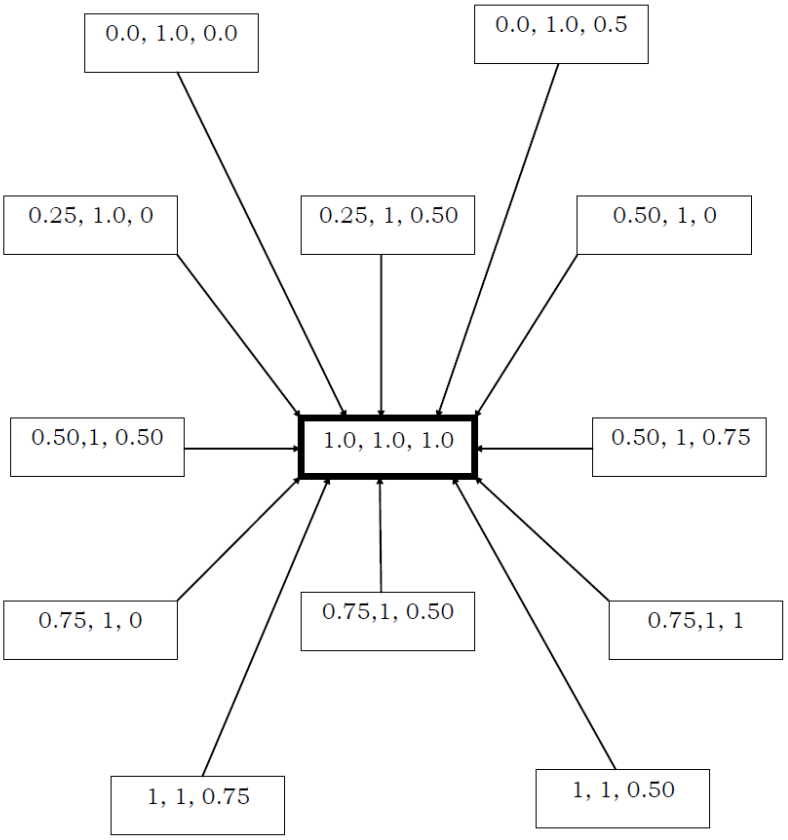


*Figure 4 :* MACA-Modified Clonal Classifier Tree with basin .75, 1, .50

The algorithms takes input as DNA sequence and the maximum population and give output as the class, matrix representation and rule specification.

*Input:* S = {S1, S2, · · · , Sl}, Training Set, Maximum Population M max).

*Output:* Matrix Representation T, F, and information of the class Begin

*Step 1:* Generate 500 new chromosomes for Initial Population.

*Step 2:* Initialize Maximum Population MM=zero; PP← IP.

*Step 3:* Compute fitness FF for each chromosome of PP according

*Step 4:* Store T, F, and corresponding class information for which the fitness value FF = 1.

*Step 5:* If FF = 1 for at least one chromosome of PP, then go to Stop.

*Step 5a:* Check the TATA box

*Step 5b:* Check the GC box

*Step 5c:* Check the CAAT box.

*Step 6a:* Construct the MACA-CC tree based on 5a,5b,5c.

*Step 6:* Order chromosomes in order of fitness.

*Step 7:* Increment Maximum Population (MM).

*Step 8:* If GC > Gmax then go to Step 11.

*Step 9:* Form NP by operations of Modified Clonal algorithm

*Step 10:* PP← NP; go to Step 3.

*Step 11:* Out Put and Store T, F, and corresponding class information for which the fitness value is maximum.

*Step 12:* Stop.

## IV. Experimental Results

The data sets are taken from Eukaryotic Promoter Database, EPDnew [10] which is a collection of promoters of human, mouse, zebrafish and D.melanogaster. A total of 75% of each data set is used for training and 25% are used for testing the promoters.

*a) Parameters for testing promoters*

The important statistics to look at include:

1. True Positives (TP): Number of correctly predicted promoters.
2. False Positives (FP): Number of incorrectly predicted promoters.
3. True Negatives (TN): Number of correctly predicted promoters.
4. False Negatives (FN): Number of incorrectly predicted promoters.

Using the above measures following are calculated

- Actual Positives (AP) = TP + FN
- Actual Negatives (AN) = TN + FP
- Predicted Positives (PP) = TP + FP
- Predicted Negatives (PN) = TN + FN
- Sensitivity (SN) = TP / (TP + FN)
- Specificity (SP) = TP / (TP + FP)

The proposed classifier is compared with standard promoter prediction programs like Promoter Inspector, Dragon Promoter Finder, Promo Predictor, CNN-Promoter, SPANN and IMC as shown in table 1. The developed front is reported in figure 5. This classifier has an inbuilt parameter for estimating the average time to predict promoters in a given DNA sequence. This classifier predicts promoter region in .7 nano seconds for a DNA sequence of length 252.

*Table 1:* Comparison of MACA-Modified CC with existing approaches

| | Sensitivity | Specificity |
|---|---|---|
| Promoter Inspector | 56.9 | 46.9 |
| Dragon Promoter Finder | 62.3 | 59.3 |
| Promo Predictor | 65.3 | 66.9 |
| CNN-Promoter | 76.3 | 82.3 |
| SPANN | 68.9 | 84 |
| IMC | 76 | 86 |
| MACA-Modified CC | 84.5 | 92.7 |

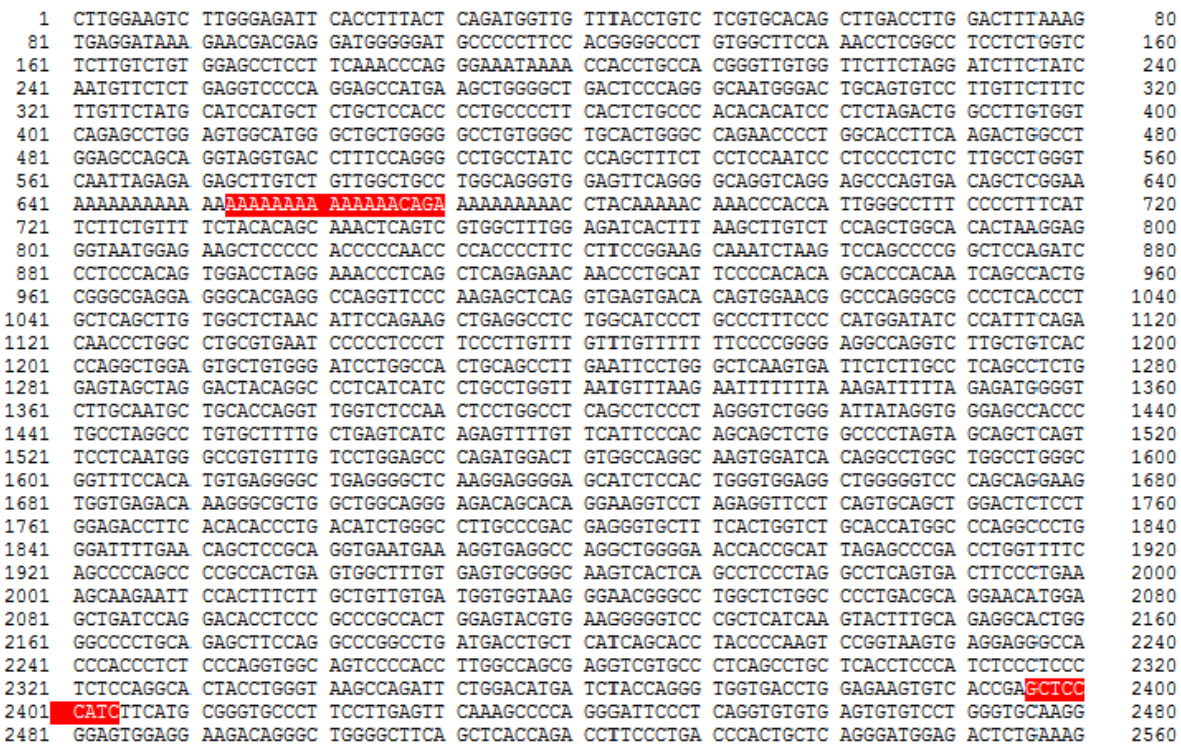


*Figure 5 :* Front End of MACA-Modified Clonal Classifier

## V. Conclusion

We have successfully developed and tested the MACA based modified Clonal Classifier for predicting promoter regions in eukaryotes. The proposed classifier is tested for specificity and sensitivity. It is compared with important promoter programs available. The results obtained are found promising and comparable. This classifier is also observed and tested for the amount of time it will be taking to predict the promoter and it was found as .7 nano seconds. A sensitivity of 84.5% and specificity of 92.7 were reported.

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# Science and Technology in Africa: The key Elements and Measures for Sustainable Development

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*Abstract-* The contributions of science and technology to foster development in the world cannot be over emphasized. Thanks to the evolution and revolution of science and technology, the world has greatly achieved advancement in various sectors such as infrastructure, energy, industrial, health, education, communication, financial, entertainment, transport, agricultural, economic and environmental protection to name a few. While the growth of science and technology is pronounced in the western continents, the pace in Africa is slow. In addition to its contribution to development, science and technology is today a menace to man, the society and ecosystem. This paper highlights on the interdependence between sciences and technology for development with emphasize on the key contributing elements such as research, biomimetics, philosophy of science, communication, innovative fields and global partnership. Possible measures to manage the detrimental effects of science and technology in the society and promote those technologies which are paramount to ensure sustainable development are enumerated. The advancement of science and technology will only be achieved if Africans can change their mentality and lay emphasis on those key elements and measures that are of priority to development.

*Keywords:* *science, technology, sustainable, research, development, Africa.*

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*Keywords: science, technology, sustainable, research, development, Africa.*

## I. Introduction

Africa, especially the sub-Sahara regions is challenged with a lot of crisis. The immediate problems have made it difficult for this continent to enjoy certain values and comfort. Due to the over grown population, people are in constant conflicts for the quest of resources and raw materials, there is an increase in disease affluence especially the communicable diseases [1]. Poor agricultural yield due to climate change has promoted hunger and starvation [2]. These impediments have led to socioeconomic problems, political instability and poverty which have prevented the development of this continent.

Science and technology is one of the major aspects in which most developed nations have highly promoted to foster their development. Though science and technology began as far back as in the time of the first existing human beings, its evolution has greatly revolutionised the world to suit man's desire, improve on his well-being and comfort. Prior to development, certain key elements such as research, biomimetics, communication, partnership etc. are very essential in the advancement of science and technology. These key elements are critical for the growth of science and development in Africa.

In spite the positive contributions of science and technology to development, the detrimental effects to the society cannot be underestimated. Global warming, depletion of natural resources destruction of the environment and ecosystem and sophisticated weapons are all outcomes of science and technology which are against the promotion and sustenance of humanity [3]. In this era, the way forward is to promote those technologies that ensure a sustainable development; that is those technologies that will eliminate the detrimental effects in the society and promote life.

## II. Evolution of Science and Technology

Sciences and technology began as early as the ancient days of the early man who produced fire from sparks of stones which he used for cooking and heating as a means for survival [4,5]. In this Paleolithic age, stone was the material used for most of the items they produced [6]. Even though an explanation on how this happened was unknown, the application was very vital for their sustenance. Since then, man continued to acquire knowledge on how to exploit his natural environment for survival until the Neolithic days of early civilization with a rise in technology where blacksmith used metal such as iron, zinc to produce weapons against wars [7].

With the gradual rise of civilizations in the river valleys of Egypt, Babylonia and other kingdoms, knowledge became too complicated to transmit directly from person to person and from generation to generation [8]. For man to thrive in this complex society, he needed some way of accumulating, recording, and preserving his cultural heritage and pass up to his generations. By 300 BC with the rise of trade, government, and formal religion, man invented writing as a way to document his activities and culture [9].

Because first-hand experience in everyday living could not teach such skills as writing and reading, a

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place devoted exclusively to learning; the school appeared. As schools appeared, a group of adults specially designated as teachers (the scribes of the court and the priests of the temple) passed across this information. The children were either in the vast majority who continued to learn exclusively by an informal apprenticeship or the tiny minority who received formal schooling.

In the early days when the word science was not coined, the discipline was term philosophy of nature which was referred to as the way of pursuing knowledge from nature [10]. The early Greek philosophers such as Socrates, Isocrates, and Aristotle thought arithmetic, astrology, philosophy, music, dancing, and gymnastics, physical health and others. Among these philosophers, some were basically interested in the knowledge of nature and the material things which are true for every community which separated them from those who used a specialized way for the pursuit of this knowledge. These two schools of thoughts shaped the field of science and philosophy respectively. By the middle age period, sciences became documented; theories were developed and tested experimentally to prove them [11]. In trying to use the knowledge of nature to imitate nature, these philosophers discovered a new field which they named called technology. From this era, scientist began to develop laws and theory such as the laws of nature, newton's laws of motion etc. It is during this period that the word "science" gradually became more commonly used to refer to a type of pursuit of knowledge which focuses on nature and material objects.

Around the 17$^{th}$ and 18$^{th}$ centuries, new laws of nature were develop which led to rapid scientific advancement and the successful development of new types of natural science namely mathematics, physics, geology, chemistry, biology etc [12]. The interdependence between science and technology in this era called the industrial revolution marked a tremendous rise in the invention of machines and creation of industries. This era was marked by great achievements in material sciences with the production of various materials that were paramount to the development of the globe.

