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**IMPACT OF SPIRULINA (*Spirulina fusiformis*) FOOD SUPPLEMENT
ON PREMALIGNANT CONDITIONS IN WOMEN**

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**Thesis submitted in partial fulfillment of the requirement
for the degree of**

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Kerala Agricultural University, Thrissur**

2002

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Dedicated to

MY FAMILY

DECLARATION

I hereby declare that this thesis entitled "Impact of Spirulina (*Spirulina fusiformis*) Food Supplement on Premalignant Conditions in Women" is a *bonafide* record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "Impact of Spirulina (*Spirulina fusiformis*) Food Supplement on Premalignant Conditions in Women" is a record of research work done independently by Ms. Seenia George. (99-16-07) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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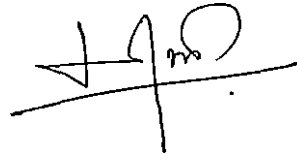
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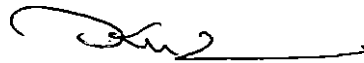
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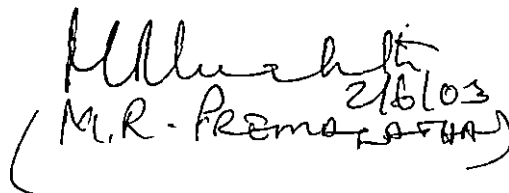


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INTRODUCTION

1. INTRODUCTION

Nutraceuticals are food extracts used as preventive drugs or food supplements. Among these, *Spirulina fusiformis*, a cyanobacterium is now considered as the richest and most complete source of organic nutrition to humans. It is also found to be a concentrated nutritional profile ideal for people preferring a food supplement and for food industry interested in the manufacture of nutraceuticals.

Spirulina is reported to have over 60 per cent protein content and is superior to meat, milk, egg and soybeans. It is a fine blend of vitamin complex containing beta carotene (Pro-vitamin A), B1, B2, B6, E and H and is considered the richest vegetarian sources of vitamin B12 (Bamji *et al.*, 1995). It has been estimated that one kilogram of spirulina is equivalent to 1,000 kg of assorted fruits and vegetables (Rasheed, 2000).

In recent years there has been considerable interest in the role of micronutrients in the genesis and prevention of cancer. Of this, beta carotene is the most effective substance for scavenging free radicals which damage the cells, leading to cancer. Studies have shown that natural β -carotene from algae and green leafy vegetables has got greater antioxidant properties.

Spirulina, apart from its high protein content is a rich source of several micronutrients, especially β -carotene and biotin. Studies conducted at NIN (1990) on bioavailability of vitamin A have shown that carotenes present in spirulina are completely available. These studies have proved that spirulina could be a low cost alternative to ensure adequate intake of vitamin A. Its antioxidant effects have been reported to prevent several epithelial cancers.

High incidence of oral cancers have been reported from Kerala and listed as the third common cancer among females. The fishermen community in the coastal areas of Thiruvananthapuram district are heavily habituated with betel quid chewing and studies conducted in

these population have shown that most of the subjects have precancerous lesions in the oral cavity.

Experimental studies have demonstrated an inhibitory effect of spirulina on oral carcinogenesis. Reports indicate that it is preferable to achieve this effect through dietary supplementation rather than pharmacological intervention.

Based on the above observations the present study was done to evaluate the beneficial effects of spirulina incorporated food supplement in alleviating precancerous oral lesions common among fisherwomen of coastal areas of Thiruvananthapuram.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The literature related to the study are reviewed in this chapter under the following headings

2.1 Cancer as a health problem

2.2 Influence of food in the development and prevention of cancer

2.2.1 Food and development of cancer

2.2.1.1 Dietary factors related to oral cancer and pre cancer

2.2.1.2 Other risk factors of oral cancer

2.2.2 Food and prevention of cancer

2.2.2.1 Role of vitamin A and β -carotene

2.2.2.2 Role of other nutrients and phytochemicals

2.3 Spirulina – Nature's super food

2.3.1 Nutritional aspects

2.3.2 Effect of supplementation

2.3.3 Other important aspects

2.1 CANCER AS A HEALTH PROBLEM

Cancer has been considered a public health problem all over the world, as this disease affects at least nine million and kills five million people every year, being currently the second cause of death in most of the countries. The incidence of cancer tends to raise about 100 per cent within the next 20 years, if prevention and control measures are not taken (WHO, 1999).

In India, cancer is reported to be the sixth leading cause of death. For India the annual estimate of cancer for the year 2001 is 0.98 million (Murthy *et al.*, 1990) and the annual mortality in 2000 is 0.7 million (Murray and Lopez, 1996).

Cancer of the oral cavity represents a major health problem, as indicated by their high incidence in many parts of the world.

According to Parkin *et al.* (1999) cancer of the oral cavity is estimated to be the 12th most frequent cancer worldwide.

Blot *et al.* (1996) and Pisani *et al.* (1999) reported that oral cancer is the fifth most common among males in developed countries and the sixth most common for males in developing countries and a cause of substantial morbidity and mortality. Oral cancer varies in frequency very greatly among different countries and geographic regions. While usually much less frequent in women, in South Central Asia it is the third most common form of cancer in females (Franceschi *et al.*, 2000).

According to Tanaka (1995) oral cancer is a multi-cancer disease and experimental studies indicated that these lesions develop through a multistage process.

Gupta *et al.* (1980) stated, the natural history of development of the disease shows that over 75 per cent of oral cancer arise from pre-existing pre-cancerous lesions. Silverman *et al.* (1984) and Gupta *et al.* (1989) reported that most invasive oral cancers arise from pre-cancerous lesions such as leukoplakia, erythroplakia and oral submucous fibrosis. According to Garewal (1991) these lesions are considered pre-malignant based on the following data: -

1. Oral cancers are very often histologically and clinically associated with leukoplakic changes.
2. Epidemiologic studies showed that almost all new cases of oral cancer in regions of the world with an endemically high incidence of head and neck cancer arise in patients with previous evidence of pre-malignant changes and
3. Prospective follow-up of leukoplakia patients has revealed a significant incidence of transformation to malignancy.

