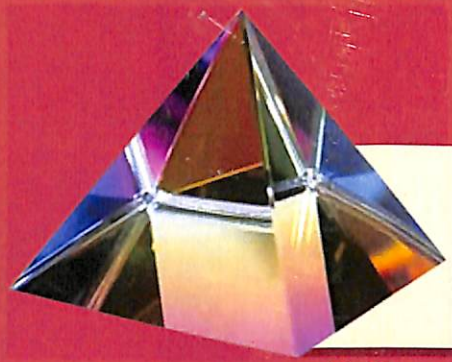

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A Comparative Study of Use of Technologically Advanced Household Appliances on Life Satisfaction & Leisure Time Activities of Earning & Non-Earning Married Women (25-40)

Sabiha Najam and Mrs. Punam Mehra

ABSTRACT

The present investigation was undertaken with the aim to study the impact of use of technologically advanced household appliances on life satisfaction & leisure time activities among 240 earning married women (120 with children and 120 without children) & 240 non-earning married women (120 with children and 120 without children) who uses everyday technologically advanced household appliances to do their daily household chores as well as for spending their leisure time activities too between the age group of 25-40 years. The obtained results reflected that there is an impact of use of technologically advanced household appliances on leisure time activities of earning & non-earning married women (with or without children) There is also a no significant relationship between leisure and level of life satisfaction obtained by using technologically advanced household appliances.

Keywords: Household Appliances, Life Satisfaction, Leisure Time Activities.

Introduction

A woman is very important and has a very integral part of every human life right from the time of birth. She plays various roles as a mother, sister, wife, and daughter so on. Every woman in some way or other is special and has a special inherent talent, only difference being that some women get to exhibit their talent and some do not. A woman is supposed to perform an important responsibility of bringing up their children. She is considered as the first institution of the society from where the child learns. Moreover, woman is also the one who is responsible for keeping intact the value system of the family unit and thus the women population holds the key to value system of the society in general. In a conservative community like ours men always dominate women and most of them cannot do what they want to and what they can do. There are a few lucky women who get to do what they want or rather have the guts to do so, even without the support of their man. But in no way earning women are superior or non-earning women are inferior.

Modern women have to face a complex life and thus play various roles. Her primary biological function of motherhood is slowly receding and its place is gradually being taken by manifold activities. Now she may be a busy woman, housewife or mother who has to look after the home when they return from their workplace. She thus has to play a very difficult and arduous role. Exposure to constant stress and strains of modern life might jeopardize their health and mental peace; which plays a deciding role in determining their quality of life and their leisure time thereby affecting the anxiety produced[6].

Household Appliances Household Appliances is the use of different technology sources at home for the combined advantages of comfort and efficiency[4].

Life Satisfaction Life Satisfaction has been defined as the function of the physical, psychological and social well being[1].

Leisure Time Activities Leisure Time Activities may be very simply defined as 'activity chosen in relative freedom for its qualities of satisfaction[5].

Therefore the management of free time is always a complex issue for busy working women whether at home or outside. The leisure behaviour of women is clearly distinctive from that of men. Free time activities of many a woman sharply differ from one another. Many women prefer spending their free time at home with family because the traditional culture advocates so, whereas as many women accommodate more outside activities.

Leisure time gives women the space in which they can experiment with different lifestyles, as well opportunities for identity development[3].

The relation between leisure and life satisfaction can be observed through an important indicator: health. The WHO considers leisure as essential for the correct development of the human being and basic for his or her psychic and social balance. It has been shown that the correct enjoyment of leisure prevents disease, increases creativity and provides a better life satisfaction. Leisure that is a satisfactory experience has a beneficial effect that goes far beyond its own existence, affects our whole

being and has an impact on other aspects of our life and our relations with our surroundings.

Thus we can say that home appliance is a necessity to help us survive in our busy lifestyles and also to enjoy more time with our family and friends and ultimately provide satisfaction[2].

The use of technologically advanced household appliances overlap with those relating to life satisfaction & leisure time activities of earning & non-earning married women, it is amply clear that there can just be no substitute to domestic technology.

Methodology

The sample consisted of earning married women who uses technologically advanced household appliances to do their daily household chores as well as for spending their leisure time (120 with children and 120 without children)and non-earning married women who uses technologically advanced household appliances to do their daily household chores as well as for spending their leisure time (120 with children and 120 without children)between the age group of 25-40 years in the city of Kolkata.The technique used for the collection of data in the present study is Questionnaire Method. The tools used in the present study were:

1. Life Satisfaction (LS-SCALE) by Dr. Promila Singh and George Joseph[7].
2. Leisure Time Activity Questionnaire Inventory (Self Prepared)

Results and Discussions

The present study was undertaken with the aim to find out, the impact of use of technologically advanced household appliances on leisure time activities among earning & non-earning married women. The results obtained from the present study with three variables, technologically advanced household appliances, life satisfaction and leisure time activities of earning & non-earning married women are cited below.

Table 1 – Showing the "t" values of the test of proportion to examine the impact of technologically advanced household appliances on leisure of earning married and non-earning married women

Groups	P= Sample Proportion	Computed t	Tabulated t at 5 %	Null Hypothesis
Earning Married Women	0.567	18.29	1.645	Rejected
Non-earning Married Women	0.555	17.32	1.645	Rejected

From the obtained result, it has been found that the use of technologically advanced household appliances has statistically significant impact on the leisure among earning & non-earning married women. Thus, it can be interpreted that in today's age technology has a major influence on everyday life activities as well as on women as it saves time energy which they can use in perusing other creative endeavor, and hence it is also found that technology also tends to override their leisure and therefore their leisure is also based on various modes of technology, as leisure helps to relive the stress and also helps to relax mentally, physically and therefore leisure is supposes to be very important aspect in a person's life but with changing times, a lot of globalization emerging and technological advancement, people in today's age use technologically advanced equipment as a mode of leisure.

Test Of Equality Proportion was also found to examine whether impact of technologically advanced household appliances on leisure is same for earning and non-earning married women. The results indicated that when considered individually, technologically advanced household appliances have statistically significant impact on the leisure among earning & non-earning married women (considering both with and without children) but there is no significant difference between the impacts of technologically advanced household appliances on the leisure of earning & non-earning married women.

Chi Square Test (Test for Independence of Attributes) was also found out of all the four groups i.e. group I – married non earning women with children, group II – married earning women with children, group III – married non earning women without children and group IV– married earning women without children on relation between leisure obtained by technologically advanced household appliances and life satisfaction. The results indicated that there exists no statistically significant association between life satisfaction and leisure (obtained by using technologically advanced household appliances).

Conclusion

Thus from the present study it may be concluded that there is an impact of use of technologically advanced household appliances on leisure time activities of earning & non-earning married women (with or without children).

Furthermore, it may be concluded that among all the four groups, i.e., Group I and II as well as Group III and IV, there is no statistically signifi-

cant association between life satisfaction and leisure (obtained by using technologically advanced household appliances).

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A Study on the Socio-Economic Problems, Mental Health and Social Stigma of Leprosy Patients in Kolkata

Mehnaaz Siddiqi and Krishnakali Bhattacharyya

ABSTRACT

The present study was undertaken to determine the Socio-economic problems, mental health and Social Stigma of leprosy patients in Kolkata. Data were collected using structured questionnaire, from 66 leprosy patients who came for treatment in the School of Tropical Medicine, Kolkata. Also 503 individuals from various walks of life were included in the study to explore the knowledge and attitude towards leprosy among general population. Leprosy patients were administered 3 questionnaires and general population two respectively. It was found that leprosy patients were suffering mental health problems like, anxiety, insomnia and depression, as well as somatic symptoms. They feel highly stigmatized although they have not reported of having socio-economic problems. Majority of literate people in the general population knew the actual cause of the disease and they do not have a tendency to keep a social distance from leprosy patients. In general, people knew the disease is infectious.

Keywords: Leprosy, Socio-Economic Problems, Mental Health, Social Stigma.

Introduction

Leprosy or Hansen's disease (HD) is a chronic infectious disease caused by the bacteria Mycobacterium Leprae. Named after physician Gerhard Armauer Hansen, leprosy is a primarily a granulomatous disease of the peripheral nerves and mucosa of the upper respiratory tract; skin lesions are the primary external sign[10].

The mode of transmission of Hansen's disease remains uncertain. Most investigators think that Mycobacterium Leprae and Mycobacterium Lepromatosis are the causative agents of leprosy. Mycobacterium Lepromatosis is a relatively newly identified Mycobacterium isolated from fatal case of diffuse Lepromatous leprosy in 2008[10]. An intracellular acid-fast bacterium, M.leprae is an aerobic and rod-shaped. It is surrounded by the waxy cell membrane coating characteristic of mycobacterium species[13].

It is estimated that due to genetic factors, only 5% of the population is susceptible to leprosy[3]. In addition, malnutrition and prolonged exposure to infected persons may play a role in development of the overt disease.

The most widely held belief is that the disease is transmitted by contact between infected persons and healthy persons[3]. In general, closeness of contact is related to the degree of infection, which in turn is related to the occurrence of disease.

Two exit routes of M.leprae from the human body often described are the skin and the nasal mucosa, although their relative importance is not clear.

According to two authors, the incubation period can vary from 2 to 20 years (average about 3 years)[4].

On the basis of various symptoms, the World Health Organization (WHO) classifies leprosy as Paucibacillary leprosy (PB) and Multibacillary (MB) leprosy [4]. According to Ridley- Jopling and Mesh leprosy is further divided into Tuberculoid ("TT"), Borderline Tuberculoid ("BT"), Mid Borderline or Borderline ("BB"), Borderline Lepromatous ("BL") and Lepromatous ("LL").

In addition to the disease's physical effects, patients historically have suffered severe social stigma and ostracism from their families, communities and even health professionals to such an overwhelming extent that leprosy has been known as "the death before death"[3].

According to Goffman, the term 'stigma' originated with the ancient Greeks. Stigma has also been defined as "an attribute that is deeply discreting leading to a spoiled identity"[5].

The research was supported by a finding of a longitudinal study, undertaken from November 1977 to January 1979, of 344 leprosy-affected persons attending a leprosy clinic in Gwalior, India showed that social stigma was present in a variety of forms, and was more prevalent among persons who were illiterate and from low socio-economic groups[12].

The stigma of leprosy in many people's lives causes psychosocial-economic problems apart from the physical ailments. The peripheral neuropathy cause gross deformities if the face and limbs of the infected individuals as well as crippling disabilities involving sight, touch and manual dexterity. Such stigma intensifies the social and economic isolation of patients[6].

They have to face negative reactions from their families, spouses and society members, they are either deserted by partners or not living with their spouses. Social prejudice and deformities due to leprosy have played a key role in socio-economic deterioration of patients[6].

Studies on leprosy involving stigma and income generation stated that 'even today social stigmatization is frequent so that affected persons with clear signs of chronic manifestations are often unable to work, or to marry, they become dependent for care and financial support, leading to insecurity, shame, isolation and consequent economic loss"[18].

World Health organization defines mental health as "a state of wellbeing in which the individual realizes his own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community". It is difficult to analyze the traumatic experience to which the leprosy patients are subjected and the tremendous changes that takes place not only in the body but also in the mind [14]. A small-unnoticed patch on his body, once medically diagnosed as leprosy, envelopes the patient with the cumulative feeling of helplessness, shame and dependency.

In one study, using the Cornell Medical Index, found the prevalence of any psychiatric disorders was around 18% in leprosy patients with depressive reaction the most common disorder [19].

Recent studies indicate the significant effects of leprosy on mental health of leprosy patients. This study by Weiss investigates the interrelationship of culture, mental health and medical illness. According to the result 50% of 56 recently diagnosed leprosy outpatient and 35% of 19 controls with another stigmatized derma logical condition met DSM-111-R criteria of an axis 1 depressive, anxiety or somatoform disorder [7].

Methodology

The sample comprised of 66 male and female leprosy patients who were undergoing treatment and surveillance for at least six months in the School of Tropical Medicines, Kolkata. They were all aware that they are suffering from leprosy. All the patients were married, having on an average at least two children. They belonged to the age group of 20-55 years.

However, this total sample was further divided into two groups for analysis on the basis of the locality the family is residing, i.e. whether living in urban locality specifically in Kolkata or in rural area.

The present investigation also studied some common people to see how much aware are they about the disease and whether they have a tendency to keep a social distance from them or not, In other words, their knowledge and their social stigma regarding the disease and the people suffering from it. For this 503 people were selected at random from different age groups, gender (male and female) religion (Hindu, Muslim and Christian), educational qualification and professions. Out of 503 individual studied, 459 (222 males and 226 females) were literate and 44 (20 males and 24 females) were illiterate.

Results and Discussion

To start with the first variable of investigation i.e. mental health, four areas of it were being explored. Percentages of high responses, considered as 'cases' are given in the figure (fig 3) below:

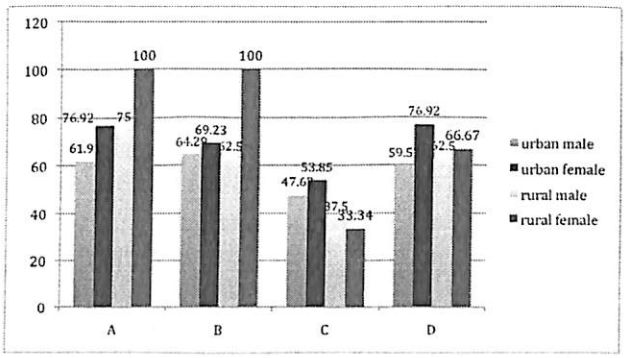

Fig 1: Percentage of 'cases' in four areas of Mental Health (A, B, C and D)

Taking into account, the first area of mental health i.e. A, which focuses on somatic symptoms it was found that more than 60 % of leprosy patients were suffering from somatic symptoms and were considered as cases. Moreover, female patients reported more somatic symptoms as compared to males (fig 1). Somatic symptoms include feeling ill, run down, pain in head, having hot or cold spells, need for tonic and so on.

Examining the next area relating to anxiety and insomnia, i.e. B, the study found that rural females reported maximum problem followed by urban males and females respectively (fig 1). They lost their sleep over worry. Male leprosy patients reported to be worried as they were they sole breadwinners of their family and were concerned about the financial responsibilities they have towards their families. Females were worried about the future of their children, as reported informally to the researcher.

Overall, from the results obtained in the area C, it was found urban female reported maximum dysfunction while rural females reported the least (Fig 1). Leprosy and the physical effects associated with it (for e.g., anesthesia, disabilities and deformities) prevented the patients from enjoying their day-to-day activities. Although the number of leprosy patients in the present sample who complained of not being satisfied with their task were not large. They tried hard to keep themselves busy and occupied over the things they do.

How pessimistic one is about life reflects the level of depression. The person may feel that life isn't worth living (Question D3 of General Mental Health Questionnaire). In other words, a person's sense of wellbeing is greatly affected by leprosy. The idea of hopelessness and giving up ones life was reported by majority of urban female patients. Other patients were also not too optimistic towards life (Fig 1).

The result obtained in the present study found that the 66 respondents all feel highly stigmatized. Analyzing the data obtained from each subscale of Internalized Stigma of Mental Illness Scale, adjusted for leprosy-affected people, the detailed results were as follows:

For the dimension A which stands for alienation which sought to measure the subjective experience of being less than a full member of society, or having a 'spoiled identity'[5], it was found that more than 62% leprosy patients in all groups were suffering from alienation.

The second dimension is stereotype endorsement (SE) measures the degree to which respondents agree with common stereotypes about people with leprosy.

Due to high self-stigma the respondents said that stereotypes about leprosy applied to them also. According to them, people with leprosy cannot live a rewarding life and their contribution to society is less as compared to normal healthy people.

Discrimination experience (DE), being the third dimension .The patients coming from rural areas experienced more discrimination than the urban .The leprosy patients of the study reported that people did not discriminate them. Neither the people patronized them nor people are treated them like a child, may be because they do not talk much about themselves and had mostly concealed the disease from others.

Very few males and female leprosy patients reported that they stayed away from social situations and avoided people. Thus they are not avoided and rejected by others. They attend social functions and ceremonies so that people do not think something unusual about them. Overall, their social withdrawal is low.

The last subscale of ISMI is Stigma Resistance (SR), which intends to portray the experience of being affected by internalized stigma. The result obtained from the present study found that except rural leprosy female patients, the stigma resistance is high among other groups.

Considering all the subscales together the mean values of the groups studied has been found to be quite high (fig 2).

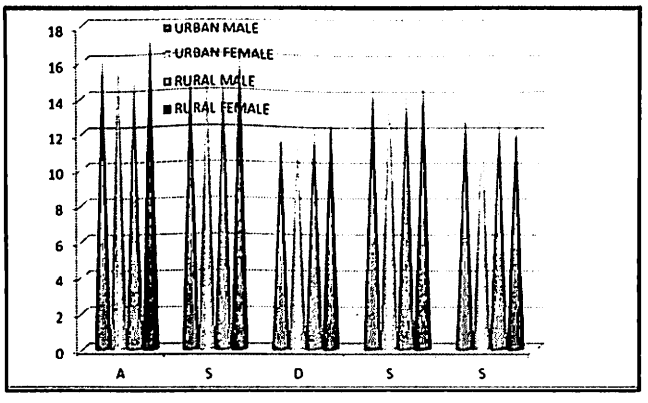

Fig 2: Mean values of various subscales of ISMI

In the present study internalized stigma of leprosy patients were quite high thus indicating that rather than experiencing stigmatization from the surrounding, most patients reported suffering mainly from internalized form of stigma. This result was further conformed by administering the socio-economic problems questionnaire.

According to the sequence of questionnaires being administered to the respondents the last was related to the socio-economic problems. The result obtained is as follows:

Table 1: Showing the mean value of socio-economic problems of leprosy patients.

Age range	Sex	N	Mean
20-55 years	Literate males	42	6.785
	Literate females	13	5.77
	Illiterate males	8	4.5
	Illiterate females	3	5.67

The above result revealed that these patients were not suffering from major socio-economic problems.

The knowledge regarding leprosy remains incomplete without knowing how common people think and feel about the disease leprosy and the leprosy patients. Keeping this view in mind the knowledge and attitude of general population regarding leprosy formed another part of the present study.

Surveying 503 people from different backgrounds and occupation who were willing to cooperate, it was found that majority of the literate people know the actual cause of leprosy. However, illiterate people attributed the cause to ill luck. Thus the findings of the study reflect that there are still some wrong conceptions prevailing among people. The earlier studies also found that bad blood[11], and past sin[1], ill luck[18] or curse[2] and even consumption of certain food items are its causes.

When asked about the transmission of the disease, surprisingly majority of people regardless of their educational qualification knew that it spreads through physical contact, which was followed by genes and respiratory tract. Again taking into consideration the peoples knowledge about the infectious nature of the disease it may be further interpreted that this may be the reason which kept the general people away from the and restricted them from buying food from them. Although infectious 70 % people was sure that it can be cured and while 37.6% reported that they knew about the present day treatment of leprosy. Thus it may be said that although infectious, 70 % people was sure that it can be cured and while 37.6% reported that they knew about the present day treatment of leprosy. Few (25%) were aware of rehabilitation of leprosy patients. On the whole the general population is quite ignorant about modern treatment facilities and still some hold the notion that it cannot be cured. Knowing their acquaintance they were given a vignette where a 23-year-old person who was treated successfully and declared cured by the doctors. The person was earning and doing well in job although crippled to some extent by the disease. When asked some questions based on the story to find out the social distance which people maintained from a leprosy patient. It was found that literate people had low prejudice as compared to illiterate ones, against the treated leprosy patients. May be the idea of, successful treatment and cure had lead to lowering of stigma response among literate people regarding leprosy.

Conclusion

Leprosy is like any other disease and any one can suffer from it. It can happen to any one irrespective of the cast, creed, sex, occupation, educational qualification and economic status.

The present study aiming to find the socioeconomic problems, mental health and social stigma explored that majority of the leprosy patients feel highly stigmatized. However they denied having any socio-economic problems, even being economically dependent on others and temporary unemployed. Often being the sole bread earner of the family and not being able to work all the time because of their disease, they were not ready to accept that they have economic problems.

Socially they said to be accepted by neighbors and family members, however, most them have concealed the disease. In the place of employment they reported to be treated at par with others, but most of them feel that they have restricted opportunities in life.

Studying the mental health it was found that, most of them were suffering from depression, anxiety, insomnia and somatic symptoms but hardly any patients reported social and cognitive dysfunction.

The present study surveying 503 people from various walks of life found that more than 50% of people will avoid them and 70% reported that they would not buy food from leprosy patient. In this context it may be further mentioned that majority of the literate people know the exact cause of leprosy, although the illiterate people attributed it to ill luck. Regardless of their educational qualification, most people studied know that the disease spreads through physical contact. May be the infectious nature of the disease are keeping the common people away from leprosy affected people and restriction them from buying food from them.

The only ray of hope in darkness is the mean value of social distance of the literate people of the general population. Surprisingly, it has been found that the literate people do not maintain very large social distance from leprosy patients when they know the person has been treated successfully and the doctors declare the persons cured, they responded positively.

So, it may be concluded by saying "the fight is not over yet. But is winnable. There are enough men and women of good will who can spread the word that leprosy is curable, and that leprosy sufferers need not, must not be shunned unless the message reaches every continent, every country, every village, every patient, the disease will prevail in dangerous pocket"[8].

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A Comparative Study on Stress and Life-satisfaction of Working and Non-working Mothers having One Pre-school Child.

Pooja Jain and Ms Geetika Sachdeva

ABSTRACT

The present investigation was undertaken with the aim to study stress and life-satisfaction of working and non-working mothers having a pre-school child. The study was designed with 200 subjects (working mothers having a pre-school child=100) and (non-working mothers having a pre-school child=100) between the age group of 25-45 years on the basis of sex, age, educational qualification, marital status and occupation. The data was collected with the help of Personal Stress Source Inventory (PSSI-SSS) by Arun K. Singh, Ashish K. Singh and Aparna Singh and Life Satisfaction Scale (LSS-SJ) by Dr. (Mrs) Promila Singh and George Joseph. Collection of data was followed by statistical analysis. The obtained results reflected that there is no significant difference between working and non-working mothers regarding level of stress and life-satisfaction. Thus Null Hypotheses was accepted in both the cases.

Keywords: Working Mothers, Non-working mothers, Stress and life-satisfaction

Introduction

The modern world broadly marching towards progress and achievement has also brought in stress and strains of life. Every new day dawns unfolding unpredictable problem and chaos[4].

Stress is defined by the National Safety Council, USA as the inability to cope with a perceived threat to one's physical, mental, emotional and spiritual well being, which in turn affects one's physical health. Stress is the mental and physical condition that occurs at any time in the environment. Stress is felt by every human being at one time or other while facing life's challenges[4].

Life satisfaction is defined as having a favorable attitude towards one's life as a whole. It is widely considered to be a central aspect of human welfare[3]. The presence of pre-school years of child (3-6 years) poses many challenges to the home makers and this appears to be the most demanding period of a homemaker's life. Pre-school children needs are quite different from grown up children that requires constant attention from home makers[2].

The balancing of motherhood and career becomes stressful as they have to manage both the domains simultaneously[1].

Methodology

The present study was conducted to compare the level of stress and life satisfaction faced by working and non-working mothers having one pre-school child.

The variables were set after conducting a detailed study of the researches already conducted in similar areas.

Survey method was used to conduct the research and the tool of investigation was questionnaire.

The scales used in the study are Life Satisfaction Scale (LSS-SJ) English by Promila Singh and George Joseph and Personal Stress Source Inventory (PSSI-SSS) by Arun Kumar Singh, Ashish K. Singh and Aparna Singh.

For the collection of data various Schools were approached officially and the questionnaires were distributed to mothers having one pre-school child. The sample consisted of 100 working and 100 non-working mothers having one pre-school child between the age group of 25-45 years and this study was carried out in Kolkata, capital of West Bengal.

Results and Discussion

Table 1- The Mean and Standard Deviation Values of Two Groups on the Various Dimensions of Stress and Life-Satisfaction

Groups	Stress		Life - Satisfaction	
	Mean	Standard Deviation	Mean	Standard Deviation
Working Mothers having One Pre-School Child	46.31	16.72	144.69	12.91
Non-Working Mothers having One Pre-School Child	47.03	17.35	143.63	15.28

Table 2- Showing " T " Values Of The Two Groups On The Various Dimensions Of Stress And Life-Satisfaction

Groups	Stress	Life Satisfaction
Working And Non-Working Mothers Having One Pre-School Child	0.298	0.527
Hypotheses	Accepted	Accepted

The present obtained results enumerates that there is no significant difference between working and non-working mothers hav3ing one pre-school child in the level of stress.(Refer table 2) This means that working and non-working mothers have

moderate stress level as both the groups are striving equally towards maintaining their household and professional lives in an amiable way.(Refer table 1)

The obtained results enumerates that there is no significant difference between working and non-working mothers having one pre-school child in the area of Life-Satisfaction.(Refer table 2) This means that working and non-working mothers are similar in regards to life-satisfaction. The mean values revealed that working and non-working mothers having one pre-school child are highly satisfied (Refer table 1) in areas seen in the data collected which they enjoy doing in their lives, like their household work, professional work, participating in community, leisure and social activities. Therefore we can conclude that working and non-working mothers having one pre-school child have no significant difference in the level of stress and life-satisfaction.

Conclusion

From the obtained results on stress, it may be concluded that there is no significant difference between the mean stress level of working and non-working mothers having a pre-school child. The obtained results lead to the acceptance of Null Hypotheses.

Again, from the obtained results on Life-Satisfaction, it may be concluded that there is no significant difference between the mean life-satisfaction level of working and non-working mothers having a pre-school child. Thus accepting Null Hypotheses.

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A Comparative Study on Stress and Anxiety faced during pregnancy by working and non-working women in the age group 25-40 years.

Keyanet Khan and Punam Mehra

ABSTRACT

The present investigation was undertaken to conduct a comparative study on stress and anxiety faced during pregnancy by working and non-working women in the age group 25-40 years. The study constituted of 64 pregnant women out of which 32 were working pregnant women and 32 non-working pregnant women in the age group 25-40 years on the basis of sex, age, educational qualification, marital status, income level and duration of pregnancy. The data was collected with the help of Personal Stress Source Inventory by Arun Kumar Singh, Ashish K.Singh and Arpana Singh and Comprehensive Anxiety Test by H.Sharma, R.L.Bharadwaj and M.Bhargava. The obtained results reflected that there exist no significant difference between the stress level of pregnant working and non-working women. The results also indicated that there exist no significant difference between the anxiety level of pregnant working and non-working women.

Keywords: Working and Non-working Women, Pregnancy, Stress and Anxiety.

Introduction

Pregnancy is a time of growth and hope (Schroeder, 1996)[1]. Pregnancy is perceived by many pregnant women as a period of happiness in anticipation of motherhood. Women hope for a smooth journey in pregnancy without any complications and a normal fetal development.[1] Pregnancy sometimes tend to make a woman experience joy and cheerfulness and at times to experience stress as well as anxiety. Anxiety is a dimension of stress (Brown, 2001) that occurs in response to internal or external stimuli and can result in physical, emotional, cognitive and behavioral symptoms (Relier, 2001). Depression, anxiety and stress are quite prevalent during pregnancy (Halbreich, 2005) and occurs in majority of the women with a prevalence rate of 30% (Matteson, 2001). Anxiety, on the other hand, can cause a stressful pregnancy that can result in fetal distress, preterm delivery, low birth weight, postpartum disorders and other delivery complications. Some aspects of personal pregnancy work involved dealing with ambivalent or competing familial or societal expectations regarding women's work particularly in relationship to the challenge of integrating career and employment with motherhood[2].

Anxiety and Stress, while common during pregnancy, is not a healthy side effect of pregnancy. There are many reasons when a woman may feel anxious during pregnancy. Along with pregnancy comes the stress during pregnancy and responsibility of carrying a new life into this often chaotic and unpredictable world. Fortunately they don't have to spend every waking moment thinking, obsessing and stressing over their pregnancies. There are several strategies one can adopt to help reduce the anxiety and stress associated with pregnancy[3].

Methodology

The present study was conducted to examine the stress and anxiety faced by pregnant working women and compare it with that of the pregnant non-working women. The variables were set after conducting a detailed study of the researches already conducted in similar areas.

Survey method was being used to conduct the research and tool of investigation was questionnaire. The scales used in the research are Personal Stress Source Inventory by Arun Kumar Singh, Ashish K.Singh and Arpana Singh and Comprehensive Anxiety Test by H.Sharma, R.L.Bharadwaj and M.Bhargava.

RESULTS AND DISCUSSIONS

In the present study, we have applied mean, standard deviation and t-test statistics to measure the values of stress and anxiety on pregnant working and non-working women.