By the 20$^{th}$ century the world observed the era of the second industrial revolution with the expansion of information science and rise in information technology. Today, the world we live in is a computerized one where any activity of human engagement can be programmed to facilitate labour and communication. Thanks to globalization of the 21$^{st}$ century, science and technology is readily made available to everyone in the society.

## III. Relationship Between Science, Technology and Development

It is obvious when one thinks of development, the terms science and technology cannot be set apart. Sometimes, the terms, science and technology are interchangeably used in normal day life to describe certain activities simply because they are interdependent on each other. To clearly understand the relationship between science, technology and development, one needs to define them individually and link them up to understand their coexistence.

Science from Latin *scientia*, meaning "knowledge" is a field that systematically builds and organizes knowledge in the form of testable explanations and predictions about the universe. In an older and closely related meaning, "science" also refers to a body of knowledge that can be rationally explained and reliably applied [13]. Two aspects of sciences that are fundamental to its definition is the ability of a concept to be tested and provide result using a scientific method. A scientific result could be such that no application is attributed but may apply to the basic underlining concepts of the field. On the other hand, some scientific studies or research may produce results which have an immediate application in real life. This application is that which drives us to the term technology.

Technology is mostly described as applied science which can be organised to have practical implication in life. However, because technology must satisfy societal requirements and values such as utility, usability and safety, technology cannot be consider as an exclusive product from science. Most technologies in the past were discovered without a scientific background. One of such was the production of fire or heat energy from sparks of stones without any scientific dependence. Also, the invention of stone weapons for wars and stones axes as agricultural tools were the technologies within the Paleolithic era when the basic concepts of sciences were not yet laid. Today, because of the advancement in science, almost all recent technologies have a scientific background. When a new technology is discovered, it needs to be made available to the society. This process of providing a technology or its product to the masses is what is known as engineering. Engineering is therefore the goal-oriented process of designing and making tools and systems to exploit natural phenomena for practical human means, often (but not always) using results and techniques from science [14]. The fundamental objective of developing a technology is to provide good or services that will meet the demands or improve on the needs of the society, hence development. This implies that for development to be achieved there must be some inputs which will have a positive impact or changes to improve on the well-being of man, some of such inputs are applied science and technology. Thus science, technology and development are symbiotic in their relationship as they are interdependent to each other (See Figure 1).

## IV. Key Elements of Science and Technology

There are certain key elements within the fields of science and technology that are critical for development. This includes the following;

*a) Philosophy of science*

Understanding the philosophy of sciences is fundamental in the quest for development. Knowing the philosophy of science will help to expose the evolution of science and shape the minds of new scholars and thinkers how to develop scientific ideas which can be transformed to technologies. Working scientists usually take for granted a set of basic assumptions that are needed to justify the scientific method. This include; (1) that there is an objective reality shared by all rational observers; (2) that this objective reality is governed by natural laws; (3) that these laws can be discovered by means of systematic observation and experimentation. Philosophy of science seeks a deep understanding of what these underlying assumptions mean and whether they are valid.

There are different schools of thought in philosophy of science. The most popular position is empiricism, which claims that knowledge is created by a process involving observation and that scientific theories are the result of generalizations from such observations. Empiricism generally encompasses inductivism, a position that tries to explain the way general theories can be justified by the finite number of observations humans can make and the hence finite amount of empirical evidence available to confirm scientific theories. This is necessary because if the number of predictions those theories make is infinite, it means that they cannot be known from the finite amount of evidence using deductive logic only.

*b) Biomimetics*

If one takes carefully look on some products of technology like aircraft, the mode of operation resembles how birds fly in the sky. So many of such technology closely resembles either the behaviour or action of a natural existing body, object or system (See Figure 2). It becomes clear that this resemblance did not just occur by chance. It is obvious that their design was an imitation of the natural occurring processes. This observation has defined a new field of studies called biomimetics. Biomimetics is a science that observes real life processes and mimics them to have applications in real life [15]. This concept is based on basic understanding how real life processes occur, their mechanism of action and how they can be applied in developing new technologies. The Renaissance genius Leonardo da Vinci dedicated himself to observing and recording natural phenomena half a millennium ago. His superb graphic renditions of plausible flying machines are based on his direct observations of birds in flight [16] and today it is a reality by the invent of aircraft. Most technologies today are thanks to biomimetics mimicking nature. Advances in computers are being made emulating the operation of the human brain. Organisation of muscle structure in man was replicated to design hanging bridges. The organisation of spider web oriented the designs of the best models of roofs for very large structures such as stadium. Some engineers are using the shell of a seaweed-eating snail as a guide in the development of a new generation of bullet stopping armor [17].

*c) Research*

Research is a process whereby natural situations are observed and translated using a systematic approach to give an understanding or explanation to them. In scientific research, observation on the natural processes is usually questioned such that they can be tested in an organised systematic manner to produce meaningful results which can answer these questions. The outcomes of a research can either have an immediate application or apply to the basic concepts of science which may not have an immediate application but contribute in developing new theories, principles or law. Based on these outcomes, research can be grouped into four categories (See Figure 3);

- Category I: Research that neither apply to the basic concepts nor have application.
- Category II: Research that apply to the basic concept but do not have application.
- Category III: Research that do not apply to the basic concept but have application.
- Category IV: Research that both apply to the basic concept and have application.

Category I research is usually repetition of studies which do not have any particular question to be addressed. Such research may lay the basis for routine mastery of techniques, principles or theories but do not contribute much in the development of a nation or community. Such research is common in Africa where researcher most of the times do not really carry out research to address particular problems or issues pertinent to the discipline or society. Rather are involved in repeating studies done elsewhere just as a means of producing articles which enhance their promotion in their various jobs and institutions.

Category II research is that which contributes to the basic concepts of that field or area of studies even though applications may not be attributed. They may either support or disagree with some of the existing concepts or postulates new concepts or theories. The concept of nuclear translation is an example of this category of research. Nuclear translation is a new paradigm that is changing the existing concept of the absence of protein synthesis (translation) in the nucleus. Recent research shows the presence of nascent

proteins in the nucleus confirming the presence of nuclear translation which is estimated to be over 11% [18].

Category III research usually does not contribute to the basic principle but have a direct application. They do not usually have an explanation to the results obtained but rather have very useful applications in the society. The researcher who discovered fluorescent light produced it just by passing current across electrodes but had no explanation to the phenomenon [19].

Category IV research is that which contributes or applies to the basic principles and equally have application. This is the most productive research which greatly contributes to development. Newton's research led to the creation of the newton's laws of motion which applied to the concepts of physics with direct application in the production of automobiles, aircraft. Louis Pasteur, the father of microbiology laid the concept of microbial growth and applied the concept of microbial culture in industries for the production of alcoholic beverages, yogurts and biopharmaceuticals such as antibiotics, vaccines etc [20]. Recent discovery of prions has changed the paradigm of viruses as the smallest infective agent. Prions which are proteins in nature are now none as the smallest infective agents [21, 22]. Due to their very small size and the ability to change conformation, this concept has now been exploited by nanotechnology for their use as drug delivery system to serve as drug protein carriers for target specific drug directed treatment of diseases such as cancer with minimum toxicity.

*d) Emerging Innovative Scientific Field or areas of Study*

The early discoveries of science were concepts and theories which were solely dependent on the natural materials found in nature. Most of these materials were both those of living things such as man, animals, plants and microorganisms and the non-living things found in the earth crust like the natural existing elements such as metals and non-metals. In understanding the fundamental principles, concepts and processes that led to their existence and functioning, the major scientific disciplines such as mathematics, chemistry, physics and biology were developed. Mathematics intercepted all cross the fields since it was fundamental laying the basic principles. Within the era of industrial revolution, technological application in the area of material sciences in combination with the existing disciplines gave rise to new sets of disciplines such as engineering (electrical, chemical, petrochemical, electronics) and architecture. After this era, around the 20$^{th}$ century other fields like biochemistry, biotechnology, and nanotechnology emerged.

Around the mid-twentieth century, the world invested more on how to improve on communication leading to the development of information science. The application of information science from the primary disciplines such as mathematics, chemistry, biology, and physics led to the creation of information technology, bioinformatics, chemioinfomatics etc. These emerging innovative fields are the key to the development of countries in the western world. This new technologies are at the forefront in meeting the demands of our present society. One of the leading technologies in the world is information technology which is very much applicable in almost all sectors of life for communication.(See Figure 4)

*e) Communication*

New concepts, ideas and research discoveries need to be documented and made accessible so that it can disseminate across the scientific community to researchers and scholars or feature generations. This could be done by writing of articles, text book, creation of journals and publication of research works, as well as calling for proposals to present new research ideas.

*f) Global Partnership for Transfer of Knowledge and technology*

Any nation that intends to improve on its level of development must be ready to welcome new technologies or permit a flexible exchange. This permits the transfer or gain of technology. Creating a global partnership whereby knowledge and technology can flow is fundamental for development. This can be done through exchange studies cross countries, training individuals from various locations to become experts, invite experts in various fields to train the younger generation. Also, the organisation of international conferences, conventions, symposium, workshops, seminars and meetings would help to share and exchange ideas across various nations and societies.

## V. Scientific and Technological Contributions to Development

For a society to be described as developed there is a minimum level of comfort or well-being that is necessary to overcome the challenges incurred so as to meet the immediate demands and needs of the society. This minimum well-being is what I describe as development. Science and technology has contributed significantly in the development of most nations and society at various sectors which are of great importance to the society.

Science and technological progress has had impact in sectors such as infrastructure, energy, industrial, health, education, communication, financial, entertainment, transport agricultural, and environmental protection to name a few. These effects have not been limited to the improvement of society's material wealth, but have also extended in altering the existing paradigms under which society operates.

Information technology (IT) is one example of a paradigm-changing technology. The world has move from an analogue to a digital system where any information can be computerise and easily diffuse into the society [23]. With information technology, new means of communication such as the internet, mobile phones have been introduced using satellite transmission in addition to the existing ones. These advances in mobility, joined with inventions in the area of telecommunications technology, such as the telephone and radio, have served to broaden the range of human activities and to expand the scope of human exchanges. Computerised programming has increase the versatility of machine to perform numerous and various functions improving on the efficiency and efficacy of production. Information technology and communication has greatly pronouns the entertainment industries in the world digitalising the products as videos, audios etc.

Another area of changing paradigm is the medical or health sector. The shift of biomedical process from structural to molecular basis has greatly contributed in identifying new disease causing agents such as viruses and prions responsible for illnesses whose origin was unknown. The mode or mechanisms of action of most diseases processes have been established and new diagnostic methods of high throughput technologies with high sensitivity and specificity have been developed for various diseases and illnesses and made possible appropriate treatment [24]. New technologies also have identified new drug targets for Drug discovery and expanded the pharmaceutical industries.

The discovery of recombinant DNA technology has boosted the biopharmaceutical industries. Biomolecules such as antibiotics, vaccines, hormones which were not available due to the cost implication and complexity of the fermentation processes are now made available to the society [25]. The improvements of various control measures such as vaccines have eradicated so many infectious diseases in the world and greatly reduce morbidity and mortality. Health care delivery system has been improved making available, diagnosis and treatment to the society.

At the molecular level of biomedical sciences, sciences and technological advancement have changed the existing paradigm. Prions; pathogenic proteins whose conformation is changed to cause an infection have been discovered to be responsible for some diseases such as the mad cow disease and are now known as the smallest infective agents and no longer viruses. Also, about 11-20% of nuclear translation is possible and it is now known that protein synthesis can occur in the nucleus. The discovery of these new concepts in biology can greatly improve the understanding of diseases mechanisms, and facilitating diagnosis and treatment.

The discovery of new sources of energy especially renewable sources has almost supress the myth of depleting ores of crude oil. Energy can readily be trapped from the sun, wind, water, vegetal organic sources and others natural renewable sources. Nuclear energy from nuclear plant and thermal energy are other new sources of energy which greatly meet the demands of the overgrown population.

Infrastructural development has risen substantially in the world. Intermesh transport systems of roads and rails as well as GPS technology have reduced traffic congestions. Cities with sophisticated buildings are well planned to reduce overcrowding and make them assessable to all.

Science and technology have laid the foundations for progress in society, and have helped to make people's lives more materially prosperous. In particular, after the industrial revolution, there has been a tremendous rise in the industrial sector. Industries of all sort of production are available today. The diversity of product has provided man with the utility to make choice to their desire. Industries have created new jobs and alleviate the state of unemployment. In addition, inventions in machine tools have been linked to advances in energy technology to achieve automation and acceleration of manufacturing processes. The result has been large-volume production of goods in ever-shorter periods of time. Moreover, progress in materials technology has resulted in the ability to produce diverse types of material items. Progress in materials technologies has given rise to a variety of new transport modes, such as the railroad, the automobile, and the airplane, vastly improving human mobility in terms of both time and space.

The contributions of biotechnology and genetic engineering have massively improved the agricultural sectors in the continent. There is improvement in agricultural yield and the quality of food stuff to be resistant to diseases. This has reduced problems of food shortage and scarcity, as well as starvation and hunger in the globe.

Furthermore, as progress in science and technology has broadened and enlivened human activity, new issues have appeared in society, and these have in turn led to demands for new sciences and technologies capable of resolving the new issues arising from the changes in society.