Boyle *et al.* (1990) reported that oral cancer account for approximately four per cent of all cancers and the incidence and

mortality from oral cancer are reportedly increasing (Daftery *et al.*, 1991).

According to Kirita *et al.* (1997) it is predicted that mortality from oral and pharyngeal cancer will continuously increase by 2010 in Japan. Su *et al.* (1999) reported that trends in mortality from oral and pharyngeal cancer among Japanese males, 1950 – 1994 found that the crude mortality rate from cancer of the lip, buccal cavity and pharynx has increased by 3.7 fold in the 45 year period.

Several investigations on mortality from oral and pharyngeal cancer have been reported in countries other than Japan (Goldbery *et al.*, 1994 and Bumdgaard *et al.*, 1995). In the United States 30,300 new cases of tongue, mouth, pharyngeal and other oral cavity cancers are anticipated in 1998 along with 8000 deaths attributed to cancers at the same sites (Landis *et al.*, 1998).

WHO (1984) reported that in some South East Asian countries such as Bangladesh, India, Pakistan and Sri Lanka, oral cancers are most common forms of cancer and constitute about a third of all cancers. Trends in oral and pharyngeal cancer incidence showed an increase in Eastern and Central Europe while declining in Bombay, India and Puerto Rico (Blot *et al.*, 1994).

The incidence rates for cancers of the oral cavity in India for both sexes are among the highest reported worldwide (Parkin *et al.*, 1997).

According to Padmanabhan *et al.* (1988) cancer of the oral cavity is the commonest intra-oral malignancy in South India. The average annual incidence rates were 3.4 and 3.7 in Bombay, 2.5 and 7.7 in Bangalore, 4.8 and 5.5 in Madras among males and females respectively.

R.C.C., Thiruvananthapuram (1987) showed that every year 15,000 new cancer cases occur in the state, of these 17 per cent among men and 11 per cent among women are oral cancer. According to

Mathew *et al.* (1993) 30 per cent of all cancers in males and 15 per cent of all cancers in females are found in head and neck cancer. Among these cancer of the oral cavity are pre-dominant.

R.C.C., Thiruvananthapuram (1988) reported that cancer of the oral cavity is the pre-dominant cancer in Kerala. It is the commonest malignancy among males and the third most common among females in Kerala, India (Nair *et al.*, 1988; Sankaranarayanan, 1996).

The apparent geographic variation in incidence trends is due to the regional differences in exposure patterns to risk factors, particularly tobacco, alcohol and diet (Blot *et al.*, 1988; Winn, 1995).

2.2 INFLUENCE OF FOOD IN THE DEVELOPMENT AND PREVENTION OF CANCER

The relationship between diet and cancer is an important growing field of research. According to Giovannucci (1999) diet is a major environmental risk factor.

Mc Leod (1987) pointed out that upto 90 per cent of all deaths due to cancer could be attributed to potentially avoidable factors and diet is one among them. Ten to seventy per cent of all cancers is attributed to diet.

Components of food or drink we consume may contain exogenous oncogens or stimulate the production of endogenous oncogens or hormones that favour oncogenesis. Further a dietary factor may be protective or a dietary deficiency may pre-dispose to cancer.

2.2.1 Food and Development of Cancer

Both epidemiological as well as experimental studies suggest dietary and more specifically nutritional influences on the development of cancer.

Franceschi (1996) carried out a study on people who consumed majority of carbohydrate as refined products such as white bread and pasta showed that high starch intake was associated with a risk of

breast cancer. Cummings (1998) reported that consumption of refined carbohydrates for prolonged periods may cause cancers of the colon and breast.

Most animal studies show that dietary protein affect carcinogenesis when fed at higher amounts than required for optimum growth. Stefani *et al.* (1998) carried out a study of diet and renal cell carcinoma risk on red meat eaters. The result showed a 3.4 increase in risk for higher intake category. Singh and Fraser (1999) reported that consumption of red meat (beef, lamb and pork) could increase the risk of pancreatic, breast and renal cancer.

The 1998 report from the World Cancer Research Fund together with American Institute for cancer research concluded that diets high in total or in saturated fat may increase the risk of cancer. Persky and Horn (1995) reported that high meat consumption, higher animal fat stores, and lower vegetable intake increased ovarian cancer risk. Stefani *et al.* (1997) observed a significant positive association between total fat intake, saturated fat intake, dietary cholesterol intake and lung cancer as well as breast cancer.

There is an association between consumption of alcohol and cancers of the upper aerodigestive tract as well as liver. Hashibe *et al.* (2000) reported that alcohol drinking was a significant risk factor for oral leukoplakia among non-smokers and non-chewers. Khan and Yatsunami (2000) investigated the effect of moderate and heavy alcohol intake on the progression of hepatic cancer. It was found that there was 1.5 to 2.5 fold greater risk of hepatic cellular carcinoma in the alcohol intake group compared to the alcohol free group.

Vatten and Loken (1990) examined the relation of daily consumption of coffee with age, body weight and Body Mass Index. The results suggested that daily consumption of coffee increased the risk of breast cancer in obese women. According to Stefani *et al.* (1998) the consumption of a beverage known as 'mate' (local tea

derived from the herb (*Ilex paraguariensis*) was associated with an increased risk of renal cell carcinoma for heavy drinkers.

Animal studies indicate that most of the mycotoxins are carcinogens. Imaida *et al.* (1982) reported that mycotoxins administered in either the initiating or the promoting stage significantly increased the induction of hyperplastic nodules. Linsell (1984) pointed out that aflatoxins has a potential role in the causation of hepatocellular carcinoma.

Among cooking trends of today deep fat frying is one of the common methods of preparing food in the Indian culinary system.

Yurkov (1991) reviewed from his studies that deep-frying of different batches of potatoes in the same frying oil every eight hours for 48 hours resulted in quality deterioration in both the oil and fried product. Francis (1994) also reported that reheating of oils for more than 12 hours might cause mutagenicity or carcinogenicity. According to Turdy and Comstock (1999) women who consistently consumed very well done meats had a four times higher risk of breast cancer than those who consumed meats cooked rare or medium done.