Table 1 : Mean, Standard Deviation and t-Value for Stress and Anxiety of Pregnant Working and Non-working Women

Dimension	Group	Mean	Standard Deviation	t-Value	Hypothesis
Stress	Pregnant Working Women	56.34	231.7	0.084	Accepted
	Pregnant Non-working Women	51.46	231.7	0.084	Accepted
Anxiety	Pregnant Working Women	42.91	296.6	0.016	Accepted
	Pregnant Non-working Women	41.68	296.6	0.016	Accepted

From the obtained result it has been found that there is no significant difference between the stress level of pregnant working and non-working women (Table 1). This means that both the groups have

similar stress level. It was found that both the groups had proportionately moderate level of stress (Table 1). From the obtained result it has been found that there is no significant difference between the anxiety level of pregnant working and non-working women (Table 1). This means that both the groups have similar level of anxiety level. It was found that both the groups had proportionately average (normal) level of anxiety (Table 1).

Conclusion

From the present study it may be concluded that pregnant working and non-working women have moderate level of stress and average (normal) level of anxiety between them irrespective of their job and household chores.

Overall, the study constituted of 64 pregnant women out of which 32 were working and 32 were non-working women, the present study found that both working and non-working women had similar level of stress and anxiety.

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Challenges Faced by Women Executives within the Age Group (25 To 40 Years) in Their Domestic Sphere

Kankana Roy and Sreyasi Chatterjee

ABSTRACT

The study was conducted on the "challenges faced by women executives within the age (25 to 40 years) in their domestic sphere". The aim of the study is to analyze different challenges faced by women executives in their domestic sphere keeping in focus that various sets of social values have exacerbated the double burden of women's responsibilities. The study focuses mainly on four different challenges that women face while keeping pace with socially assigned roles, while maintaining proper health , fulfilling commitment towards relationships and also while fulfilling occupational demands. The study was conducted by interviewing 210 women executives from eight different sectors like bank, IT, telecom, media, hospitality, tourism, healthcare and retail. Further the results were drawn using statistical method like standard normal test at 5% level of significance that rejected the proposed hypotheses and hence the alternative hypotheses were accepted. And thereafter the individual responses were also taken into account which suggested that women executives do face challenges while keeping pace with socially assigned roles, while maintaining proper health, fulfilling commitment towards relationships and also while fulfilling occupational demands.

Keywords: Women Executives, Challenges, Domestic Sphere.

Introduction

The era of economic reform in India began in the year 1990 as a strategy to become a part of the global economy. Women with specialized skills and technical knowhow are today employed in large numbers causing a dramatic shift in the demography of the work force. This category of women workers forms a class of professional and technical executives[7]. Therefore women executives in Indian context is defined as a person or group having administrative and managerial authority in an organization or supposes to be the chief officer of the government, state, or political division that constitutes around 20 percent of the total female workforce in India (human development report 2002) and hence the present study aims to analyze different challenges faced by women executives in their domestic sphere.

Among the numerous challenges faced by women executives one of them is 'Challenges while keeping pace with socially assigned roles'. Even though a huge proportion of women are found working at an executive level in various government and non government sectors but the social ideologies are patriarchally structured[4]. The expectations associated with the ideal employee and the ideal parent is linked to the idea that workforce should be comprised mostly of men and women should be largely confined to household duties and childcare and hence due to this social endorsement of the outdated roles a female member or a female parent endures a great pressure from the family. And therefore the pervasiveness of stereotypical sex role expectation molded by social constructs tend to always predominate in the women's life and creates challenges for women to secure and maintain their position in the workforce[2].

Another important challenge where the focus has been drawn in the study is about 'fulfilling commitments towards relationship'. Confining women's identity to a domestic sphere is one of the most important factors that restrict her entry into a workforce despite her having all kinds of credentials to suite a particular job profile. The double burden of family responsibilities hinders mobility of the woman and restricts her career opportunities. Concern for children, parents, and husband and in laws and other important relationships sometimes creates a conflict and overrides the passion for career. The problem of dual productivity and reproductive role of women has been highlighted each time because work in the home are always considered to be exclusively performed by the woman as the society expects a woman to perform duties of a wife , mother , daughter or a potential partner first and therefore many women executives face challenges in her professional career.

Women have expanded their career aspirations but women executives still encounter several 'challenges while fulfilling occupational demands'. There are some researches that confirm that employment has a positive effect for the women and their families. Despite such findings women still encounter a number of difficulties and misperceptions in their domestic sphere that affects their level of performance at their workplace. One of the foremost difficulties that women face is the work/ family conflict particularly among fulltime women employees

with regard to the working hours, late night shifts and the like and the women do not receive tangible support from their family. Women also face tremendous pressure while they have to make numerous occupational adjustments and bring about contemporary changes within themselves to adapt to various aspects of work place culture such as communication pattern, hierarchy, and dress code and food habits and especially if it does not adhere to family constructs and their particularities.

The last challenge which the study aims to analyze is the 'Challenges faced by women executives while maintaining proper health This section will consider some of the aspect of women's roles as defined by their involvement in the production , reproduction and domestic duties which at times increases their exposure to potentially deliberating or life threatening conditions.' The Indian situation is no different from one in the developed countries, where even today women carries the responsibility for some 80 to 90 percent of the household chores (United Nations 1991)and these chores are much more labor and energy intensive. Occupational demands along with certain domestic confinement and sole responsibility for a range of back breaking domestic activities increases the risk of exposure to health imbalance among women executives[3].

There are various existing researches that show that women who are within the age group 25 to 40 years face tremendous problems while maintaining dual role and face tremendous obstacles while achieving their career goals. To take this body of research a step further, the present study focuses on women executive from a varied range of sectors like banking, IT, telecom, media, tourism, healthcare, hospitality and retail. The sample under this study in the present research comprises of women from both urban and semi urban areas of Kolkata. Two communities namely Bengali and Marwari have been taken into account for the present study to explore whether the challenges differs based on an individual's community – identity. Both married and unmarried women have been studied to explore how the institution of marriage impacts the above mentioned challenge. The women executives under study have a monthly income in the range Rs 25, 000/- to Rs 50, 000/- and they have work experience of at least two years.

Methodology

Research methodology is a systematic way to solve the research problem. It may be understood as a science of studying how research is to be done scientifically. Research methodology thus is a logic behind the methods or techniques that are to be used in a research.

The survey method has been used to carry out the study on the challenges faced by women executives within (25 to 40 years) in their domestic spheres to get the precise and the possible information that the researcher wants. The questions are clearly understood by the respondents and it is also pleasing to the respondent so that they are willing to answer a question engaging them sufficiently to the answer and are not giving any superficial or misleading answers. This helps to generate a valid measurement of the concept being studied.

An interview method was used as a mode of eliciting information. The respondent's answers were written down while the interview was carried out face to face and also on the telephone. The questionnaire that has been used or the questions that has been used while carrying out the interview was structured in such a way to get the underlying attitude and the disposition (orientation) surrounding a piece of information and measures were taken so that it does not lead to any ambiguity or misleading data[1]. The questionnaire was designed and was further approved by two eminent feminists.

The methods of sampling used in the study were snow ball sampling and purposive sampling both of which involves a deliberate selection of a particular unit of population for constituting a sample which represents the population and hence using these methods the study was conducted on 210 people from eight different sectors namely banking, IT, telecom, media, hospitality, tourism, healthcare and retail.

Results and Discussion

Table 1 – Showing the Hypothesis and Result

Null Hypotheses	Alternative Hypotheses	Result
$H0_1$ - Women executives do not have to face any significant challenge while keeping pace with the socially assigned roles.	H_a - Women executives have to face significant challenge while keeping pace with the socially assigned roles.	$H_0 1$ is rejected and alternative is accepted
$H0_2$ - Women executives do not have to face any significant challenges in maintaining proper health.	H_b - Women executives have to face significant challenges in maintaining proper health.	$H_0 2$ is rejected and alternative is accepted
$H0_3$ -- Women executives do not have to face any significant challenges while fulfilling commitments towards relationships.	H_c – Women executives have to face significant challenges while fulfilling commitments towards relationships.	$H_0 3$ is rejected and alternative is accepted
$H0_4$ – Women executives do not have to face any significant challenges while coping with the occupational demands.	H_d – Womenexecutives have to face significant challenges while coping with the occupational demands.	$H_0 4$ is rejected and alternative is accepted

It was found that women do face significant challenge in keeping pace with the socially assigned roles. This challenge is faced to a greater extent by the women from sectors like IT, telecom and media. Women from the health care sectors face emergency conditions and this poses a unique challenge while keeping pace with the socially assigned roles. Women themselves often feel guilty of not being able to fulfill their role of a mother, nurturer and a caregiver. It was found that women working in the bank face this challenge to a lesser extent.

It was also found that women executives are prone to stress, anxiety burnouts and exhaustion primarily because of the overload they face while attempting to balance their dual roles. Women executives working in IT, telecom and banking are more burdened because of intense competition in such sectors. Women working in media are more prone to stress because of their ever changing and ever demanding work schedules.

This study also proves that women executives do face a challenge while fulfilling commitment towards relationships. Both married and unmarried women face this challenge. Women executives from hospitality and media sectors feel this to be a big challenge as they are of the opinion that their partners and their family members do not understand the nature of their job. Unmarried women states that often because of the nature of their job they are unable to meet expectations of potential partners and often even refrain from getting married.

When exploring that whether women executives face challenge in meeting occupational demands, it was found out that women executives do face this challenge specially with regards to their work – schedule and working hours. Women executives from sectors like IT and telecom face family objection while meeting with occupational demands of adhering to a particular dress code.

Conclusion

The study was conducted on the "Challenges faced by women executives within the age (25 to 40 years) in their domestic sphere. Although women and men should enjoy equal status under the constitution along with the right of equal participation. But reality is that women are being handicapped by virtue of their gender role in the family and the society. Women are being disadvantaged often not because of any unfair policies and practices but because of family demands, cultural tradition, and social behavior[5].The study concludes that women executives still face significant challenge in keeping pace with the socially assigned roles, in maintaining proper health while fulfilling commitment towards relationships and in coping with occupational demands.

It was found that women do face significant challenge in keeping pace with the socially assigned roles. This challenge is faced to a greater extent by the women from sectors like IT, Telecom and Media. Women from the health care sectors face emergency conditions and this poses a unique challenge in them while keeping pace with the socially assigned roles. Women themselves often feel guilty of not being able to fulfill their role of a mother, nurturer and a caregiver. It was found that women working in the bank face this challenge to a lesser extent.

It was also found that women executives are prone to stress, anxiety burnouts and exhaustion primarily because of the overload they face while attempting to balance their dual roles. Women executives working in an IT, Telecom and Banking are more burdened because of intense competition in such sectors. Women working in media are more prone to stress because of their ever changing and ever demanding work schedules.

It was concluded that women executives do face a challenge while fulfilling commitment towards relationships. Both married and unmarried women face this challenge. Women executives from hospitality and media sectors feels this to be a as big challenge as they are of the opinion that their partners and their family members do not understand the nature of their job. Unmarried women states that often because the nature of their job they are unable to meet expectations of potential partners and often even refrain from getting married.

When exploring that whether women executives face challenge in meeting occupational demands, it was found out that women executives do face this challenge. Especially with regards to their work – schedule and working hour's women executives from sectors like IT and Telecom face family objection while meeting with occupational demands of adhering to a particular dress code.

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A Study of Parental View on the Role of Private Tuition for Their Adolescent Children

Aritri Ghosh and Krishnakali Bhattacharya

ABSTRACT:

The present study was undertaken to find out the parental view on the role of private tuition for their adolescent children residing in south Kolkata. The study was designed with 234 Bengalee parents [viz. Fathers (N = 109) and Mothers (N = 125)] of adolescent children [age group 13 to 16 years] going to schools affiliated to the West Bengal Board of Secondary Education. The obtained results reflected that irrespective of the gender of the child, there is no significant difference between fathers and mothers regarding their view on private tuition and in their level of anxiety. Both fathers and mothers preferred private tuition for their adolescents along with school education. The level of anxiety of these parents was average. However, a significant positive correlation between preference for private tuition and anxiety was found for both fathers and mothers, indicating preference for tuition related to parental anxiety.

Keywords: Private Tuition, Anxiety, Parents and Adolescents

Introduction

In the world of human affairs, there is no nuisance than a boy at the age of 14. He is neither ornamental, nor useful[10]. These great lines by Rabindranath Tagore express the awkward position of an adolescent, who is neither considered an adult nor a child. This also brings about certain dilemma in the minds of parents who gradually loosen their reins as their school aged children become adolescents but they by no means cease to set rules and monitor their children's behaviour.

The parent-adolescent relationship is truly a partnership and its quality depends on what both parents and their children renegotiate their relationship. Apparently, most parents and their teenagers maintain positive feelings for one another and also rework their relationship, so that it becomes equal[12].

In parent-child/adolescent relationship, the very parental role may give rise to some expectations which the adolescent may think unrealistic and oppressive. Adolescence being a period of change, the parents must also develop realistic expectations. Parents share the responsibility of bringing up their children in a manner so that as adults they become effective members of the respective society.

Being a parent to a child is not an easy task to handle. It brings about huge responsibility which in turn at times gives rise to tension and anxiety. Children's health, education, safety, etc bother the parents enormously. As parents play a vital role in moldering a child's behaviour and personality development, it can also lead to parental anxiety. Parental anxiety for their children extends to the kind of expectations they have for their child's school achievements and their expectations tend to persist throughout the school years.

It seems that parents choose the every time appropriate strategy of regulation, based on their own feelings, needs and preferences than the ones of their children. In other words, in the process of choosing regulating strategy, parental attitudes do interfere[9].

Parental expectations directly affect parent- child communication as well as the adolescent's own aspirations[6]. The most consistent predictor of academic achievement among adolescents is parent's expectations of an adolescent's academic attainment. When parents play an active role in the education of adolescents, adolescents become more successful and confident in their abilities to achieve and accomplish goals[8]. The relationship between parental assets with their expectations and involvement of children's education and educational performance was examined by a researcher[14]. It was found that after controlling the family income and other parental characteristics, parental assets were positively related to children's math and reading scores. Parental assets positively associated with their expectations partially mediated the relationship between assets and children's educational performance. These findings imply that parental assets enhance children's education.

Parental involvement in their children's education has been seen as a mechanism for raising standards, developing new partnership between school and parents and promoting social inclusion. Parental involvement refers to parent's behaviours at home and at school intended to assist with children's overall learning experiences [4]. Parental involvement has mostly been viewed as a positive aspect regarding child's development. The impact of student's perception of parental involvement on their levels of achievement was studied by various authors[2]. By assessing the levels of achievement of 127 senior students in a diverse suburb high school, it was found that the perception of a high level of parent involvement led them

to perform significantly better in the National Act exams than students who perceived a low level of parent involvement.

Roe, an early theorist proposed that early childhood experiences play an indirect role in shaping later career behaviour[5]. Parenting style, parental support and guidance can include specific career or educational suggestions as well as experiences that support career development[1]. The authoritative parenting balances clear, high expectation with emotional support and recognition of children's autonomy. The absence of support, guidance and encouragement can lead to "floundering", the inability to develop and pursue a specific career focus[1]. Lack of support can take the form of conflict, when a parent pressurizes a child toward a particular career.

Parental expectation regarding their children's education and parental participation in moldering their children's career leads them to enroll their children for private tuitions for the betterment of education.

Private tutoring can be seen as a mechanism through which pupils extend their learning and gain additional human capital, which benefits not only themselves but also the wider societies of which they are a part[3]. Tutors are commonly perceived as people who help pupils to carry the heavy academic load of formal classrooms. The forms of private tutoring may be varied. Some tutoring is provided on one to one basis in home of either the tutor or his/her student. Other tutoring is in small groups, in large classes or even huge lecture theatres with video screens to cater for overflow[6]. Students are more likely to demand for tutoring at the secondary level than at the primary level[6]. Private tuition can help students by giving him some extra time and necessary push for relatively not so brilliant students. Private tuition also has its disadvantages. Sometimes tutors provide additional homework on top of those given by teachers in school. The extra homework from tutor is an added burden to the students[13]. Tutoring may significantly affect the dynamics of teaching and learning in mainstream classes. For example, where all students receive tutoring provided by outside agencies, mainstream teachers may not need to work so hard[3].

Thus it is good to have private tuition provided that the parents can afford and students find the tuition session beneficial to them. The benefits are plentiful if parents manage to find a good tutor, who can help the students achieve academic success which ultimately leads to some good grades and finally a good career[13].

Methodology

In the present study, data were collected from 234 Bengalee parents (109 fathers and 125 mothers respectively) who have adolescent children studying in schools under West Bengal Board of Secondary Education. They all belonged to extended family. The fathers either having business or in service had minimum educational qualification of graduation, while mothers were all graduates and housewives. The sample of fathers having single adolescent child were independent of that of the mother. Two Questionnaires were used - a standardized questionnaire on Anxiety – C.A. Test by Dr. Harish Sharma, Dr. Rajeev Lochan Bharadwaj and Dr. Mahesh Bhargava and a Self Prepared Questionnaire on parental view on the role of private tuition.

Results and Discussion

The present study focused on two variables, viz. parental view on the role of private tuition and anxiety. The parental view on private tuition was assessed through a self prepared questionnaire while anxiety was measured with a standardized test.

Table 1: Mean, Standard Deviation (SD) And "T" Value Of Parental View Regarding Private Tuition And Their Level Of Anxiety

Variables	Group	Mean	SD	Group	"t"
Private Tuition	Father	28.80	4.11	Father and Mother	0.115
	Mother	26.68	4.16		
Anxiety	Father	36.009	9.03	Father and Mother	0.095
	Mother	36.88	9.30		

From the obtained result regarding the parental view on role of private tuition.

It was found that majority taking tuition in all subjects as stated by both fathers and mothers while all of 234 adolescents were taking tuition in Math and Physical Science followed by Bengali and Life Science.

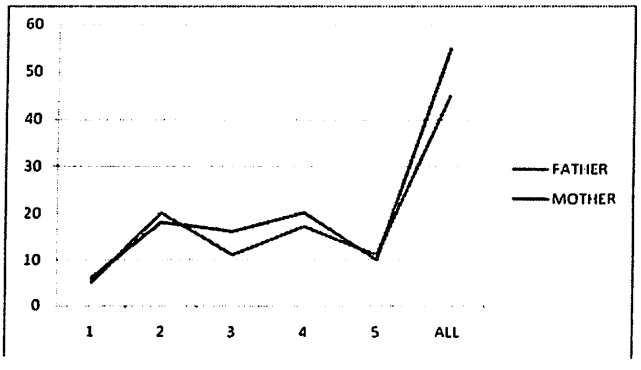

Fig 1: Showing The Number Of Subjects In Which Adolescents Take Tuition As Reported By Parents (Frequencies)

This is also line with the parental aspiration that they want their child to become doctors or

professors or engineers. They also said that their children will not go against their will rather their child will pursue the same. Even the parents think that their children are academically capable to take up these professions.

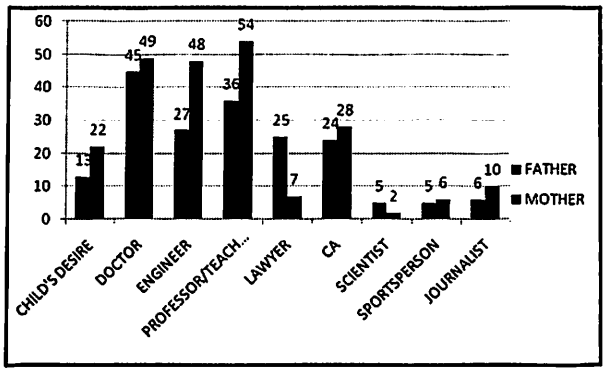

Fig 4: Showing The Career Parent Wants Their Child To Pursue (Frequencies)

Another interesting finding is that none of their parents want their child to pursue careers like fashion designing, modeling and acting. These two opposing view - what they want their child to be and what they never want their child to be is a reflection of preference towards traditional professions for their children.

Finally the results of the present study found a significant positive correlation of parental view on the role of private tuition and anxiety level of parents, indicating more preference for private tuition or more importance given to the role of private tuition is associated with more anxiety.

Table 5: Value of "r" between Private Tuition and Anxiety of Parents

GROUP	"r"
Father	0.4529
Mother	0.306

Conclusion

Overall, it was found from the results of the study that parents with average level of anxiety do not discriminate between boys and girls when it comes to taking private tuition along with school education. Parents having adolescent boy are not more anxious than those having daughter contrary to the common Indian preference for boys. Further analysis, reflected that irrespective of the gender of the child, there is no significant difference between fathers and mothers regarding their view on private tuition and their level of anxiety

Finally, to explore the relationship between private tuition and anxiety it was found that there is a positive correlation between these two.

At a glance, it may be concluded that along with preference for private tuition, all parents volunteering for this study unanimously viewed that although private tuition is supplementary not a substitute for school education, but it is a necessity. Parents all agreed that "all work and no play make Jack a dull boy" and majority accepted that private tuition after school leaves little time for their children to socialize.

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Comparative Study on Heavy Metal Contamination in Organic and Non-Organic Vegetables in Markets of Kolkata

Debalina Paul and Vipasha Chakraborty

ABSTRACT

A survey was conducted to check the availability of different organic vegetables in various stores of Kolkata. After the survey two stores were selected from where organic vegetables were collected and two markets from where the respective non-organic vegetables were collected. The vegetables were then cut into small pieces and prepared for freeze drying. After the samples were freeze dried they were crushed into powder and pellets were prepared by using a pelletizer. The elemental analysis of each sample was then done using the EDXRF (Energy Dispersive X-Ray Fluorescence) and the results were analyzed to compare the elemental concentration of organic and non-organic samples. Variable data was obtained showing the heavy metal contamination in organic and non-organic vegetables. The data obtained was compared with the standard values of Indian Council of Medical Research and Tolerable Upper Limits.

Keywords: - EDXRF (Energy Dispersive X-Ray Fluorescence), Freeze Drying, Heavy Metal, Non-organic Vegetables, Organic Vegetables, Pelletizer.

Introduction

Nutrition is a basic human need and a prerequisite to healthy life as it is the sum total of the processes involved in the taking in and the utilization of food substances by which growth, repair and maintenance of the body are accomplished. In the process of acquiring proper nutrition vegetables provide exceptional nutritional benefits. Technically; the vegetable realm consists of any edible part including the leaf, stem, tuber, root, bulb, berry, and seed of a plant. However with the introduction of green revolution technologies agriculture is getting increasingly dependent upon the steady supply of artificial fertilizers and pesticides and Because of these problems organic vegetables have progressively become an alternative by increasing number of consumers who are worried about the presence of chemicals residues and the detrimental effects on the health and environment caused by such chemical intensive production methods.

Heavy Metals, in general are not biodegradable, have long biological half-lives and have the potential for accumulation in the different body organs leading to unwanted side effects. Heavy metals are natural components of the Earth's crust and cannot be degraded nor can be destroyed[1]. Heavy metals frequently reported in literature with regards to potential hazards and occurrences in contaminated soil are cadmium (Cd), Chromium (Cr), Lead (Pb), Zinc (Zn), Iron (Fe), Copper (Cu)[5]. Soil serves as a basic source of heavy metals in case of vegetables. Sources of heavy metals in soil include fertilizers, pesticides, waste water, metal mining milling processes and industrial waste, air borne sources etc. Plants may adsorb heavy metals from the deposits on the parts of the plants exposed to the polluted air due to emission of heavy metals from the industries and vehicles and contaminated soils. Heavy metal contamination of vegetables may also occur due to irrigation with contaminated water in addition of fertilizers and metal based pesticides[7].

Organic foods are foods that are produced using methods that do not involve modern synthetic inputs such as synthetic pesticides and chemical fertilizers, do not contain genetically modified organisms, and are not processed using irradiation, industrial solvents, or chemical food additives. The market for organic food has increased considerably over the last decade due to consumer's increasing awareness of both health and environmental issues[11]. The popularity of organic foods continues to grow dramatically: organic foods now constitute more than 2% of all food sales[14]. Organic food consumption continues to increase as consumers seeks foods perceived as healthier (greater nutritional value and fewer toxic chemicals, while the amount of vitamin and minerals will obviously vary from crop to crop and from farmer to farmer[2].

Methodology

Sample collection

For the collection of Organic vegetables a survey was conducted to check the availability of organic vegetables in Kolkata. After the survey two spots Spencer's (South city) and Arambagh organic farm was finalised for the collection of organic vegetables. Three types of vegetables namely cabbage, cauliflower and brinjal was collected. The respec-

tive vegetables were collected from non-organic sources Jadavpur local market and Dhapa.

Sample Preparation

The vegetables were cut into very small pieces and put into a glass vial and a rubber cork was attached to it. The vial was then dipped in liquid nitrogen and was attached to the freeze drier. Liquid Nitrogen is used as a cryogenic liquid which can cause rapid freezing on contact with living tissue.

Freeze drying

The samples in the vial were then attached to a freeze drier for the purpose of drying. Freeze drying is based upon the dehydration of the frozen product through sublimation. Freeze Drying, or lyophilisation is a dehydration technique, which enables liquid or slurry products, which have previously been frozen to be dried under a vacuum, allowing the ice to directly from solid to vapour without passing through a liquid phase.

Pellet Preparation

The freeze dried sample was grinded to fine powder using a mortar pestle. 1mm thick and 13mm diameter pellets were prepared using a table top pelletiser. 150mg of the powdered sample was taken and a high pressure of 120kg/cm^2 was applied in the pelletiser for 2 minutes. 5 identical pellets were made from each sample.

Elemental Analysis of the Samples by EDXRF (Energy Dispersive X-Ray Fluorescence) The elemental concentration was determined by using an EDXRF spectrometer. In the present study, the Xenemetrics (previously Jordan Valley) EX3600 EDXRF Spectrometer has been used for elemental analysis. This consists of an X-Ray tube with a Rh anode as a source of X-Rays with a 50V, 1mA power supply, Si (Li) detector with a resolution of 143eV at 5.9keV. The measurements were carried out in vaccum using Ti filter in front of the source with an applied voltage of 20kV and current 400mA. A 10 sample turret enables mounting and analyzing 10 samples at a time, The inbuilt software nEXT carries out the quantitative analysis[8].

Results and Discussion

The elemental analysis of the vegetable samples carried out by the EDXRF spectrometer has shown the signature of many elements. The concentration of certain heavy metals like Manganese, Iron, Copper, and Zinc were measured in the sample.

Manganese

Manganese in small amount is essential to several critical enzymes involved in energy production bone formation and protein metabolism. The concentration of manganese in cauliflower is 0.10mg/100gms[4]. In the present study the concentration of manganese is found to be in the range of 1.3-2.3mg/100gms.The concentration is little high in the non-organic vegetables collected from dhapa. The concentration of manganese in cabbage is 0.18mg/100gms[4]. In the present study it is found that the concentration of manganese in cabbage ranges from 2.9-16mg/100gms.The concentration of manganese is very high in the non-organic sample collected from Jadavpur local market. The concentration of manganese in brinjal is 0.13mg/100gms[4]. In the samples collected the concentration of manganese ranges from 1.1-2.3mg/100gms. The concentration are almost in the same range in case of both organic and non-organic samples.

The Recommended Dietary Allowances for manganese is 1.8-2.3mg/day. The upper limit for manganese is 11mg/day. In the present study the concentration found in the non-organic cabbage exceeds the Tolerable Upper Limits (TUL)[3]. Hashmi et al in their study found a similar result and they attributed this to the fact that in leafy vegetables, leaves are most exposed part of plant to the environmental pollution because of their large surface areas[6]. In the case of the organic cabbage the concentration is much lesser than the tolerable limit. The concentration of manganese in Organic and non-organic cauliflower samples are within the tolerable limits. The concentration is high in the organic cauliflower samples which can be because of higher uptake of cauliflower as compared to cabbage and brinjal samples. In case of Brinjal also the concentration is below the tolerable limits. The concentration is high in the non-organic samples. The high concentrations in the non-organic sources may be contributed by excessive use of chemical fertilizers. A study conducted on Soil and applied manganese by Schulte et al (2004) states that manganese sulphate and chelated manganese are the most common manganese source in the soil[12].

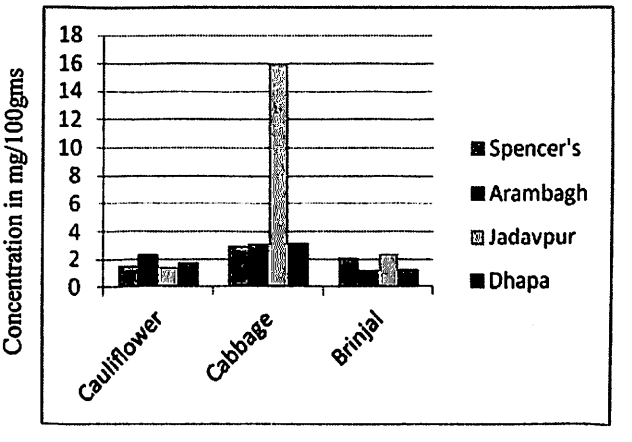

Fig 1 Analysis of concentration of Manganese in the collected samples (Concentration in mg/100gms.)