The advancement of science and technology has promoted education. New technologies have permitted the creation of new innovative academic field. The interdependence of science and technology, and the evolution of material to information science, new professional disciplines from the basic scientific fields such as chemistry, biology, physics, mathematics, geology have emerge to permit a mastery of the new technology and make them available to meet the demands of the challenging economic society. The new

innovative academic fields include genetic engineering, nanotechnology, engineering, information technology, computer engineering etc (Figure 4). The creation of these new disciplines has greatly promoted literacy. More peoples can now read and write, manipulate phones and computers and get connected in the global village.

Above every other thing, the overall success of science and technology has been the economic and financial bloom of the entire globe. The most developed countries which are economically and financially stable are those ones with a strong scientific and technological background. The gross national income as well as the per capital income of this countries are usually high and sufficient enough to foot the bills of their basic needs.

## VI. The Destructive Aspects of Science and Technology

Meanwhile, progress in science and technology has contributed enormously to the growth and development of the society, the detrimental aspects cannot be minimized. Science and technology, though worth meaning is one of the greatest challenges the world is facing. Advancement in nuclear technology has not only promoted the production of nuclear energy but also more sophisticated nuclear weapons which are responsible for major destructions. The rise in industries has increased the level of greenhouse gases that are released to the ecosystem and depleted the ozone layer [26]. Global warming is one of the major problems of the society today as a result of technological growth. The ecosystem has been destabilised; there is loss of variety and species of various biological flora, deforestation is highly promoted, natural resources are depleting and diminishing, environmental pollution is increasing all as a result of technology. In the health care and biotechnological sector, there is high risk of creating new infective strains of diseases with new available technology of genetic engineering in vaccine production and gene therapy [27]. Genetically modified products continued to be questioned from the ethical point of view against nature [28]. The rise in information technology and communication has increase crime wave using the internet and other social networks of communication. There is a discriminative balance of wealth and technology across the globe. The great nations that have developed the new technologies continue to exploit other nations who are in need.

## VII. Sustainable Development through Science and Technology

With all the contributions in various sectors that promote development, science and technological also contain some negative aspects within the society. To make the world a better place, there is need to separate the good from the bad technology and propel just the good ones for sustainable development. Sustainable development implies those qualities and values that can continue to sustain the society in future will be properly managed and propagated. Science and technology can be made sustainable by managing and promoting those technologies that do not have detrimental impact in the environment and society but also eliminating the bad technologies and providing solutions. The following measures can be implemented to ensure sustainable development.

*a) Education and Public Awareness*

Educating and teaching the society on the nature of this interdependence of the ecosystems and human sustenance is fundamental for sustainable development [29]. This understanding of the origins of the components of our urban environment can result in more careful utilization of natural resources and enable individuals to take informed and responsible decisions and actions, now and in the future by also realizing the impacts of their decisions on others. Improving awareness of sustainability includes such issues as the life-cycle impacts of human activities on Earth systems, control of greenhouse gases, land and energy use, consumption patterns, pollution and transport, all of which have direct connections to education for sustainable and responsible development. Promote new academic discipline in the area of sustainable development to develop idea on how technology can be managed to ensure sustainable development.

*b) Promote new Approaches to Sustainable Energy*

New forms of renewable resources of energy from water, land, air, soil, etc. form the basis of the entire living processes in the present and in the future. More emphasis and focus should be laid on these new sources rather than relying on the non-renewable existing resources. This can be achieved by promoting sustainable scientific research which encourages more active and responsible investment in alternative energy even though this may not be currently profitable, but its continued development would be an important gift to future generations.

*c) New Technologies for Pollution Reduction and Environmental Protection*

Most existing technologies usually have negative impact on the environment especially by pollution. New technologies which can manage and reduce pollution should be promoted and made mandatory in industries as part of their quality control units. Technologies which can reduce the release of greenhouse gases should be introduced in the industrial sectors, automobiles industries and other sectors of related application.

*d) Science, Technology and Ethics for Sustainability*

Ethics; the philosophical study of the moral value of human conduct and the rules and principles

that ought to govern it allows us to better analyse such intergenerational interactions in the critical context of the social, economic and natural environment. Ethics monitors how the values at the core of our social contracts are evolving and can address the crucial issue of consumption of the present versus the needs of future generations. Promoting ethics both in research and education will guide scientist and technologist to consider human and ecological moral values when developing new technologies.

## VIII. The Status and Challenges of Science, Technology and Development in Africa

Science and technology among other things have contributed substantially to the development of the world. However, the distribution of this development is not evenly spread across the globe because some nations have intensively exploited this knowledge to enhance their well-being. These parts of the world, termed to be developed are well grounded with this knowledge of science and technology and practically manifest it to enhance their economic and financial status. Other emerging economies like China, United Arad Emirates, and Brazil are current utilising this knowledge to improve on their level of the development.

In Africa, except for South Africa and a few countries of the south African region which are exploiting the knowledge of science and technology to foster their development, most parts of Africa especially the sub-Sahara region are among the least developed nations with less focus and interest in science and technology. Africa is one of the richest continents with natural reserves of raw materials, yet this resources are either exploited by foreigners of the developed world simply because the necessary technology to convert them to finish products are not available. This is as a result of certain challenges which impede the growth of science and technological advancements in the continent.

The nature of African political systems and governance greatly limits the growth of science and technology. Most polices of the government do not encourage invests and promotion in this sectors. Scientific projects are hardly supported due to the high cost of implementation. Corruption and poor governance is the key to the underdeveloped state of most of these nations.

Another critical factor for poor scientific and technological development is generally due to the African man's mentally towards science and technical education and scientific research. The African mentally has mostly projected issues of social science than those of natural science and technology. Even when these nations promote natural science and technical education, most studies are theoretical and the practical applications are usually not implemented. One of such mentally is in the investment of scientific research. Scientific research is one of the key aspects of development in the developed worlds. Billions of dollars are invested yearly in research both by the government and private institutions, organization or industries in most of the leading economies like, USA, England, Germany, France, Italy, Japan and in some emerging economies like China, India, Brazil etc. In Africa, such investment is equality observed in South Africa which is developed and has the strongest economy. One may things the other nations in the underdeveloped world do not have programmes that sponsor research or promote it at all but it is not the case. Most research in underdeveloped world are usually repetition of previous works or studies which do not contribute to the basic concepts or have an application and fall under category I research.

## IX. Measures to Improve and Ensure Development in Africa through Science and Technology

To acquire a significant development through science and technology, the African people must be willing and ready to change certain existing factors which restrain the progress of technology in the African society. For this to be achieved, African people should change the mentally of their immense phobia against sciences and technology. This should be accompanied by improving on the governance systems and policies which can promote and financially sponsor scientific and technological processes including research and scientific projects. Research especially empirical research needs to be encourage and promoted to identify new technologies that can manage their immediate resources and place the economy in competition with others nations Means by which future scientists can be trained and scientific ideas are projected and shared such as conference, seminars, convention, workshops should be promoted.

Africans need to improve on the ways to communicate scientific ideas. The creations of scientific journals, documenting information in books and the internet will spread scientific information across borders and also more science journalist should be employed to communicate scientific information. This will help to popularize scientific ideas and create public awareness.

Science and technological education which are practically oriented along with industrial attachments should be encourage at the level of basic education on subjects such as physics, chemistry, mathematics, biology and at the secondary and tertiary level. Technical courses such as engineering and applied sciences at the tertiary level should be introduced as well as new emerging innovative academic fields.

A global partnership with mother institutions, well establish industries and associations which can

permit the transfer of information across will encourage technology transfer. Above all, the participation of everybody and not only the government is paramount to foster development. With all this suggestion and the full commitment of the society, development can be achieved through sciences and development.

## X. Conclusion

Science and technology cannot be denied to have immensely contributed to development. The evolution of science and technology has witnessed so many revolutions from the Neolithic to industrial and now to the present computer age. There have been great achievement of science and technology in various sectors such as the medical, industrial, entertainment, education, infrastructure for development whose success lies on the key elements such as research, global partnership, new disciplines etc. Most of the countries that have exploited this knowledge and technique have seen their socioeconomic and financial status improved along development. Though science and technology has some detrimental effects to the society, the way forward is to promote sustainable development; a strategy to separate the bad from the good technology and promote good one is presently been encourage worldwide for effective development that will support the sustenance of man and his environment. In addition to the challenges faced in Africa, the advancement of science and technology will only be achieved if Africans can change their mentality and lay emphasis on those key elements and measures that are paramount for development.

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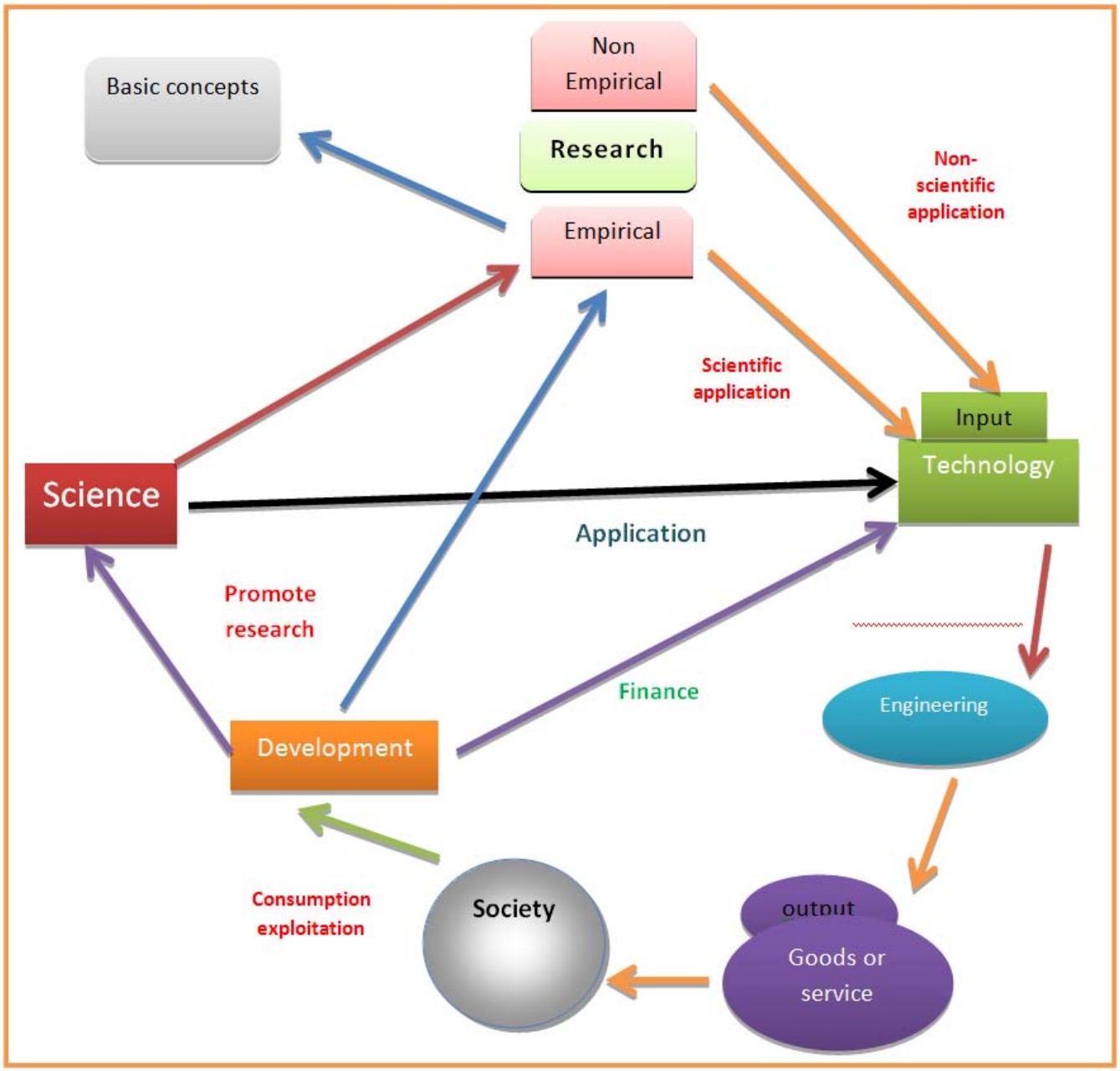


*Figure 1 :* Relationship between Science, Technology and Development

| NATURE | TECHNOLOGY |
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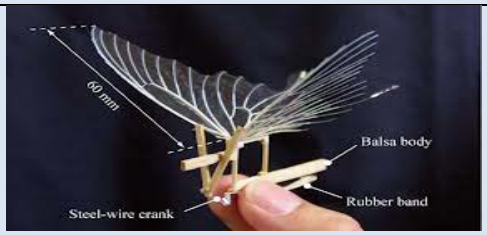
|  BIRD |  AIRCRAPT |
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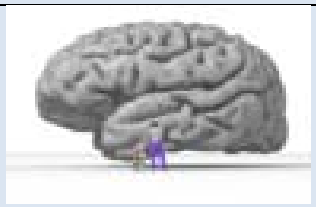
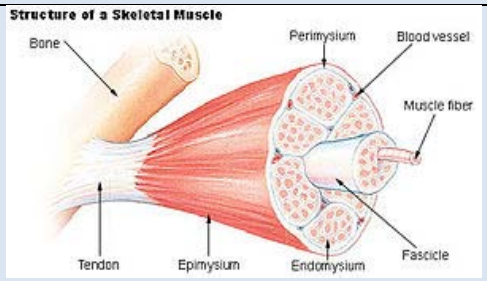
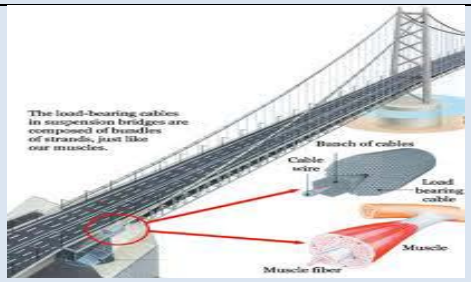
|  BRAIN |  COMPUTER |
|  MUSCLES |  BRIDGE |