Permitted food additives such as colour, flavour and preservatives contribute significantly to the overall risk of cancer in humans among which nitrate is the most important. Badawi (1998) reported that increased consumption of dietary nitrate and nitrite is associated with elevated levels of salivary nitrate and the levels were higher in oral cancer patients. According to Vinceti *et al.* (1999) higher incidence of nasopharyngeal cancer in South Eastern China was largely due to consumption of canned salted fish, which is high in N-nitroso compounds. They also found that compared with lowest, subjects with highest consumption of preserved meat had over four times the risk of nasopharyngeal cancer.

2.2.1.1 Dietary Factors Related to Oral Cancer and Precancer

In the area of oral cancer and pre cancer, there has been a surge of reports on associations of diet and oral cancer and pre cancer. Low intakes of fresh fruits and vegetables has been consistently linked with an increase in cancer prevalence/incidence. (Benndich *et al.*, 1989; Garewal, 1991; Zheng *et al.*, 1993)

Chandrasekaran and Sujatha (1989) reported that the frequency of consumption of green leafy and yellow vegetables and fresh fruits was rare among cancer patients. Prasad *et al.* (1995) stated that vegetable intake green or otherwise was significantly low in oral cancer patients compared to controls.

According to Zeigler (1991) vegetable and fruit intake may reduce the risk of cancers of the mouth, pharynx, larynx, oesophagus.

Wattenberg (1983) stated that macro and micronutrient deficiencies or imbalances have long been considered as probable causes of cancer of the oral cavity. Chandrasekaran and Sujatha (1989) reported that cancer patients had a deficient serum level of vitamin A, zinc, iron and protein.

According to Comstock (1991) among different cancer cases only oral carcinoma had a strong and significant association with low levels of serum β -carotene. A study carried out by Stahelin *et al* (1991) showed that subjects with low carotene had a significantly higher risk of death from cancer. For all cancers the combination of low carotene and low vitamin A was associated with a significantly elevated risk.

Study conducted by Prasad *et al.* (1995) on oral cancer patients showed that serum albumin and fat-soluble vitamins both vitamin A and vitamin E were significantly lower. Ramaswamy *et al.* (1996) have shown that serum vitamin A, B₁₂, C, E, β -carotene and foliate were decreased in leukoplakia patients as compared to controls.

A study was carried out by Zain *et al.* (1999) on serum antioxidant micronutrients in Malaysia revealed that serum levels of

alpha-tocopherol, zeaxanthin/ lutein and β -carotene were found to be significantly lower in the subjects with oral cancer and precancer as compared to the control subjects. In a recent report by Nagao *et al.* (2000) on serum micronutrients and oral cancer and precancer in the Asia Pacific region, the serum levels of lycopene and β -carotene were significantly lower in those with leukoplakia as compared to the control.

2.2.1.2 Other Risk Factors of Oral Cancer

Betel quid chewing is a widely prevalent oral habit in Taiwan, India, Papua New Guinea, South Africa and other South East Asian countries (IARC, 1985b) (Thomas and Mc Lennan, 1992).

According to Sharan (1996) it has been estimated that there are about 600 million betel quid chewers living in different regions of the world.

WHO (1984) and IARC (1985a) reported that in developing countries, chewing of tobacco is by far the most important cause of oral cancer. Epidemiological data suggested that the high incidence of oral cancer in some South East Asian countries such as Bangladesh, India, Pakistan and Sri Lanka may be closely related to the high prevalence of betel quid chewing (Ko *et al.*, 1995)

Stich (1986) stated that a summary of various observations in different parts of the world suggests that tobacco is the predominant carcinogenic source in betel quid. Elevated frequencies of micronucleated cells were observed in the oral mucosa of chewers of various tobacco mixtures (Stich *et al.*, 1989). Warnakulasuriya (1995) and Gupta *et al.* (2000) also reported chewing of betel quid with the inclusion of tobacco within the ingredients have shown to be a major risk factor.

In developing countries, the habit of quid chewing is wide spread especially among the older generation. However in Taiwan, the

younger generation is practicing the habit of chewing areca quid (Chen and Shaw, 1996).

Gupta and coworkers (1980) reported that the use of tobacco in various forms is widespread in India with between 47 per cent and 73 per cent of the population indulging in the habit, compared with approximately 36 per cent in the United States. Oral cancer is increasing in India due to young people chewing panmasala products often containing tobacco, which is responsible for the carcinogenicity of the betel quid (Gupta *et al.*, 1982). It is estimated that in India about 60,000 new oral cancers occur in every year, out of which over 40,000 could have been averted if tobacco habits were avoided (Nair *et al.*, 1993). According to Ko *et al.* (1995) the incidence of oral cancer for individuals who use tobacco and chew betel quid has been 123 fold higher than abstainers.

Sankaranarayanan (1988) reported, in the Southern Indian States of Kerala and Tamil Nadu, 40 per cent to 70 per cent of adult men and 20 per cent and 40 per cent of adult women chew pan. According to the Hospital Cancer Registry, R.C.C. (1988) cancer of the oral cavity is the predominant cancer among men in Kerala. Sankaranarayanan and Duffy (1989) reported that studies in Kerala have indicated a frequency of 34 per cent to 36 per cent for chewing among adults and among women pan tobacco chewing is the predominant habit. According to Nair *et al.* (1993) in India tobacco related cancers constitute 50 – 55 per cent of cancers among men and 20 – 25 per cent among women. Tobacco chewing was a stronger risk factor for oral leukoplakia among women than for men (Hashibe *et al.*, 2000).

IARC (1985b) reported that an average of 15-20 quids is chewed daily by betel quid chewers. Mathew *et al.* (1993) observed that in tobacco chewers cancer of the oral cavity develops because of the carcinogenic effects of various ingredients in chewing.

Arecanut which is widely chewed has been linked with the development of oral submucous fibrosis (Trivedy *et al.*, 1999). Jeng *et al.* (1999) observed that arecanut or betel nut contains numerous chemical ingredients of which arecoline has been suggested as a possible carcinogen.