Iron

The concentration of iron in Cauliflower is 1.23mg /100gms[4]. In the present study the concentration of iron in cauliflower ranges from 5.4-9.2mg/ 100gms.The concentration is comparatively high in the organic sample collected from arambagh. The concentration of iron in cabbage is 0.8/100gms[4]. In this study the concentration ranges from 5.4-15.5mg/100gms.The concentration is high in both the non-organic samples collected from Jadavpur local market and dhapa.The concentration of iron in brinjal is 0.38mg/100gms[4]. In the collected samples the concentration ranges from 5.9-11.17mg/ 100gms.The concentration is comparatively high in the organic samples collected from Spencer's as well as non-organic samples collected from dhapa.

The Recommended Dietary Allowance for iron is 15-10mg/day. The Tolerable Upper Limit (TUL) of iron is 45mg/day[3]. The concentration found in the present study does not exceed the TUL but the concentration of iron is surprisingly high in all the three vegetables irrespective of organic and non-organic aspect. The high concentration of iron as compared to the standard values in all the samples may be attributed to high iron content in the water used for irrigating the agricultural soil. The high concentration of iron in the samples can also be due to the high load of iron in the atmosphere of Kolkata. This was supported by a study conducted by Majumdar et al (2009). From the findings it can be said that the concentration of iron in organic cauliflower samples is higher as compared to the non-organic samples. The same findings can be seen in the case of brinjal samples also. The higher uptake of cauliflower and brinjal from the soil and the water used for irrigation can be a contributing factor for the above. The concentration of iron is found higher in the non-organic cabbage samples as compared to the organic ones. The concentration of iron in the soil of the site where the vegetable was cultivated and the iron content of the water used for irrigation may be a contributing factor.

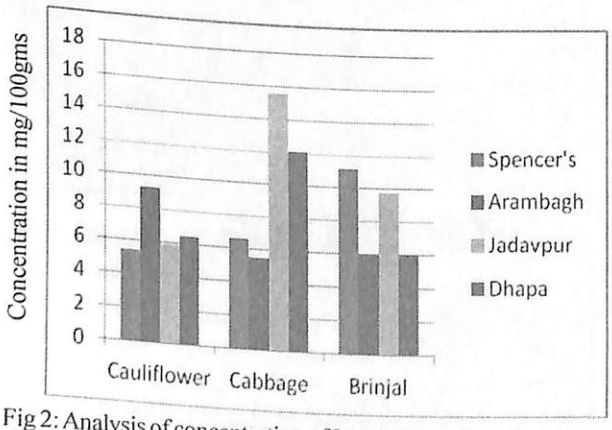

Fig 2: Analysis of concentration of Iron in the collected samples.

Copper

Copper is a trace element, important for the function of many cellular enzymes. The concentration of copper in cauliflower is 0.13mg/100gms[4].In the present study the concentration of copper in cauliflower ranges between 0.17-0.65mg/100gms.The concentrations is higher in the non-organic sample collected from dhapa as compared to other samples. The concentration of copper in cabbage is 0.02mg/ 100gms[4]. In the present study the concentration in cabbage is found in the range of 0.2-0.6mg/ 100gms.In case of cabbage also the concentration is higher in the non-organic samples collected from dhapa as compared to other samples. The concentration of copper in brinjal is 0.12mg/100gms[4]. In the collected samples copper was found to be in the range of 1.08-2.15mg/100gms.In this case also the concentration is more in the non-organic samples as compared to the organic samples. The concentration of copper in the brinjal samples was more as compared to cauliflower and cabbage.

The Recommended Dietary Allowance for copper is 0.9mg/day. The Tolerable Upper Limit (TUL) for copper is 10mg/day[3]. The values obtained from the study do not exceed the TUL in case of all the three types of vegetable samples. It can be concluded from the collected data that the concentration of copper is high in all the non-organic samples as compared to the organic samples. The high concentration of copper in the non-organic samples can be because of the chemical copper fertilizers used. A study on Soil and Applied copper states copper chelate, copper sulphate, cupric oxide and cuprous oxide as main sources of copper in the soil[13]. A study conducted on 'Heavy metals in selected edible vegetables and estimation of their daily intake' found high concentration of copper because of application of micronutrient fertilizers and Cu based fungicides. From the obtained values it can be concluded that the retention of copper in brinjal samples is more as compared to cauliflower and cabbage samples. This finding is similar to a previous study done by Hashmi et al (2007)[6].

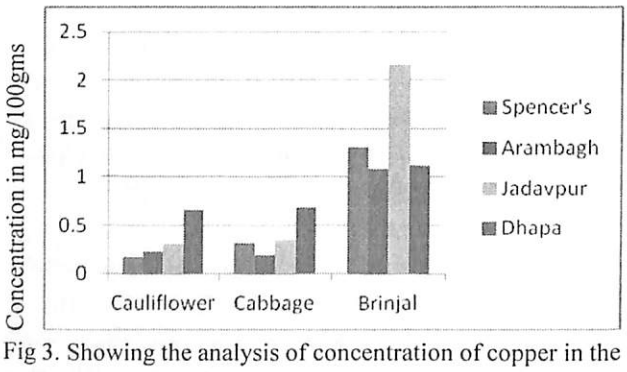

Fig 3. Showing the analysis of concentration of copper in the collected samples (Concentration in mg/100gms).

Zinc

Zinc is an essential trace element for all forms of life. Zinc, an essential trace mineral, is required for the metabolic activity of 300 of the body's enzymes, and is considered essential for cell division and the synthesis of DNA and protein. These enzymes are involved with the metabolism of protein, carbohydrate, fat and alcohol. The concentration of zinc in cauliflower is 0.40mg/100gms[4].In the present study it was found that the concentration of zinc in cauliflower ranges from 2.48-3.44mg/100gms.The concentration is near about same in both the organic as well as non-organic samples. The concentration of zinc in cabbage is0.30mg/100gms(4). In the collected samples the concentration ranges from 1.63-10.30mg/100gms.The concentration is significantly high in the non-organic sample collected from jadavpur local market. The concentration of zinc in brinjal is 0.22mg/100gms(4). Concentration of zinc in the collected samples ranges from 2.45-4.93mg/100gms.The concentration is high in the non-organic samples collected from jadavpur local market and surprisingly high in the organic samples collected from Spencer's.

The Recommended Dietary Allowances for Zinc is 12-15mg/day. The Tolerable Upper Limits (TUL) for zinc is 40mg/day(3). The values obtained are below the TUL for all the three types of vegetables. The concentration of zinc is surprisingly high in the organic cauliflower samples as compared to the non-organic samples. This can be attributed to more inputs of zinc fertilizers in the organic farming procedures. But to very contrary to this the concentration of zinc is found to be high in the non-organic cabbage samples as compared to the organic ones. The high concentration of zinc in non-organic samples is attributed to more use of zinc fertilizers in the soil. A review on zinc fertilization by Lindenmayer (2007) states that zinc sulphate has been the major source of zinc in the soil(9). The zinc concentration is particularly high in the cabbage samples as the uptake of zinc by leafy vegetables is higher. This finding is supported by an earlier study done by Mapanda et al (2005) where leafy vegetables were irrigated with waste water(10). The concentration of zinc is high in the non-organic brinjal samples and very interestingly in the organic samples collected from Spencer's The possible contributing factor for this can be excessive use of zinc containing fertilizers in case of non-organic farming as well as in case of organic farming also.

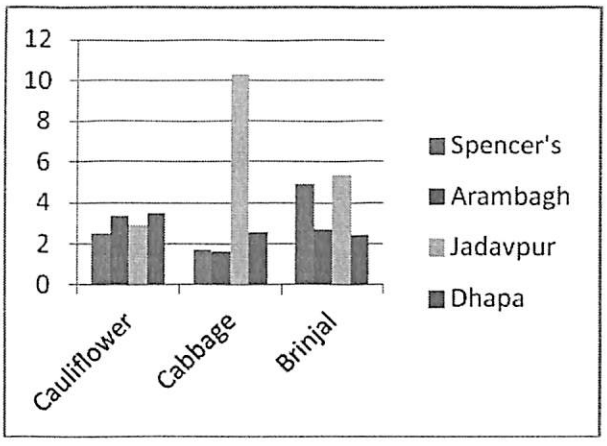

Fig 4: Analysis of concentration of Zinc in the collected samples (Concentration in mg/100gms).

Conclusion

The contamination of heavy metals to the environment i.e. soil, water, plant and air is of great concern due to its potential impact on human and animal health. In present study the concentration of heavy metals and other elements in all the samples organic as well as non-organic exceeds the standard values given by ICMR. The contributing factor behind this could be that vegetables are often grown in polluted and degraded environmental conditions in agricultural zones and are subject to further pollution from vehicles and industries during packaging, supplying and marketing. However, it may be mentioned that the values were within the Tolerable Upper Limit (TUL) especially for heavy metals like Manganese, Iron, Copper and Zinc. The concentration of Manganese, Copper and Zinc is high in certain non-organic samples as compared to the organic samples. But the concentration of iron was very high in all the samples and especially in some organic samples.

With the present scenario of very limited published data available on heavy metal concentrations in the vegetables from the market sites in India, the present work stresses upon regular monitoring of heavy metal concentration in different vegetables from the market especially the organic products so as to diminish the possible health risks that can be posed on the human subjects.

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Quality Analysis of Milk Based Indian Sweets from Renowned Retail Outlets of Kolkata

Mariyah Irfan and Alifiya Nomanbhoy

ABSTRACT

A total of 30 samples of milk based Indian sweets including 15 samples of Sandesh and 15 samples of Pedha were collected from 5 renowned retail outlets of Kolkata. The milk based sweets were examined for microbial quality that includes enumeration of Total Plate Count, Escherichia coli, Klebsiella, Mucor, Aspergillus sp. and Penicillium sp. *The results revealed that all samples had high Total Plate Counts and* E.coli *counts. All samples also showed the presence of* Klebsiella, Mucor *and* Aspergillus sp. *Chemical analysis of samples revealed that all samples were high in nutritive value due to their content of fat, protein, sugar, calcium and phosphorus although they were poor sources of iron. Tests for permissible food colour in Pedha samples showed that colour was present in all samples above permissible limits. Sensory evaluation of the samples revealed that all sweets were highly acceptable. The present study concluded that milk based Indian sweets from renowned retail outlets of Kolkata are acceptable in terms of nutritive and sensory qualities but they were inferior in microbiological quality.*

Keywords: Microbial Quality, Chemical Quality, Sensory Eevaluation

Introduction

Access to good quality, safe and nutritious food is considered a basic right of the people. Consumption of unsafe, contaminated food leads to food-borne diseases. The foods most commonly involved in food-borne disease are meat and meat products, poultry, eggs, milk and milk products and sweetmeats[16]. Khoa and Channa serves as the base for many popular traditional products such as Pedha and Sandesh. Sandesh is one of the most popular channa based sweet of the Eastern part of India[1]. Contamination of these products is largely due to human factor and unhygienic conditions. The manufacture of these products is based on traditional method without any regard to the quality of raw material used and the hygienic quality of the products. Under such conditions many microorganisms can find access to the milk products. Among all micro-organisms *Escherichia coli* is frequently contaminating organism, and is a reliable indicator of fecal pollution[14].

Milk and dairy products are also an excellent source of calcium and phosphorus. These minerals in optimum ratio are present in milk and are required for optimum growth and maintenance of bones[9]. But adulteration of milk and milk products, and the use of colours to mask product quality are quite common. Several cases of adulteration of food with colours have been recorded[4]. The trend of consumption of food products coloured with synthetic dyes has been increasing over the years[2].

Keeping in view the public health importance of the consumer and the significant contribution of milk-based sweets to the supply of nutrients to human beings, hygienic measures and examination of microbial load in milk-based Indian sweets is of utmost importance so as to increase the quality of the product[3,11].

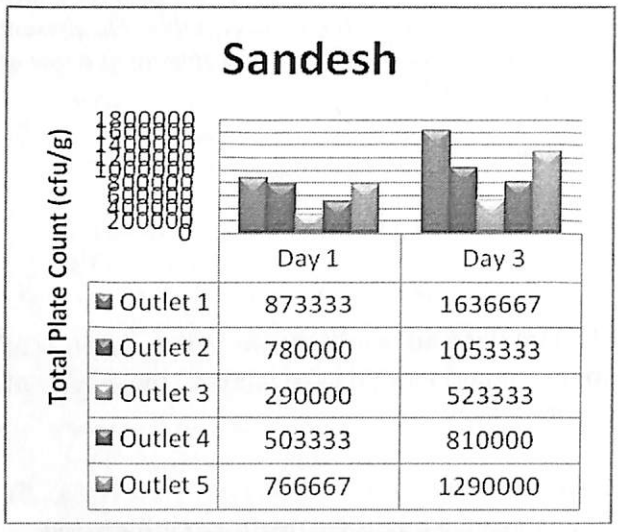
Methodology

To analyze the microbial quality of milk-based Indian sweets, namely Sandesh and Pedha:
15 samples of Sandesh and 15 samples of Pedha from 5 different renowned retail outlets of Kolkata were collected. Each Sandesh and Pedha sample from the 5 outlets were collected on separate days. All Sandesh and Pedha samples were analyzed on the day of collection, that is, Day1 as well as after two days of storage at ambient conditions, that is, Day3 for their microbial content including total plate count, E.coli count and fungal count.
Chemical analysis of milk based Indian sweets:
15 samples of Sandesh and 15 samples of Pedha from 5 different renowned retail outlets of Kolkata were collected. All samples were chemically analyzed for their moisture, iron, calcium, phosphorus, fat, protein, and sugar content. Presence of adulterants was detected using rapid methods. Samples were also analyzed for the content of permissible food colour.
To find out the acceptability of Milk-based Indian sweets from Renowned retail outlets of Kolkata by sensory evaluation:
The Milk Based Indian sweets namely Sandesh and Pedha were evaluated for acceptability using nine-point Hedonic scale. Attributes to be scored were colour, appearance, texture, taste, odour and overall acceptability. The evaluation was done by a panel of 20 members.

Results and Discussion

Total Plate Count:

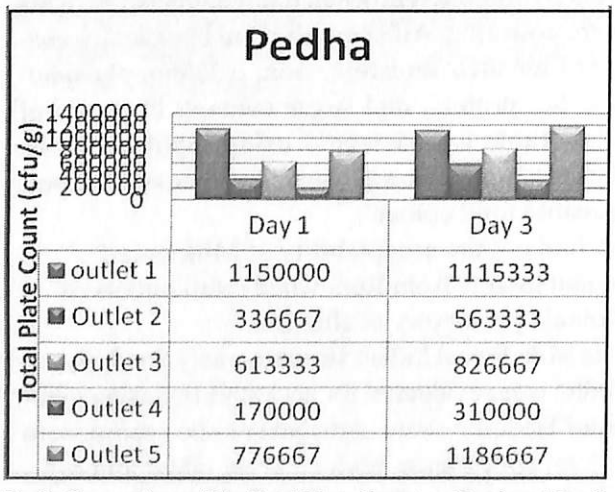
Total Plate Count was determined for all samples of Sandesh and Pedha from 5 renowned retail outlets of Kolkata. The test for Total Plate Count for each sample was conducted on Day1 as well as on Day3. A comparison of the Total Plate Count on Day1 and Day3 for Sandesh and Pedha samples from 5 renowned retail outlets are shown in Figure 1 and Figure 2 respectively.



	Day 1	Day 3
Outlet 1	873333	1636667
Outlet 2	780000	1053333
Outlet 3	290000	523333
Outlet 4	503333	810000
Outlet 5	766667	1290000

Fig 1: Comparison of the Total Plate Count on Day 1 and Day 3 for Sandesh samples.

From the above figure it is indicated that Total Plate Count has exceeded 3, 00,000 cfu/g in case of sandesh samples from 4 out of 5 retail outlets on Day1 itself, that is, on the day of collection of the sample. On storage under ambient conditions, the Total Plate Count increased in case of all samples. According to the Food Safety and Standards Authority of India, the Total Plate Count of Channa, the base product for Sandesh preparation, should not exceed 3, 00,000 cfu/g[5].



	Day 1	Day 3
outlet 1	1150000	1115333
Outlet 2	336667	563333
Outlet 3	613333	826667
Outlet 4	170000	310000
Outlet 5	776667	1186667

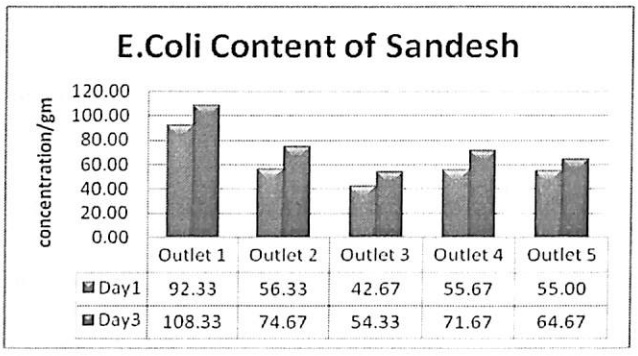
Fig 2: Comparison of the Total Plate Count on Day1 and Day3 for Pedha Samples

From the above figure it is indicated that Total Plate Count has exceeded 5, 00,000 cfu/g in case of pedha samples from 3 out of 5 retail outlets on Day1 itself,. On storage, the Total Plate Count increased in case of all samples. According to the Food Safety and Standards Authority of India, the Total Plate Count of Khoa, the base product for Pedha preparation, should not exceed 5,00,000 cfu/g[5].

The high total plate count maybe due to the unhygienic conditions of preparation of these sweets using traditional methods without any regard to the quality of raw materials and water used for washing of utensils[11].

Estimation of *Escherichia coli*

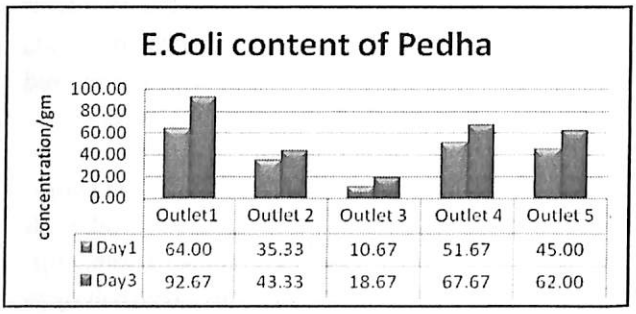
Samples of Sandesh and Pedha from 5 renowned retail outlets were tested for the presence of *Escherichia coli* on Day1 and Day3. The results are shown in Figure 3 and Figure 4 respectively.



	Outlet 1	Outlet 2	Outlet 3	Outlet 4	Outlet 5
Day1	92.33	56.33	42.67	55.67	55.00
Day3	108.33	74.67	54.33	71.67	64.67

Fig 3: Comparison of the *E.coli* content on Day1 and Day3 for Sandesh samples.

The above figure indicates that *E.coli* count was the highest in the samples of Sandesh collected from outlet 1. It can be seen that *E.coli* counts exceeds 10/g in case of samples from all outlets and that the microbial growth increases on storage. According to the Food Safety and Standards Authority of India, the *E.coli* content of Channa, the base product for Sandesh preparation, should be less than 10/g[5].



	Outlet1	Outlet 2	Outlet 3	Outlet 4	Outlet 5
Day1	64.00	35.33	10.67	51.67	45.00
Day3	92.67	43.33	18.67	67.67	62.00

Fig 4: Comparison of the *E.coli* content on Day1 and Day3 for Pedha samples.

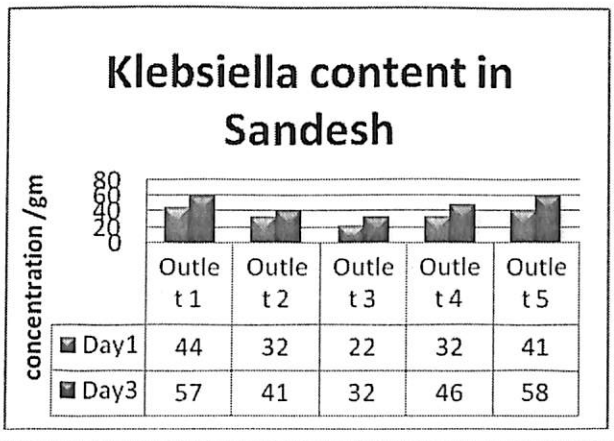
The above figure indicates that *E.coli* count was the highest in the samples of Pedha collected from

outlet 1 and lowest from those samples collected from outlet 3. It can be seen that *E.coli* counts exceeds 10/g in case of samples from all outlets and that the microbial growth increases on storage. According to the Food Safety and Standards Authority of India, the *E.coli* content of Khoa, the base product for Pedha preparation, should be less than 10/g[5].

Escherichia coli is a frequently contaminating organism in milk products[3]. It is a reliable indicator of fecal pollution generally in unsanitary conditions. Recovery of *E.coli* from food is an indicator of the possible presence of enteropathogenic microorganisms which could constitute a public health hazard[14]. Consumption of contaminated sweet milk products with *E.coli* may cause food poisoning.

Estimation of *Klebsiella* Content

Samples of Sandesh and Pedha from 5 renowned retail outlets were tested for the presence of *Klebsiella* on Day1 and Day3. The results are shown in Figure 5 and Figure 6 respectively.



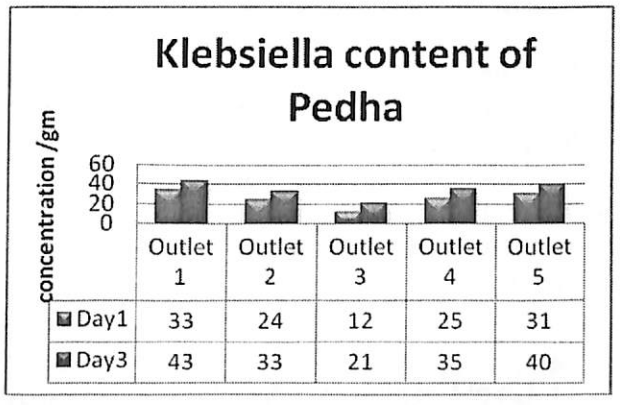
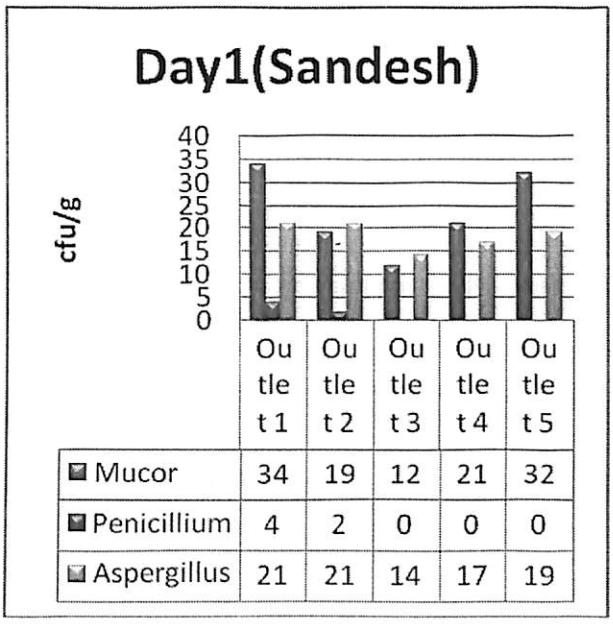


Fig 5 and 6: Comparison of the *Klebsiella* content on Day 1 and Day 3

Presence of *Klebsiella* was detected in all samples of both Sandesh and Pedha and the microbial content increased on storage for all samples from all the outlets.

Estimation of *Fungi* Content

Samples of Sandesh and Pedha from 5 renowned retail outlets were tested for the presence of *Fungi* on Day1 and Day3. The results are shown in Figure 7 and Figure 8 respectively.



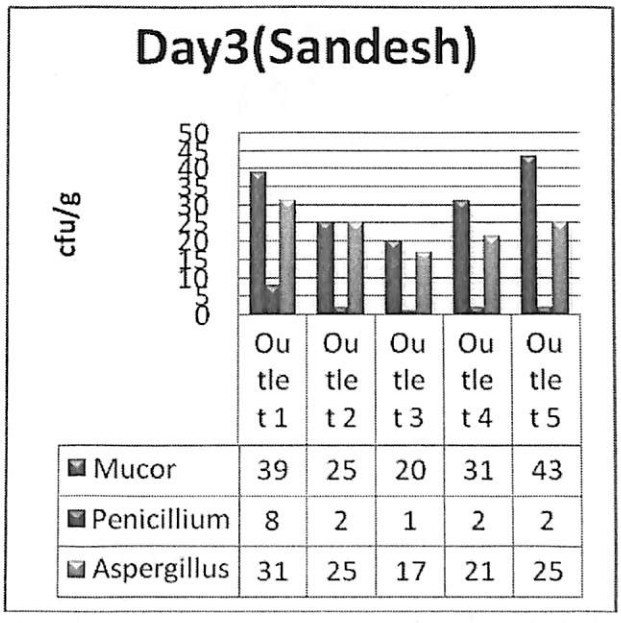
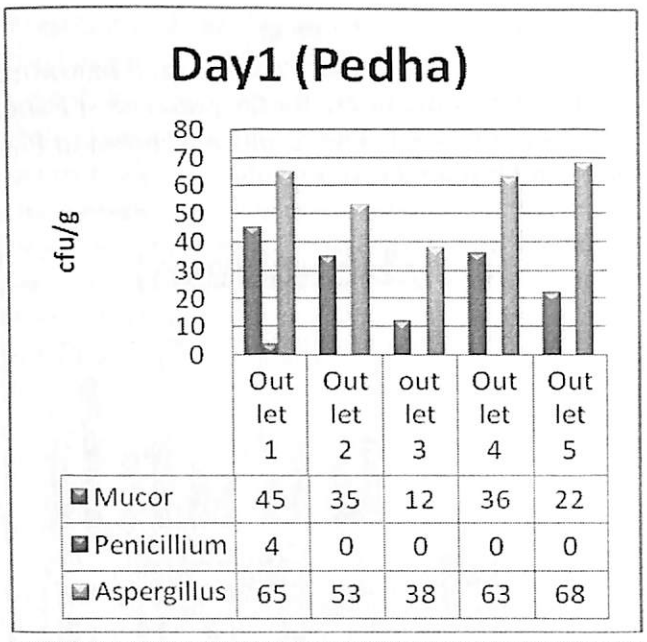
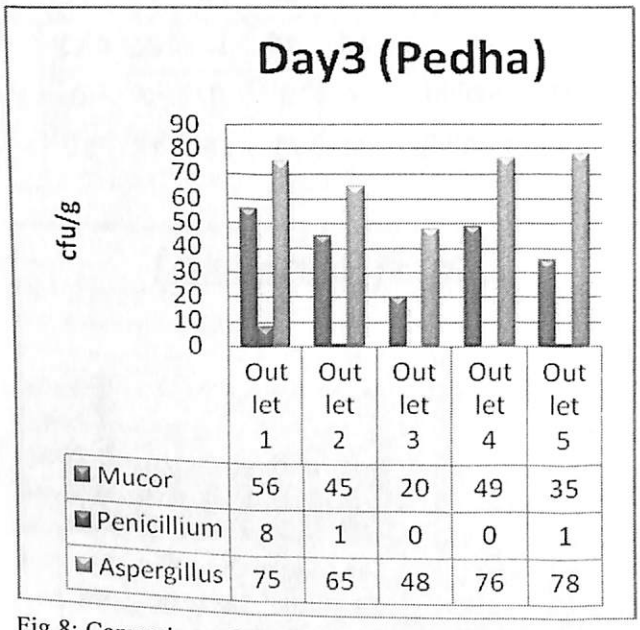


Fig 7: Comparison of the *Fungi* content on Day 1 and Day 3 for Sandesh samples.

Presence of *Mucor* and *Aspergillus sp.* was detected in Sandesh samples from all 5 outlets. Whereas *Penicillium sp.* was detected in Sandesh samples from 2 outlets only. The microbial counts increased after two days of storage.



	Outlet 1	Outlet 2	out let 3	Outlet 4	Outlet 5
Mucor	45	35	12	36	22
Penicillium	4	0	0	0	0
Aspergillus	65	53	38	63	68



	Outlet 1	Outlet 2	Outlet 3	Outlet 4	Outlet 5
Mucor	56	45	20	49	35
Penicillium	8	1	0	0	1
Aspergillus	75	65	48	76	78

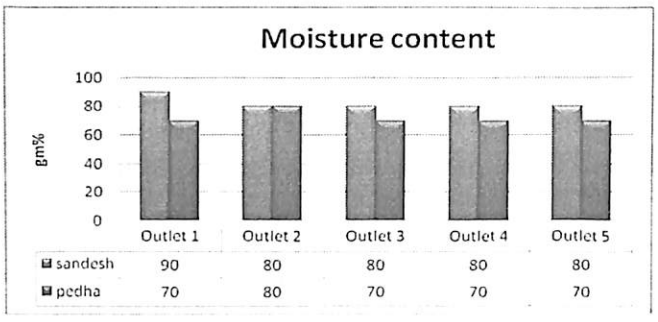
Fig 8: Comparison of the *Fungi* content on Day1 and Day3 for Pedha Samples

Presence of *Mucor* and *Aspergillus sp.* was detected in Pedha samples from all 5 outlets. Whereas *Penicillium sp.* was detected in Pedha samples from 1 outlet only. The microbial counts increased after two days of storage with *Penicillium sp.* appearing in one additional outlet.