*Figure 2 :* Technologies Mimicked from Nature

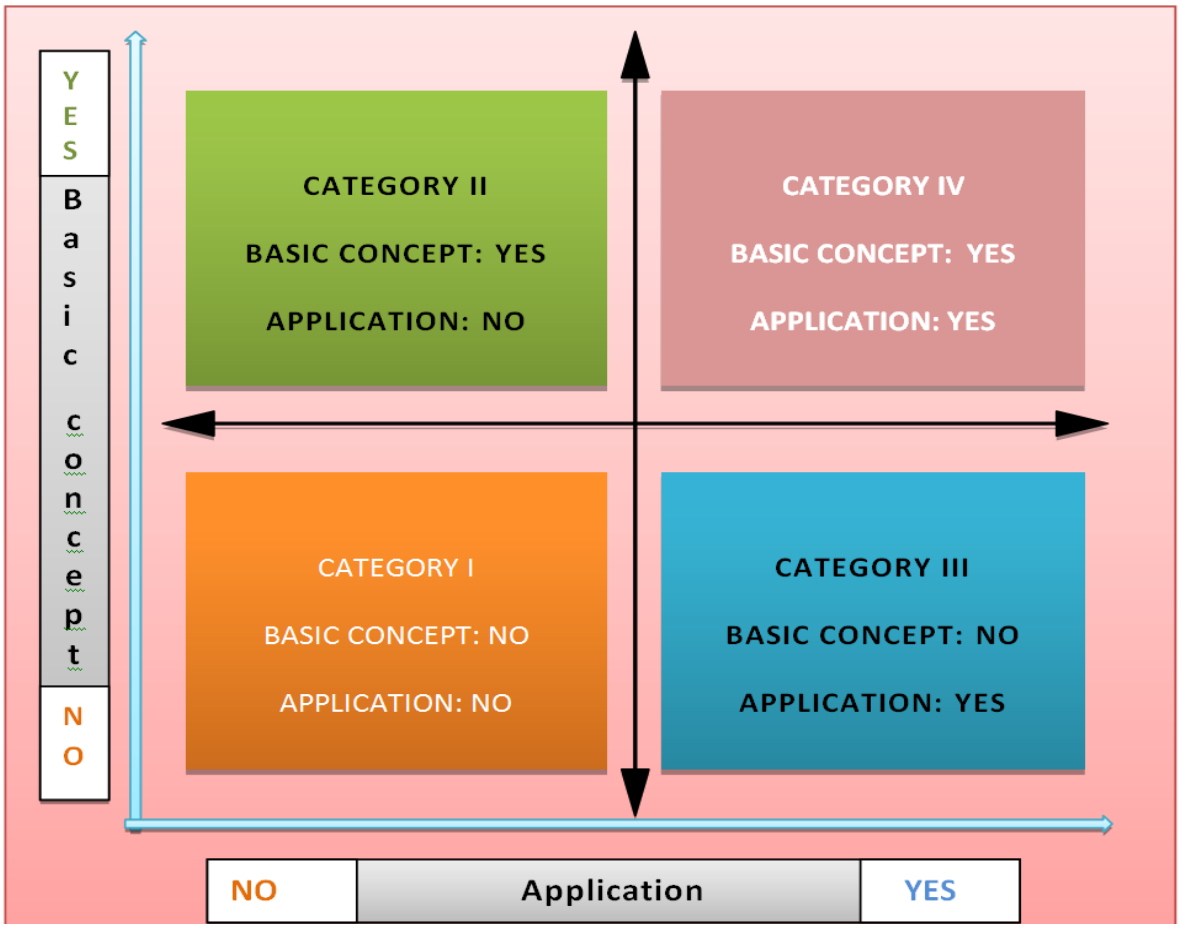



*Figure 3 :* Categories of Empirical Research

| | | | | |
|---|---|---|---|---|
| | BASIC SCIENCES | | | |
| BASIC CONCEPT | BIOLOGY | CHEMISTRY | PHYSICS | GEOLOGY |
| | MATHEMATICS | | | |
| | MOLECULAR BIOLOGY/ BIOCHEMISTRY | | GEOPHYSICS | |
| APPLICATION | APPLIED SCIENCES/ TECHNOLOGY | | | |
| MATERIAL SCIENCE | BIOTECHNOLOGY/ GENETIC ENGINEERING | CHEMICAL ENGINEERING<br>INDUSTRIAL CHEMISTRY | NANOTECHNOLOGY ELECTRICAL ENGINEERING | PETROCHEMICAL ENGINEERING |
| INFORMATION SCIENCE | BIOINFORMATICS | CHEMIOINFORMATICS | INFORMATION TECHNOLOGY | |

*Figure 4 :* Innovative Academic Fields

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# Urine Cytology Screening among Renal Transplant Recipients for Presence of Atypical Changes

By Emmanuel E.Siddig Bsc, LIBMS, M.B., B.S, Magdi M. Salih Phd, Mutaz A Dsc & MIMLS, Abdullah H. Elnabi

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*Abstract-* *Objectives:* The aim of this study was to screen transplant patients for the presence of inflammatory and atypical cytological changes, and to correlate cytological finding with demographical, clinical data and type of immunosuppressive drug in use.

*Methods:* A total of 300 voided urine samples were collected from patients , 242 males and 58 female, there ages were ranged from 11 to 71 and mean age 41years. All patients were using immunosuppressive drugs including cyclosporine, Tacrolimus, mycophenolate motifel, with highest ratio of patient taking tacrolimus (38.6%), and the least were taken cyclosporine (13%). The average period of transplant was 8.4 years, with the highest group (64.3%) were 0 – 4.2years.

*Results:* 300 cases were identified, including 262(87.34%) reported with normal cytology, the remainder 38(12.6%) were inflammatory, in which 6(2%) have a nonspecific inflammation, 17(5.67) consist from viral infections that includes (3 BKV, 2 CMV and 12 HPV), 10(3.34%) Bacterial infections, 3(1%) fungal infections constitute from candida albicans and 2 were mixed of bacterial and fungal infections.

*GJSFR-G Classification :* *FOR Code: 100499*


*Strictly as per the compliance and regulations of :*

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*Conclusion:* The study concluded that urine cytology is an excellent tool for routine follow up of renal transplant recipients to detect a variety of inflammatory and infectious agents, and need to be a widely recognized among physicians.

## I. Introduction

Renal transplantation has markedly increased over the years. The two major factors for successful renal transplantation are better control of rejection and better prevention and treatment of infection. Infectious complications are frequent in renal transplant recipients. The term opportunistic infections are applied to an infection occurring in an immunocompromized host with impaired defense mechanisms. Such infections have been on the increase for a variety of reasons. New immunosuppressive drug that, can foster the genesis of new opportunistic infections (1).

Most of the opportunistic infections occurring in renal allograft recipients are Polyoma virus, Cytomegalovirus, Epstein - Barr virus, Herpes zoster virus; Asparagellus, Candida and other infection many of them have characteristic appearance in the urine cytology. Urine cytology can be used to detect, infection, atypical changes and follow patient with bladder cancer. Despite many attempts to develop another test with greater sensitivity and specificity, cytology remains the single best inexpensive, quick and reliable way to diagnose a variety of bladder lesions (2).

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## II. Material and Methods

During a period of 6 months from November 2011 to April 2012, a total of 300 Patients were attained to renal transplant hospital in Khartoum state, the data were taken from patients by questionnaire asking them about their names, age, and transplant period as well as any clinical signs and symptoms. Then we took a voided urine sample from each ones. The samples were centrifuges for 5 minutes, followed by addition of suspending media and leaved it for 30mins, followed by centrifugation and discard the supernatant and we added to sediment an acid alcohol and leaved it for 30mins and followed by centrifugation and discard the supernatant and then from the sediment we make the smears and allow it to dry. Then the smears are fixed and stained by Papaniclaou procedures (3). The staining was assessment by expertized cytologist the nucleus display blue color while the cytoplasm displays different shades of green color.

The cytological diagnosis was grouped in four categories: 1: negative benign or reactive changes, 2: cases showing acute inflammatory changes, 3: inconclusive case and 4: malignant.

## III. Results

Three hundred (300) renal transplant recipients were screened using urine cytology, 242 (80.7%) male and 58 (19.3%) were females. Their ages were ranged from 11 to 71 years the age was mean 41 years. The study population were classified in to age groups, large number of them were within the age group 41 to 50 years age (36%) and few number were more than 71 years (0.3%).

All patients were using immunosuppressive drugs including cyclosporine, Tacrolimus (prograf), mycophenolate motifel (cellcept) and steroids which includes predinsoline and imuran. With highest ratio of patients taken prograf and steroids (38.6%) and the least were taken cyclosporine and cellcept (13%). The

average period of transplant was 8.4 years with the highest group 193 (64.3%) were 0-4.2 years.

The cytological results of study groups showed that 262 (87.34%) reported with normal cytology, the remainder 38 (12.6%) were inflammatory in which 6 (2%) found to have none specific inflammation and cast, 17 (5.67%) viral infections that include (Three BKV, two CMV and 12 were HPV), 10 (3.34%) Bacterial infections, three (1%) fungal infections and two (0.67%) were mixed bacterial and fungal infections.

The association between inflammatory conditions and gender were 17(5.6%) among male and 21(7%) female patients these results analyzed statistically found to be significant using a Chi square test ($P<0.00$) This result was consistent with literature where infectious agents such Cytomegaly virus, herpes simplex, candida and HPV were all detected in large series of more 7000 samples (4). Compare to our study of less than 300 samples we were able to correctly detect candida, HPV, BKV and other inflammatory conditions. These considered as satisfactory indicator for the usefulness of urine cytology as a daily test for patients with renal transplantation in Sudan.

With regard to relation between age groups and urine cytology results we found the inflammatory condition was most frequently in patients ageing between 41to 50 years ( 13 / 38), and less frequent for patient above 71 years (1/38) these results is statistically significant ($P<0.00$).

The results of urine cytology and types of immunosuppressive drugs showed that most frequently positive in patients who received Tacrolimus and mycophenolate (Prograf and cellcept respectively) (14.4%) and least in patients with cyclosporine and steroids (9.67%) these results shown to be insignificant ($P>0.05$).

Cytomorphology of inflammatory/degenerative changes in general were described comparable to what has already mentioned in literature. In addition to karyorrhexic changes, and nuclear enlargement, we have reported somewhat inflamed changes in the chromatin were definitely washed away homogenously. Cell degeneration was alsoevident. In candida changes presence of organism in lytic background was observed. In this instance this smear considered taken at late secretary phase.

Human Papiloma-virus changes were observed in 12 patients (4%) the number and rate of occurrence were 11(3.67%) in females and 1(0.34%) in males and interpreted based koilocytic changes cytomorpho-logically. These cells contain large cytoplasmic vacuoles, with clear glassy background and display dyskaryotic chromatin. Which of HPV reflex testing this confusion could easily solved by the aid of molecular testing. In this study, HPV changes were reported correctly, the urologist however can refer his patients to gynecologist for more information.

As opportunistic findings “Clue cell” for gardnerella vaginalis was detected as a supporting findings to help physicians for further workup, in fact the same changes due presence of cytoplasm filling bacteria of epithelial cells.

Decoy cells nuclei appeared as uniform washed chromatin, large or small cell and the nuclei. No prominent nuclei were present. Decoy cell structure ranges from small to large cell with bizarre appearances such comet-like, oval and tall columnar cells. The identification of these cells was difficult when marked degeneration was observed. The chromatin clumps were large and there degenerative spaces which lead to confusion of chromatin window in cancer cells. Also the clue for accurate interpretation is that their nuclear membrane is uniform. Hyprechromasia due to diffuse and washed DNA content also a confusing factor for false positive diagnosis. Here a well prepared Hematoxylin is required; Harris Hematoxylin is somewhat strong and might lead false positive interpretation, and however this is not the case for skilled cytologist. However Gill.

Hematoxylin is recommended since it outlines the chromatin crisply and less heavy (5).

## IV. Conclusion

Urine cytology is a safe, noninvasive, and reliable diagnostic tool for identifying viral cytopathic effects in urothelial cells, and deserves more widespread use in the monitoring of renal transplant patients. It is now seems that urine cytology need to be implemented in clinical settings for the benefit of patients and urologists. Polyoma virus infection detection among transplant patients helps urologists to early manage renal graft rejection which of invaluable benefit for patients, community, physicians and economy. We also acknowledge regular screening for transplant patients with urine cytology as a part of routine check with other laboratory findings.

Also we have observed that urine cytology procedure need to be enhanced for harvesting well preserved cells. Improving techniques support accurate screening and diagnosis. We also found the Papanicolaou’s stain is superior in many instances to all other relative stains including those for air-dried preparations. Each laboratory however, needs to be equipped with good personnel and standard procedures.