Similar mutagenicity of arecoline on buccal, gingiva and oral submucous fibrosis fibroblast is supported by the study of Chen *et al.* (1995).

Nair *et al.* (1987) and Stich *et al.* (1989) observed that polyphenolics, a group of arecanut derived compounds on chewing are released into the saliva. Their genotoxic action on the oral mucosa appears to be mainly due to their ability to generate H_2O_2 and reactive oxygen species. Deve *et al.* (1991), Ramachandani *et al.* (1998) and Kumar and Saiyed (1999) reported that arecanut a major constituent of panmasala contains several alkaloids, which are found to be carcinogenic in nature.

Wenke *et al.* (1985) and Nair *et al.* (1985) reported that potential carcinogens and promoters originally from betel quid ingredients can be quantitated in saliva. Stich (1986) stated that all the examined compounds appear in the saliva of betel quid chewers and could penetrate directly into the oral mucosa.

Thomas and Kearsley (1993) and Warnakulasuriya (1995) have also briefly reviewed the carcinogenesis, mutagenicity and cytotoxicity of betel quid ingredients.

2.2.2 Food and Prevention of Cancer

The geographic distribution of types of cancer tumours, the changing cancer patterns of migrant group, the varying incidence of specific tumours by socio-economic group and data from experimental animal studies all point out, diet and nutrition as important factors in the possible control and prevention of human cancer (Shantha, 1988).

These components may have an important role to play as protective agents against carcinogenesis (Griffiths *et al.*, 1996).

Ross (1990) reported that fibre content of the diet protects not only against colorectal cancer but also against other cancers such as mammary cancer.

According to Willeft (1989) the formation of complexes between the fibre and bile acids has been suggested as a factor implicated in the protective process. Insoluble fibres such as wheat bran and cellulose are known to prevent the development of colon cancer. In a study by Faivre and Giacosa (1998) supplemental fibre was used in four chemopreventive trials as wheat bran. The result provided evidence for the inhibition of adenoma growth through high fibre diets. Challier (1998) examined the association between breast cancer risk and intake of dietary fibre. The result showed decreased risk with increased consumption of dietary fibre.

2.2.2.1 Role of Vitamin A and β -carotene

Greenwald and Nixon (1990) stated that chemoprevention is complementary strategy cancer prevention where in certain naturally occurring or synthetic substances are used to arrest the process of carcinogenesis.

According to Steinmetz and Potter (1991) and Shklar and Schwartz (1993) the rationale behind chemoprevention comes from the findings from epidemiologic studies that a diet rich in vegetables and fruits protect against several epithelial cancers. Gao *et al.* (1994) reported that studies provided sufficient evidence of a protective effect of vegetables and fruits in cancers of the oral cavity, pharynx, larynx and oesophages. Both vegetables and fruits are rich in micronutrients like β -carotene, vitamin C and vitamin E, glutathione and flavanoids.

Increasing evidences suggests that certain antioxidant compounds may act as preventive or protective factors (Bertran and Kolonel, 1987).

Consistent observations across wide population groups in different countries strongly support vitamin A and β -carotene as candidates for chemoprevention agents (Malone, 1991; Scully, 1995; Tanaka, 1995 and Sanakaranarayana *et al.*, 1996).

Besides having pro-vitamin A activity, dietary intake of carotenoids has been reported to have an inverse relationship with cancer occurrence (Tesoriere *et al.*, 1993).

Paganini (1987) investigated the association between intake of fruits and vegetables and the risk of lung cancer. The study showed a decreased risk with increased intake of fruits, fruit juice and green salads which are rich sources of β -carotene. According to Connett *et al.* (1989) there is a decreased risk of all cancers with increased intake of β -carotene.

Flagg *et al.* (1995) assessed the relationship of carotenoids with different sites of cancer. The result showed a protective effect of carotenoids on lung, upper aero-digestive tract and breast cancer. According to Speek *et al.* (1988) carotenoids possess anti-ageing and anti-ulcer properties. In animal models, β -carotene is effective in preventing carcinogenesis, including that of oral cavity (Suda and Schwartz, 1988). According to Prasad *et al.* (1995) vitamin A and β -carotene appears to be protective in the promotional aspects of cancer.

Bendich and Olson (1989) stated that β -carotene is unique only because it is the most abundant of the carotenoids that humans can metabolise of vitamin A. Several hundred carotenoids exist in the food supply and many of these carotenoids are antioxidants. According to Garewal (1991) β -carotene fulfills all the criteria for a suitable chemopreventive agent in that it is non-toxic, cheap and is a nutrient. It suppress micronuclei in exfoliated oral mucosal cells from subjects at risk for oral cancer and recently has been shown to be active in reversing leukoplakia.

According to Steinmetz *et al.* (1991) β -carotene are thought to protect against oral cancers through an antioxidant effect. Both human and animal studies with dietary supplementation of β -carotene have demonstrated that β -carotene can protect tissues against oxidative damage (Franke *et al.*, 1994). There are suggestions that the antioxidant activity of retinol (Preformed vitamin A) include quenching singlet oxygen (O_2), neutralizing thiyl radicals and combining with and stabilizing peroxy radicals (Palace *et al.*, 1999).

Jyothirmayi *et al.* (1996) reported that chemoprevention with vitamin A is an acceptable treatment with no troublesome side effects. Observational studies have found that persons who ingest more β -carotene exhibit a reduced risk for several chronic diseases including cancer (Edge *et al.*, 1997).

According to Stich *et al.* (1988) β -carotene and vitamin A intake appear to result in high rates of regression of leukoplakia among heavy chewers, smokers and alcohol users. Chemoprevention trials were carried out with vitamin A and β -carotene in a population group with increased risk of oral cancer by Regional Cancer Centre, Thiruvananthapuram and Environmental Carcinogenesis Unit, University of British Columbia, Canada. The result showed that smaller doses of β -carotene (90 mg / week and 60 mg/ week) were found to be effective in maintaining the protective effect obtained by heavy doses of vitamin A and β -carotene (Mathew *et al.*, 1992).

Calhoun *et al.* (1989) reported that administration of two chemopreventive agents together was more effective than one alone in the inhibition of carcinogenesis.