The mould growth is favored by the presence of high moisture content in products like Pedha and Sandesh, high humidity and sufficient aeration in storage rooms[8].

Estimation of Moisture Content

15 Samples of Sandesh and 15 samples of Pedha from 5 renowned retail outlets were estimated for their moisture content. The results are as shown in figure 9.



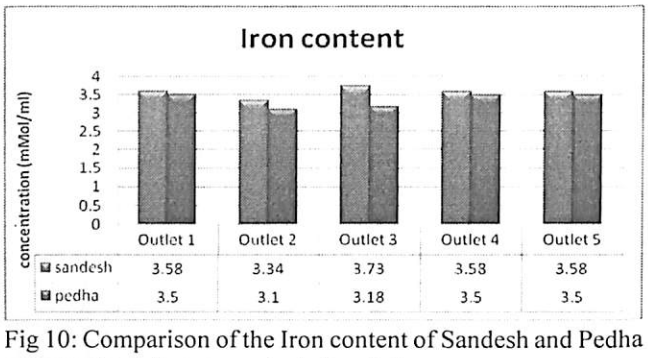
	Outlet 1	Outlet 2	Outlet 3	Outlet 4	Outlet 5
sandesh	90	80	80	80	80
pedha	70	80	70	70	70

Fig 9: Comparison of the Moisture content of Sandesh and Pedha samples from 5 renowned retail outlets.

Moisture content of the Sandesh samples from the 5 outlets ranges from 80-90% and that of Pedha samples ranges from 70-80%. Moisture % in the product affects the shelf life of the product[15]. Due to the high moisture content, milk based sweets serve as an excellent medium for the growth of many kinds of microorganisms which can contaminate the product[13].

Estimation of Iron Content

15 Samples of Sandesh and 15 samples of Pedha from 5 renowned retail outlets were estimated for their iron content. The results are shown in figure 10.



	Outlet 1	Outlet 2	Outlet 3	Outlet 4	Outlet 5
sandesh	3.58	3.34	3.73	3.58	3.58
pedha	3.5	3.1	3.18	3.5	3.5

Fig 10: Comparison of the Iron content of Sandesh and Pedha samples from 5 renowned retail outlets.

Figure 10 indicates that the iron content of Sandesh is slightly more than that of Pedha. Milk and milk products are a poor source of iron[10]. Khoa and Channa, which are used as the base products for the preparation of Pedha and Sandesh, also contain negligible quantities of iron[6]. Thus, the iron content of the Sandesh and Pedha samples are also very low.

Estimation of Calcium and Phosphorus Content

15 samples of Sandesh and 15 samples of Pedha from 5 renowned retail outlets were estimated for their calcium and phosphorus content. The results are shown in figure 11 and 12.

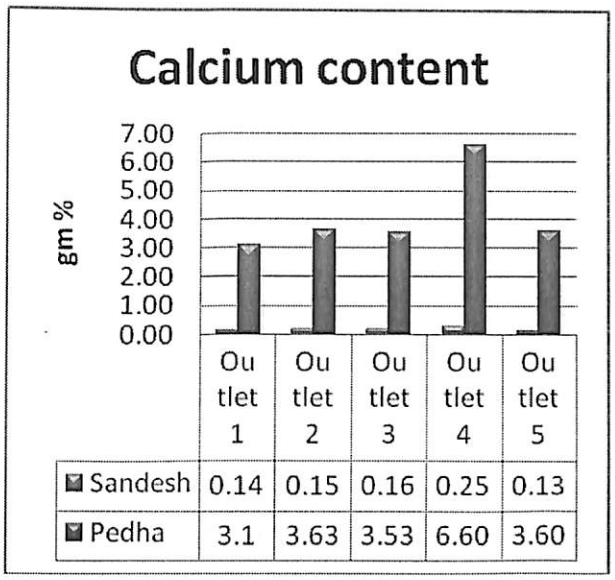


Fig 11: Comparison of the Calcium content of Sandesh and Pedha samples from 5 renowned retail outlets.

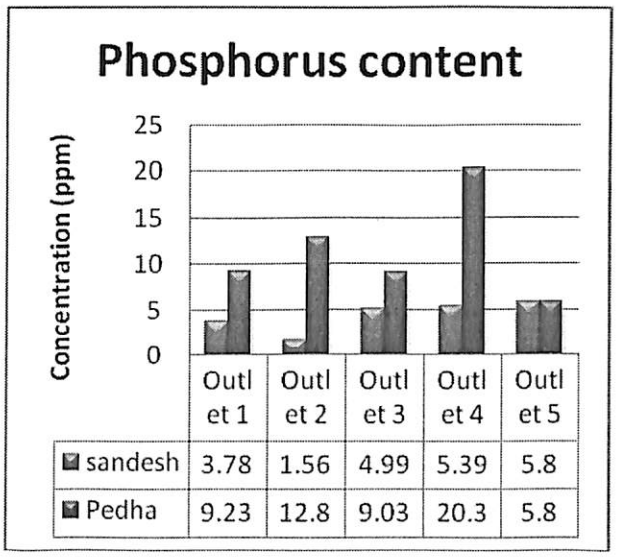


Fig 12: Comparison of the Phosphorus content of Sandesh and Pedha samples from 5 renowned retail outlets.

From the figures 11 and 12 it is evident that Calcium and Phosphorus content of Pedha is higher than that of Sandesh due to the higher quantity of calcium as well as phosphorus present in Khoa compared to Channa.[6]Calcium and Phosphorus content of sweets from outlet 4 are highest compared to all other outlets.

Estimation of Fat Content

15 samples of Sandesh and 15 samples of Pedha from 5 renowned retail outlets were estimated for their fat content.

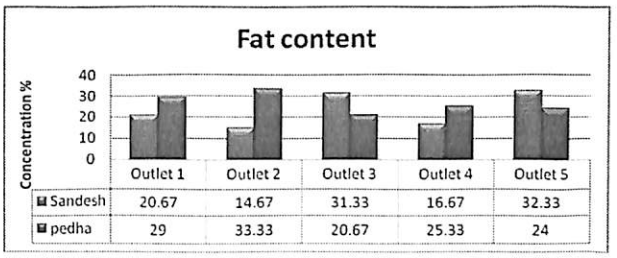


Fig 13: Comparison of the Fat content of Sandesh and Pedha samples from 5 renowned retail outlets.

From figure 13 it can be seen that the fat content of Pedha is greater than that of Sandesh in only 3 out of 5 outlets. The fat content of Khoa is greater than that of Channa[6]. But, samples of Pedha from outlets 2 and 4 contain a less proportion of fat than the samples of sandesh which maybe due to the quality of raw material used being lower in fat content.

Estimation of Protein Content

15 samples of Sandesh and 15 samples of Pedha from 5 renowned retail outlets were estimated for their protein content.

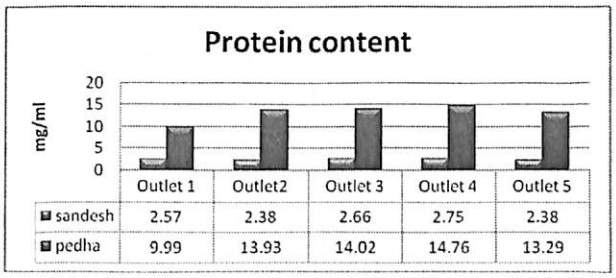


Fig 14: Comparison of the Protein content of Sandesh and Pedha samples from 5 renowned retail outlets.

From figure 14 it can be seen that the protein content of Pedha is greater than that of Sandesh in all 5 outlets. The protein content of Khoa is greater than that of Channa[6]. Milk and milk products are very rich sources of protein required for growth and development[15].

Estimation of Sugar Content

15 samples of Sandesh and 15 samples of Pedha from 5 renowned retail outlets were estimated for their sugar content.

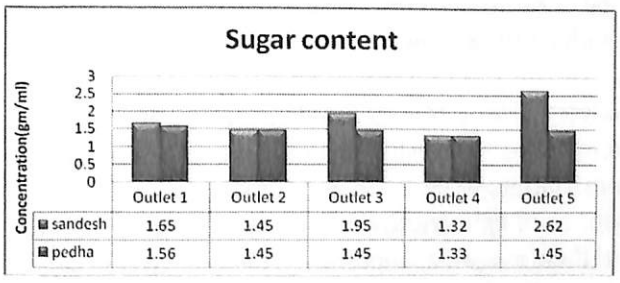


Fig 16: Comparison of the Sugar content of Sandesh and Pedha samples from 5 renowned retail outlets.

Figure 16 indicates that sugar content of Sandesh is greater than that of Pedha. Indian sweets are rich in sugar[7]. Pedha is prepared by mixing khoa with sugar and Sandesh is prepared by mixing Channa with sugar. Sugar along with providing sweetness and increasing the calorific value of the sweets also exerts a preservative effect[1].

Estimation of Food Colour

15 Samples of Pedha from 5 renowned retail outlets were estimated for their permissible colour content.

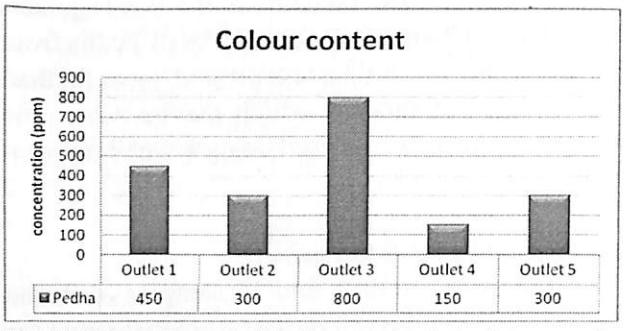


Fig 17: Colour Content of Pedha samples from 5 renowned retail outlets.

From figure 17 it is evident that the pedha samples from outlet 3 has the maximum concentration of permissible colour present in it. According to the Food Safety and Standards Authority of India, the maximum permissible level of permissible food colour that can be added to food items 100ppm[5]. From the figure it is seen that all the renowned retail outlets use food colours above the permissible limit. Due to the widespread prevalence of fraudulent practices, permitted colours are also being misused and are being added above permissible limits. Such malpractices constitute a serious public health hazard[2].

Detection of Adulterants by Rapid Method

Among the adulterants, the presence of vanaspati was detected in the samples of Pedha from outlet 1 only. No other samples of Sandesh or Pedha tested positive for adulterants.

Vanaspati being much cheaper than ghee is often used to adulterate ghee. Vanaspati contains trans fats which increases the risk of cardiovascular diseases.

Sensory Evaluation of Sandesh and Pedha

Samples of Sandesh and Pedha from 5 renowned retail outlets were evaluated for their acceptability in terms of appearance, colour, taste, texture, odour and their overall acceptability. On the basis of sensory evaluation of the sandesh and pedha samples from 5 renowned retail outlets of Kolkata, all the samples of milk based sweets scored above 6 according to the Nine-point Hedonic scale[12] in terms of all the attributes, that is, appearance, colour, taste, texture, odour as well as overall acceptability. Hence, the milk based sweets from the renowned retail outlets are high in terms of public acceptability and sensory quality.

Conclusion

Milk based Indian sweets have played a significant role in the socio-economic as well as nutritional well being of people since time immemorial[11]. The results of the above study show that the renowned retail outlets of Kolkata produce milk based Indian sweets like Sandesh and Pedha which are nutritious due to their fat, protein, calcium and phosphorus content; as well as acceptable in terms of sensory quality but the hygienic quality of these sweets is poor.

It is therefore essential for the public health authorities to take necessary steps in strictly enforcing the hygienic concept, which is lacking, so as to avoid contamination at various stages of processing, storage and handling[16].

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A Study on Enrichment of Tea Using Baker's Yeast as a Source of Vitamin D

Megha Jalan and Annalakshmi Chatterjee

ABSTRACT

A tea beverage was prepared using baker's yeast as a source of vitamin D to reduce the incidence this micronutrient deficiency. The results obtained show that enrichment of this vitamin could be carried out by fermentation. The results obtained showed that yeast when exposed to UV and sunlight could be a potential source of vitamin D. Also sunlight can be used as a source in domestic household. Yeast after exposure to UV showed maximum vitamin D content followed by exposure to sunlight. A reduction in total residual sugar and increase in yield of ethanol was observed. Black and Earl Grey tea was well accepted as a fermented beverage in comparison with other varieties. Total phenolic content greatly varied with normal tea followed by the fermented beverages. The phenolic content decreased with time of fermentation. This makes tea less bitter and more consumable. The antioxidant and antimicrobial activity of the beverages were found to be promising in comparison with standard antioxidant and antibiotic respectively.

Keywords: MNM : Micronutrient Malnutrition; PEM : Protein-energy Malnutrition; DNSA : Dinitrosalicylic Acid; TAE : Tannic Acid Equivalent; IZD : Inhibition Zone Diameter.

Introduction

Micronutrient malnutrition (MNM) is widespread in the industrialized nations, but even more so in the developing regions of the world. Micronutrient malnutrition has many adverse effects on human health, not all of which are clinically evident. Even moderate levels of deficiency can have serious detrimental effects on human function. Thus, in addition to the obvious and direct health effects, the existence of MNM has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and the loss of human capital formation.

From a public health viewpoint, MNM is a concern not just because such large numbers of people are affected, but also because MNM, being a risk factor for many diseases, can contribute to high rates of morbidity and even mortality. It has been estimated that micronutrient deficiencies account for about 7.3% of the global burden of disease, with iron and vitamin A deficiency ranking among the 15 leading causes of the global disease burden[44].

In the poorer regions of the world, MNM is certain to exist wherever there is undernutrition due to food shortages and is likely to be common where diets lack diversity. Generally whereas wealthier population groups are able to augment dietary staples with micronutrient-rich foods (such as meat, fish, poultry, eggs, milk and dairy products) and have greater access to a variety of fruits and vegetables, poorer people tend to consume only small amounts of such foods, relying instead on more monotonous diets based on cereals, roots and tubers. The micronutrient content of cereals (especially after milling), roots and tubers is low, so these foods typically provide only a small proportion of the daily requirements for most vitamins and minerals[40]. Fat intake among such groups is also often very low and given the role of fat in facilitating the absorption of a range of micronutrients across the gut wall, the low level of dietary fat puts such populations at further risk of MNM. Consequently, populations that consume few animal source foods may suffer from a high prevalence of several micronutrient deficiencies simultaneously. Micronutrient vitamin D falls under such group micronutrient deficiencies.

Vitamin D is one of the most important regulators of calcium and phosphorus homeostasis. It also plays many roles in cell differentiation and in the secretion and metabolism of hormones, including parathyroid hormone and insulin. Vitamin D (calciferol) is synthesized in the skin of most animals, including humans, from its precursor, 7-dehydrocholesterol, by the action of sunlight. This produces a naturally-occurring form of the vitamin known as vitamin D3. Vitamin D can also be obtained from the diet, either as vitamin D3 or as a closely related molecule of plant origin known as vitamin D2 (ergosterol). Since both forms are metabolized by humans in much the same way, from a nutritional perspective, vitamin D3 and vitamin D2 can be considered to be equivalent. Vitamin D3 is metabolized first in the liver to 25-hydroxyvitamin D (25-OH-D3), and then in the kidney to 1,25-dihydroxyvitamin D (1,25-(OH)2-D3), which is the biologically active form of the vitamin[3].

Severe vitamin D deficiency produces the bone disease called rickets in infants and children, and osteomalacia in adults, conditions which are characterized by the failure of the organic matrix of bone to calcify[4]. The global prevalence of vitamin D deficiency is uncertain, but it is likely to be fairly common worldwide, and especially among infants and young children, the elderly and those living at high latitudes where daylight hours are limited. Vitamin D synthesis in the skin will also be inadequate if the body is consistently covered by clothing, a probable factor in the high prevalence of deficiency among veiled women (e.g. Kuwaiti women) and their breast-fed infants and children[11]. Several studies have shown that the effects of poor vitamin D status are exacerbated by low calcium intakes. This has been demonstrated in adults from India[20] and in children from Nigeria[43].

The control of vitamin and mineral deficiencies is an essential part of the overall effort to fight hunger and malnutrition. The aim is for all people to be able to obtain from their diet all the energy, macro- and micronutrients they need to enjoy a healthy and productive life. Policy and programme responses include food-based strategies such as dietary diversification and food fortification, as well as nutrition education, public health and food safety measures, and finally supplementation[8,9]. Of the three options that are aimed at increasing the intake of micronutrients, programmes that deliver micronutrient supplements often provide the fastest improvement in the micronutrient status of individuals or targeted population groups. Food fortification tends to have a less immediate but nevertheless a much wider and more sustained impact[10].

Food fortification refers to the addition of micronutrients to processed foods. In many situations, this strategy can lead to relatively rapid improvements in the micronutrient status of a population, and at a very reasonable cost, especially if advantage can be taken of existing technology and local distribution networks. Since the benefits are potentially large, food fortification can be a very cost-effective public health intervention[6].

Being naturally present in relatively few foods, dietary sources of vitamin D usually supply only a small fraction of the daily requirements for the vitamin. Salt-water fish such as herring, salmon, sardines and fish liver oil is the main dietary sources. Small quantities of vitamin D are found in other animal products (e.g. beef, butter), and if hens are fed vitamin D, eggs can provide substantial amounts of the vitamin[12,31]. Because the consumption of these foods tends to be relatively low, in industrialized countries most dietary vitamin D comes from fortified milk and margarine. Milk only provides small amounts of vitamin D unless it is fortified. In many locations, the addition of vitamin D to selected foods has proved to be a prudent public health measure[14]. The vitamin has been added to milk in Canada and the United States since the 1920s, a policy that has been largely responsible for the elimination of vitamin D deficiency rickets in children[49].

Either vitamin D2 (ergocalciferol) or D3 (cholecalciferol) can be added to foods. The two forms have similar biological activities and both are very sensitive to oxygen and moisture, and both interact with minerals. For a fortification programme to be effective, the chosen food vehicles have to be available nationwide or, at least, in the specific geographical areas targeted by the programme. In practice, this means that the product must be available and accessible to the targeted segments of the population[27]. The ultimate purpose of a fortification programme is to ensure that the fortified product, of the desired quality, is made available and is accessible to consumers in sufficient amounts. This in turn could be studies through evaluation of the effectiveness and the impact of a programme on the target population. Enrichment is synonymous with fortification and refers to the addition of micronutrients to a food irrespective of whether the nutrients were originally in the food before processing or not.

Information on vitamin D fortification in foods other than milk is limited. In the 1930s, food and beverage manufacturers began to fortify milk, breads, hot dogs, sodas, and even beer with vitamin D[17]. The stability of vitamin D has been reported in bread[29], processed dairy products[18], and orange juice[5]. However, the outbreak of vitamin D intoxication in Europe in the 1950s and the strict regulations issued by the US Food and Drug Administration limited fortification to only milk and cereals in the 1950s; these policies have persisted to this day[17,41]. Fortified milk is not suitable for preventing vitamin D insufficiency in the general population because of the high prevalence of lactose intolerance in Asians, blacks, and Native Americans[22] and because of milk allergies[52]. However, individual studies have often proven contradictory, and it appears that vitamin D stability depends upon the composition, processing, and local environment (e.g., pH and temperature) of the food.

In this study, tea was chosen as a food vehicle for enrichment because it is one of the most widely consumed beverage after water and well ahead of coffee, beer, wine and carbonated soft drinks world wide including the Indian subcontinent. Present day India is the largest producer of tea in the world with the output of some nine hundred thousand tons a year. In India, Assam, Sikkim, Dooars, Darjeeling, Nilgiri are the areas where tea is grown and harvested. Generally, tea is consumed with spices and milk. It has a cooling, slightly bitter, astringent flavour which many people enjoy[47]. Consumption of tea (especially green) is beneficial to health and longevity given its significant antioxidant, flavanols, flavonoids, and polyphenols content[7,26]. Tea is known to have anticarcinogenic[48], antimitogenic[23], anti-oxidative[16,51], hypocholesterolemic effects[50] and antibacterial action against a wide range of bacteria[45]. Teas can generally be divided into categories based on how they are processed. There are at least six different varieties of tea like white, yellow, green, oolong, black, and post-fermented teas[33] of which the most commonly found on the market are white, green, oolong, and black.

Evidence through studies has demonstrated that tea can be fermented with the help of yeast or the symbiotic culture of bacteria and yeast. Fermentation of tea can take place within three to seven days of preparation[32]. Black tea is a good fermentation medium because the infusion contains proteins, amino acids, volatile compounds, lipids, enzymes and polyphenols. Microbial fermentation of black tea leads to value addition in terms of taste, flavour and health components. Fermented tea decoctions such as 'Kombucha' have been prepared by cofermentation with yeast and acetic acid bacteria and are known to have health benefits[13,53]. Black tea fermented with yeast accumulates the vitamins A, C and B complex, making it a nutritious and a therapeutic agent, besides increasing shelf life.

In the present study yeast was employed as a food fortificant, since yeast cells have the ability to synthesize vitamin D2 (ergocalciferol) that have similar biological activities like of vitamin D3 (cholecalciferol) synthesized by the human skin cells[34]. The species Sacchromyces Cerevisae is also known as Baker's yeast used in fermentation for baking and preparation of alcoholic beverages was considered as an ideal source for vitamin D.

Since this research work revolves around micronutrient vitamin D deficiency, fortification method employing fermentation technique using yeast as source for enrichment of the vitamin was attempted with the popular local beverage tea.

Methodology

Materials

Black tea from Tata, Green, Oolong, White and Earl Grey tea varieties from Chamong, Baker's yeast (Sacchromyces Cerevisae).

Chemicals

Ferric ammonium sulphate, Potassium ferri-cyanide, Sulphuric acid, Phosphate buffer (Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium chloride), Trichloro-acetic acid, Ferric chloride, folin-ciocalteau reagent, gallic acid, tannic acid, sodium carbonate, ferrozine, peptone, Beef extract, sodium chloride, agar-agar, molybdic acid, sodium hydroxide, phosphoric acid, sodium sulphate, copper sulphate, sucrose, dinitrosalicylic acid, sodium potassium tartarte, 95% ethanol, potassium dichromate, s-diphenylcardazide, antimony trichloride, acetyl chloride, chloroform, methanol, potassium chloride, magnesium chloride, furfural solution, sucrose.

Tea extraction

The tea extract was prepared by brewing tea concentration of 1.0 % (w/v) for 3 min in sucrose / sugar sweetened water (5°Brix) to near boiling (80–100°C). This product was sieve filtered and dispensed into loosely plugged sterile glass bottles (500 ml capacity with a 400 ml working volume). The extracts were cooled to 25°C before inoculation[21].

Tea fermentation

The fermentation of the tea infusion was conducted at sucrose level of 5°Brix and tea concentration of 1 % (w/v). The tea infusions (in triplicate) were inoculated with a 24 h culture of Sacchromyces cerevisiae at an already standardized inoculum concentration of 0.25 % (v/v) and incubated at 25±2°C. Fermentation was monitored by periodically taking triplicate samples for the estimation of residual sugar and ethanol production[21].

Estimation of sugar content by DNSA method

To one ml of sample, two ml of distilled water was added; one ml of dinitrosalicylic acid reagent was added. Incubated in the water bath for 20 minutes and then nine ml of water was added. The absorbance was checked at 540 nm. The experiment was performed in triplicate sets[33].

Estimation of sugar content by phosphomolybdic method

To one ml of sample, one ml of copper reagent was added. The test tube was incubated in the water bath for 20 minutes and then three ml of phosphmolybdic reagent was added. The absorbance was checked at 610 nm. The experiment was performed in triplicate sets[10].

Estimation of Ethanol content

To one ml of sample, one ml of Potassium dichromate reagent solution was added and one ml of saturated s-Diphenylcarbazide solution was added. The mixture was incubated for 15 minutes at 90° C. One ml of potassium sodium tartarate solution was added to stabilize the colour. After cooling the absorbance was checked at 575 nm. The experiment was performed in triplicate sets[22].

Isolation of whole cell lipids from yeast cells

One g dry yeast was dissolved in five ml of distilled water. The solution was centrifuged at 2000 rpm for seven minutes. The supernatant was discarded and the cells were washed with distilled water three times. The harvested cells were mixed with 10 ml methanol. Glass beads were added to the suspension and centrifuged at 2000 rpm for seven minutes. Chloroform was added to the suspension to give a ratio of chloroform:methanol (2:1), and stirred for one hour on flat bed stirrer at room temperature. The extract was filtered through a sintered glass funnel and the glass beads was washed off with chloroform:methanol. The extract was mixed with 0.034% magnesium chloride solution and it was stirred for 10 minutes. The upper layer was removed and the organic phase was washed off with 2N potassium chloride: methanol (4:1 ratio). The solution was centrifuged at 3000 rpm for five minutes. The upper layer was removed. The organic phase was washed with chloroform:methanol:water (3:48:47 ratio). The solution was centrifuged at 3000 rpm for five minutes. The upper layer was discarded and the organic phase was washed with distilled water. The organic phase was transferred into a beaker and the solvent was evaporated. The lipid film was dissolved in chloroform:methanol (2:1 ratio) and stored in glass vials for further studies[36].

Estimation of vitamin D

The samples were taken in different concentrations of 0.2 ml - 1 ml. Required amount of distilled water was added to make up the volume of the solution. To this one ml of test sample, 0.5 ml of furfural reagent was added. The test tubes were kept in ice bath and one ml of concentrated sulfuric acid was added slowly every minute for three and half minutes. After cooling the solution the absorbance was measured at 430 nm[30].

Sensory Evaluation

After completion of fermentation, the tea beverages produced were decanted by siphoning, filled into drinking glass of 100 ml capacity and presented for sensory analysis using a 9 point hedonic scale on the basis of appearance, colour, taste, odour and overall acceptability to a panel (semi-/untrained) of 10 tasters (see Annexure 1a-1d for details). The sensory scores were calculated and a mean was taken for each taster for every beverage. Finally scores were compared to find out the best accepted beverage[21].

Determination of phenolic compounds by Folin-Ciocalteau method

0.2 ml of solution was pipette into a test tube and one ml of Folin-Ciocalteau was added. To the above mixture 0.8 ml of sodium carbonate was added. The mixture was stored at room temperature for 30 minutes and the absorbance was read at 700 nm. Total phenolic compounds were calculated using a standard curve prepared with dilutions of tannic acid in terms of tannic acid equivalent (TAE %)[35].

Determination of phenolic compounds by prussian blue method

16 µl of sample was added to different test tubes followed by addition of five ml of distilled water. 0.3 ml of ferric ammonium sulphate was added to the above solution and mixed thoroughly. Additions were timed. After 20 minutes of addition, 0.3 ml of potassium ferricyanide was added. Further after incubating for 20 minutes, the absorbance was read at 700 nm. Blank was prepared by addition of reagents without the sample. Tannic acid was used as standard. The experiment was carried out in triplicate set[15].

Ferric reducing antioxidant assay

0.12 - 0.60 ml of the standard and sample was made up to one ml by addition of distilled water. 2.5 ml of phosphate buffer (pH 6.6) was added followed by addition of 2.5 ml of 1% potassium ferricyanide. This mixture was incubated at 50° C for 20 minutes. After incubation, 2.5 ml of 10% of trichloroacetic acid was added and then it was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and then 0.5 ml of 0.1% of ferric chloride was added. The solution was vortexed and the absorbance was measured at 700 nm. Blank was prepared by addition of reagents without samples. Tannic acid was considered as standard. The experiment was carried out in triplicate set[1].

Metal chelating activity

Standard and test samples were added in different concentrations 0.2 - 1 ml to a solution of 1 mM ferric chloride (0.05ml). The reaction was initiated by addition of ferrozine (0.1 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 minutes. After incubation period absorbance was read at 562 nm[33].

Disc diffusion susceptibility assay

For microbial susceptibility testing of unknown samples, an assay format was developed for screening of crude samples like normal and fermented tea. Standardizations of methods and protocols were carried out essentially according to Glupczynski (1996). Basically, 0.5 ml innoculum (~10^8 cfu/ml) for each of the microbial strain tested was flooded on freshly prepared nutrient agar plates. Excess culture was removed and the plates were dried for 2-3 minutes. Sterilized disks (5 mm diameter), each containing a test sample, or a standard antibiotic, was placed on the agar surface. The plates were incubated for 24 h in a B.O.D. incubator at 37°C. At the end of the incubation period, the diameter of the zone of inhibition was measured in cm. For comparison sensitivity of each microbe was also tested against a commercial antibiotic amoxicillin[25].

Results and Discussion

The fermentation experiment on tea infusion prepared by brewing tea leaves at 5% sugar and 1 (w/v) tea at 60°, 80° and 100°C revealed no significant difference with respect to drop in Brix and alcohol produced. From the table, it is clear that sugar utilization and ethanol production was not that significantly differed with 60° and 80°. However, the tea extracted at 100°C recorded lower post fermentative residual total sugar (0.11%), sensory score (6.4) and at pH 3.7 when compared to tea extracted at 60° and 80°C (Table 1).