Our findings also showed that other inflammatory/infectious conditions also are frequent cytological findings, however fungal infections such as candida albicans, viral infections such as HPV and other infectious agents readily identified in urine cytology from transplant patients.

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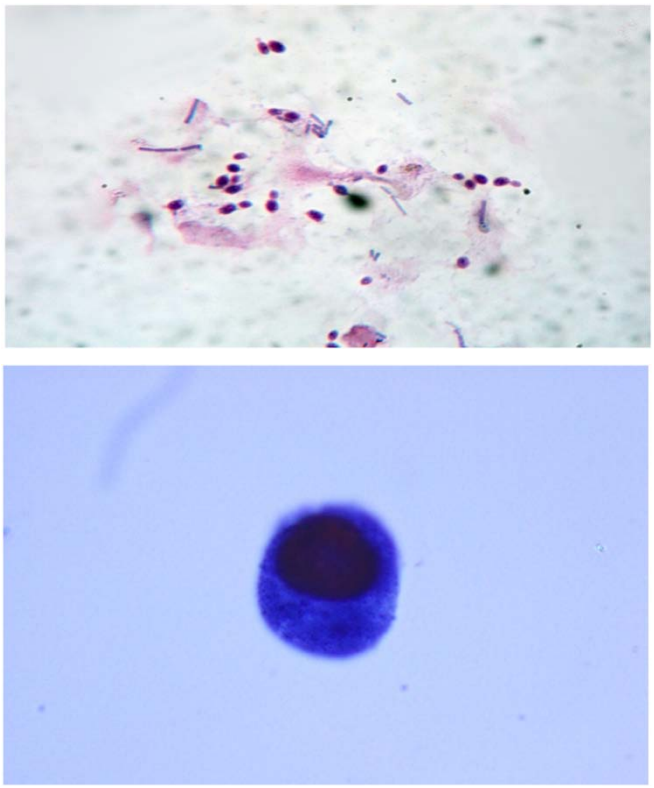
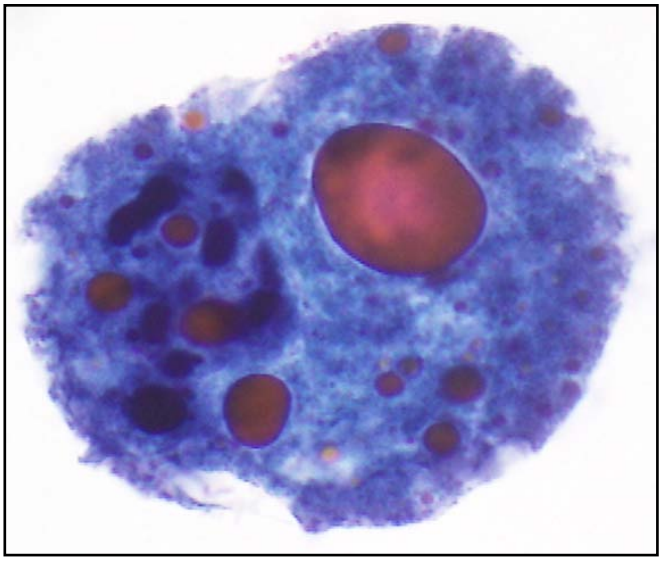


*Picture 3 :* Typical "classical decoy" cell, the cell is large with dense uniform "washed" chromatin, with inflamed purplish zones. The nuclear membrane is regular and the cytoplasm is thick but slightly vacuolated and granulated and abundant. (LBC X40)



*Picture 4 :* Melamed-Wolinska Bodies accompany DeCoy cell present in the same case mentioned in Fig 3

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# Study on Cell Concentration in the Production and Optimization of High Temperature Alkaline A-Amylase Enzyme by Bacillus Lichenoformis using Low Cost Medium Derived from Agricultural byproducts

By M. Sathiyamoorthy & Dr. S. Theneshkumar
*Himalayan University, India*

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*Keywords:* *α-amylase, bacillus lichenoformis, low cost medium, agricultural by products, fermentation, alkaline enzyme.*

*GJSFR-G Classification :* *FOR Code: 100101p*


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M. Sathiyamoorthy [α] & Dr. S. Theneshkumar [σ]

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*Keywords:* *α-amylase, bacillus lichenoformis, low cost medium, agricultural by products, fermentation, alkaline enzyme.*

## I. Introduction

Enzymes are proteins which catalyze variety of reactions in the biological system. When enzymes were first intensively studied in the last two centuries this chemical nature was obscure and even the reactions catalyzed were frequently ill defined. It was natural and therefore, that individual enzymes were given names by their discoverers. Most enzymes are studied and need to be named before any significant information about their structures exists. Whenever the 'same' enzyme from different organism is studied, it is found that Proteins different in detailed structure (and some times in gross structure) can have essentially the same catalytic properties. In the recommendations of the "International Union of Biochemistry Nomenclature Committee (1984), therefore, an enzyme name does not specify a structure but instead defines the Principal reaction catalyzed.

Enzymes are classified in to six classes. Enzymes in the first three classes all catalyze transfer reactions, with stoichiometry A+B → P+Q, but differ in other respects. Oxidoreductases catalyze reaction in which one or more electronics (usually two) are transferred from a donor (reducing agent) to an acceptor (Oxidizing agent). In many oxidoreductases the oxidized substrate can be regarded as a hydrogen donor, and for these enzymes the term dehydrogenase is preferred. Hydrolases catalyze hydrolytic reaction, i.e. reactions in which water is the acceptor of the transferred group. The transferases thus comprise all enzymes catalyzing transfer reaction that are not oxide reductases or hydrolases. Lyases catalyze elimination reaction, where the bond is broken without oxidoreduction or hydrolysis and in most cases have stoichiometry. A → P+Q.

The six classes are further sub divided in to subclasses, to specify the type of reaction more fully and to indicate the reactants. All the enzymes have a property of either intra cellular or extra cellular in nature. But most of them are extra cellular in nature.

### a) *Intracellular Enzymes*

Enzymes occur in all living cells, where they catalyze and regulate reactions of Biochemical pathways essential to the existence of the living system. In general substrates for these enzymes are small molecular weight molecules, e.g. Sugars, amino acids, carboxylic acids, which are able to permeate the

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membrane. Their catalytic properties are regulates by conformational changes in their three dimensional structure accomplished by allosteric cofactor molecules.

*b) Extracellular enzymes*

Extra cellular enzymes were originally defined as enzymes which are external to the cell wall and in contact with surrounding medium. At present we consider transport the membrane as the primary secretion event. Thus for the purpose of this review the term & erection is used to refer to the transmembrane passage of protein and the term extra cellular to those proteins that have undergone this process. The biological function of this kind of enzymes may be seen in the hydrolysis of macro molecules which are too large to be transported in to the cell.

*c) Animal tissue Enzyme*

Enzymes used in Industry are isolated from animal and plant tissues, as well as from Micro organisms. One of these three sources may be favored for a given enzyme. For example, some proteolytic enzymes isolated from animals may be advantageous in special fields of application. The enzyme chymosin, also known as rennet, is an acid protease used in the milk-clotting step of cheese production. A mixture of chymosin and its zymogen prochymosin, which may be converted chymosin by low pH treatment, are currently obtained from the abo-masum of an unweaned calf. Animal glands, e.g. the pancreas, are sources for hydrolyzing enzymes used as a digestive acids. The pancreas is a very rich sources of enzymes. It contains about 23% of trypsinogen and 10 -14% of chymotrypsinogen. So called pancreatin, a digestive aid, contains several enzymes such as amylase, lipase and protease.

*d) Plant tissue enzymes*

Plant protease isolated from pineapple (bromelain) and the papaya plant (papain) have been used for meat tenderizing and chill proofing beer. Useful amylolytic enzymes occur in plant tissues such as barely, wheat, rye, Potatoes, sweet potatoes, beans, soy beans, α - amylase, β - amylase, which starts at the non-reducing ends of the outer chains of the starch and proceeds by gradual removal of maltose units and de branching enzyme which hydrolyzes the α -1 - 6 linkages of starch, were detected in these plants.

*e) Microbial enzymes*

Microorganisms have become increasingly important as producers of industrial enzymes and in fact most enzymes used in industry today are of Microbial origin. Attempts are now being made to replace enzymes which traditionally have been isolated from animal tissue and plant tissues with enzymes from Microorganisms. Examples for partial replacement of plant and animal enzymes in dudes. Amylases and endo - β - glucanases of malted Barley and wheat by enzymes from Bacillus and Aspergillus in the beer, distillery, baking and textile industries. Plant and animal proteases by Aspergillus and Thermoactinomyces protease for meat tenderization and for chill proofing beer.

*f) Uses of α – amylase*

The enzyme α–amylase is used as a biocatalyst in many small scale and large scale industries some of the uses are.

- The Bacterial α–amylase used in starch hydrolysis industries, Brewing industries, Detergents industries and textile industries.
- The fungal α–amylase used in starch industries and baking industries.
- The α–amylase from Malt used as a digestive aid and supplement to bread.
- The α–amylase from Aspergillus Orygaze is used to Iproduce starch liquefying syrups.
- The α–amylase from Bacillus Subtillis used in Desizing textile industries, Alcohol fermentation industries and glucose producing industries.
- The α–amylase produced from Aspergillus Niger is highly acid resistant is used as a digestive acid at pH-5.
- The α–amylase from Bacillus lichenoformis is used in all starch industries and detergent industries and to produce starch sizing pastes for use in paper coatings.

## II. Objective of the Study

Enzymes are Proteins which catalyze variety of reaction in the Biological systems. There are many methods used to produce the enzymes among that the biological methods are widely used. In this type of biological method of production, solid state fermentation is applied for the production. In all the types of fermentation processes, the cultures has been prepared using yeast extract and peptone etc. These are added to the culture in terms of nutrients as a carbon and nitrogen sources for the microorganism. The cost of these chemicals are much expensive. So the alternative method has been proposed for the preparation of culture medium using some low cost agricultural byproducts such as defatted cotton seed, defatted soybean, mustard seed etc. The fermentation has to carryout using these type of low cost medium to check the productivity and enzyme activity.

## III. Experimental Setup

*a) Biostat E fermentor*

The fermentation was carried out in a B. BRAUN CO, Biostat E fermentor. It is a compact and comprehensive fermentation system on a laboratory

scale, which can be used in microbiological and biotechnological research and development. Biostat E fermentors are designed for use in discontinuous fermentation (Batch operations) as well as in continuous process. The measurement and control system used in compatible with computers. The Biostat E is protected against unauthorized use with a main key. All modules of the measurement and control section are separately switched on. Therefore they can be installed or removed independently from the control in spite of the central mains switch. Additional modules can be inserted without interruption or disturbance of operations.

The lower front panel of the basic device is provided with installation ports for at least 4 dosing pumps of the four, three are peristaltic pumps for the supply of acid, alkali and antifoam agent, the fourth is prepared to install precision dosing pumps.

The arrangements of the various technical appliances in the basic devices are:

- Thermostat system which containing heating and cooling water circuit for tempering as well as for sterilization.
- Gas supply system including exhaust equipment.
- Motor and drive system for the stirrer shaft drive.

The recorder, of 6 channels dot printer records the following measurement values in the basic devices.

- Temperature
- Speed
- pH Value & Antifoam consumption

The culture vessel is mounted on the console laterally fixed at the fermentor where there are the corresponding borings for the feet of the culture. Simultaneously the connection to the stirrer drive is guaranteed. For starting operating the device the filling state of the fermentor thermostat is to be checked. The set point temperature is adjusted at the corresponding digital switch of the module. A good mixing of the culture vessel is a prerequisite. For that a stirrer system is provided which is driven by a controlled DC motor. The stirrer speech can be directly adjusted by the digital switch of the speed controlled module. The adjustable speed range is 50 – 1500 minutes-1.

The pH – value in the culture medium can be electro chemically determined via a combined – glass electrode. The pH set point desired can be adjusted with the digital switch of the pH controller.

*b) Dimensions of the fermentor*

| | | |
|---|---|---|
| Total volume of the fermentor | : | 6 lit. |
| Working volume | : | 5 lit. |
| Max working temperature | : | 138° C |
| Max working pressure | : | 124° C |
| Diameter of the fermentor | : | 17.5 cm |
| Height of the fermentor | : | 40 cm |
| Agitator type | : | 6 Blade, Paddle type Agitator |

## IV. Materials and Methods

*a) Microbial strain*

*Bacillus Lichenoformis,* NCIM 2051 Received from National Chemical Laboratory, Pune, India.