According to Stahelin *et al.* (1991) low plasma carotene which is known to reflect carotene intake is associated with increased cancer risk. Dixon *et al.* (1994) evaluated the effect of consuming a low carotene diet (60 μ g carotene/ d) on oxidative susceptibility and superoxide dismutase activity in women living in metabolic research

unit. The study revealed that dietary carotenes seems to be needed, not only as a precursor of vitamin A, but also to inhibit oxidative damage and decrease oxidation susceptibility. The study strongly encourage, a higher intake of dietary carotenoids and carotene containing supplements as a preventive measure against cancer.

2.2.2.2 Role of Other Nutrients and Phytochemicals

There are many micronutrients in the diet with anti-mutagenic and anti-carcinogenic property. Intake of food rich in bioactive compounds may trigger detoxification enzymes which inturn reduce the exposure of DNA to carcinogens. Foods rich in micronutrients such as vitamin C, vitamin E, selenium, glutathione and flavanoids have shown a protective effect against tumour promotion.

Vitamin C play a predominant role in free-radical scavenging and protection against lipid-peroxidation (Frei *et al.*, 1989). Block (1991) reported strong protective effect of vitamin C for non-hormone dependent cancers. According to Verhoeven *et al.* (1997) breast cancer reduced with the intake of vitamin C.

Vitamin E has anti-cancer effects as a lipid antioxidant and free-radical scavenger (Chow, 1991). Knekt *et al.* (1991) reported that individuals with low level of α -tocopherol has about 1.5 fold risk of cancer compared with those with a higher level. The results showed protective association of vitamin E with lung cancer and gastro-intestinal cancers. Similarly a study by Comstock *et al.* (1991) showed that serum Vitamin E levels had a protective association with lung cancer. In a recent publication, researchers confirmed that tocotrienols especially the delta- tocotrienol are potent inhibitors of human breast cancer cells.

Selenium has shown to counteract to varying degrees some of the carcinogenic effects of chemicals and inhibit skin, liver, mammary gland and intestinal tract tumours. Finley (2000) demonstrated the

effect of selenium in preventing colon cancer. The results showed that selenium supplied as high selenium broccoli significantly reduced pre-neoplastic lesions in the colon. Rathnasinghe *et al.*, (2000) carried out a study on tin miners and a significant gradient of decreased lung cancer with increased selenium intake was apparent. High consumption of seaweed which is a rich source of selenium is associated with low incidence of malignant breast disease (Cann and Van, 2000).

A review by Kasperzak and Waalker (1986) reveals that zinc and magnesium supplementation tends to inhibit carcinogenesis. The mechanism involved is that there is inhibition of carcinogenesis and the reduction of carcinogen binding to cells and DNA. Carter (1997) reported that zinc is a direct inhibitor of the microsomal metabolism of methyl benzyl nitrosamine an Oesophageal carcinogen. According to Thiagarajan *et al.*, (1998) feeding additional calcium may reduce the early stages of colon cancer. Garland *et al.* (1999) reported an inverse association of calcium intake and colon cancer risk. The findings which are consistent with laboratory results, indicate that most cases of colon cancer may be prevented with regular intake of calcium in the range of 1,800 mg/day. According to Kempman *et al.*, (2000) dietary calcium was inversely associated with colon cancer risk in men and women.

Phytochemicals have protective effects against various forms of cancers such as breast, lung, prostate and uterine (Dragsted, 1993).

Challier *et al.* (1998) reported that breast cancer decreased as consumption of garlic and onions increased. Extracts of Chinese green teas as well as Japanese green teas were proved to have anti-carcinogenic activity *in vivo* (Bu, 1999). Isoflavonoid, a group of biologically active phytochemical present in soy food reduced the risk of breast cancer (Zheng *et al.*, 1999). According to Nishino *et al.* (2000) Curcumin a major constituent of turmeric yellow exert anti-carcinogenic activities in various organs.

2.3 SPIRULINA- NATURE'S SUPER FOOD

Christopher Hills- the father of Spirulina is the pioneer in the field of using Spirulina as a human food. Spirulina is a multicellular, filamentous Cyanobacterium belonging to an algae of class cynophyta and is established as a highly nutritious food (Robert, 1990).

2.3.1 Nutritional Aspects

Spirulina is one of the richest sources of natural β -carotene, the Provitamin A (Annapurna *et al.*, 1991a). Many health effects of *Spirulina fusiformis* were reviewed in an Ecology, Taxonomy, Technology and Applications (ETTA) Symposium on spirulina organized by the Departments of Science and Technology and Biotechnology of the Government of India. The effects claimed include antioxidant activity because of high content of superoxide dimutase and β -carotene (Seshadri and Dai, 1992).

Annapurna *et al.* (1991b) examined the bio-availability of total carotenes and β -carotene from spirulina in apparently healthy pre-school children and found that it is comparable to those values reported for other plant sources like leafy vegetables and carrots. Researchers concluded that spirulina can be used as a source of Vitamin A in the diet, is relatively inexpensive, has higher β -carotene than any other plant source and can be cultivated through out the year.

Spirulina is very rich in iron, the total iron content is around 89mg/100g (Johnson and Shubert, 1986). Reccolina (1995) pointed out that spirulina is free from iron absorption inhibitors and is rich in enhancers like proteins, folic acid and Vitamin B₁₂.

Robert (1989) reported that spirulina contains good amount of bio chelated Ca, Mg, Mn, K, Cu, Zn, Cr and Se. A study by Youghuang (1994) among children suffering from zinc deficiency revealed that spirulina with a high zinc content is twice as effective as a zinc supplement in curing zinc deficiency. They theorized that high zinc

spirulina had many bio-active and nutritious substances which improved mineral absorption, general health and the immune system.

Spirulina has over 60 per cent protein content, and is superior to meat, milk, egg and soyabeans. The Net Protein Utilization volume of spirulina is 62 per cent (Switzer, 1982). According to Ciferri (1983) spirulina has the highest protein content among the natural foods ranging from 46-70 percent.

Spirulina contains a rapid acting and efficient carbohydrate called rhamnase (Ray, 1985). According to Dillon *et al.* (1995) spirulina has about 5-6 per cent essential fatty acids, of which gamma linolenic acid is 30 per cent, one of the highest natural source available.