Thus the infusion temperature of 100°C was selected for further experimental analysis. Hot water is known to extract amino acids, vitamins etc from tea leaves and these nutrient are used for yeast fermentation[42]. Therefore, greater extraction at 100°C could have resulted in better fermentation and the resultant lower residual sugars. The fermentation efficiency was examined with decrease in the Brix drop rate i.e., decrease in the sugar concentration with time of fermentation.

Table 1. Effect of extraction temperature on fermentation of black tea

Fermentation period (days)	Extraction Temperature		
	60°C	80°C	100°C
0	5	5	5
7	4	4.2	3.7
14	3.6	3.8	2.4
21	2.4	2.8	1.9
° Brix drop/day for first 7 days	0.8	0.84	0.74
Further ° Brix drop/day up to 21 days	0.48	0.56	0.38
Alcohol % (v/v)	0.4	0.4	0.5
Sensory Score	4.4	5.0	6.4
Residual total sugar	0.2	0.24	0.11
Final PH	2.5	2.8	3.7

Fermentation parameters : tea concentration 1% (w/v); Initial pH 4.0; Initial Brix 5°; Temperature 25±2°C; Inoculum 0.25% (w/v).

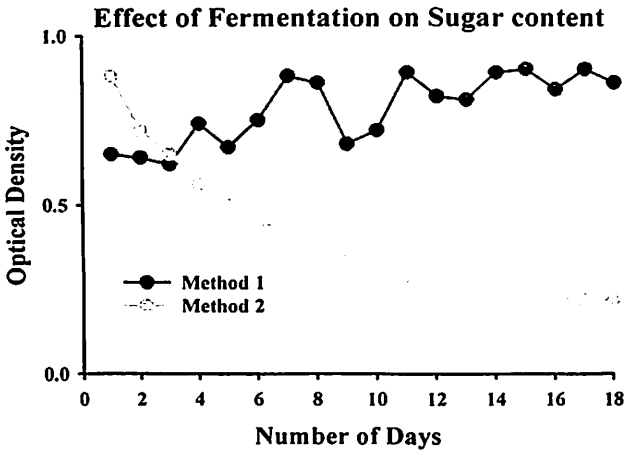

Fig 1: Effect of fermentation on sugar utilization. Fermentation parameters : tea concentration 1% (w/v); Initial pH 4.0; Initial Brix 5°; Temperature 25±2°C; Inoculum 0.25% (w/v).

Sugar concentration was analyzed primarily by two methods employing DNSA and phosphomolybdic method (Fig.1). Based on the fermentation efficiency outcome the method employing phosphomolybdic reagent was found more suitable for estimation of sugar during fermentation process. The time of addition of sugar during the fermentation experiment was analyzed and the results demonstrate no significant difference with sugar addition time. The result clearly demonstrates that no significant difference was noted with time of addition of sugar for preparing the tea beverage. The rate at which sugar was utilized to form alcohol was equivalent with both the experimental data (Figs. 2a & 2b)

The yeast cells were treated for synthesis of ergosterol (vitamin D2) by exposing directly to sunlight and/or to UV c light with the help of a UV lamp. The experimental data clearly indicates that yeast can synthesize vitamin D on exposure to sunlight and UV light and the duration of exposure to sunlight did not affect the vitamin synthesis in the cells (Fig. 3). Thereafter the fermentation efficacy was analyzed through fermentation experiments in comparison with normal yeast cells. The drop in brix concentration as well as increase in ethanol production clearly indicates that exposure to UV or sunlight did not alter any functional variation in the fermentation process (Fig. 4).

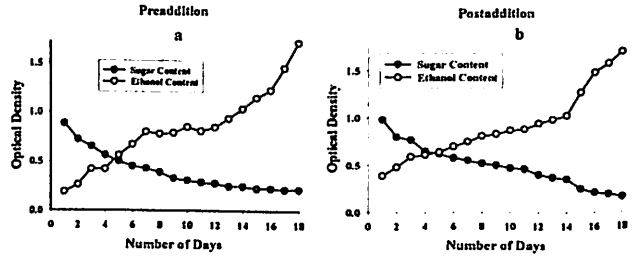

Fig 2: Effect of sugar addition time on fermentation. Fermentation parameters : tea concentration 1% (w/v); Initial pH 4.0; Initial Brix 5°; Temperature 25±2°C; Inoculum 0.25% (w/v).

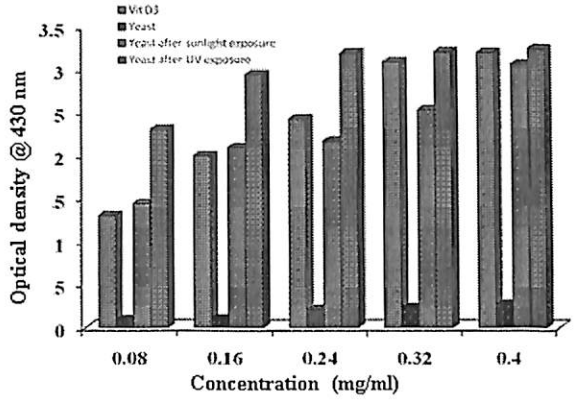

Fig 3: Vitamin D content of yeast cell. Comparative analysis was carried out with commercially available vitamin D3. Cells without any treatment were employed as negative control in the experimental set up.

Further the acceptability of the above prepared beverages was examined by the panel of ten members (Table 2). The sensory score were analyzed employing nine point hedonic scale and the results indicates that tea prepared from one hour exposure to sunlight was showed almost equivalent score to that of beverage prepared with normal yeast cell. Result indicates that the beverage prepared with treated yeast cell showed equivalent score to that of beverage prepared with normal yeast cell and tea prepared from yeast cell exposed to sunlight was considered best in terms of sensory analysis.

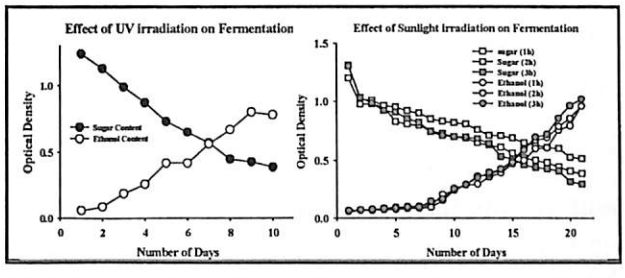

Fig 4: Fermentation of tea with yeast cells after exposed to UV /sunlight. All parameters were similar to normal fermentation process. Yeast cells exposed to UV / sunlight with different time duration before the experiment.

Table 2. Sensory analysis of the fermented beverage after storage

		Accrued score points Fermented tea (black)		
Character	Maximum score point	Normal	After UV irradiation	After sunlight irradiation
Appearance	8	4.6	5.6	6.5
Colour	8	4.5	5.5	6.3
Taste	7	4.4	4.2	5.0
Odour	7	4.4	4.5	5.2
Overall	7	4.4	4.6	5.4
Total	37	22.3	24.4	28.4

Mean of 10 tasters. Tasting parameters: 37 point sensory analysis scale, tea beverage bottles stored at room temperature, 50 mL glass tumblers used for tasting.

A comparative sensory analysis was also carried out with normal fermented, UV exposed fermented and sunlight exposed fermented tea after three weeks of experimentation (Table 2). The fermented tea after sunlight treatment was best in terms of total sensory score and it is worth mentioning that the elevation of score point emanated from the general quality character and it was much ahead of other varieties respectively.

Further the tea beverages were stored at room temperature and their chemical qualities in terms of sugar utilization, alcohol yield and pH were assessed over a period of three weeks (Table 3). The consistent alcohol levels however indicate that the residual yeast cells do not contribute to significant alcohol production at the room temperature. Also, there was insignificant change in pH of the beverage, suggesting low or no contamination by microorganisms or at least activities at the room temperature which was not expected.

Table 3. Effect of aging on the chemical quality of fermented tea

	Duration of storage after fermentation					
Chemical characteristics	Fermented		Fermented after after UV irradiation		Fermented after sunlight irradiated	
	0 week	3 week	0 week	3 week	0 week	3 week
Alcohol (%)	7.3	7.2	7.56	7.7	8.02	8.3
Residual sugar (%)	0.058	0.008	0.06	0.006	0.059	0.009
pH	3.9	3.5	3.8	3.7	3.9	3.8
° Brix	5	3	5	3.5	5	3.4

The quality of tea as drunk is largely determined by its polyphenols content, which is responsible mainly for the strength and colour of the infusion, its mouth-feel and the ability to form tea-cream – a precipitate of complexes of polyphenols with caffeine[33]. These polyphenols are formed during conversion of fresh leaf to black tea by oxidation of flavanols, flavandiols and theogallin. The polyphenols in tea are astringent, these compounds are chemically quite distinct from tannins. Their reaction with protein, unlike those of tannic acid and other commercial tannins are reversible and there is no evidence that they damage the intestinal mucosa[19].

Of total solids extracted from tea, some 40% are polyphenols, often referred to incorrectly as tannins, 20% are proteins and amino acids, 5% caffeine, 5% inorganic ions and 3% miscellaneous substances including lipids, carbohydrates and vitamins. Since it is clear that the largest

constituents is the polyphenols, the experimental tea beverages were examined for the total phenolic content employing two methods (Fig. 5).

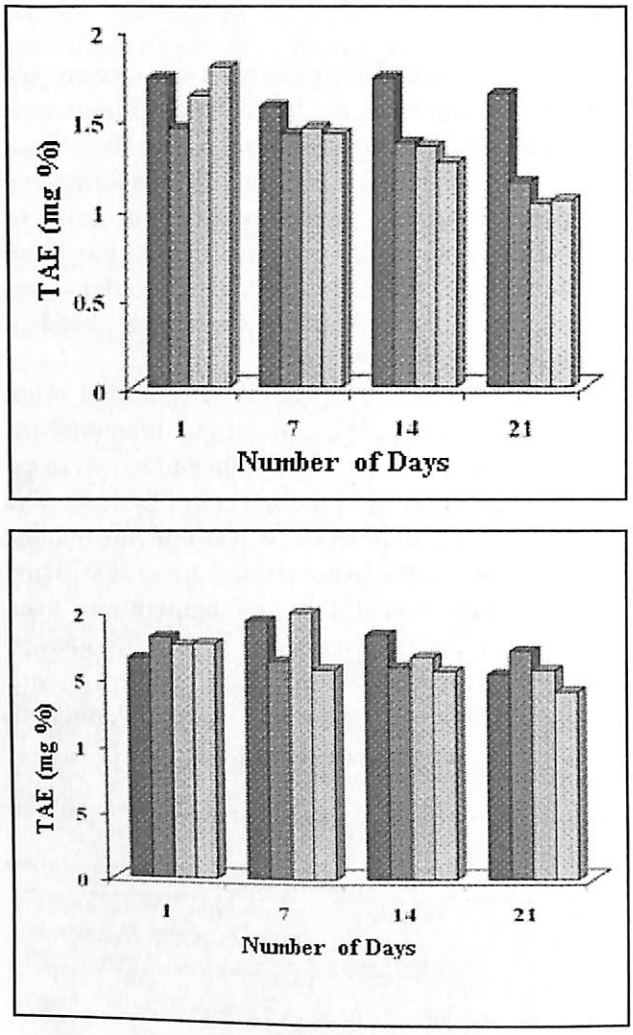

Fig 5: Total phenolic content. Normal tea ■; Fermented tea ■; Fermented tea after UV exposure □; Fermented tea after sunlight exposure □.

Comparative study was carried out with tannic acid and the results are expressed in terms of percentage of tannic acid equivalent. Left and right panel indicates TPC by folin-ciocalteau and Prussian blue methods.

The total phenolic content in the beverages varied significantly between the normal and fermented after UV or sunlight treatment. The normal tea without fermentation showed maximum polyphenol content, followed by fermented beverages. The phenolic content decreased with increase in time of fermentation period with respective to tannic acid equivalent. The lowest polyphenol content was observed towards three weeks of fermentation. The above data revealed that the fermentation by yeast definitely decreases the phenolic compounds compared to the normal tea beverage and makes the fermented ones less bitter and more consumable.

Phenolic phytochemicals exhibit a wide range of biological effects and one of the broad categories is there property of scavenging free radicals. Reactive oxygen species have been implicated in the development of several oxidation-linked diseases such as cancer, CVD and diabetes. Recent epidemiological studies have indicated that diets rich in phenolic compounds like green tea are associated with lower incidences of oxidation linked diseases such as above. These protective effects of tea beverage are now linked to the presence of bioactive phenolic phytochemicals which support the body's antioxidant defense system[39]. The well described mode of action of these phenolic phytochemicals in managing oxidation stress-related diseases are due to the direct involvement of the phenolic phytochemicals in quenching the free radicals from biological systems[46].

The antioxidant potential of experimental beverages was analyzed using the ferric reducing antioxidant assay and metal chelating assay (Table 4). The results indicate the percentage reducing power of the beverages followed the order: normal tea (96±3) > fermented tea (87±1) > fermented tea after UV treatment (80±4) > fermented tea after sunlight treatment (75±3). But the reducing power of the beverages was less than that of standard tannins (90 - 95%).

Table 4. Antioxidant activity of tea beverages

Sample	Ferric reducing activity (%)	Metal chelating activity (%)
Normal tea	96±3	85±1
Fermented tea	87±1	80±3
Fermented Tea after UV exposure	80±4	81±2
Fermented tea after sunlight exposure	75±3	78±1
Tannic acid	95±0.5	92±2
Gallic acid	97±1	94±0.5
Catechin	90±0.5	91±0.5

Transition metal has played a pivotal role in the generation of oxygen free radicals in living organisms. Chelating agents may inactivate metal ions and potentially inhibit the metal-dependent processes. Transition elements like iron and copper are powerful catalysts of oxidation reactions because they contain one or more unpaired electrons that can enable to participate in electron transfer reactions. They can participate in the conversion of H_2O_2 to OH in the Fenton reaction and in the decomposition of alkyl peroxides to the heavy reactive alkoxyl and hydroxyl radicals[28].

Due to this property, transition metal chelation to form low redox potential complexes can be an important antioxidant property. The metal chelating activity of tea beverages are shown in Table 4. At the same concentration, the beverages showed a comparatively equivalent chelating activity like

that of the control tannins. The normal tea beverage chelate metal with 85% and the fermented beverages chelate around 78 to 81%. Among the fermented tea, the beverage after UV treatment of yeast showed a metal chelating activity of nearly 81%, followed by normal fermented tea (80%) and fermented tea after sunlight treatment of yeast (78%). Overall, both normal and fermented beverage possesses a better metal chelating property. This metal chelating property of the tea may be attributed to their endogenous chelating agents, mainly phenolic compounds that have properly oriented functional groups, which can chelate metal ions.

Tannin containing plants have a significant evolutionary advantage over their enemies, since tannins deter herbivors from predator, also deter, microorganism, either by increasing resistance against pathogens. The antimicrobial property of tannin has been well documented[38]. Tannins have been reported to be bacteriostatic and bactericidal against various microbial pathogens. Since antimicrobial activity is also accounted as a property for biological effect of tea tannins, therefore the beverages were examined for their antimicrobial effect on various known microbes. Antimicrobial activity of the beverages was evaluated employing disc diffusion susceptibility assay against an array of microbial strains (Table 5). It is indicated that normal tea beverage showed inhibition zone diameter (IZD) around 1.4 -1.6 at dose of 40 µl/disc. The fermented beverages of tea showed IZD around 1.0 - 1.3 cm at 40 µl/disc and the inhibition was equivalent against four bacterial and one fungal strain. The beverages showed maximum activity against *E. coli* and *A. niger* species.

Table 5. Disc diffusion susceptibility assay of tea beverages

	Inhibition Zone Diameter (cm)				
Samples (µg/disc)	S. aureus	E. coli	B. subtilis	Pseudosomas sp.	A. niger
Amoxicillin (2) (40 µl/disc)	1.8	1.9	1.8	1.9	1.7
Normal tea	1.6	1.5	1.4	1.4	1.5
Fermented tea	1.2	1.2	1.0	1.1	1.2
Fermented tea after UV exposure	1.1	1.2	1.1	1.2	1.3
Fermented tea after sunlight exposure	1.2	1.3	1.2	1.0	1.2

Discs impregnated with samples (40 µl/disc) were placed in plates flooded with clinical strains and the inhibition zones were recorded after confluent growth (24 h). Antibiotic amoxicillin was employed for comparative analysis.

Further to scale up the studies, different varieties of tea were analyzed for the preparation of fermented beverage. For the experimental purpose four tea varieties like Green, Oolong, White and Earl Grey were employed. The fermentation efficiency of the cells treated with UV or sunlight were analyzed through experiments with the treated yeast cells employed in the tea infusion for carrying out the fermentation process in comparison with the normal yeast cells without any treatment (Fig. 6). The figures clearly indicate that exposure to UV and sunlight did not alter any functional variation in the cells in carrying out the fermentation process. The treated yeast cells utilized sugar available for their growth and converted it to ethanol very efficiently like the normal cells (compare with Fig. 2). Further to confirm its acceptability, sensory evaluation for the different tea beverages were carried out with help of ten panel members (semi-trained). The tea solutions were analyzed for sensory score employing a nine point hedonic scale to a panel of ten tasters (Table 6). From the data it was observed that tea prepared from black and Earl grey varieties were considered best in terms of sensory score than other three varieties.

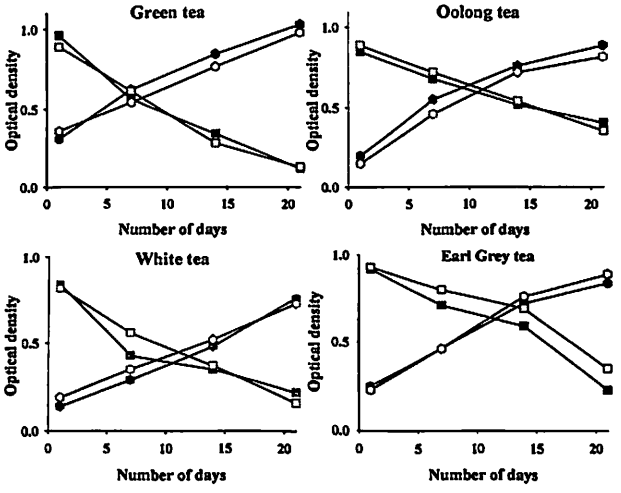

Fig 6: Effect of fermentation on tea varieties

Effect of fermentation with yeast was studied for green (a), oolong (b), white (c) and earl grey (d) tea. Fermentation efficiency was estimated by analyzing sugar and ethanol content of the resultant beverage. ■: Sugar content after UV exposure; □: Sugar content after sunlight exposure; ●: Ethanol content after UV exposure; ○: Ethanol content after sunlight exposure.

Table 6. Sensory analysis of the fermented beverage for tea varieties

Character	Maximum score point	Accrued score points				
		Fermented tea				
		Black	Green	White	Oolong	Earl Grey
Appearance	9	7.1	4.3	4.5	4.1	6.1
Colour	9	7.2	4.2	4.5	4.1	6.0
Taste	8	7.0	6.0	4.6	4.3	6.4
Odour	9	5.9	5.1	4.5	4.4	6.5
Overall	8	6.9	5.5	4.2	4.4	6.3
Total	43	34.1	25.1	22.3	21.3	31.3

Mean of 10 tasters. Tasting parameters: 43 point sensory analysis scale, tea beverage bottles stored at room temperature, 50 mL glass tumblers used for tasting.

Further the tea preparations were subjected to analysis of phenolic content, antioxidant activity and antimicrobial effect if any present (Table 7). The normal tea beverage of green and Earl grey showed the highest phenolic content with > 90 % followed by normal oolong and white tea preparations. The lowest phenolic content was observed with white tea fermented beverages. The reducing effect of normal green and Earl grey beverage (86 %) was potential than other beverages. In brief, the percentage reducing power of the beverages followed the order: green tea > Earl grey tea > oolong tea > white tea (Table 7). Preliminary antimicrobial studies indicated that normal tea beverage of four varieties showed inhibition of the microbes at dose of 40 µl/disc (Table 7). The fermented beverages of green and Earl grey tea showed positive IZD against four bacterial and one fungal strain.

Table 7. Antioxidant, antimicrobial and TPC of tea varieties

Sample	Total phenolic content (%)	Ferric reducing activity (%)	Disc diffusion susceptibility assay
Green tea			
Normal	96±3	86±2	++
Fermented	80±1	82±0.5	+
Fermented after UV exposure	74±2	76±1	-
Fermented after sunlight exposure	71±3	70±0.5	-
Oolong tea			
Normal	66±0.5	65±2	+
Fermented	61±2	59±2	-
Fermented after UV exposure	60±2	52±2	-
Fermented after sunlight exposure	62±1	56±4	-
White tea			
Normal	62±2	58±4	+
Fermented	50±1	49±2	-
Fermented after UV exposure	47±5	46±0.5	-
Fermented after sunlight exposure	41±6	40±4	-
Earl Grey tea			
Normal	90±1	86±0.5	++
Fermented	81±0.5	80±2	+
Fermented after UV exposure	75±1	77±2	-
Fermented after sunlight exposure	72±3	64±2	-

Tannic acid, gallic acid and catechin were used as standard controls for TPC and antioxidant activity and amoxicillin for antimicrobial activity.

The major objective of this study was to prepare a fermented tea beverage and to enrich the same with the micronutritient vitamin D for the benefit of the common population. Studies from above experiments showed that yeast cells on exposure to UV or sunlight radiation could synthesis the vitamin D2 (ergosterol) which can be utilized as an alternative for the vitamin D3 (cholecalciferol) synthesized by human skin cells. Therefore it could be hypothesized that using yeast cells after treatment with UV or sunlight for fermentation process would result in enrichment of the micronutrient (vitamin D) in the prepared beverage. Thus considering this statement, the various tea preparations were examined for the vitamin D content.

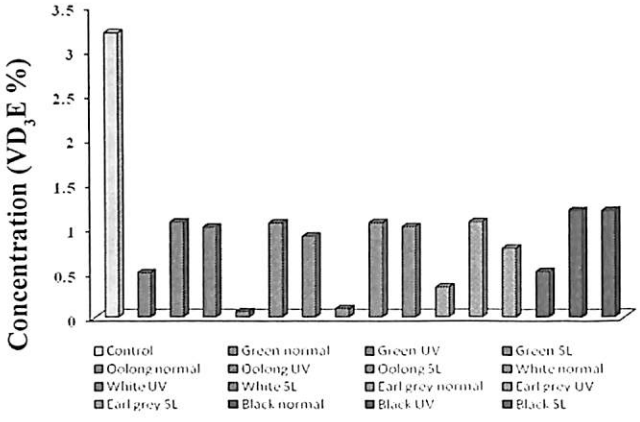

Fig 7: Vitamin D content of the fermented tea beverage. For comparative study commercial vitamin D3 was employed as standard.

On comparison with the commercially vitamin D3, it is clearly observed that normal tea beverages showed negligible presence of the vitamin in comparison with the standard control (Fig. 7). The fermented tea preparations employing UV as well as sunlight exposed yeast cells definitely showed the presence of vitamin but in moderate concentration when compared with the standard vitamin D3.

Conclusion

Nevertheless, the results obtained show that there is enrichment of vitamin D in the prepared beverages. Based on the data gathered so far, it is reasonable to state that yeast when exposed to sunlight certainly possess the ability of synthesizing the vitamin D which can be exploited in making dietary therapeutic management of diseases related to this vitamin deficiency, and also to be explored in a more focused manner towards delineating the such observations. This technique could be employed at commercial house hold level which would help the community to combat the micronutrient insufficiency by the domestic application.

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A Study on the Effect of Antioxidants on Stability of Lipid

Suchanda Chatterjee and Banani De

ABSTRACT

Biologically important n-3 *polyunsaturated fatty acid enriched fish lipid is highly susceptible to oxidative deterioration. To delay oxidation six spices - fennel, black-pepper, ginger, cinnamon, celery,* panchforon *(mixture of fennel, celery, cumin, black-cumin, fenugreek), and BHT a synthetic antioxidant were mixed separately with homogenized tilapia fish muscle. Total lipid composition, oxidative stability and antioxidant activity of fish oil, at interval of one week over a span of six weeks was evaluated on basis of amount of cholesterol, phospholipid, fatty acid, peroxide,* p-*anisidine, thiobarbituric acid (TBA), iodine, conjugated diene-triene values, total phenol content, inhibition to peroxidation and metal chelating activity. Overall increasing trend in most values except phospholipid was observed. Values were higher in control. Contrary to other spices cinnamon and* panchforon *decreased the amount of malonaldehyde accumulation in oil. The highest phenolic concentration and inhibition to peroxidation was recorded in cinnamon extract. Ginger mixed fish oil recorded highest inhibition to peroxidation but least metal chelating activity. Cinnamon was maximum efficient in controlling oxidation, followed by* panchforon. *Celery and pepper were effective for a shorter span. Antioxidant activity of fish oil revealed that long storage of spiced-fish enriches it with antioxidants which are beneficial to human body.*

Keywords: n-3 Polyunsaturated Fatty Acid, Fish Lipid, Oxidative Stability, Antioxidant

Introduction

Lipids are an assorted group of naturally occurring compounds insoluble in water but soluble in organic solvents and encompass monoglycerides, diglycerides and triglycerides[25]. The most abundant lipid in the body is triglycerides and their constituent fatty acids as well as cholesterol, phospholipids, sterols, and fat-soluble vitamins (such as vitamins A, D, E, and K).

Fatty acids are the building blocks of these lipids. The fatty acids which cannot be produced within the human body and must come from dietary sources are known as essential fatty acids. These are required for the formation of healthy cell membranes, for proper development and functioning of the brain and nervous systems, and for the production of hormone-like substances called eicosanoids.

Lipids are susceptible to oxidative processes like autoxidation, photo-oxidation, thermal oxidation, and enzymatic oxidation under different conditions in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms. It gives rise to off-flavors and loss of essential amino acids, fat-soluble vitamins, and other bioactives[19, 23].

Fish lipids are well known to be rich in long chain *n*-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Compared to beef and chicken, fish meat contains higher levels of *n*-3 PUFAs[2], hence are usually susceptible to attack by the oxygen of air.[20] Autoxidation is the oxidative deterioration of unsaturated fatty acids via an autocatalytic process consisting of a free radical chain mechanism. This chain includes initiation, propagation, and termination reactions that could be cyclical once started. An antioxidant is defined as a substance which, in relatively low concentration, markedly inhibits the rate of the reaction with oxygen[20, 12]. Due to the toxic effects of synthetic antioxidants use of spices and herbs are now encouraged to control the rate of lipid oxidation. In this study *oreochromis mossambicus* was judiciously chosen as the source of lipid because of its considerable lipid availability along with few popular Indian spices.

Methodology

Preparation of sample: Total amount of 6 Kg of Tilapia fish (each having an average weight of 300 gram, length 6.5 inches and age of 3 months) was procured from Chowbaga bheri located in east Kolkata wetland. Fish muscles were minced and homogenized in a blender. The mass was further divided in 41 equal portions each weighing 100 gms for mixing six spices, fennel seeds (*Foeniculum vulgare)*, black pepper (*Piper nigrum)*, ginger (*Zingiber officinale)*,cinnamon (*Cinnamomum verum*), celery seed (*Apium graveolens*) and *Panchforon* (a mixture of cumin seed (*Cuminum cyminum L*), fenugreek seeds (*Trigonella foenum-graecum*), celery (*Apium graveolens)*, fennel seeds (*Apium graveolens*), black cumin (*Nigella sativa*) seeds in equal proportion), [all grinded and sieved at125 micron], a synthetic antioxidant BHT, and for using as control, were put in the press-and-lock polythene freezer bag and stored in the freezer chamber of a refrigerator at 0-4°C. Samples were kept in five batches.

Extraction of lipid

It was done by Bligh and Dyer method (1959)[1]

Estimation of lipid composition

Lipid composition was monitored through thin layer chromatography.

Estimation of cholesterol

Cholesterol was estimated by CHOD – PAP method.

Direct estimation of phospholipids

This colorimetric method is based on the formation of a complex between phospholipids and ammonium ferrothiocyanate.[21]

Estimation of Oxidation in fish lipid

Acid value

Acid value was calculated using IUPAC method 2.201.

Peroxide value

Peroxide value was calculated using IUPAC method 2.501

Thiobarbituric acid value

Thiobarbituric acid value was calculated using IUPAC method 2.531.

Para-anisidine value

Para-anisidine value was calculated using IUPAC method 2.504.

Iodine value

Iodine value was measured using IUPAC method 2.205.

Conjugated diene and triene value

These values were calculated using IUPAC method 2.505

Estimation of antioxidant activity in fish lipid and spices

Test for antioxidant in fish sample

Determination of total phenolic compounds:

Total phenolic compound was determined using Folin-Ciocalteu reagent

Antioxidant activity in a linoleic acid system

Antioxidant activity was evaluated by the thiocyanate method

Metal chelating activity

The chelation of ferrous ions by the extracts was estimated by the method of Dinis *et al.* (1994).[5]

Results and Discussion

Evaluation of variation in total lipid composition: Cholesterol: Cholesterol can undergo autoxidation in air to form cholesterol oxide products (COPs) which increase the total cholesterol concentration. It is observed that cholesterol concentration increased in all the cases from initial week except cinnamon. This indicates that possibly cinnamon has most successfully controlled the oxidation of oil.