*b) Chemicals*

Beef extract
Peptone
NaCl
$MgSO_4$
$KH_2PO_4$
$CaCl_2$
Yeast extract
Agar
Corn Starch
Defatted Cotton Seed
Defatted Soya flour
Mustard Seed

*c) Medium*

i. *Universal medium for bacteria*

| | | |
|---|---|---|
| Beef extract | : | 1.0 % |
| Sodium Chloride | : | 0.5 % |
| Peptone | : | 1.0 % |
| pH | : | 7.0 - 7.2 |

Sterilize the medium, and adjust the pH at 7.2. Add 2% Agar for making slants.

ii. *Corn starch medium: (Basal Medium)*

| | | |
|---|---|---|
| Corn starch | : | 1 % |
| Yeast extract | : | 0.2 % |
| Peptone | : | 0.5 % |
| $MgSO_4$ | : | 0.05 % |
| $KH_2PO_4$ | : | 0.05 % |
| NaCl | : | 0.15 % |
| $CaCl_2$ | : | 0.015 % |

iii. *Low cost medium*

| | | |
|---|---|---|
| Corn starch | : | 1 % |
| $MgSO_4$ | : | 0.05 % |
| $KH_2PO_4$ | : | 0.05 % |
| NaCl | : | 0.15 % |
| $CaCl_2$ | : | 0.015 % |
| Soya bean | : | 0.5 % |
| Mustard Seed | : | 2 % |
| Cotton seed | : | 3 % |

*d) Procedure*

Shake flask cultures were operated at constant temperature of 37$^0$C and fixed rpm with 100 ml of medium in a 500 ml Erlenmeyer flask and inoculated with the culture. Fermentation studies were carried out in above described B. Braun Biostat E fermentor with the cultural conditions of 37$^0$C, pH 7, and 300 rpm. Since it is an aerobic fermentation, the aerobic rate was maintained at 1 vvm. For every six hours the sample were collected from the sampling point provided in the top of the culture vessel, and analyzed.

i. *Stock Culture*

Bacillus Lichenoformis NCIM 2051 was maintained in an Agar slant at 40C.

ii. *Sub Culture Maintenance*

Subculture was prepared using a universal Bacteria medium and it was maintained in an incubator at 37oC.

iii. *Pre inoculum*

Take 100 ml of the Universal medium inoculate this with a stock agar culture in a 500 ml Erlenmeyer flask and kept in a shaker at 300 rpm and 370C. It is also called as seeding of culture.

iv. *Biomass*

Biomass was estimated by the method of dry weight for every sample. It was expressed in terms of 1 dry weight/lit.

## v. Results and Discussion

*a) Biomass estimation*

The Biomass for the sample, which got from the fermentation broth was determined by the dry weight method. Take some known amount of liquid from the fermentation of liquid from the fermentation broth in the centrifuge test tube, and kept in a centrifuge for 20 minutes at 5000 rpm. The supernatant liquid was collected and kept for α– amylase activity determination. The cells settled in the bottom of the centrifuge tube was transferred to a funnel contains the gravimetric filter paper (ash less), and washed thoroughly with distilled water. Transfer this gravimetric filter paper in to the known weight silica crucible, and incinerate for 30 min. Cool the contents and measures the weight from this calculate the cell concentration.

*Sample calculation*

| | | |
|---|---|---|
| Weight of empty crucible | : | 15.8603 gms. |
| Volume of fermentation liquid taken: | | 11.4 ml |
| Weight of crucible with cells | : | 15.8895 gms. |
| Cell mass/volume of liquid taken | : | |
| | : | 15.8895 – 15.8603 |
| | : | 0.0292 gms |
| For 1 litre, cell concentration weight. | : | 2.57 gm dry |

*b) Production of enzyme*

The growth pattern of Bacillus Lichenoformis NCIM 2051 and α–amylase production was observed for three days in basal medium with 1% cornstarch as a carbon source. The formation of α–amylase started from 4 hours. The maximum enzyme production was achieved at 24 hours. The pH of the broth increased from 7 at the beginning to 8.9 at the end of fermentation. The maximum yield was achieved at 350C.

*c) Effect of corn starch concentration*

The effect of corn starch concentration was further studied. The α–amylase production was studied, by changing the Corn starch concentration at 0.5%, 1% and 2.5%. It was found that with an increase of starch concentration in the medium beyond 1%, enzyme production did not increase. At higher starch concentration, enzyme production was comparatively lower and the time required to reach the maximum enzyme level was longer.

*d) Effect of pH*

The bacterium was found to grow at pH 3-11, with growth resulting in an increase of the patient's media's pH. Enzyme production started at 5.0 and ceased at pH 10.0. Maximum enzyme production occurred at pH 6-9. Very little enzyme production in the medium at initial pH of 3 - 4. At higher pH values (10-11), growth was quite high, but the amount of enzyme production was very low.

*e) Effect of temperature*

The strain was found to grow and produce enzyme at temperatures from 25 to 500C. Maximum enzyme production was observed at 350C. Growth and enzyme production both started decreasing drastically above 400C.

*f) α–amylase production in low cost medium*

The α–amylase production was further studied by using the low cost medium which containing the carbon and nitrogen sources like corn flour, mustard seeds. Since the cost of yeast extract and peptone in the Basal medium is very high, we can replace the yeast extract and peptone with the above mentioned things. The low cost medium produced 2 times more enzyme than the high cost synthetic medium (yeast extract and peptone). The medium containing 0.5% defatted, 2% mustard seed in the place of yeast extract and peptone, was found to yield the maximum amount of α–amylase. The experiments were conducted for 6 different batches with various concentrations, which are given in the below table and graph.

*Table 1 :* Enzyme production for 1% corn starch concentration

| Time hours | pH | Temp $^0$C | %$PO_2$ | rpm | Cell concentration gm dry wt per lit |
|---|---|---|---|---|---|
| 0 | 7.0 | 36.9 | 104.7 | 300 | 0.052 |
| 3 | 7.1 | 36.8 | 101.8 | 300 | 0.236 |
| 6 | 7.2 | 37.0 | 100.2 | 300 | 0.480 |
| 12 | 5.8 | 37.1 | 22.6 | 300 | 1.215 |
| 18 | 7.3 | 35.7 | 89.3 | 300 | 2.882 |
| 24 | 8.2 | 35.6 | 86.8 | 300 | 2.843 |
| 48 | 9.0 | 35.6 | 96.4 | 300 | 2.745 |
| 72 | 8.9 | 36.2 | 98.7 | 300 | 2.461 |

*Table 2 :* Enzyme production for 2.5% corn starch concentration

| Time hours | pH | Temp $^0$C | %$PO_2$ | rpm | Cell concentration gm dry wt per lit |
|---|---|---|---|---|---|
| 0 | 6.3 | 37.0 | 100.8 | 300 | 0.035 |
| 3 | 5.8 | 36.4 | 100.1 | 300 | 0.21 |
| 6 | 6.1 | 35.9 | 92.7 | 300 | 0.53 |
| 12 | 6.7 | 35.7 | 95.6 | 300 | 0.78 |
| 18 | 6.9 | 35.4 | 97.9 | 300 | 1.52 |
| 24 | 7.1 | 35.3 | 98.3 | 300 | 2.61 |
| 48 | 7.9 | 35.1 | 88.5 | 300 | 2.48 |
| 72 | 8.5 | 34.8 | 84.3 | 300 | 2.57 |

*Table 3 :* Enzyme production for 0.5% corn starch concentration

| Time hours | pH | Temp $^0$C | %$PO_2$ | rpm | Cell concentration gm dry wt per lit |
|---|---|---|---|---|---|
| 0 | 6.1 | 37.0 | 120.3 | 300 | 0.026 |
| 3 | 6.1 | 36.7 | 110.8 | 300 | 0.21 |
| 6 | 6.9 | 36.6 | 102.6 | 300 | 0.58 |
| 12 | 7.5 | 35.9 | 100.9 | 300 | 0.97 |
| 18 | 7.8 | 35.8 | 98.7 | 300 | 1.38 |
| 24 | 7.9 | 35.6 | 98.5 | 300 | 1.85 |
| 48 | 8.3 | 35.7 | 98.1 | 300 | 1.61 |
| 72 | 8.8 | 35.4 | 83.6 | 300 | 1.43 |

*Table 4 :* Enzyme production using Basal medium + 0.5% defatted soya flour

| Time hours | pH | Temp $^0$C | %$PO_2$ | rpm | Cell concentration gm dry wt per lit |
|---|---|---|---|---|---|
| 0 | 7.1 | 37.0 | 98.3 | 300 | 0.042 |
| 3 | 7.8 | 36.8 | 90.7 | 300 | 1.19 |
| 6 | 8.3 | 36.1 | 83.6 | 300 | 2.25 |
| 12 | 8.5 | 35.7 | 85.9 | 300 | 3.37 |
| 18 | 9.2 | 35.4 | 70.2 | 300 | 3.93 |
| 24 | 9.8 | 35.3 | 56.3 | 300 | 4.89 |
| 48 | 9.7 | 35.4 | 79.3 | 300 | 4.91 |
| 72 | 9.8 | 35.3 | 73.2 | 300 | 4.87 |

*Table 5 :* Enzyme production using Basal medium + 3% defatted cotton seed

| Time hours | pH | Temp $^0$C | %$PO_2$ | rpm | Cell concentration gm dry wt per lit |
|---|---|---|---|---|---|
| 0 | 7.0 | 37.0 | 120.3 | 300 | 0.02 |
| 3 | 7.1 | 38.3 | 110.4 | 300 | 1.25 |
| 6 | 7.3 | 37.1 | 93.6 | 300 | 2.31 |
| 12 | 8.1 | 36.3 | 83.9 | 300 | 3.80 |
| 18 | 8.7 | 36.1 | 70.8 | 300 | 4.93 |
| 24 | 8.9 | 35.9 | 64.7 | 300 | 5.29 |
| 48 | 8.7 | 36.3 | 53.9 | 300 | 5.3 |
| 72 | 8.9 | 37.8 | 48.7 | 300 | 5.32 |

*Table 6 :* Enzyme production using Basal medium + 2% mustard seed

| Time hours | pH | Temp $^0$C | %$PO_2$ | rpm | Cell concentration gm dry wt per lit |
|---|---|---|---|---|---|
| 0 | 7.0 | 37.0 | 120.1 | 300 | 0.01 |
| 3 | 7.3 | 38.1 | 117.3 | 300 | 1.23 |
| 6 | 7.9 | 37.5 | 93.5 | 300 | 2.56 |
| 12 | 8.5 | 36.3 | 83.8 | 300 | 3.19 |
| 18 | 9.1 | 35.3 | 77.9 | 300 | 4.84 |
| 24 | 9.7 | 35.5 | 64.2 | 300 | 6.71 |
| 48 | 9.9 | 36.1 | 56.9 | 300 | 6.68 |
| 72 | 10.3 | 38.5 | 28.5 | 300 | 6.70 |

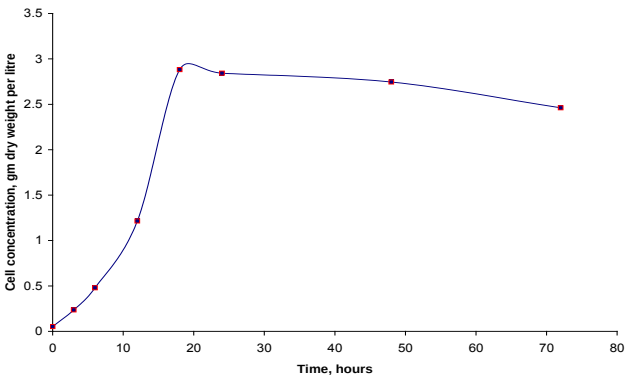


*Figure 1 :* Biomass cell concentration for 1% corn starch

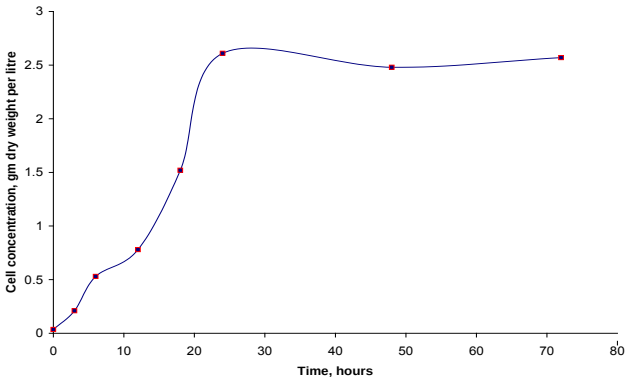


*Figure 2 :* Biomass cell concentration for 2.5% corn starch

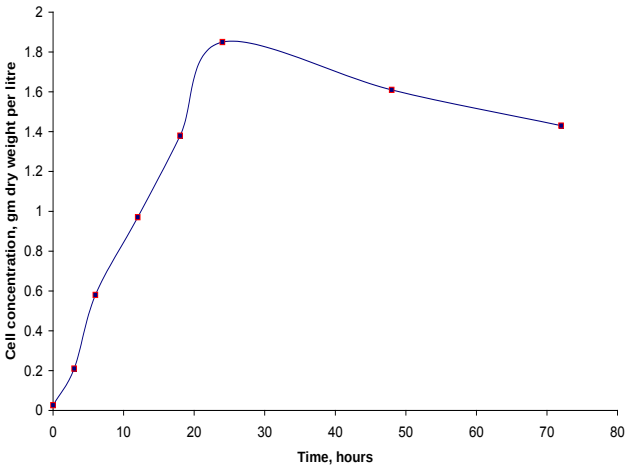


*Figure 3 :* Biomass cell concentration for 0.5% corn starch

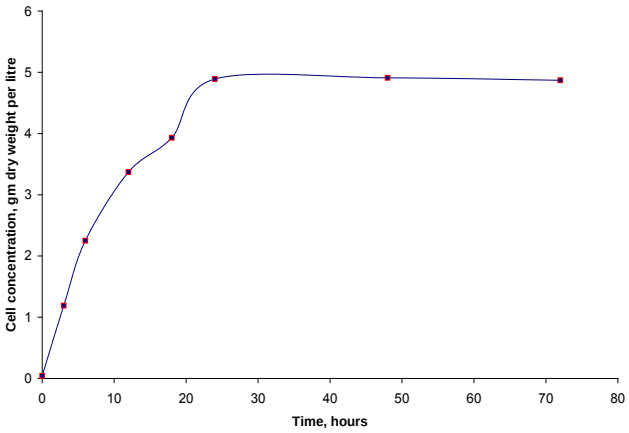


*Figure 4 :* Biomass cell concentration for Basal medium with 0.5% defatted soya flour