The pigment phycocyanin present in spirulina helps to strengthen the body's defence system (Robert, 1994).

Weisburger (1991) reported that spirulina contains Vitamin B₁, Vitamin B₂, vitamin B₃, vitamin B₆, vitamin E, folic acid, biotin, inositol and pantothenic acid. Sharma (1992) opines that spirulina is a rich source of natural bio-chelated vitamins containing all the vitamins in highly bio-available form. According to Bamji *et al.* (1995) spirulina is a good source of vitamin B₁₂ and it is the only food of plant origin which contains B₁₂ vitamin.

2.3.2 Effect of Supplementation

The blue-green algae spirulina which are rich in micronutrients have been studied in animal models.

Study carried out by Tokai (1987) by feeding rats a diet with five per cent spirulina for 100 days revealed that the weight of the caecum increased by 13 per cent, lactobacillus increased by 32 per cent, vitamin B₁ inside the caecum increased by 43 per cent. The study suggests eating spirulina increases lactobacillus and may increase efficient absorption of vitamin B₁ and other vitamin from the entire diet.

Yamane (1988) observed the effects of spirulina on acute nephritis caused by inorganic mercury in rats. The study showed kidney detoxification suggesting that spirulina may have a beneficial effect for humans suffering from heavy metal poisoning. Mittal *et al.* (1999) found a significant reduction in the hepatic cytochrome P₄₅₀ content in Swiss Albino mice treated with spirulina in comparison with control group. Study carried out by Juvekar and Nimbkar (2000) on rodents revealed that spirulina plantensis algal extract has potential hepatoprotective activity.

According to Shklar *et al.* (1985) and Schwartz and Shklar (1987) the algae extracts has been shown to be more effective than single nutrients like β -carotene or canthaxanthine, in inducing regression of tumour in hamster buccal pouch cancer. The same extract resulted in regression of hamster buccal squamous cell carcinoma.

A study carried out by Fica (1984) on patients with various nutritional deficiencies *viz.*, weight loss in conjunction with gastric resection, tubercular infection, chronic pancreatitis and gastritis, rheumatoid arthritis, anaemia and diabetes mellitus revealed that with spirulina, the patients gained weight and their proteinograms improved. In Nanjing children's hospital, 27 children 2- 6 years old, recovered in a short period from bad appetite, night sweats, diarrhoea and constipation from a baby nourishing formula containing 1.5 g spirulina, 12 g baked sprout, vitamin B₁ and Zinc. The clinical effects showed spirulina is a genuine health food for children (Miao Jian Ren, 1987). Spirulina fed among 5000 rural pre-school children with one gram a day had reduced the prevalence of bitot's spot and prevented the occurrence of severe form of vitamin A and also β -carotene deficiency with the supplementation (Seshadri and Jayam, 1991). A study carried out by Fathima and Salma (2001) to find out the role of spirulina in ameliorating nutritional deficiency among malnourished children showed that over a period of three months of supplementation with one

gram spirulina per day, the mean weight of the subjects in the experimental group increased to 15.4 kg from 15.1 kg.

A study conducted in Japan by Takeuchi (1988), with eight women who were treated with four gram of spirulina for 30 days showed increase in their average blood haemoglobin content by 21 per cent. Study of haemoglobin levels of humans fed on spirulina supplement (2g/d for 36 days) revealed an average increase in haemoglobin level by 1.33 gm / dl and average increase in body weight by 1.25 kg (Seshadri and Valliammai, 1992). Another study by Mani *et al.* (2000) on young anemic girls revealed that supplementation with five gram of spray dried spirulina powder for 30 days increased the blood haemoglobin levels and could be effectively used to combat iron deficiency anaemia.

The effect of spirulina on hypercholestralmic patients was carried out by Ramamoorthy and Kumari (1996). Thirty ischaemic heart disease patients without any compliance of the disease and with hypercholesterolemia, two grams and four grams of spirulina was supplemented for 3 months. Results indicated that spirulina plays a key role in weight reduction, lowering blood cholesterol levels and improving the lipid profile of the patients. Anuradha and Gayathri (1999) noted weight reduction in hypertensives after supplementation of three grams of spirulina for 60 days. There was also significant reduction in BMI among male and female patients after the supplementation of spirulina.

According to Babu (1989) and Anuradha and Vidya (2001) spirulina has a hypoglycemic effect on non-insulin dependent diabetic patients.

The radio protective effect of an extract of spirulina was studied using the micronucleus test in polychromatic erythrocytes of bone marrow in mice. The extract caused a significant reduction of the micronucleus frequencies induced by gamma radiation (Quishen and

Kolman, 1989). According to Loseva and Dardynskaya (1993) spirulina a natural food supplement, reduced urine radioactivity levels by 50 percent in only 20 days. This result was achieved after giving 5 g a day to children at the Institute of Radiation Medicine in Minsk. The study concludes that spirulina is favourable for normalizing the adaptive potential of children's bodies in conditions of long-lived low dose radiation.

Mathew *et al.* (1995) evaluated the chemopreventive activity of *Spirulina fusiformis* (1g per day for 12 months) in reversing oral leukoplakia in pan-tobacco chewers in Kerala, India. Complete regression of lesions was observed in twenty of fortyfour evaluated subjects supplemented with spirulina. Arora (1996) reported that spirulina has rich chlorophyll, which has the ability to heal wounds.

2.3.3 Other Important Aspects

Spirulina has a long history of human usage in Mexico and Central Africa where it grows naturally in the alkaline lake.

Seshadri and Dai (1992) reported that commercially viable processes for out door cultivation of spirulina have been developed.

Spirulina is now available in powder and tablet form. In Japan, snack chips are also available. Fruit and snack bars enriched with spirulina are also available nowadays. In Vietnam, spirulina is sold as lactogil to increase lactation on nursing mothers (Venkataraman, 1993).

Spirulina has been promoted as a natural health and slimming food in US and Europe. Spirulina is used as sports nutrient and space food as reported by Mc kelvey (1990).