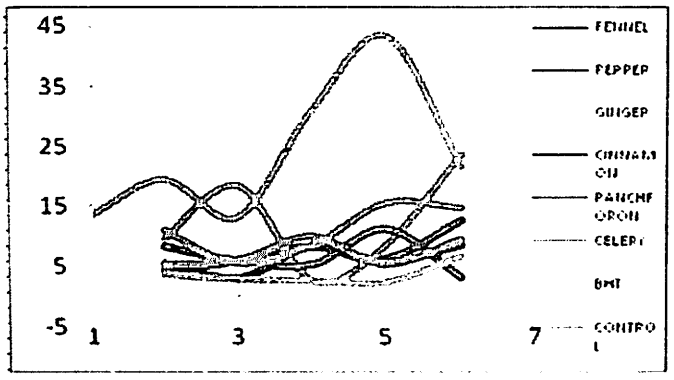

Fig 1: CHOLESTEROL

Phospolipid: From the above Graph 2 it is found that the concentrations of phospholipids have decreased remarkably when mixed with spices whereas the control recorded considerably higher value than others. This indicates that either the phospholipids have undergone hydrolytic reactions at a greater extent in spices or has reacted with the flavor components in spices. Phospholipid concentration also reduces due to presence of phopholipase. Concentration of phospholipids was found to be more or less constant throughout in case of BHT while cinnamon sample showed a gradual and steady decrease in the phospholipid content. Finally it dropped down to a value which is comparable with that of BHT. *Panchforon* and celery also revealed a remarkably low phospholipid concentration.

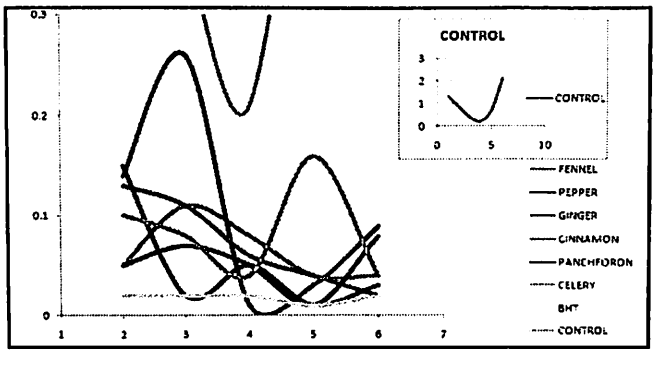

Fig 1: PHOSPHOLIPID

Parameters showing oxidation status of oil [Table 1]

ESTIMATION OF ACID VALUE

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt	Control
1								0.310
2	0.256	0.659	0.742	0.612	0.299	0.375	0.020	0.715
3	0.273	0.342	0.239	0.560	0.616	0.166	0.029	0.950
4	0.664	0.500	0.197	0.533	0.436	0.743	0.119	1.354
5	0.610	0.880	0.51	0.300	0.390	1.247	0.159	1.957
6	0.556	1.253	1.536	0.065	0.347	1.264	0.192	2.059

ESTIMATION OF PEROXIDE VALUE (/mg oil)

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt	Control
1								0.714
2	4.289	4.126	7.937	5.455	2.663	2.465	1.214	8.824
3	2.195	1.677	5.674	4.702	2.023	1.186	1.683	11.300
4	2.959	3.185	0.658	2.532	2.591	2.941	1.593	13.793
5	3.764	3.261	7.692	6.723	2.985	11.111	1.401	44.186

ESTIMATION OF TBA (value/mg oil)

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt	Control
1								0.884
2	0.549	0.619	0.661	0.705	0.358	0.528	0.008	1.176
3	0.560	0.351	0.709	0.392	0.378	0.522	0.014	1.264
4	0.710	0.637	0.804	0.222	0.336	0.515	0.120	1.638
5	0.774	0.761	1.049	0.210	0.317	0.667	0.140	1.744
6	0.867	1.006	1.250	0.191	0.308	1.130	0.182	1.835

ESTIMATION OF p-ANISIDINE VALUE

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt	Control
1								0.001
2	0.0059	0.028	1.063	1.239	0.323	0.976	0.6	0.024
3	18.271	9.351	43	35.739	14.939	12.014	11.127	0.025
4	66.09	74.95	183.85	28.410	50.07	14.200	7.538	0.149
5	5.988	118.109	27.517	14.250	6.589	17.533	2.503	0.273
6	0.061	0.153	0.235	0.211	0.321	0.122	0.047	0.424

ESTIMATION OF IODINE VALUE (value /mg oil)

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt	Control
1								0.185
2	0.191	0.209	0.275	0.213	0.191	0.237	0.058	0.170
3	0.201	0.197	0.288	0.223	0.202	0.254	0.066	0.141
4	0.210	0.185	0.405	0.233	0.243	0.271	0.073	0.125
5	0.239	0.266	0.524	0.650	0.275	0.305	0.078	0.118
6	0.248	0.241	0.740	1.06	0.284	0.339	0.130	0.012

ESTIMATION OF CT

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt	Control
1								0.001
2	0.002	0.003	0.004	0.008	0.001	0.002	0.003	0.001
3	0.011	0.011	0.029	0.029	0.011	0.010	0.007	0.021
4	0.235	0.109	0.027	0.036	0.040	0.013	0.012	0.022
5	0.015	0.029	0.014	0.066	0.009	0.036	0.016	0.037
6	0.0002	0.0004	0.0008	0.0001	0.0003	0.099	0.017	0.039

ESTIMATION OF CD

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt	Control
1								0.00005
2	0.001	0.027	0.009	0.002	0.001	0.001	0.005	0.002
3	0.028	0.066	0.079	0.043	0.030	0.022	0.017	0.054
4	0.077	0.128	0.019	0.073	0.062	0.002	0.025	0.063
5	0.047	0.053	0.011	0.091	0.038	0.001	0.033	0.210
6	0.0002	0.0004	0.0009	0.092	0.0003	0.0005	0.063	0.213

ESTIMATION OF FATTY ACID IN SPICES [TABLE 2]

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt
1.	0.545	0.858	0.723	0.871	1.041	0.875	0.603
2.	0.684	0.674	0.77	1.02	0.902	0.764	0.887
3.	0.801	0.519	0.801	2.158	0.852	0.444	0.957
4.	0.863	0.521	1.342	3.013	0.523	0.233	0.965
5.	0.93	0.711	1.44	3.21	0.321	0.21	1.071
6.	1.04	0.78	1.6	4	0.01	0.11	1.2

Acid value: The acid value measures the amount of carboxylic acid groups in free fatty acids generated in the fat due to storage. Increase in this value leads to formation of off-flavour as a result of degradation of fat[3,4]. A steady increase in values was observed in case of control as well as BHT, whereas in case of cinnamon there was a gradual decrease (Table 1). Though an overall increase in the fatty acid content occurred in all spices in the duration between the initial and final week, the value reached its peak at third week in *Panchforon,* and at fourth week in case of fennel before it finally declined. Fatty acid value in celery, ginger and black pepper underwent a depression at third week and ultimately increased in 4th, 5th and 6th week in celery, pepper and ginger respectively. This decrease in the fatty acid value might be attributed to the higher antioxidant activity of these spices in these respective weeks. It is to be noted that cinnamon could lower the fatty acid value most effectively and the value recorded in the final week (0.065) is even lower than the synthetic antioxidant BHT. The acid values recorded in 3rd and 4th week in celery and ginger respectively though dropped down to an appreciable range ultimately turned out to be less effective for a longer period of time. Whereas the values recorded for spices in Table (2) reveals that the fatty acid content gradually decreased in *panchforon* and celery throughout the period contrary to increasing trend of fennel, ginger, cinnamon and BHT. The acid content in pepper though reduced till 4th week increased in the later period. The highest increase was recorded for cinnamon while *panchforon* showed the least.

Peroxide value: Peroxide value measures the degree of oxidative rancidity of the oil and the amount of oxidized substance formed during lipid oxidation[8]. The primary oxidation products are mainly hydroperoxides which results due to interaction of singlet oxygen with the fat[6, 10]. The primary oxidation products hydroperoxides were unstable during long storage and readily decompose into mixtures of volatile and non-volatile compounds[7, 22].

The increasing peroxide value in control indicates higher rate of oxidation (Table 1). In case of other spices the peroxide values although increased initially underwent a reduction in the consecutive one or two weeks. This was followed by an increase in value in all the cases. This trend indicates that the antioxidant activity of the spices must have been higher in the 3rd or 4th week which helped in controlling the rate of peroxidation. In BHT the peroxide value showed a gradual an initial increase followed by a gradual decrease. Though the peroxide value increased in the 5th week in all the cases (except BHT) possibly due to reducing antioxidant activity, the final week recorded no peroxide value. This might be due to decomposition of peroxides to secondary oxidation products on long storage.

Though celery seeds and pepper most effectively reduced the peroxide formation in the initial level, the most effective overall decrease was revealed in case *'Panchforon'*.

Thiobarbituric acid value: Thiobarbituric acid value measures the rate of oxidative rancidity by the formation of oxidized lipids – malonaldehyde which is a non-volatile aldehyde[14, 15]. The TBA values (Table 1) delineate a gradual increase in malonaldehyde concentration in case of fennel, ginger, BHT, and control. Only in black pepper and celery a sudden drop in value occurred at third week which was again followed by a gradual rise. This shows that these spices act as antioxidant effectively for a shorter period of time. The most interesting result was observed in cinnamon and *panchforon* where the TBA value gradually decreased thereby arresting the formation of malonaldehyde. In cinnamon at 6th week the value dropped down to 0.191 which is comparable with BHT antioxidant activity. However in the initial level it was not very effective. Moreover it was noted that much variation in the antioxidant activity of *panchforon* was not found and hence can be concluded to be effective to same extent throughout the storage.

Para-anisidine values: *Para*-anisidine value reflects the magnitude of aldehydic secondary oxidation products and rate of formation of these product[26]. It reacts with volatile carbonyl compound. All the spices underwent dramatic increase in value higher than control in 3rd, 4th and 5th week (Table 1). Cinnamon and BHT reached their peak in 3rd week while fennel, ginger and *panchforon* recorded their highest in 4th week. Pepper and celery showed maximum value in 5th week. The values decreased noticeably in the last week. Fennel measured lowest followed by celery. The unusual high value observed in spices may be due to presence of volatile compound in the spices.

Iodine value: Iodine value measures the degree of unsaturation of oil[11]. In case of polyunsaturated fatty acids the double bonds can be conjugated and/or non-conjugated[17, 18]. The values gradually increased in case of celery, *panchforon*, ginger, fennel (Table 1). In case of black pepper, cinnamon, BHT the values suddenly decreased in third week, and again increased from fourth week. In case of control the values increased in second week, but gradually decreased from third week.

Conjugated dienes and trienes value: The formation of conjugated dienes and trienes as a result of long storage requires presence of unsaturated fatty acids with at least two double bonds and with more than two double bonds respectively in the lipid samples. The UV absorbance measurement at 233 nm and 268 nm indicates the formation of conjugated dienes and trienes. In case of BHT and control these values gradually increased. In case of celery and ginger CT increased upto fourth week and CD increased upto third week but the decreased from fifth week and fourth week respectively. [Table (1)] In all other cases CD and CT increased upto fourth week then it gradually decreased. [Table (1)] In all other cases CD and CT increased upto fourth week then it gradually decreased.

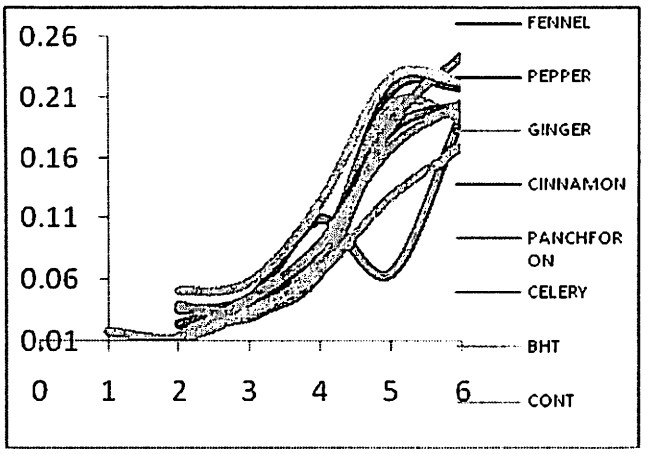

Fig 3: Total phenol in fish (Tannic acid equivalent)

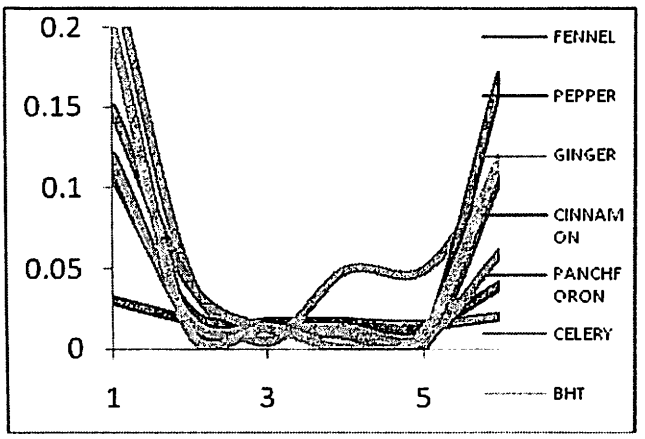

Fig 4: Total phenol in spices (Tannic acid equivalent)

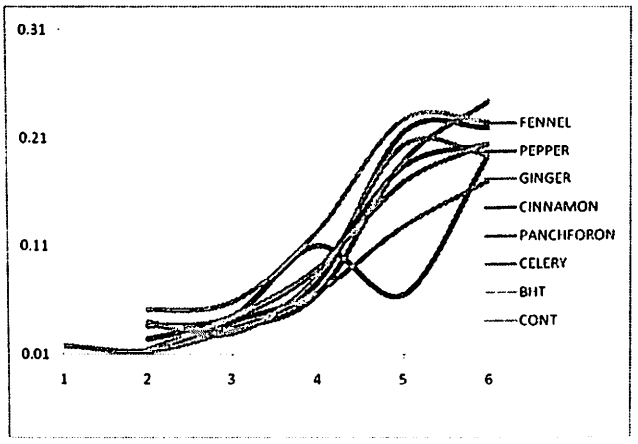

Fig 5: Total phenol in fish (Gallic acid equivalent)

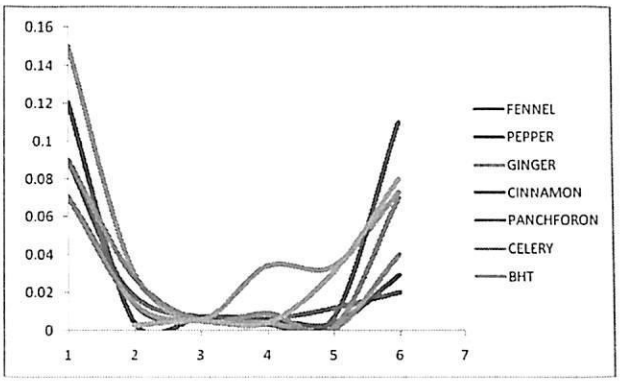

Fig 6: Total phenol in fish (Gallic acid equivalent)

Total phenol content: The total phenolic content in the fish sample mixed with various spices were determined spectrophotometrically with Folin-Ciocalteu reagent using the modified method of Wolfe et al (2003)[24]. Phenolic compounds are very important plant materials because of their inhibitory effect on autoxidation of oils[16] and their radical scavenging ability[9]. Therefore, it is important to determine the total phenolic compound in the spices as well as in the fish oil. The total phenol content was found to increase in all cases with time thereby indicating accumulation of phenolic compounds in the fish oil. [Graph 3 & 5] In the spices though concentration increased slightly in the last week, a major drop in value was observed from 2nd to 5th week.[Graph 4 & 6] It is interesting to note that though the phenolic concentration in fish oil is highest in *'panchforon'* and pepper, the highest concentration was recorded in cinnamon extract.

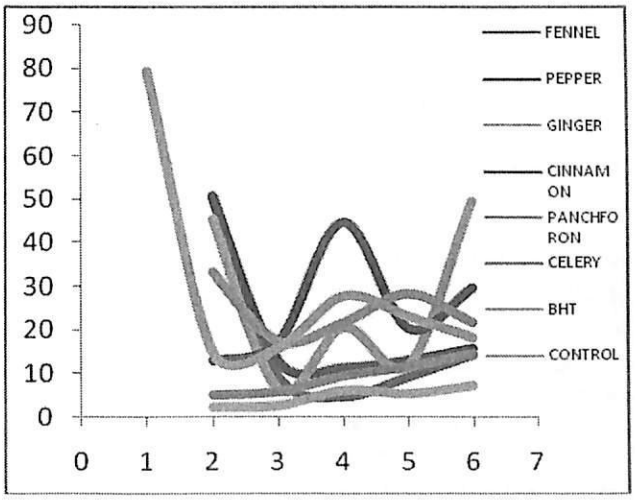

Fig 7: Antioxidant activity in linoleic acid system in fish oil

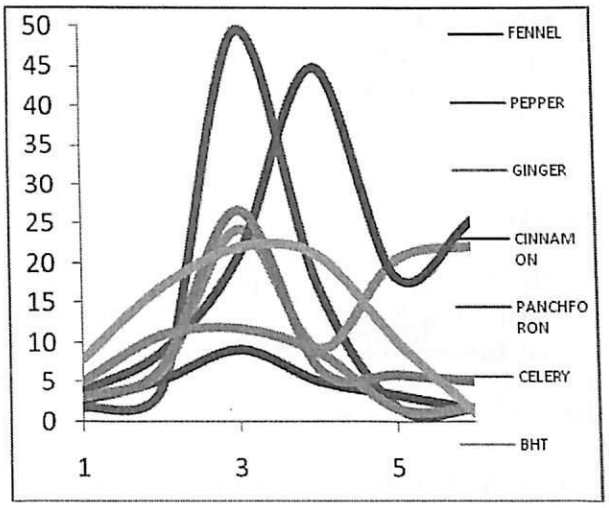

Fig 8: Antioxidant activity in linoleic acid system in spices

Antioxidant activity in linoleic acid emulsion: Antioxidant activities of fish sample mixed with various spices were determined according to the thiocyanate method[13]. The lower values in the Graph 7 indicates that there is a decrease in inhibition to peroxidation of linoleic acid in these spices. Maximum activity was observed in ginger followed by cinnamon. On the other hand an increase in the values in all the spices in the middle weeks indicates higher antioxidant activity of these spiced fish oil which gradually reduced with time. Cinnamon recorded highest activity in this period. It is interesting to note that among the values recorded (Graph 8) for individual spice extracts, fennel and cinnamon showed highest inhibition to peroxidation.

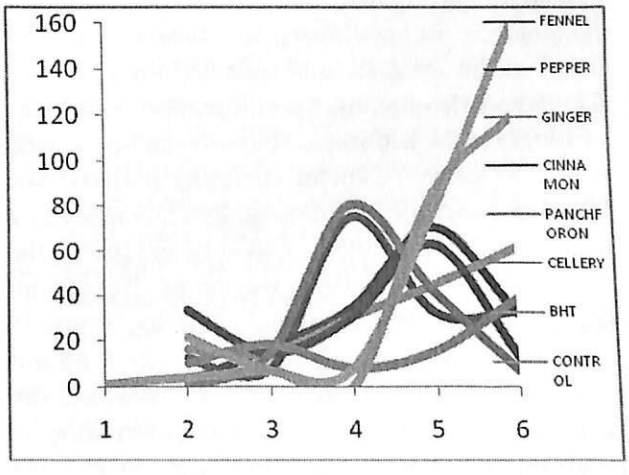

Fig 9: Metal chealating activity in fish oil

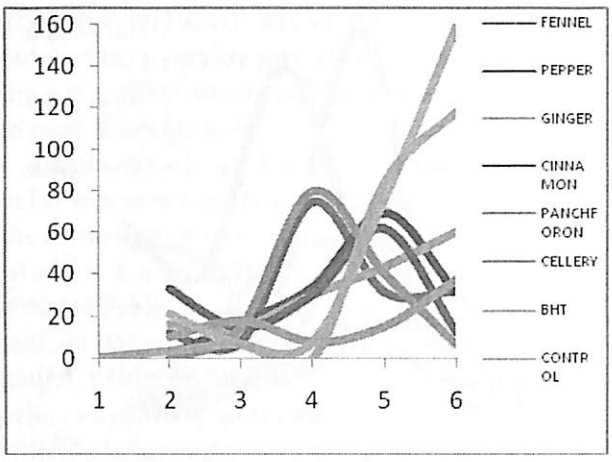

Fig 10: Metal chealating activity in spices

Metal chelating activity: Ferrozine can quantitatively form complexes with Fe^{2+}. However, in presence of other chelating agents this complex formation is disrupted. This results in the decrease in red colour intensity of the solution. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The ferrous ions possess the ability to move single electrons by virtue of which it triggers propagation of many radical reactions, even with relatively non-reactive radicals[2]. The main strategy to avoid reactive oxygen species generation is through chelating of the metal ions. The above Graph 9 exhibits that the value has increased in case of control and BHT, though the later decreased slightly in the 2nd week. Cinnamon, celery, *panchforon* pepper and fennel showed reduction in colour intensity in the later part of the time span. *Panchforon* dropped down to a lowest value of 0.557 in the 2nd week and 6.557 in the 6th week. Cinnamon also recorded a comparable low value of 13.031 in the last week. Hence it can be inferred that most effective metal chelating activity was found in *panchforon*, followed by cinnamon. The least activity was noted in case of ginger. In the spice extract (Graph 10) it was noted that though fennel showed very low metal chelating ability in the initial week gradually improved with time and recorded highest activity in the 6th week. On the contrary extracts of cinnamon and celery in spite of exhibiting a considerable low value in 4th week and *'panchforon'* in 3rd week could not maintain its activity in the final week.

Correlation study: Correlation between various parameters of lipid oxidation like peroxide value, TBA and p-anisidine value have been depicted in the following figures. *'Panchforon'* and cinnamon was found to record higher positive correlation value (0.66 and 0.61 respectively) in Graph 11. Others show little correlation between TBA and peroxide value. In Graph 12 BHT and celery recorded high positive correlation (0.75 and 0.724 respectively) between *p*-anisidine and peroxide value whereas other did not show a high correlation except pepper which recorded a moderate value of 0.506. In Graph 13 high positive correlative is observed in pepper, fennel and panchforon while a higher negative co relation is observed in case of control. The positive correlation in spices indicate that in spite of increase in peroxide value the fish oil is capable if inhibiting further peroxidation due to diffusion of spices.

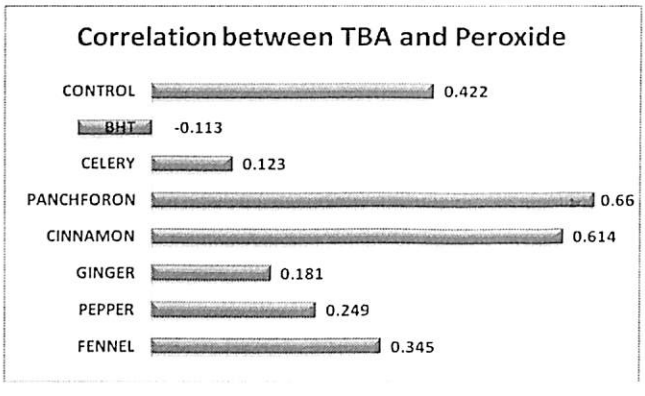

Fig 11: Correlation between TBA and peroxide

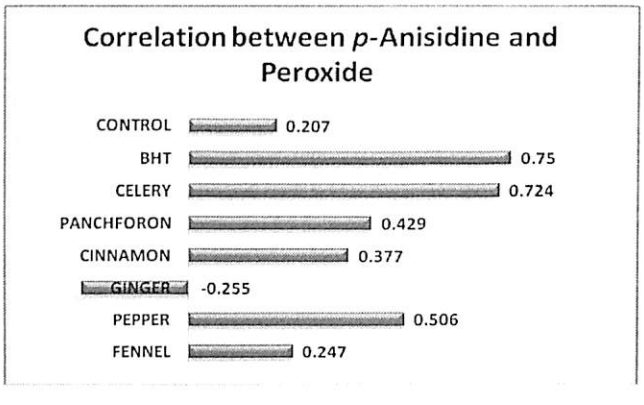

Fig 12: Correlation between p-anicidine and peroxide

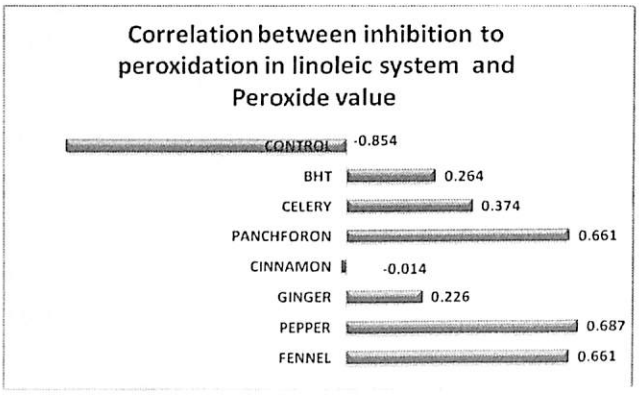

Fig 13: Correlation between inhibition to peroxidation in linoleic system and peroxidation

Conclusion

This study has provided us with some interesting and important findings which can help us in storage of fish under domestic refrigerating condition. Lipid composition varied in all the cases as monitored through thin layer chromatography and individual estimation of cholesterol, phospholipid and free fatty acid. Cholesterol and free fatty acid increased considerably while phospholipid decreased significantly. The interesting finding was that cinnamon and *panchforon* reduced the malonaldehyde accumulation which is attributed to the antioxidants of the spices that has reacted with the malonaldehyde. Conjugated diene-triene value indicated the effectiveness of the spices in ultimately reducing the values of otherwise normally increasing trend. Antioxidant activity of the spices in the fish oil revealed that the fish on long storage with spices becomes enriched with antioxidants which are beneficial to human body. The spices showed fluctuating antioxidant activities which sometimes increased in the initial weeks or in the later part of the time period. The highest phenolic concentration and inhibition to peroxidation was recorded in cinnamon extract. Finally it can be concluded that cinnamon showed the maximum efficiency in controlling oxidation as well as high antioxidant activity. Panchforon also exhibited considerable activity as an antioxidant. Other spices like celery, *panchforon* were found to be effective in short term storage.

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A Study on Efficiency of Edible Food Packaging on Soft Fruits

Anuradha Sharma and Mrs. Ahalya Pai

ABSTRACT

Edible food packaging was prepared using gum arabic and was tested for increase in post-harvest life of the sample (tomato). The results obtained show that the coated fruit had a longer ripening period as compared to control fruit, which increased with the proportion of the coating solution. Functional properties of the fruit like the antioxidant activity and vitamin C content was also seen to be maintained. Most of the best results were seen in the 10% gum arabic coated fruits in the 18 day cycle though it was expected in the 15% coated fruit. Due to much delayed ripening of the 15% gum arabic coated fruit, the sensory characteristics, lycopene and vitamin C content did not develop in the fruit, and thus even other characteristics like titratable acidity and soluble solids content were affected.

Keywords: Edible Food Packaging, Post Harvest Quality, Shelf Life, Gum Arabic, Soft Fruit

Introduction

In India, there is a vast scope for growing fruit and vegetable throughout the year in one or other part of the country because the climatic conditions are highly suitable for growing various types of fruits and vegetables. Fruits and vegetables are highly perishable but most important commodity for human diet due to their high nutritional value. They are the cheapest and other source of protective food supplied in fresh or processed or preserved form throughout the year for human consumption.

Fruits and vegetables are available in surplus only in certain seasons and availability in different regions. In peak season due to improper handling practices, marketing, storage problems around 20-25% fruit and vegetable are spoilt in various stages. Fruit and vegetable are living commodities as they respire. Hence, proper post harvest management handling and processing is required in horticulture crops. In general, an inverse relationship has been shown between respiration rates of fruits and vegetables and their postharvest shelf-life.

The concept of using edible coatings to extend shelf life of fresh and minimally processed produce and protect them from harmful environmental effects has been emphasized based on the need for high quality and the demand for minimal food processing and storage technologies. By regulating the transfer of moisture, oxygen, carbon dioxide, aroma, and taste compounds in a food system, edible coatings have demonstrated the capability of improving food quality and prolonging shelf life of fresh produce. Edible coatings may also be used to advantage on processed fruits and vegetables for improving structural integrity of frozen fruits and vegetables and preventing moisture absorption and oxidation of freeze-dried fruits or vegetables.