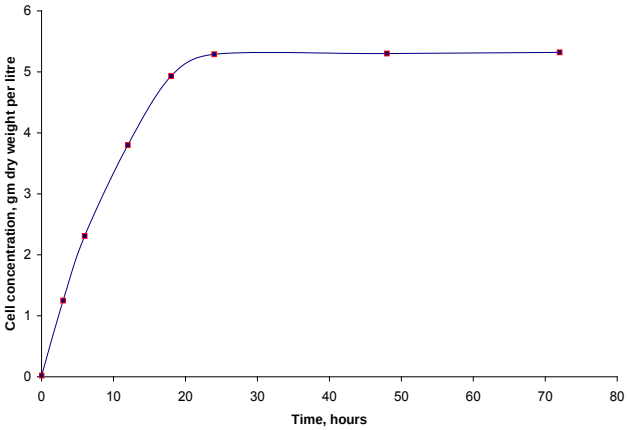


*Figure 5 :* Biomass cell concentration for Basal medium with 3% defatted cotton seed

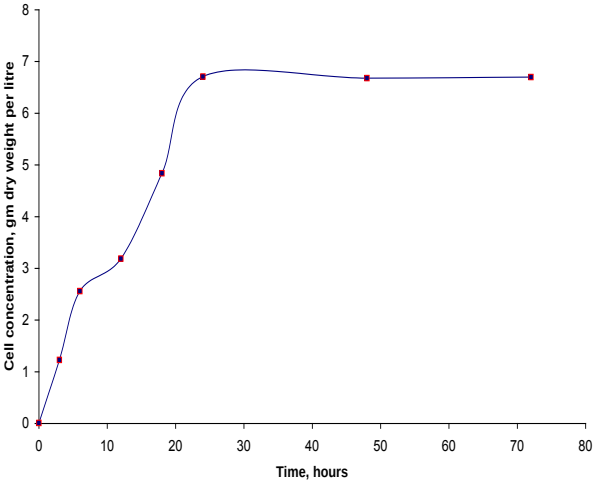


*Figure 6 :* Biomass cell concentration for Basal medium with 2% mustard seed

## VI. Conclusion

The Bacterial strain, *Bacillus lichenoformis* NCIM 2051 was obtained from National Chemical Laboratory, Pune, which produced high temperature alkaline α–amylase enzyme. The optimum cultural conditions are found to be 35oC, pH 7 and 300 rpm. The α–amylase produced from this Bacterial strain, *Bacillus lichenoformis* was quite active even at 1000C, however it showed optimum activity at 90oC, and also it exhibited optimum activity in the broad pH range 5.5 – 10, thus α-amylase of *Bacillus lichenoformis* seems to have a very broad pH range. A low cost synthetic medium producing large quantities of α–amylase has been developed from *bacillus lichenoformis* was used for α–amylase production. The α–amylase of this strain showed excellent stability at high temperatures and over a wide pH range. The cell mass concentration and the enzyme activity were determined and optimized. The low cost medium which contains, Defatted soya flour, Defatted cottonseed, and Mustard seed, produces two

times more enzyme than the high cost synthetic medium using yeast extract and peptone in the B. Braun Biostat E fermentor. So it is further suggested to change the cheapest different nitrogen sources components in this low cost medium like corn steep liquor etc.

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# Application of Protected L-Carnitine in Dairy Cows During Transition and High Lactation Period

By Heiko Scholz, Elke von Heimendahl, Frank Menn & Andreas Ahrens
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*Abstract-* 262 dairy cows were fed either 0 or 10g of a rumen protected carnitine product (containing 2g Carnitine) per cow and day supplemented via TMR. Milk yield and ingredients were investigated in all cows. For the investigation of blood parameters 55 cows per group were selected. Carnitin supplementation significantly decreased blood NEFA concentration one week a.p. and there was a trend for decreased NEFA one week p.p. and 5 weeks p.p.. GLDH in blood was significantly reduced one week a.p. and remained on a lower level throughout the trial in cows fed carnitine. Cholesterol level in blood was significantly decreased one week p.p. in heifers supplemented with carnitine. Cows in the supplemented group had also lower insemination index and improved conception rate.

*Keywords:* *l-carnitin, dairy cow, lactation.*

*GJSFR-G Classification :* *FOR Code: 270299*


*Strictly as per the compliance and regulations of :*

# Application of Protected L-Carnitine in Dairy Cows during Transition and High Lactation Period

Heiko Scholz [α], Elke Von Heimendahl [σ], Frank Menn [ρ] & Andreas Ahrens [ω]

*Abstract-* 262 dairy cows were fed either 0 or 10g of a rumen protected carnitine product (containing 2g Carnitine) per cow and day supplemented via TMR. Milk yield and ingredients were investigated in all cows. For the investigation of blood parameters 55 cows per group were selected. Carnitin supplementation significantly decreased blood NEFA concentration one week a.p. and there was a trend for decreased NEFA one week p.p. and 5 weeks p.p.. GLDH in blood was significantly reduced one week a.p. and remained on a lower level throughout the trial in cows fed carnitine. Cholesterol level in blood was significantly decreased one week p.p. in heifers supplemented with carnitine. Cows in the supplemented group had also lower insemination index and improved conception rate.

Carnitine supplementation led to an improved metabolic status of dairy cows during transition and high lactation period and increased fertility. Carnitine, in particular in a rumen protected variation can support metabolic health of dairy cows during the critical period of transition and high lactation.

*Keywords: l-carnitin, dairy cow, lactation.*

## I. Introduction

The main goal of milk production is a further increase in milk yield, and at the same time maintaining animal health. The transition and high lactation period is a very critical phase for dairy cows. Energy requirements for milk production in early lactation of dairy cows exceed the available energy from feed intake resulting in a more or less severe negative energy balance, which the cow tries to compensate by fat-mobilization from adipose tissue. Excessive mobilization of fatty acids can exceed the liver's capacity for degradation and results in elevated formation of ketone bodies and accumulation non esterified fatty acids (NEFA) in the liver where they are converted to triglycerides and stored. About 50% of the dairy cows within the first 4 weeks p.p. suffer from the so called fatty liver syndrome (Bobe et al., 2004; Jurritsma et al., 2003). An impaired liver function favors the development of other postpartum disorders like ketosis, metritis, displaced abomasum and immune suppression as well as a poor reproductive performance.

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L-Carnitine serves as a Co-factor for activated fatty acids and therefore has direct impact on fat-metabolism (Drackley et al., 1991a,b; Owen et al., 2001). In addition, carnitine acts as a buffer for acetyl-residues originating from fat-mobilization, thereby reducing ketone body formation (Harmeyer & Schlumbohm, 1997).The altered ratio of acetyl-CoA:CoAas a result of this buffer function further improves ß-oxidation and also stimulates carbohydrate metaboslism(Rebouche&Seim, 1998).As approximately 80% of dietary carnitine is degraded by rumen microbes (LaCount et al., 1996; Harmeyer, 1995).

The aim of this study was to showtheimpact of a rumen protected L-carnitine supplementation on performance and metabolic parameters during the transition and high lactation period.

## II. Material and Methods

262 dairy cows (German Holstein) were allotted to two treatments according to lactation number and milk yield. Animals in the carnitine group were marked by ear tags.Cows were fed a TMR as presented in Table 1 and 2. The composition and nutrient content of the TMR is presented in Table 1 and 2. Diets were formulated to meet the nutrient requirements of dairy cows according to GfE Guidelines (GfE 2001).In the carnitine group (CP), cows were fed 10g of a rumen protected carnitine product (Lohmann Animal Health, containing 2g Carnitine) per cow and day whereas the animals in the Control group (C) received 10 g of barley groat instead (table 3). Carnitine supplementation was given individually.

*Table 1 :* Composition of the transition and fresh cow diet (% and kg/day)

| | Transition | Fresh cows |
|---|---|---|
| Grass silage | 37,3 % (12,0 kg) | 21,2 % (13,3 kg) |
| Corn silage | 43,5 % (14,0 kg) | 41,1 % (25,8 kg) |
| Hay | 3,1 % (1,0 kg) | 1,0 % (600 g) |
| straw | 3,9 % (1,3 kg) | 0,6 % (350 g) |
| Concentrate 18/4* | | 0,4 % (250 g) |
| Sugar beet pellets | | 1,8 % (1,1 kg) |
| Protein concentrate** | 1,2 % (400 g) | 3,9 % (2,4 kg) |
| UDP concentrate*** | 6,2 % (2,0 kg) | 4,6 % (2,9 kg) |
| Barley groats | 2,3 % (750 g) | 2,8 % (1,8 kg) |
| Mineral feed | 2,3 % (750 g) | 0,3 % (200 g) |
| Protected fat | | 0,3 % (200 g) |
| salt | 0,1 % (40 g) | 0,1 % (50 g) |

* 18% crude protein, energy level 4 (7.4 MJ NEL)

**7,5 MJ NEL, 22.0% crude protein, 195g usable crude protein, 4g RNB

***7.2 MJ NEL, 40%CP, 3% crude fat, 8.5 %crude fiber, 260g usable crude protein, 22g RNB

*Table 2 :* Analyzed nutrient content of the transition and fresh cow diet

| | Transition | Fresh melking cows |
|---|---|---|
| NEL (MJ) | 6,0 | 6,6 |
| DM (g/kg) | 452 | 443 |
| Crude protein (g/kg) | 135 | 158 |
| Crude fiber(g/kg) | 218 | 192 |
| Ether extract (g/kg) | 27 | 31 |
| sugar(g/kg) | 24 | 34 |
| starch (g/kg) | 199 | 200 |
| Sugar + starch (g/kg) | 223 | 234 |
| Crude ash (g/kg) | 78 | 78 |
| usable rumen escape protein (g/kg) | 138 | 152 |
| Ruminal N-balance | -0,4 | 1,0 |
| Kalzium (g/kg) | 5,9 ± 0,3 | 7,7 ± 2,0 |
| Phosphor (g/kg) | 3,6 ± 0,4 | 4,5 ± 0,6 |
| Natrium (g/kg) | 2,9 ± 2,0 | 3,2 ± 0,9 |
| Magnesium (g/kg) | 2,3 ± 0,2 | 3,0 ± 0,5 |
| Kalium (g/kg) | 16,4 ± 1,3 | 14,4 ± 1,8 |
| Chlor (g/kg) | 6,6 ± 3,7 | 6,9 ± 1,2 |
| Schwefel (g/kg) | 1,8 ± 0,1 | 2,2 ± 0,3 |
| DCAB (meq/kg) | 248 ± 52 | 183 ± 40 |

*Table 3 :* design of investigation

| | Carnipass | Control |
|---|---|---|
| Dry period | Last milking – 22 days ante partum withoutCarnipass | |
| Transit period | 21 days ante partum with 10 g Carnipass | 21 days ante partum withoutCarnipass |
| Fresh milking | 1.-60. daypost partum with 10 g Carnipass | 1.-60. daypost partum withoutCarnipass |
| High milking | 61.-100. daypost partum withoutCarnipass | |

All cows were kept in a free stall under the same conditions. Milk yield was determined at 3 subsequent monthly milk controls (MC) after starting carnitine supplementation from all 262 cows. At the same time, milk samples were taken and investigated for milk yield, milk fat, milk protein and urea. Blood samples were taken from 110 cows out of the group of 262 cows 1 week a.p., 1 week p.p., 5 weeks p.p. and 9 weeks p.p (BS 1-4) and analyzed for NEFA, BHB, Cholesterol, GLDH and Bilirubin. Only cows with 3 complete milk and 4 blood samples were included in statistical evaluation.Data were analyzed by SPSS, Version 20 using one-way randomized block analysis of variance (ANOVA) and Kolmogorow-Smirnow-Test (KS) with a significant level set at $p \leq 0.05$. Results were expressed as mean ± standard deviation (s). Bivariate correlations procedure of SPSS with the PEARSON option (2-tailed) was used to determine correlations between parameters.

## III. Results and Discussion

249 of the 262 cows completed the trial, 131 for the control and 118 for the carnitine group. Blood parameters were investigated in 89 cows (51 control and 38 in CP). The exclusion of animals from the trial was not treatment related and mainly due to inappropriate calving date of heifers which reduced the period of carnitine supplementation significantly.

Carnitin supplementation did only slightly but not significantly influence milk yield in the first 100 days of lactation (Table 3-5). As a result of slightly decreased fat but increased protein percentage in carnitine supplemented cows, fat/protein ratio was a significantly reduced in the carnitine group.Reduced fat percentage in the milk and lower fat/ protein ratio indicate reduced fat mobilization probably as a result of improved fat metabolism and thereby reduced NEB by carnitine (Tasdemir et al., 2011).