The acceptability of spirulina incorporated at a level of 1-2 g in common preparations such as sambar, bengal gram laddus, chatney and brinjal curry was found to be satisfactory in organoleptic trials (NIN, 1991a). According to Venkataraman (1992) the best way to use spirulina is to grind the sun dried material and mix the powder in the

flour of wheat, rice, maize, millets and dhal or in soups, curries and other food preparations.

The NIN has conducted toxicological studies and has proved spirulina to be safe. Extensive research in toxicology, carried out in Japan as well as under the UNIDO programme in Mexico show that spirulina is as safe a food as anything known to mankind (UNIDO Report, 1980).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The "Impact of spirulina (*Spirulina fusiformis*) food supplement on premalignant conditions in women" is a study carried out with an objective to formulate food supplements incorporating spirulina and to assess its impact on premalignant conditions of the oral cavity.

Materials and methods chosen for the study are presented under:

- 3.1 Selection of area and subjects
- 3.2 Assessment of socio-economic and dietary pattern of the subjects
- 3.3 Nutrition and health education to the subjects
- 3.4 Formulation of spirulina incorporated food supplements
- 3.5 Conduct of the feeding experiment
- 3.6 Evaluation of the feeding experiment
- 3.7 Statistical analysis

3.1 SELECTION OF AREA AND SUBJECTS

Puthukuruchy and Maryanad villages of fishermen community in Thiruvananthapuram district was selected for the study. These villages are located about 20 km away from Thiruvananthapuram city along the Arabian sea coast.

Basic criteria for selection of these villages were:

- i. Fisherfolk in these villages are at higher risk for oral precancerous condition (Sankaranarayanan, 1996).
- ii. Villages are approachable to conduct the research study.
- iii. Willingness of the villagers to provide all the necessary information during survey and further follow up programmes (Shukla and Saxena, 1988).

At Puthukuruchy with the help of the parish priest and women volunteers and at Maryanad, with the support of Panchayat members, the local women were approached. Through an informal discussion, the significance of the present trial was explained and they were persuaded



Fig. 1. Conduct of medical camp

to attend a medical camp so as to identify women suspicious of having oral precancerous signs and who were known to be heavily habituated with betel quid chewing. Person to person explanation was also carried out by the volunteers to persuade them to attend a medical camp.

Subjects were identified through clinical examination by conducting two medical camps in the villages with the help of a medical team consisting of three doctors belonging to the Regional Cancer Centre, Thiruvananthapuram. During the camp, blood samples were collected from the subjects for further biochemical analysis.

Criteria for the selection of the subjects for the study were :

1. Only women in the age group of 40 to 55 years.
2. Women with symptoms of precancerous condition of the oral cavity and known to be heavily habituated with betel quid chewing.
3. Women not undergoing any other treatment for the condition, at present.

A specially designed check list (Appendix-I) used to elicit information on the family history of cancer incidence, type of oral precancerous condition *viz.*, leucoplakia and submucous fibrosis, difficulty in swallowing, talking and intolerance to spices in food was designed.

Through the medical camp, from among 118 women habituated with betel chewing at Maryanad, 42 were screened to have clinical symptoms. Similarly 18 women were clinically identified from among 92 tobacco habitues at Puthukuruchy. These sixty symptomatic women were in the age group of 40 to 55 years and were selected for the feeding trial.

Out of the 60 women, 30 were kept as control group (CG) and the remaining 30 as experimental group (EG). Written consent of the subjects to participate in the study was also obtained.



Fig. 2. Selection of respondents through clinical examination

A sub sample of ten each from the EG and CG were further identified at random for detailed study.

3.2 ASSESSMENT OF SOCIO-ECONOMIC AND DIETARY PATTERN OF THE SUBJECTS

The socio-economic level of the subjects such as social, economic, religion and the family background in general have a very distinct part to play in determining the attitude and food consumption, health and behavioural pattern of the individual (Arora, 1991 ; Nayga, 1994).

According to Sirshi (1985) to assess the socio-economic status, details pertaining to the type of family, family size, monthly income and caste are to be ascertained.

According to Swaminathan (1993), through diet surveys information on nutrient intake levels, sources of nutrients, food habits and preferences could be collected.

Meer *et al.* (1995) had opined that the social and economic condition in which one lives is said to have a direct impact on food habits and nutritional status.

Considering all these aspects a specially designed and pretested proforma was structured for the study and the details are presented in Appendix-II. A food use frequency score sheet for different food items and for β -carotene rich foods was also included in the diet survey schedule since the frequency of use of different food groups would give an indication to the adequacy of the daily diet pattern as observed by Nelson (1995).

Food use frequency for different food items was measured on a six point scale and for β carotene foods on a four point scale. On the basis of the frequency of use, the food was scored as given below:

Frequency of use	Scores
Never	1
Occasionally	2
Once in a week	3
Twice in a week	4
Thrice in a week	5
Daily	6

The total scores for each of the food groups are calculated (Reaburn *et al.*, 1979) and formula is given in Appendix-III.

To study the actual food intake of the subjects with oral precancer, 24 hour recall method was conducted with suitably structured questionnaire (Pennigton, 1988). In this recall method of oral questionnaire diet survey, the individuals were asked a series of questions to ensure recollection and description of all foods before the interview with emphasis on food consumption meal by meal. The schedule used for recording actual food intake is presented in Appendix-IV. The nutritive values of foods consumed were calculated using the food consumption table of ICMR (1999).

Interview method was selected for collecting all the details mentioned above, since this method was reported to be a suitable way to proceed systematically and quickly to collect information. According to Gupta (1987) interview is a two way method which permits the exchange of ideas and information and explains that the information received from an interview schedule is more reliable as the accuracy of the statement can be checked by supplementary questions whenever necessary.

Nutritional status of the selected subjects were evaluated through evaluation of anthropometric measurements and biochemical investigation.

Anthrometric measurements are considered as an effective measure for assessing the nutritional status of the subjects. Cole (1993)



Fig. 3. Biochemical assessment

reported that anthropometry is widely used as a screening tool for diseases in adults. In this study height and weight of the subjects were measured. From the data, Body Mass Index (BMI) was worked out using the formula Wt/Ht^2 (Beaton *et al.*, 1990).