Methodology

Preparation of Fruit:

Freshly harvested tomato fruits at the mature-green stage of ripening were selected. The fruits were visually selected for uniformity in size, colour, absence of blemishes and fungal infection, and transported to the laboratory. Before treatment was applied, fruit were washed with a solution of sodium hypochlorite (0.05%) for 3min, and air-dried at ambient temperature.

Preparation of Edible Food Packaging Material:

To prepare gum Arabic coating solutions at 5, 10, and 15 % (w/v), 5, 10 and 15 g of powder was dissolved in 100mL purified water. The solutions were stirred with low heat (40 °C) for 60 min on a magnetic stirrer, and then filtered to remove any undissolved impurities using a vacuum flask. After cooling to 20 °C, glycerol monostearate (1.0%) was added as a plasticizer to improve the strength and flexibility of the coating solutions. The pH of the solutions was maintained at 5.6 using 1N NaOH. The coating treatments were selected according to preliminary experiments in tomatoes to assure adherence and steadiness of the coatings.

Six fruits were immersed in each concentration of gum Arabic coating solution (5, 10, and 15%) for 2-3 min and the coating solution was applied uniformly on the whole surface, while control fruit

were dipped in purified water. After treatment, fruit were air-dried, packed in cardboard boxes and stored at room temperature for 18 days. The data were recorded before treatment (day 0) and at 3-day intervals for 18 days. The entire process was repeated for 3 times and average of the 3 readings was taken the analysis.

Sensory Evaluation:

12 panel members were asked to perform the sensory evaluation throughout the assessment period and the same panel was maintained. They were asked to compare the given sample with fresh tomato used in salads. The tomato pieces were presented in front of the panel members and they were asked to evaluate and fill the sensory evaluation form. Sensory evaluation of the fruit for pulp colour, texture, flavour and overall acceptability for all the samples was done at each interval of 3 days. The evaluation was scored on the basis of 9 point hedonic scale.

Weight Loss Percentage

Tomato samples were weighed at day 0 and at the end of each storage interval. The difference between initial and final fruit weight was considered as total weight loss during that storage interval and calculated as percentages on a fresh weight basis by the standard AOAC (1984) method.

Weight loss percentage = Difference with Day 0 / Initial Weight X 100

Colour: Lycopene Test

The low volume hexane extraction method (LVHEM) was performed as in Fish et al. (2002). Approximately 0.6 g (determined to the nearest 0.01 g) duplicate samples were weighed from each puree into beakers that contained 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% USP grade ethanol, and 10 ml of hexane. Purees were stirred on a magnetic stirring plate during sampling. Samples were extracted by rotating by hand in orbital manner for 15 min on ice. After shaking, 3 ml of deionized water were added to each beaker and the samples were shaken for an additional 5 min on ice. The solution was then transferred to separating flask through filter paper and was then left at room temperature for 5 min to allow for phase separation. The absorbance of the upper, hexane layer was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was then estimated using the absorbance at 503 nm and the sample weight. The lycopene content of the tissue was estimated by the following relation:

Soluble Solids

The tomatoes from each treatment were ground in a blender and juice from the fruit was used to determine the soluble solids concentration (SSC) using a Pocket Refractometer. The machine was standardized using purified water before readings were taken.

Titratable Acidity

10mL of the tomato juice was pipetted into a 50mL beaker. The pH probe was inserted into the tomato juice. It did not fully immerse, however once the titration began it was covered. The initial reading of the NaOH 0.1M on the burette was noted to an accuracy of 0.1ml. Titrating was done with NaOH from the burette, swirling the beaker so that the juice and NaOH mix, and that the pH probe was fully wetted. Titrating was continued until the pH is approached 7.5. Titrating was stopped and the pH was allowed to stabilise. Then NaOH was added drop wise until the pH approached 8.2. The final burette reading was noted down to an accuracy of 0.1ml. The initial was subtracted from the final to calculate the volume required for the titration. This volume was multiplied by 0.64, to determine the titratable acidity expressed as g/L of citric acid at and end point of pH 8.2.

Ascorbic Acid Test

10 ml standard ascorbic acid solution was taken in the pipette and 1 ml of the dye in the test tube. To the test tube, 1-2 drops of glacial acetic acid was added. Then the dye was titrated with the standard ascorbic acid solution till the colour disappeared. This pink colour has to persist to 30 seconds. The volume of the required ascorbic acid solution was recorded to standardize the dye. The titration was repeated 3 times to get concordant value.

Titration of unknown tomato sample: Tomato paste was centrifuged in cold for 15 min at 15000 rpm. The superficial liquid was poured in a beaker and 10 ml of it was taken in the pipette and 1 ml of the dye in the test tube. To the test tube, 1-2 drops of glacial acetic acid was added. Then the dye was titrated with the sample till the colour disappeared.

The volume of the required sample was recorded to standardize the dye. The titration was repeated 3 times to get concordant value.

Microbiological Analysis

Serial dilutions of the tomato samples were prepared. The tubes were mixed well before each transfer. Transfer of 1 ml of the dilutions (10-5) was carried out to nutrient agar plates (sabaraud media), and the plates were labeled according to the sample being used. The inoculum was spread evenly on the entire surface of the nutrient agar plates until the medium no longer appears moist. The petri plates were inverted and kept in incubator at 37° C for 72 hours and total plate count was taken and recorded.

Antioxidant Activity

2 gm Tomato puree with 20 ml distilled water was centrifuged at 8000 rpm for 15 minutes and supernatant was separated. Various measures of supernatant (0.5, 1, 1.5, 2, 2.5 ml) in deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50 °C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (0.5-2.5 ml) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Results and Discussion

Sensory Evaluation

The overall acceptability has been towards 10% coated sample till the end of cycle and the other samples had similar scores as for taste and texture. Overall acceptability depends on the different parameters like taste, colour, texture, and smell, and the results show that the taste, smell and texture were maintained best in the 10% sample.

Microbial Contamination

Microbial contamination was seen maximum in the control sample and 15% coated sample till the end of cycle. It was expected that control sample will develop microbial contamination, but 15% sample was expected to have minimum plate count. There was visible contamination on the fruit by the end of cycle with cottony growth. Some of the fruits were eaten up by the contaminants to some extent. This led to more weight loss, off smell and probably texture loss also in the 15% samples. 10% mostly had low scores except for day 12. May be this was due to manual error as one of the readings showed 114 total plate count which increased the average. 5% sample also had considerable amount of contamination seen after day 6.

Weight Loss Percentage

Weight loss percentage was highest in 15% sample, though weight loss increased gradually in all samples. As explained earlier microbial contamination had eaten up the fruit with 15% coating till some extent which also caused severe weight loss to occur. 10% sample always had lower weight loss percentage but strikingly most of the times 5% sample had more weight loss percentage as compared to control sample beyond day 12. This shows that 5% coating is not sufficient in counteracting the vapour pressure of the atmosphere. Though 10% sample had lower percentage it was not considerably lower than control sample.

Ascorbic Acid Content

Earlier studies show that in tomato fruit, ascorbic acid content increases with maturity and stage of ripening. However once fruit reach the full ripe stage, ascorbic acid content starts to decline. Similar trend was seen in control sample. After day 12 the vitamin C content started deteriorating. Same trend was seen in 5% coated sample, but the vitamin C content always remained much lower. This trend came because the average of day 9 for 5% was lower as compared to other samples as shown in the table below. 10% was always on the increasing trend and has not reached the peak in the 18 day cycle. A longer cycle had to be tested to know proper results in this regard. 15% sample too had good scores but peak is not visible in the results because the day 15 reading came lower and proper anticipation cannot be done in this regard.

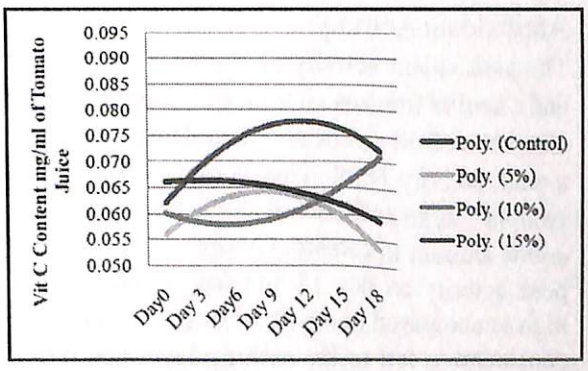

Fig 1: Vitamin C Content Treadline

Soluble Solid Content

In general, there was a gradual increase in Soluble Solids Content during the 18 day cycle. The Soluble Solids Content was significantly higher in control compared to coated fruit and the reduction in Soluble Solids Content in coated fruit was directly proportional to the concentration of the coating. The lowest Soluble Solids Content at the end of the storage period was recorded in fruit coated with 15% gum arabic.

Titratable Acidity

The values show that minimum deviation was seen in 10% sample. The low level of TA in control fruit compared to coated fruits suggests that the gum arabic coating delayed ripening by providing a semi-permeable film around the fruit. Since organic acids, such as malic or citric acid, are primary substrates for respiration, a reduction in acidity is expected in highly respiring fruit.

Lycopene Content

Lycopene is the red pigment and antioxidant in tomato which is known to increase as the fruit ripens. The obtained data shows that control sample had highest lycopene content as compared to coated samples and the lower lycopene content was always proportional to the percentage of the edible coating. Most of the 15% coated samples remained green till 15th or 18th day of the cycle. This clearly shows that ripening was delayed with the help of edible coating.

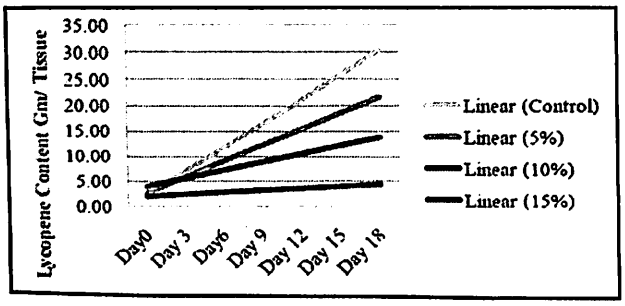

Fig 2: Lycopene Content Treadline

Antioxidant Activity

The antioxidant activity as shown by the sample had a similar trend as vitamin C. It increased in the samples as fruit ripens and then deteriorated after a peak activity level. This is due to the fact the vitamin C is an antioxidant and is found in considerable amount in tomato. Control sample showed peak activity on day 12 and the activity was not high as compared to day 0. After day 12 there was considerable fall in the activity level and day 18 had the lowest values. 5 % coated sample showed peak activity on day 15 and day 18 activity level was almost similar to day 9. It was seen that there was considerable reduction on day 3 then activity increased thereafter. The 10 % coated sample showed peak on day 12 and 15 and the day 18 activity level was also considerably high. The 15% coated sample had even higher antioxidant activity till the end of cycle as compared to 10% sample and its peak occurred in day 12. Vitamin E and ß-carotene are other antioxidants present in tomato which should be responsible for high antioxidant activity in 15% coated sample. Day 15 values were little less than 10% values and day 18 antioxidant activity was also considerable. The results show that edible food packaging helps to increase the post-harvest life of the rapidly perishable fruit, tomato. It has not only extended the ripening period but has also helped in maintaining good levels of functional properties of the fruit. In all cases the results were proportionately better with the increase in edible coating percentage, with little exceptions for 15 % coated sample.

5% coated sample had better results than control fruit but there wasn't a considerable difference in most of the cases and weight loss was seen higher than control fruit. Therefore 5% solution of the edible food packaging was not sufficient enough to control fruit deterioration. Although the results for 5% coated sample indicate that edible food packaging does help but a higher concentration will be required.

10% coated sample had best results in most of the experiments. Most importantly it was the preferred sample in the sensory evaluation till the end of the 18 day cycle. Consumer preference is one of the most prominent factors to decide whether the technology can be commercialized or not. Apart from sensory qualities, it has also extended ripening considerably well, improved functional qualities (Vitamin C, Antioxidant activity), and there was considerable less microbial contamination seen.

The only setback is that 10% sample did not counteract the vapour pressure of atmosphere thus there was not considerable difference with regard to weight loss percentage. A combination of gum arabic with another edible packaging material may help to overcome this problem. Thus, further research is required.

15% coated sample had very delayed ripening, probably due to this, this sample never scored high in the organoleptic properties in the sensory evaluation. It also showed high microbial contamination and low vitamin C content. Thus, this concen-

tration cannot be accepted for edible food packaging even though the results were comparably better in regards to lycopene content, titratable acidity, soluble solids content and antioxidant activity content.

Therefore, it can be concluded that 10% coated sample showed the most acceptable results and further research can be done in combination with 10% gum arabic solution and other components to overcome the vapour pressure.

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Tannase Extraction from Agro-waste and its Application in Debittering of Fruit Juice

Shweta Singh and Sonali Ghosh

ABSTRACT

An extracellular tannase was extracted from the fermented agricultural waste like red gram husk, sugarcane bagasse and rice straw using Aspergillus niger and Aspergillus oryzae. Tannase production was evaluated using solid-state fermentation (SSF) at different pH and incubation period. The optimum condition of pH and incubation period was found to be 5.5 and 72 hrs of incubation. The crude enzyme was purified using acetone precipitation followed by DEAE cellulose chromatography which leads to 21.36 fold of purification and 23% yield. SDS–polyacrylamide gel electrophoresis revealed the band of tannase at 72 kDa. During gel localization study, the native form of the purified enzyme was detected at 180 kDa (approx). The present work was aimed to lower the bitterness and astringency and thereby to enhance the quality of juice using tannase. The apple juice was treated with purified tannase (10% v/v) with an activity of 100 U/ml at room temperature for 2 hrs. Tannase treatment resulted in 81% degradation of tannins in the treated fruit juice when compared with the control juice. The results were encouraging as the overall acceptability of the juice was satisfactory with minimal loss of nutritional constituents in the treated juice.

Keywords: Apple Juice: Bitterness: Nutritional Constituents: Red Gram Husk: Rice Straw: Sugarcane Bagasse: Tannase and Tannin Degradation.

Introduction

Apples are widely consumed, rich source of phtyochemicals and excellent source of dietary fiber. The epidemiological studies have linked the consumption of apples with reduced risk of cancer, asthma and diabetes[1, 17 & 18]. The potential health benefits of apples are numerous but the processing of apples for juice do not effectively decrease tannins(polyphenols) which when exceeds the limits interact with other constituents of apple resulting in haze and bitterness. Therefore there is an increased concern in the fruit juice industry about the loss of market due to bitterness and haziness in the juice[16].

Industrial clarification procedures for juices typically involve physico-chemical precipitation of sediments and haze-active components. All these industrial applications are arborious and expensive, considered harmful and require special handling and disposal procedures. Enzymatic treatment of fruit juice has got advantage over conventional industrial procedures to produce lower haze in juice and increase its shelf life[13].

Tannin acyl hydrolase, commonly referred to as tannase (E.C: 3.1.1.20), is an enzyme that cleaves ester linkages in hydrolysable tannins [2, 6] producing glucose and gallic acid [3] by various filamentous fungi. Several agro-industrial waste and by-products such as orange bagasse, sugarcane bagasse, wheat bran, red gram husk, rice straw and other food metabolites are effective substrates for depolymerizing enzyme production Tannase is industrially used as a catalyst in manufacture to gallic acid. It is also potentially utilized as a clarifying agent in instant tea, wine, beer, beverage and fruit juice processing.

An attempt was undertaken using solid state fermentation of agricultural waste residues with Aspergillus niger and Aspergillus oryzae for the production of tannase. Based on the potential use of tannase to reduce tannin levels in fruit juice, first time efforts were made for debittering of apple juice by controlling its bitterness without significant loss of quality and increase the mean life expectancy of the product.

Methodology:

Chemicals

Agar agar from Qualigens Fine Chemicals, Dextrose from HiMedia Laboratories Pvt.Ltd, Urea. Magnesium sulphate, Potassium chloride, Potassium phosphate, Ammonium nitrate, Sodium chloride, Magnesium sulphate, Tannic acid, Citrate buffer (Citric acid, Sodium citrate), Tris HCl, Hydrochloric acid, Acetone, Gallic acid, Ethanol, Acetate buffer (Acetic acid, sodium acetate), ß mercaptoethano, Coomassie blue, Tetramethylethylenediamine, Bromophenol blue, Methanol, Sodium dodecyl sulphate-Triethanolamine solution, Ferric chloride, 2,6 di chlorophenol indophenols, Bovine serum albumin, Copper sulphate, Sodiun potassium tartarate and Folin-Ciocalteau reagent from Merck Ltd. Diethylaminoethyl cellulose, Acrylamide, Sodium dodecyl sulphate, Ammonium persulphate, PMSF, Rhodanine, Methyl gallate and Quinine hydrochloride from Sigma Aldrich. Molecular weight marker SMO671 from Fermentas

Sample collection

Fresh apple, canned apple juice was purchased from Real (Dabur food products). Red gram husk, rice straw and sugarcane bagasse were procured from the local markets of Kolkata. Aspergillus niger from college laboratory and Aspergillus oryzae (MTCC 634) from IMTECH Chandigarh.

Substrate pre- treatment

Sugarcane bagasse and rice straw and red gram husk from were procured locally, sun dried, pulverized and stored in air tight containers for further use[11].

Preparation of spore inoculums

A strain of Aspergillus niger and Aspergillus oryzae MTCC 634 was used for the study. The inoculum was prepared by adding 10 ml of sterile distilled water to the sporulated slants and dispersed the spores [11]. 1 ml of the respective spore suspension was inoculated in to the respective sterilized production medium.

Substrate and Solid State Fermentation

5 gram Red gram husk and 5 gram mixed substrate of rice straw powder with sugarcane baggase powder (1:1 ratio) was moistened with 10 ml of salt solution respectively. The composition of the salt solution was NH_4NO_3 0.5 %, NaCl 0.1 %, $MgSO_4 \cdot 7H_2O$ 0.1% and Tannic acid 4% at pH 5.5[11]. The contents were sterilized by autoclaving at 121°C, for 20 min. The solid substrates were inoculated with 1 ml spore inoculum of A.niger and A.oryzae (MTCC 634) respectively and were incubated at 37°C for 72 hrs.

Extraction and analysis of crude enzyme

Tannase was extracted from the fermented substrate by adding pre-cooled 0.05 M citrate buffer, pH 5.0 and PMSF added and crushed with mortar and pestle in cold, the crude enzyme was centrifuged at 8000 rpm at 4°C for 20 min[11]. The filtrate was collected and stored for estimation of tannase activity.

Tannase activity assay

For determining the tannase activity time kinetics was performed based on gallic acid production. 0.35% (w/v) Tannic acid solution in 0.05 M pH 5 citrate buffer was prepared. To 25 µl of enzyme 100 µl of tannic acid solution was added and the volume of the reaction mixture was made up to 200 µl with citrate buffer 0.05 M pH 5. The reaction mixtures were allowed to incubate for 0, 10 and 30 mins. Reaction was stopped by addition of 1 ml ethanol to 10 µl of reaction mixture at different time interval[3,5]. Absorbance was measured at 310 nm. Gallic acid was used as standard. One unit of tannase is the amount of enzyme which liberated 1 µl of gallic acid in one minute.

Protein assay

Protein was estimated following Lowry's method[12].

Purification steps:

Acetone Precipitation & concentration of tannase

To the crude enzyme four times the sample volume, cold (-20°C) acetone was added drop by drop with constant stirring. After 60 mins of incubation the enzyme protein was centrifuged for 10 mins at 6000 g and was concentrated by ultafiltration on centricon membrane filter with a 50 kDa molecular mass cut off[12]. The concentrated extract was dialyzed in 0.02M acetone buffer pH 5 overnight. Tannase activity and protein were assayed. The proteins in each fraction were monitored by SDS-PAGE.

Anion exchange chromatography on DEAE Cellulose column

Dialyzed diluted sample was applied to DEAE cellulose chromatography column, equilibrated with 20 mM acetate buffer (pH 5). It was eluted with 50 mM acetate buffer (pH 5) by linear gradient of NaCl containing 0.1-1 M NaCl at the rate of at 1ml/10mins. The eluted fractions were collected and absorbance of the fractions was measured at 280 nm. The fractions with high optical densities were then assayed for tannase activity, and only the fractions possessing tannase activity were pooled together[12].

Molecular mass determination by Sodium Dodecyl Sulfate Polyacrylamide Gel electrophoresis (SDS-PAGE)

The samples were analyzed in 10% SDS-PAGE based on the protocol of Laemmli[12]. It was stained with coomassie blue stain and molecular weight was determined by comparing with prestained molecular weight marker.

Gel localization of tannase activity

100 µl of sample with tannase activity was mixed with 25 µl of sample buffer (3.1ml 1 M Tris HCl pH 6.8 + 5ml 50% glycerol +.5 ml 1% bromophenol blue and 1.4 ml water). The samples were loaded on 10 % gel and run at 70 V for 2.5 hrs. After running the gel was washed for 1 hour in 100ml 2.5% Triton X-100, followed by two 45 mins

washes with 100 ml 10 mM acetate buffer pH 5.5 with constant shaking. This was followed by incubation of the native gel containing tannic acid 0.5% (w/v) in 0.1 M acetate buffer pH 5.5 at 30° C with constant shaking. After 1 hour the tannic acid solution was replaced by 100 ml of 0.5% quinine hydrochloride solution in 0.05 M acetate buffer pH 5.5 at room temperature[12]. Tannase activity appeared as a clear band on a white background approximately at 180 KDa.

Juice preparation

Fruits were washed with water to remove any adhering substances. It was cut to pieces, seeds removed and juice was extracted by homogenizing in a blender followed by filtration through strainer. The extracted juice was stored at 4°C until use[13].

Treatment of juice with purified tannase

To 10 ml of juice of fruit juice taken in a test tube, 1 ml of tannase (100U/mL) was added. The control test tube received only 1 ml of water instead of tannase. Test tubes were then incubated at 37°C with gentle shaking up to 120 mins. The test tubes were then placed in water bath at 50°C for 10 mins to deactivate the enzyme. 1ml of fruit juice was taken at different time intervals and their tannin content was measured[13].

Tannin assay

The tannin content in the fruit juice was measured following the protein precipitation method by tannins[15].

Measurement of total phenolics

Total phenolics estimation was carried out with Folin-ciocalteu reagent[13].

Measurement of total sugar

To 20 µl of test sample, 1500 µl of working glucose reagent and 20 µl of standard glucose reagent was added. The reaction mixture was incubated at 37°C for 10 mins. The solution was diluted with 1500µl of distilled water and mixed well[19]. Absorbance was measured at 505 nm.

Measurement of total titrable acidity

The juice was centrifuged for 10 min at 5,000 rpm, to obtain a supernatant. To 10ml of the soup, slowly NaOH solution by the burette was added and when the pH reaches about 6, NaOH was added drop wise up to pH 8.3. The volume of NaOH used was recorded[13]. The titrable acidity is expressed as gm/lt of mallic acid at end point of pH 8.2.

Determination of pH

pH of the fruit juice was measured by pH meter

Ascorbic acid content

10 ml of centrifuged supernatant of the juice was taken in pipette and 1 ml of dye in test tube. To the test tube 1-2 drops of glacial acetic acid added. The dye was titrated with sample till colour disappears. The volume of sample recorded to standardize the sample. Titration was carried for 3 times[15].

Sensory evaluation

The organoleptic evaluation of controlled and test sample of fresh apple juice was performed[13], based on nine point hedonic scale.

Statistical Analysis

Data was subjected to analysis by standard deviation and mean comparison was carried by student's t - test.

Results and Discussion

Estimation of Tannin in Apple juice

Apple juice is a refreshing treat, but lags behind as a beverage due to its appearance and bitter taste. The bitterness factor in apple juice is result of their tannin content thus, it was essential to record the tannin content of both fresh and packed apple juice. The tannin levels present in fresh apple juice was 32 mg/100 ml and packed juice was 23 mg/100ml. The packed juice which has already undergone processing is also showing high tannin content. This can be attributed to polymerization of the tannins in presence of air. Therefore, it is essential to remove the tannins by a more superior process[14].

Effect of increasing substrate concentration

The enzyme activity was altered with the change in the substrate concentration. The concentration of the substrate tannic acid varied from 25 µl to 100 µl. It was observed, that within a time span of 10 mins all the available substrate i.e. 0.1ml of tannic acid was hydrolyzed to gallic acid and glucose. As the concentration of the substrate was increased simultaneously the activity also increased. Therefore, it was observed that the enzyme tannase extracted from the fermenting medium of red gram husk with Aspergillus niger has a significant ability to utilize the available substrate within a short time interval (Fig 1).

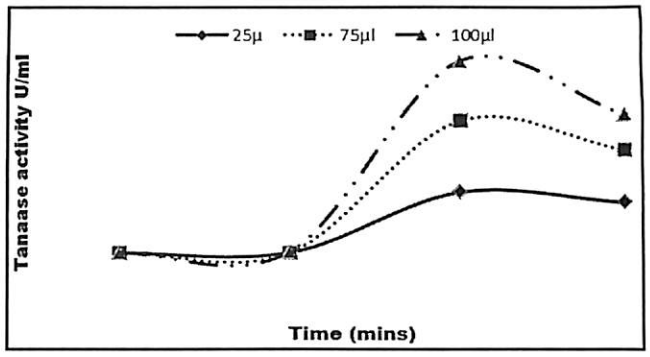

Fig 1. Effect of substrate concentration on tannase activity. With gradual increase in the substrate concentration the activity of the enzyme also increased gradually.

Tannase production by different substrate

The result in the present study may be varied due to the agro waste used as the fermenting mass. It was observed that redgram husk yielded a higher activity when fermented with Aspergillus niger (14.4U/ml) and Aspergillus oryzae (12U/ml). Whereas, the activity was considerably low in the fermenting mass of sugarcane bagasse: Rice straw (1.4U/ml). The rise in activity may be attributed to solid material's dual roles, supply of nutrients to the microbial culture and maximum enzyme production with red gram husk (Fig 2).

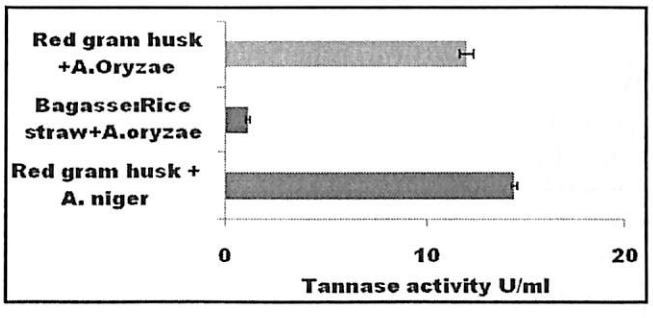

Fig 2. Extracellular tannase produced by different fungal strains Aspergillus niger fermented with redgram husk, Aspergillus Oryzae fermented with sugarcane bagasse : rice straw and *Aspergillus niger* fermented with red gram husk. T=37p, pH=5.5; t=72 hrs. Error bars indicate standard deviation from triplicate determinations.

Effect of pH and incubation period

To obtain maximum tannase production various parameters viz. pH and incubation period for two different fermenting medium and pure culture were optimized. It was found that tannase obtained from the fermenting mass consisting red gram husk and Aspergillus niger was higher as compared to Aspegillus oryzae and sugarcane bagasse: rice straw. The present study indicated optimum tannase production condition for Aspergillus niger and redgram husk was found to be pH 5.5 and 72 hr. The activity measured in the crude extract was found to be 14.4 U/ml. The activity of crude tannase produced extracellulary is similar to the earlier report[11]. One more set of experiment was conducted to confirm the viability of red gram husk as a fermenting substrate, where it was inoculated with Aspergillus oryzae at pH 5.5 for 72 hrs. This fermenting mass resulted in an activity of 12U/ml (Fig 3).

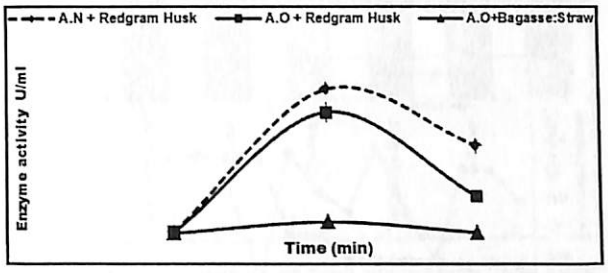

Fig 3. Extracellular tannase produced by different fungal strains *Aspergillus niger* fermented with redgram husk, *Aspergillus Oryzae* fermented with red gram husk and *Aspergillus Oryzae* with bagasse:straw . *T=37p, pH=5.5; t=72 hrs. Error bars indicate standard deviation from triplicate determination*

Table 1. Purification of Tannase extracted from the fermentation medium containing red gram husk and Aspergillus niger at each step.