*Table 3 :* milk yield, fat, protein and fat-protein-ration (FPR) of MC1

| | Milk yield | Milk fat (%) | Milk protein (%) | FPR |
|---|---|---|---|---|
| Control | 34,7 ± 7,9 | 4,23 ± 0,77 | 3,23 ± 0,32 | 1,3 ± 0,2 |
| Carnipass | 35,7 ± 8,3 | 4,09 ± 0,83 | 3,22 ± 0,36 | 1,3 ± 0,2 |

*Table 4 :* milk yield, fat, protein and fat-protein-ration (FPR) of MC2

| | Milk yield | Milk fat (%) | Milk protein (%) | FPR |
|---|---|---|---|---|
| Control | 37,5 ± 8,0 | 3,83 ± 0,69 | 3,05 ± 0,27 | $1,3^{a}$ ± 0,3 |
| Carnipass | 38,7 ± 8,8 | 3,75 ± 0,64 | 3,10 ± 0,28 | $1,2^{b}$ ± 0,2 |

a, b: signifikantbei p=0,06

*Table 5 :* milk yield, fat, protein and fat-protein-ration (FPR) of MC3

| | Milk yield | Milk fat (%) | Milk protein (%) | FPR |
|---|---|---|---|---|
| Control | 35,8 ± 7,9 | 4,02 ± 0,63 | $3,25^{a}$ ± 0,27 | $1,3^{a}$ ± 0,2 |
| Carnipass | 35,9 ± 7,5 | 3,92 ± 0,66 | $3,31^{b}$ ± 0,24 | $1,2^{b}$ ± 0,2 |

a, b: signifikantbei p≤0,07

Somatic cell counts at first and second milk control after calving were lower in the carnitine group than in the control group (figure 1). Even though these differences were not significant, it is known that a negative energy balance and subclinical ketosis have an influence on udder health (Leslie et al., 2000; Suryasathaporn et al, 2000).

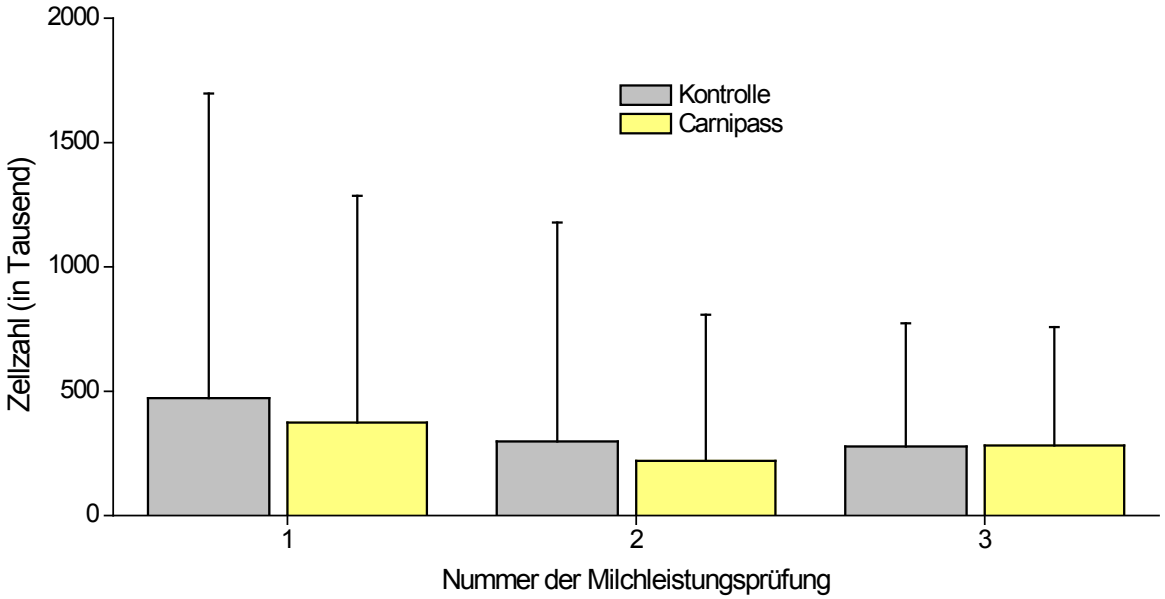


*Figure 1 :* influence of carnitine supplementation on somatic cell count of dairy cows

Carnitine supplementation tended ($p<0.15$) to decrease blood NEFA concentration during the whole trial period(Table 6). In multiparous cows the difference was significant one week ante partum (0.37in control versus 0.29 in the carnitine group).There was no significant influence on BHB. However, there was a trend for decrease BHB concentrations in week 5 and 9 p.p. (table 7). Even though there was no influence of carnitine supplementation on cholesterol levels in blood when all cows were considered, Cholesterol was significantly decreased one week p.p. in heifers supplemented with carnitine (2.7 versus 2.1mmol/l) (table 10).These findings are confirmed by other authors, when carnitine supplementation in dose levels similar to the one in this study were used (Carlson et al., 2007) or given intravenously (Erfle et al., 1971). Carlson et al. (2007) were also able to prove that carnitine supplementation significantly reduced the total lipid and the triglyceride content in the liver while simultaneously increasing glycogen concentration.

GLDH in blood was significantly reduced one week a.p. ($p=0,040$) and remained on a lower level throughout the trial in cows fed carnitine (table 8). According to Obritzhausen (2009) and Kraft &Dürr (2005) an increase in GLDH is an indicator for increased liver load which is the case in lipomobilization related fatty liver (Rehage 1996).Values for bilirubin were significantly different in week 5 p.p.. Increased bilirubin concentrations are always observed in relation to fatty liver syndrome and NEB (Kraft &Dürr, 2005; Rehage 1996). Lower levels of NEFA in carnitine supplemented group and therefore reduced liver loads are likely to be the cause for the lower GLDH activity and blood bilirubin concentration in this group.

*Table 6 :* Influence on carnitine supplementation on NEFA (mmol/l)

| | Control | | Carnipass | |
|---|---|---|---|---|
| | means ± s | min - max | means ± s | min - max |
| BS 1 | 0,37 ± 0,18 | 0,19-1,12 | 0,31 ± 0,09 | 0,19-0,64 |
| BS 2 | 0,60 ± 0,34 | 0,22-1,78 | 0,51 ± 0,28 | 0,21-1,52 |
| BS 3 | 0,36 ± 0,19 | 0,18-1,25 | 0,31 ± 0,12 | 0,18-0,67 |
| BS 4 | 0,27 ± 0,09 | 0,18-0,68 | 0,26 ± 0,07 | 0,17-0,46 |

*Table 7 :* Influence on carnitine supplementation on BHB ($\mu$mol/l)

| | Control | | Carnipass | |
|---|---|---|---|---|
| | means ± s | min - max | means ± s | min - max |
| BS 1 | 663 ± 223 | 298-1.501 | 668 ± 165 | 405-1.096 |
| BS 2 | 731 ± 578 | 258-3.526 | 739 ± 605 | 306-3.856 |
| BS 3 | 659 ± 620 | 321-4.880 | 575 ± 224 | 214-1.391 |
| BS 4 | 802 ± 428 | 278-2.508 | 737 ± 400 | 311-2.644 |

*Table 8 :* Influence on carnitine supplementation on GLDH (nkat/l)

| | Control | | Carnipass | |
|---|---|---|---|---|
| | means ± s | min - max | means ± s | min - max |
| BS 1 | 294 ± 330 | 63-1.768 | 178 ± 82 | 77-404 |
| BS 2 | 355 ± 344 | 73-1.708 | 288 ± 245 | 101-1.412 |
| BS 3 | 575 ± 1.005 | 123-5.439 | 412 ± 683 | 65-4.282 |
| BS 4 | 417 ± 397 | 93-2.610 | 391 ± 402 | 114-2.267 |

*Table 9 :* Influence on carnitine supplementation on Bilirubin (µmol/l)

| | Control | | Carnipass | |
|---|---|---|---|---|
| | means ± s | min - max | means ± s | min - max |
| BS 1 | 3,5 ± 1,6 | 1,2-8,6 | 3,4 ± 2,3 | 0,6-12,5 |
| BS 2 | 4,4 ± 3,0 | 1,0 – 17,6 | 4,3 ± 3,0 | 1,3-13,6 |
| BS 3 | 3,4 ± 2,0 | 1,4-11,4 | 2,6 ± 0,9 | 1,2-4,5 |
| BS 4 | 2,8 ± 1,9 | 1,1-14,2 | 2,5 ± 0,8 | 1,1-5,1 |

*Table 10 :* Influence on carnitine supplementation on Cholesterol (mmol/l)

| | Control | | Carnipass | |
|---|---|---|---|---|
| | means ± s | min - max | means ± s | min - max |
| BS 1 | 2,5 ± 0,9 | 1,5-6,1 | 2,3 ± 0,6 | 1,3-3,5 |
| BS 2 | 2,3 ± 0,8 | 1,0-4,4 | 2,1 ± 0,8 | 0,9-4,8 |
| BS 3 | 4,2 ± 1,0 | 2,3-6,7 | 3,9 ± 0,9 | 2,1-5,6 |
| BS 4 | 5,1 ± 1,1 | 3,4-8,7 | 4,8 ± 1,1 | 2,4-6,7 |

Carnitine supplementation also influenced fertility parameters in the cows. Whereas there was no difference in days from calving to first insemination, there was a trend for a lower insemination index in the carnitine group then in the control group. The conception rate was significantly improved in the carnitine supplemented group as compared to the control (Table 5). Supplementation of carnitin showed by Pirestaniet. (2011) a lower level on service to pregnancy than the control group. Also, there was a decreased significantly days open in carnitine compare to the control group (Pirestaniet., 2011).

*Table 5 :* Influence of Carnitin to fertility on cows

| | Control | Carnipass |
|---|---|---|
| Days open | 75 ± 35 | 74 ± 33 |
| Insemination index | 2,3 | 1,9 |
| pregnant rate | 70[a] % | 86[b] % |

237 cows of all 249 cows in the investigation had minimal one medication in the first 100 days of lactation. Between the Carnitin supplementation and the control group were found significant differences (Table 6).

*Table 6 :* Influence of Carnitin on medication of cows in the first 100 days of lactation

| | Control | Carnipass |
|---|---|---|
| Sum of medication | 1,64[a] | 1,17[b] |
| Medication fertility | 0,28[a] | 0,17[b] |
| Medication udder | 1,32[a] | 0,93[b] |

[a, b] Means within a row with different superscripts differ ($P < 0.05$).

Health and fertility of dairy cows are strongly related to NEB (Jorritsma et al., 2003). Fertility is mainly influenced by alterations in the IGF system during the period of NEB (Llewellyn et al., 2007). It is supposed that the impact of carnitine on fat and carbohydrate metabolism helped to reduce the period of strong NEB in this trial as indicated by less fat immobilization. In addition, carnitine might have had a direct impact on the IGF system as has been shown for other species (Waylan et al., 2005).

## IV. Conclusion

In conclusion, Carnitine supplementation tend toincrease milk yield during the first 2 month after calving improved the metabolic situation and in consequence also led to increased fertility in dairy

cows.Carnitine, in particular in a rumen protected variation can support metabolic health of dairy cows during the critical period of transition and high lactation.

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The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

**(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.**

**(II) Choose corresponding Journal.**

**(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.**

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Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

# Preferred Author Guidelines

**MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)**

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

**You can use your own standard format also.**
**Author Guidelines:**

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

**1. GENERAL**

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

**Scope**

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

**2. ETHICAL GUIDELINES**

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

**Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission**

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

**Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.**

**Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.**

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

**3. SUBMISSION OF MANUSCRIPTS**

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

**4. MANUSCRIPT'S CATEGORY**

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

**5.STRUCTURE AND FORMAT OF MANUSCRIPT**

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

**Papers**: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

**Format**

*Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.*

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

**Structure**

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

*Abstract, used in Original Papers and Reviews:*

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

*Acknowledgements: Please make these as concise as possible.*

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

*Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.*

*Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.*

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

*Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.*

**6. AFTER ACCEPTANCE**

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

**6.1 Proof Corrections**

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

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**6.4 Author Material Archive Policy**

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

**6.5 Offprint and Extra Copies**

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

## TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

**1. Choosing the topic:** In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

**2. Evaluators are human:** First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

**3. Think Like Evaluators:** If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

**4. Make blueprints of paper:** The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

**5. Ask your Guides:** If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

**6. Use of computer is recommended:** As you are doing research in the field of Computer Science, then this point is quite obvious.

**7. Use right software:** Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

**8. Use the Internet for help:** An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

**9. Use and get big pictures:** Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

**10. Bookmarks are useful:** When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

**11. Revise what you wrote:** When you write anything, always read it, summarize it and then finalize it.

**12. Make all efforts:** Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

**13. Have backups:** When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

**14. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

**15. Use of direct quotes:** When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

**16. Use proper verb tense:** Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

**17. Never use online paper:** If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

**18. Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

**19. Know what you know:** Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

**20. Use good quality grammar:** Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

**21. Arrangement of information:** Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

**22. Never start in last minute:** Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

**23. Multitasking in research is not good:** Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

**24. Never copy others' work:** Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

**25. Take proper rest and food:** No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

**26. Go for seminars:** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

**27. Refresh your mind after intervals:** Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

**28. Make colleagues:** Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

**29. Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

**30. Think and then print:** When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

**31. Adding unnecessary information:** Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

**32. Never oversimplify everything:** To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

**33. Report concluded results:** Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

**34. After conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

## Informal Guidelines of Research Paper Writing

**Key points to remember:**

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

**Final Points:**

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

**General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

**Title Page:**

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

**Abstract:**

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

**Introduction:**

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

**Procedures (Methods and Materials):**

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

**Results:**

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

**Discussion:**

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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| ***References*** | Complete and correct format, well organized | Beside the point, Incomplete | Wrong format and structuring |

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