Week	Fennel	Pepper	Ginger	Cinnamon	*Panchforon*	Celery	BHt Control	
Purification steps	Volume	Protein	Activity	Total protein	Total activity	Sp activity	Purification	Yield
	(ml)	(mg/ml)	(U/ml)	(mg)	(U)	(U/mg)	(Fold)	(%)
Crude Extract	30	4	14.4	120	432	3.6	1	100
Acetone precipitation	10	5.8	42	58	420	7.2	2	97
Centricon concentration	1.5	8.8	70	13.2	105	7.9	2.19	24
DEAE-C	1	1.3	100	1.3	100	76.9	21.36	23

Purification of Tannase from the fermented mass

The crude extract obtained by fermenting red gram husk with Aspergillus niger was partially purified by acetone precipitation at 4p C. The fractional purification procedure increased the activity of the enzyme to 42U/ml and the protein content was 5.8 mg/m. Other method of precipitation like ammonium sulphate precipitation was also tried but it yielded very less volume of enzyme with lower activity (3.8U/ml). This was followed by ultra filtration (50 kDa cut off) which resulted in a higher activity (70U/ml) and protein (8.8 mg/ml). Centricon concentration is an alternative step for gel filtration chromatography which separates protein according to their molecular weight.

Tannase was further purified by DEAE-cellulose column chromatography which led to an overall purification of 21.3 fold with a yield of 23% (Table 1). The elution profile (Fig.4) of the tannase extract obtained from the DEAE cellulose column showed eight protein peaks, tannase activity being found in five of the peaks. The active

fractions were pooled and used for studying the activity of the tannase. The fifth fraction pooled showed an activity of 100 U/ml which was used to run the native gel for localization of purified tannase activity. The tannase yield of 23% obtained in the present work was higher as compared to 20% yield reported by others[4, 7, 9].

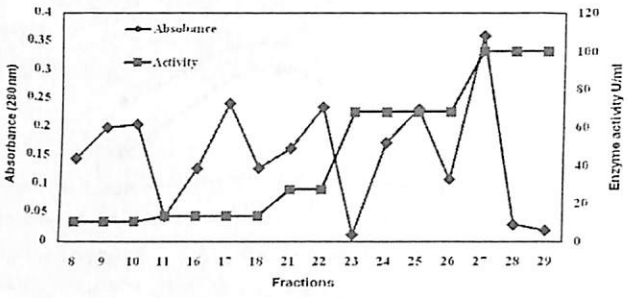

Fig 4. Elution profile of tannase produced by A. niger on DEAE cellulose using NaCl solution as eluant. The enzyme solution obtained from acetone precipitation and concentrated by ultrafication by centricon membrane was loaded onto DEAE cellulose column equilibrated in 0.2-M acetate buffer, pH 5.0. The flow rate was adjusted to 1 ml/min and 0.5-ml fractions were eluted with NaCl solution of linearly increased molarity (0.1 to 1 M)

Characterization of Purified protein by SDS-PAGE

Tannase containing fractions were pooled and were subjected to SDS-PAGE. The purified enzyme migrated as a single protein band corresponding to molecular mass of 72 kDa.

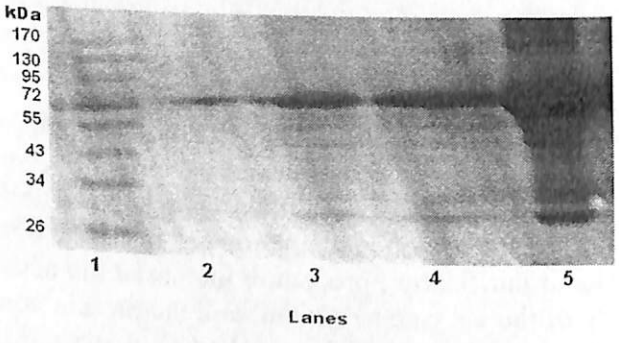

Fig 5. SDS –PAGE showing the Molcular weigh marker (Lane 1), crude extract (Lane 2), acetone precipitated sample (Lane 3), centricon concentration sample (lane 4), after purification by DEAE –C column (Lane 5). The SDS-PAGE analysis shows different fraction of enzyme obtained through subsequent purification.

Gel localisation of Tannase activity

Native gel detection method of tannase concluded that the native purified tannase is a dimeric protein of 180 kDa (approx). The insoluble tannic acid – quinine complex gave a white colouration to the gel with a transparent band confirming the existence and position of tannase.

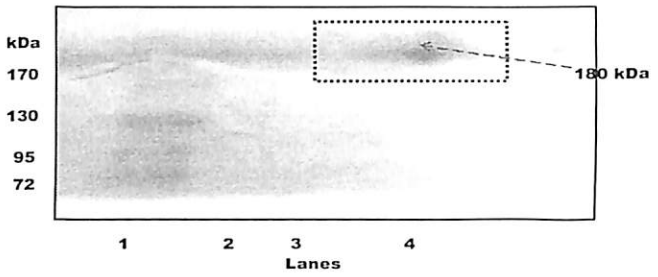

Fig 6. Gel localisation of tannase acitvity. Prestained protein marker (lane 1), crude extract (lane 2), acetone precipitation fraction (lane 3) and DEAE-C sample (lane 4). The fourth lane indicates the presence of native tannase of 180 kDa (approx) molecular weight.

Debittering of Fresh and Packed Apple Juice with purified Tannase

An 81% decrease in the tannin content of fresh apple juice was observed after 2 hrs treatment of the juice by purified tannase at 37°C (Fig 7). It was observed that the debittering efficiency of tannase gradually increased with time interval, reaching maximum after 2 hrs of incubation. The effect of purified tannase was in packed apple juice was also recorded (Fig 8). Earlier, a few studies were done to clarify the juice by partially purified tannase. There was a 35% reduction of tannin content in pomegranate juice by partially purified tannase[3, 13] resulting debittering. Moreover, only 24% reduction of tannins was reported in annola juice[14]. Whereas, the present study led to a higher percentage of tannin degradation (81%) and thereby was found to be more efficient for reducing bitterness.

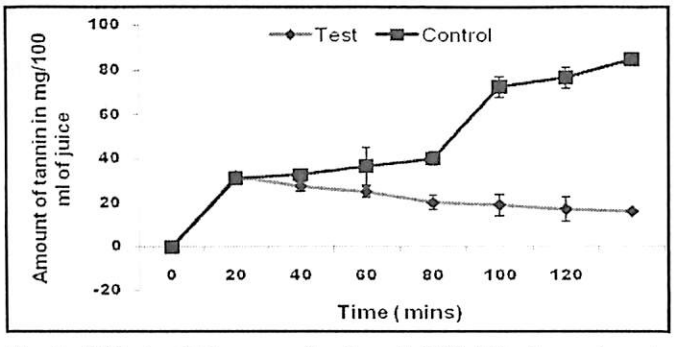

Fig 7. Effect of Tannase (1ml and 100 U/ml) on tannin degradation at different time in Fresh apple juice. Error bars indicate standard deviation from triplicate determination.

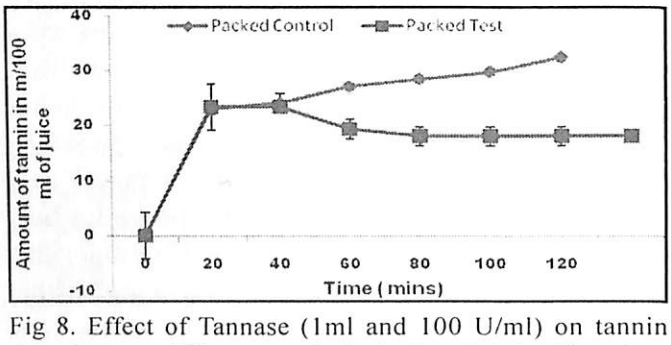

Fig 8. Effect of Tannase (1ml and 100 U/ml) on tannin degradation at different time in Packed apple juice. Error bars indicate standard deviation from triplicate determination.

Chemical and sensory evaluation

In order to determine the change in quality due to treatment with debittering aids, the non-treated(control) and treated(test) samples of both fresh and packed juice was subjected to analysis of ascorbic acid content, total phenol, pH, total sugar and titrable acidity.

The total sugar content was increased significantly by 20% and 30% in both packed and fresh juice respectively. Hydrolysis of tannins yields glucose and gallic acid. Therefore, increase of sugar levels in the juice is due degradation of tannin in apple juice.

The quality index showed 11% loss in fresh tested sample whereas packed test sample showed a loss of 10%. However, the vitamin C content in citrus juices after filter aids treatment leads to a measurable loss of up to 33% depending on types of filter aids and processing condition[13].

Successful tannin degradation was supported by the fact, as there was an increase in the total phenol levels indicating that the purified tannase acted on the available tannins in the juice and hydrolyzed it to gallic acid.

Sensory evaluation of the juice before and after treatment was performed, based on the quality parameters. Sensory score for appearance, colour, taste, astringency, odour and overall acceptability of the fresh apple juice treated with purified tannase protein by the trained panel are shown in Fig 9. It was evaluated that the acceptance of the juice has increased after treatment.

Table 2. Effect of tannase on the chemical parameters of fruit juice

Parameter	Non-treated juice		Treated juice	
	Fresh	Packed	Fresh	Packed
Total sugar (mg\100 ml)	83	37.5	100	50
Vit C mg\ml	0.81	0.19	0.72	0.17
Titrable acidity (gm\lt)	2.17	2.08	2.09	1.97
Total phenol (gm\100ml)	0.017	0.029	0.042	0.042
pH	4.8	3.9	4.3	3.7

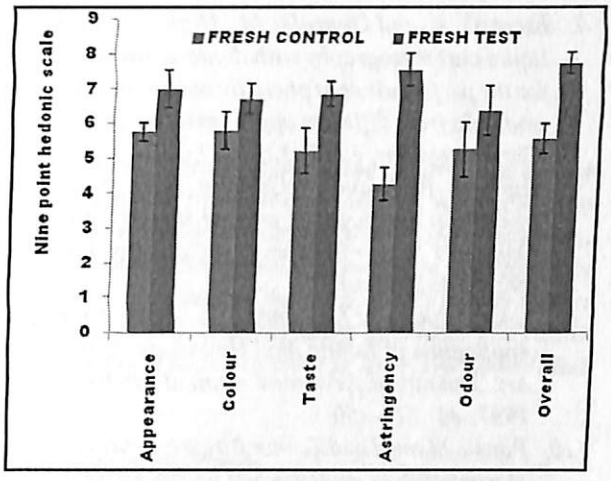

Fig 9. Sensory evaluation of fresh treated juice. The Apple juice was incubated with purified tannase of an activity of 100 U/mlfor 2 hrs. Sensory parameters were evaluated on the basis oh Nine point hedonic scale by semi trained pannel members . Error bars indicate standard deviation from triplicate determination.

Conclusion

The present study suggests that tannase produced by Aspergillus niger under optimized condition from a novel agro residue could be successfully used as a clarifying as well as debittering agent in apple juice. It also clearly states that the treatment done had no negative impact on the biochemical and quality attributes of the fruit juice.

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Heavy Metal Contamination in Street Foods of Kolkata

Debalina Ghosh and Vipasha Chakravarty

ABSTRACT:

Contamination of food and food products by heavy metals has made dietary intake as one of the major routes of these harmful elements to human beings. The human dietary intake of heavy metals from street foods (rice, vegetable chow, potato fry, sada vada) in Kolkata were determined. The cooked food was bought from the street food-vendors at 3 city locations (Dalhousie, Parkstreet, and Sector- 5) and analyzed. Heavy metal concentrations were determined by EDXRF (Energy Dispersive X-Ray Fluorescence) Spectroscopy after freeze drying of the samples. The results showed significant variation in heavy metal concentration among the foodstuff and at the different locations. The results were compared to the standard elemental concentrations of foodstuffs given by ICMR and the Tolerated Upper Limit of the elements to estimate risk of toxicity.

Key Words: Dietary Intake, EDXRF, Heavy Metal, Kolkata, Pelletiser, Street Foods

Introduction

Heavy metals represent a class of omnipresent pollutants, with toxic potential, in some cases even at low exposure levels. They concentrate in each trophic level because of their weak mobility. Systemic pollutants with such heavy metals are very dangerous because of their accumulation and retention by plants and animals. Most heavy metals are nonbiodegradable and their bioavailability and long biological half-life accounts for their bioaccumulation[16]. These can combine with minerals and oligominerals becoming blockers for these and depriving the living organisms of those elements essential for a healthy life. Heavy metals accumulate in the body and block the intracellular biochemical processes; they do not decay during food preparation. Zn, Cu and Pb are three of the most common heavy metals emitted by vehicle traffic[18]. Leaded petrol has caused more exposure to Pb than any other source worldwide. By contaminating air, dust, soil, drinking-water and food crops, it has caused harmfully high human blood lead levels around the world, especially in children. The nervous system of the foetus and infant is especially susceptible to lead, which can cross the placenta and penetrate the blood-brain barrier. Among the other heavy metals copper (Cu) is an essential element but excess exposure can cause hepatic and kidney damage, hemolytic anemia and methanoglobinemia[3]. Symptoms of acute zinc toxicity include nausea, vomiting, epigastric pain, abdominal cramps and diarrhea[6]. Manganese is more frequently of toxicological concern because overexposure to the metal can lead to progressive, permanent, neurodegenerative damage, resulting in syndromes similar to idiopathic Parkinson's disease[8, 27]. Iron toxicity cause haemorrhagic necrosis and sloughing of areas of mucosa in the stomach with extension into the submucosa [3]. Heavy metals are given special attention throughout the globe due to their toxic and mutagenic effects even at very low concentration[3].

Street foods are sold in almost every country in the world. The FAO (1989) defines street food as any ready-to-consume food that is sold in public places. In most towns and cities in India, selling of snacks and whole meals on the streets is an important way to obtain income. Street foods have a long tradition in most countries. Rapid urbanization is breaking down traditional family ties throughout the world and the street food sector is widely understood as an inevitable phenomenon tied to urban growth. This urbanization and the associated social and structural changes have caused the demand for street food to increase. Longer traveling times between living and working places are likely to lead to further increases in demand. Street food accounts for a part of the daily diet and so contributes towards meeting nutritional requirements, although the contribution varies. The foods sold in the streets are mostly not covered properly which is the most important risk factor for heavy metal contamination. Heavy metals emitted by vehicles and the street dusts thus tend to deposit in street foods. Food contamination monitoring is, therefore, important as it provides information on the levels of environmental contaminants in food thus ensuring the safety of food[16].

Methodology:

Sample collection:

A survey was conducted in various parts of Kolkata and it was found out that many of the office goers both the low income group as well as middle income group consume their whole meals from the street food stalls on a regular basis.

The selected areas for collection of samples were Dalhousie, Park Street Crossing and Saltlake Sector-5 since

All these areas are office areas.

People consume large quantities of street food regularly from these areas.

At peak hours, the traffic densities of these areas are very so the vehicular emissions are also high.

Similar foods are available in these areas.

The samples were: rice, vegetable chow, sada vada and potato fry. These foods were collected because they are most commonly consumed in large quantities. The food samples were collected on week days and weekends.

Sample preparation:

The samples were dried using freeze drier after cutting them into very small pieces to increase their surface area. They were put in clean glass vials and fitted with rubber cork. The glass vials were dipped in liquid nitrogen for pre-freezing the samples before freeze drying.

Pellet preparation:

After freeze drying dried samples were then ground to powder using mortar pestle. Pellets were made using the pelletiser from 150 mg of the powdered sample at high pressure of 120 kg/cm^2 for 2 min. However pressure to be applied and the duration depend upon the sample type. 5 identical pellets were prepared from each sample.

Elemental analysis of the samples by EDXRF:

In the present study, the Xenemetrix EX- 3600 EDXRF spectrometer has been used for the trace element analysis. This consists of an X- ray tube with an Rh anode as the source of X- rays with a 50 V, 1 mA power supply, Si (Li) detector with a resolution of 143 eV at 5.9 keV. The measurements were carried out in vacuum using Ti filter in front of the source with an applied voltage of 20 kV current 400mA. 10 sample turrets enable mounting and analysing 10 samples at a time. The in-built software nEXT carries out the quantitative analysis.

Determining the presence or absence of metal contamination by comparing the elemental concentration of the samples with the standard values:

After the elemental analysis of the samples by EDXRF, the concentration of each element present in the samples were compared with the standard elemental concentrations of Indian foods given by Indian Council of Medical Research (ICMR). At first a study was done to determine how much raw ingredients are required to prepare 100 gm of the cooked sample. It was found out that 60 gm of rice is required to prepare 100 gm of cooked rice; 70 gm of refined flour, 30 gm of carrot, 30 gm of onion, 30 gm of cabbage, and 30 gm of beans are required to prepare 100 gm of vegetable chow; 200 gm of raw potato is required to prepare 100 gm of fried potato and 100 gm of black gram dal is required to prepare 100 gm of sada vada.

Standard elemental concentrations for 100 gm of samples were calculated and compared with the elemental concentration of 100 gm of collected samples to check whether there is any difference between the two values, which will later help to determine the presence or absence of metal contamination.

Results and Discussion:

The elemental analysis of the street food samples carried out by the EDXRF spectrometer has shown the signature of many elements. The concentration of certain heavy metals like Manganese, Iron, Copper and Zinc were measured in the sample.

Iron:

Estimates of the Recommended Dietary Allowance (RDA) for iron depend on age, sex, physiological status, and iron bioavailability and range from about 10 to 50 mg/day. The average lethal dose of iron is 200–250 mg/kg of body weight[26].

The standard concentration of iron given by ICMR in 100 gm of rice, chow, vada and potato are 0.6 mg, 2.7 mg, 3.8 mg and 0.96 mg respectively[7]. In the present study the concentration of iron in rice sample collected on weekdays and weekends were found to be in the range of 3-5 mg per 100 gm. It was noticed that the concentration of iron in the rice sample collected from Dalhousie area on weekday and from Parkstreet area on weekend were relatively higher as compared to other locations. In case of chow the concentration was found to be in the range of 5-10mg/100 gms. In this case the concentration was highest for the weekday sample from sector 5. The iron concen-

tration in vada samples were found to be in the range of 6-7 mg per 100 gm. It was noticed that there was no significant difference in the iron concentration of the samples collected from all the three locations on both weekday and weekend. In case of potato fry the range of iron concentration was 6-18 mg per 100 gm. It was noticed that the concentration of iron in the potato fry sample collected from Parkstreet and Dalhousie area on weekday were significantly higher as compared to other locations.

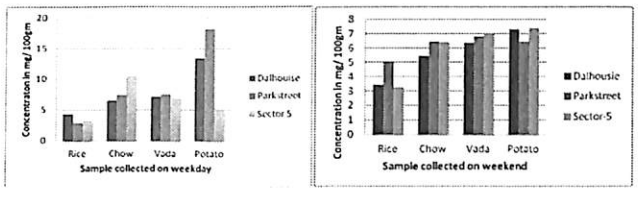

Fig. 1: Showing analysis of Iron concentration of samples collected on weekdays and weekends

After comparing the iron concentration of the collected samples, with the standard iron value of the particular food product, it was seen that the iron concentration of all the foods were significantly exceeding the standard values. An earlier report by Majumdar et. al. (2009) on accumulation of minor and trace elements in lichens in and around Kolkata states that, sites which are burdened with heavy vehicular load together with pollution from various industries have significantly higher concentration of iron in the atmosphere [14]. This iron can get deposited in the uncovered street foods. Another study by Ram S.S et. al. (2010) which deals with the characterization of dust particles deposited on the plant leaves of Kolkata also showed the high iron level in the street dusts [19]. Deposition of street dusts on the uncovered food can be another contributing factor for the increased iron level. A study carried out by state water investigation department (SWID) West Bengal (2011) revealed that iron content in water at some wards in the city is 10 times higher than the prescribed tolerance level. Another study by Sudarshan et. al. on PIXE measurements of drinking water of Saltlake, Calcutta (2000) showed that the range of iron in ground water of Saltlake area exceeds the certified limits [24]. This could be a contributing factor of increased iron content in the samples collected from Saltlake Sector-5. The water that is used to prepare the food products, if that has very high iron load that can contribute to high iron level of the street foods.

Copper:

The Recommended Dietary Allowance (RDA) for copper is 0.9mg/day and the Tolerated Upper Limit of copper intake is 5mg per day[4].

The standard concentration of copper given by ICMR in 100 gm of rice, chow, vada and potato are 0.10 mg, 0.22 mg, 0.93 mg, 0.32 mg respectively[7]. In the present study the concentration of copper in rice sample collected on weekdays and weekends were found to be in the range of 0.3-0.45 mg per 100 gm. It was noticed that the iron concentration in the rice sample collected from Sector-5 area on weekday and from Dalhousie area on weekend were relatively higher as compared to other locations. In case of chow the iron concentration falls within the range of 0.22- 0.38 mg per 100 gm. It was noticed that the concentration of Copper in the chow sample collected from Dalhousie area on weekday was relatively higher as compared to other locations. Copper concentration in vada samples were found to be in the range of 0.42- 0.58 mg per 100 gm. Here it was found out that samples collected from Sector-5 area showed the highest concentration of Copper on both weekdays and weekends. The concentration of Copper in potato fry samples were found to be in the range of 0.14- 0.40 mg per 100 gm. In this case the concentration of Copper in the potato fry sample collected from Sector-5 area on weekend was relatively higher as compared to other locations.

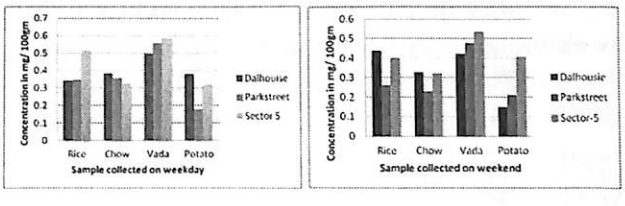

Fig. 2. Showing analysis of Copper concentration of samples collected on weekdays and weekends

After comparing the Copper concentration of the collected samples, with the standard Copper value of that particular food product, it was seen that the Copper concentration of vada was well within the standard value. Copper concentration of chow and potato fry slightly exceeded the standard value and copper concentration of rice significantly exceeded the standard value. A study by Amusan et. al. showed that within the city concentration of copper is a function of traffic density[1].This fact is also supported by a study by Ormod et. al. (1984). This atmospheric copper can get deposited on the street foods which are not covered[17]. While collecting the street foods it was seen that the rice

sample was not covered while preparing and selling. This might be the reason for high copper content of rice in relation to other samples.

Zinc:

The Recommend Dietary Allowence (RDA) of zinc is 11 mg/day and 8 mg/day for men and women respectively. Prolonged intakes of zinc supplements ranging from 50 mg/day up to 300 mg/day have been associated with a range of biochemical and physiological changes[6].

The standard concentration of Zinc given by ICMR in 100 gm of rice, chow, vada and potato fry are 0.78 mg, 0.73 mg, 3 mg and 1.06 mg[7]. In the present study the concentration of Zinc in rice sample collected on weekdays and weekends were found to be in the range of 0.9-1.2 mg per 100 gm. It was noticed that the concentration of Zinc in the rice sample collected from Parkstreet area on both weekday and weekend were relatively higher as compared to other locations. Zinc concentration in chow samples were found to be in the range of 1-1.5 mg per 100 gm. The concentration of Zinc in the chow sample collected from Parkstreet area both on weekday and weekend were relatively higher as compared to other locations. In the case of vada the concentrations were found to be in the range of 1.9- 2.6 mg per 100 gm. Samples collected from Parkstreet area on weekday and Sector-5 area on weekend showed a relatively higher concentration of Zinc. The concentration of Zinc in potato fry sample collected on weekdays and weekends were found to be in the range of 0.8- 1.7 mg per 100 gm. Zinc concentration in the potato fry sample collected from Parkstreet area on weekday and from Sector-5 area on weekend was relatively higher as compared to other locations.

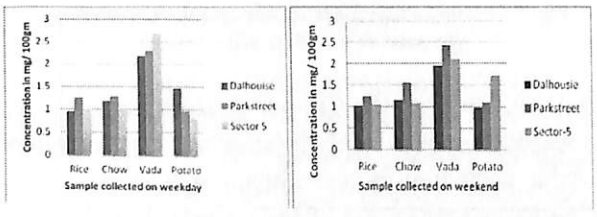

Fig. 3. Showing analysis of Zinc concentration of samples collected on weekdays and weekends

After comparing the Zinc concentration of the collected samples, with the standard Zinc value of that particular food product, it was seen that the Zinc concentration of vada was well within the standard value. Zinc concentration of potato fry slightly exceeded the standard value and Zinc concentration of rice and chow significantly exceeded the standard value. This high level of zinc in both the cereal based product in relation to the potato and can be due to relative higher uptake of zinc of the cereals than the potatoes[2]. A study by Popescu et. al. states that zinc is one of the most common heavy metals emitted by vehicle traffic[18]. Another study by Edward et. al. shows tire- wear particles are a major source of zinc in the environment[5]. This can contribute to the high zinc load by getting deposited on the street foods. Zinc can also come from the accumulated street dusts on the food particles as the zinc content of the street dusts is high[13,11,19]. Here it can be seen that the zinc concentration of rice and vegetable chow is higher than the other two food products. While collecting the food products for sampling it was noticed that only rice and chow were not covered for a very long period of time while preparing and selling of the food product. This can be one of the main causes of dust particle and heavy metal accumulation on the food product.

Manganese:

The Recommended Dietary Allowance (RDA) of manganese ranges from 2 to 5 mg per day[21] and The Tolerated Upper Limit of manganese intake is 12mg per day[8].

The standard concentration of Manganese given by ICMR in 100 gm of rice, chow, vada and potato fry sample are 0.39 mg, 0.57 mg, 0.96 mg and 0.26 mg[7]. In the present study the concentration of Manganese in rice sample collected on weekdays and weekends were found to be in the range of 0.4-0.6 mg per 100 gm. It was noticed that the concentration of Manganese in the rice sample collected from Dalhousie area on both weekday and weekend were relatively higher as compared to other locations. In case of chow the manganese were found to be in the range of 0.56-0.86 mg per 100 gm. The concentration of Manganese in the chow sample collected from Parkstreet area both on weekday and weekend were relatively higher as compared to other locations. Manganese concentration in vada samples were found to be in the range of 1.3-2 mg per 100 gm. It was noticed that samples collected from Parkstreet area on weekday and Sector-5 area on weekend showed a relatively higher concentration of Manganese. Concentration of Manganese in potato fry samples were found to be in the range of 0.5-1.1 mg per 100 gm. It was noticed that the concentration of Manganese in the potato fry sample collected from Sector-5 area on both weekday and weekend were relatively higher as compared to other locations.

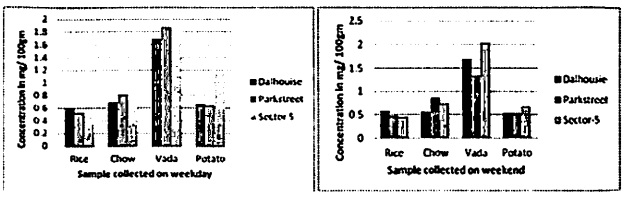

Fig. 4. Showing analysis of Manganese concentration of samples collected on weekdays and weekends

After comparing the Manganese concentration of the collected samples, with the standard Manganese value of the particular food product, it was seen that the Manganese concentration of all the foods were relatively higher than the standard values. The high level of manganese can be contributed by the high level of manganese in the street dusts[8], and the atmospheric manganese[1].

The increased heavy metal concentration of the food products can also be due to the contamination of soil where the raw materials of the cooked food products were harvested. This is supported by a study by Sharma et. al. (2007) and Khan et. al. (2008) showing, one important dietary uptake pathway could be through crops irrigated with contaminated wastewater[22]. Soils irrigated by wastewater accumulate heavy metals Zn, Cu, Mn, Fe in surface soil. When the capacity of the soil to retain heavy metals is reduced due to repeated use of wastewater, soil can release heavy metals into ground water or soil solution available for plant uptake[10]. The contamination of agricultural soils is often a direct or indirect consequence of anthropogenic activities (McLaughlin et al., 1999)[15]. Sources of anthropogenic metal contamination in soils include - urban and industrial wastes; mining and smelting of non ferrous metals and metallurgical industries (Singh, 2001)[23]. Commercial and residential vegetable growing areas are often located in urban areas, and are subject to anthropogenic contamination. Other sources of anthropogenic contamination include the addition of manures, sewage sludge, fertilizers and pesticides to soils, with a number of studies identifying the risks in relation to increased soil metal concentration and consequent crop uptake (Whatmuff, 2002; McBride, 2003)[25, 9].

Conclusion

The study has shown that there were variations in concentration of heavy metals in street foods among the study locations in Kolkata. Though certain heavy metals like lead and cadmium were not detected in the street foods, among the other heavy metals iron and manganese content were higher than the standard metal concentration given by Indian Council of Medical Research (ICMR) in all the food products. Copper and zinc were high in rice, potato fry and vegetable chow.

It was noted that though the elemental concentration of certain samples exceeded the standard elemental concentration of that particular product given by ICMR, none of the elemental concentrations exceeded the Recommended Dietary Allowances and the Tolerated Upper Limits.

Overall, the study shows that the levels of the heavy metals and other elements studied are generally within safe limits. The data here obtained will be valuable in estimating dietary intakes of heavy metals in Kolkata. A detailed study to look into the cumulative effects of intake of such food in connection to heavy metal accumulation in the concerned subjects may help to comment conclusively on the safety of street food.

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