Biochemical investigation as one of the most important tool for assessing the nutritional status of the subjects, included in the study were:

- i. Haemoglobin – Park and Park (1991) states that haemoglobin level is an useful index of the overall state of nutrition irrespective of its significance in anaemia.

Haemoglobin concentration g/100 ml of all the subjects in the EG and CG was estimated by Sahlis method using haemoglobinometer (Wordsworth, 1979).

20 μ l. of blood was taken for the measurement of haemoglobin.

- ii. Serum β -Carotene - The direct measurement of β -carotene in serum is extremely useful in detecting deficiency status. Serum β -carotene was estimated on a sub-sample of ten each from EG and CG (Bassey *et al.*, 1946).

8 ml of blood samples were taken from 10 women for serum β -carotene estimation. The estimation was carried out by the investigator in the laboratory.

3.3. NUTRITION AND HEALTH EDUCATION IMPARTED TO THE SUBJECTS

Education is one of the major means of bringing about the transformation of society (Merchant, 1999).

ICN (1992) stressed that nutrition education is a primary means of protecting and promoting the nutritional well being of the public.

According to Park and Park (1991) the aim of health education in nutrition is to guide people to choose optimum and balanced diets, which contain nutrients necessary for energy, growth and repair.



Fig. 4. Nutrition and health education class

Women in the EG were exposed to nutrition and health education sessions with special reference to the nutritional significance of spirulina to alleviate their present health condition. Motivation towards withdrawing the habits classified as risk factors was also attempted. The education programme was evaluated with reference to gain in knowledge.

The lecture cum discussion programme was well supplemented with charts and posters depicting the importance of a balanced diet, nutritional importance of spirulina and also the effect of personal habits and hygiene on health for the benefit of the subjects participating in the programme.

For evaluating the education programme an education schedule with statements on cancer and diet, importance of antioxidant vitamins, significance of spirulina, personal habit and cancer was formulated and the schedule is appended in Appendix-V.

The education schedule consisting of 25 statements was administered prior to the education programme. Correct answers were given a score of '1' and wrong answers were scored as '0'. The score awarded for each statement was summed up to work out the total score. This was repeated after the education programme. Difference in the scores was considered as "gain in knowledge".

Among the risk factor associated with personal habits, tobacco consumption seems to be the most important and widely reported (Thomas and Kearsley, 1993; Gupta *et al.*, 1996).

Details on personal habits *viz.*, chewing and oral hygiene of the women were collected before and after the education programme using a checklist. The checklist used is presented in Appendix-VI.

3.4. FORMULATION OF SPIRULINA INCORPORATED FOOD SUPPLEMENTS

Spray dried spirulina powder required for the study was supplied free of cost by "Parry Nutraceuticals Ltd., Chennai". The collected



Fig. 5. Spray dried spirulina powder



Fig. 6. Spirulina based recipes

spray dried spirulina powder was stored under refrigerated condition throughout the study period.

Information related to spirulina based recipes were collected from the available literature. The criteria for the selection of recipe for the study were familiarity of dish, ease of preparation in bulk, shelf life, easiness in apportioning and serving. Attention was given while selecting recipes, so that there would not be any heating process after the addition of spirulina powder in the course of its preparation.

Spirulina based recipes listed below were studied in the laboratory following standard procedure.

- Lemon-ginger squash
- Rava laddu
- Coconut chutney powder
- Rice balls
- Bengal gram balls
- Green gram chutney powder

Recipes standardized are detailed in Appendix-X.

Apportioning of products for a days supplement was done in such a way that each portion size of the food item was ensured to contain 1 g of spirulina (spray dried) contributing 2038 μg of β -carotene

Tests listed below were administered on the products for ranking:

- i. Nutritional composition
- ii. Organoleptic quality and acceptability
- iii. Preference studies

Nutritional composition of the standardized products were computed using the food composition table (ICMR, 1999) and the major components analysed were calories, proteins, calcium, iron, β -carotene and vitamin C.

The six spirulina incorporated food supplements were subjected to sensory evaluation by a panel of fifteen judges with the help of a score card at five-point scale. The panel members were selected from a group

of 25 healthy women in the age group of 20 to 25 using the triangle test. Hence out of the 25 women participated, 15 very sensitive women were selected by the triangle test. Evaluation card used for the triangle test is presented in Appendix-VII.

The food supplements were evaluated based on quality attributes like appearance, colour, flavour, taste, overall acceptability. The score card used is presented in Appendix-VIII.

While tasting, the judges were requested to drink water in between for the removal of any after taste carried over from sample to sample. The tasting session was held in the afternoon, since this time was considered as the ideal time for conducting the acceptability studies (Swaminathan, 1975).

The six spirulina incorporated food supplements were tested for preference at the field level among the subjects in the EG. For this fifteen subjects were selected at random. The food supplements were served to these women and were requested to rank them according to their preference. They were permitted to take enough time to rank the samples leisurely. Rank orders of the food supplements by the subjects were considered to determine the preference level of each product. The schedule used for ranking is presented in Appendix-IX.

Three most preferred spirulina incorporated food supplements were identified according to the preference grading done by the subjects.

The selected three food supplements were processed in large quantities for feeding experiment.

The quantity of each supplement required to feed sixty women altogether for a period of 56 days was worked out using the following formula.

$$R = P \times S \times D,$$

Where,

R= Total amount needed for 56 days

P = Quantity of supplements needed for a subject per day



Fig. 7. Selected spirulina supplements for feeding trial

S = Number of subjects

D = Number of days of feeding

Details of products processed for the feeding experiment is given below

Sl. No.	Spirulina food supplement	1 portion	1 portion (g) x 60 (subjects) x 56 (days)	Total quantity
1.	Lemon-ginger squash	30 ml	30 x 60 x 56	100.8 l
2.	Rava laddu	72 g	72 x 60 x 56	241.9 kg
3.	Rice balls	60 g	60 x 60 x 56	201.6 kg

The required quantity for one week was prepared on every Saturday. Individual portion of the spirulina food supplement for a week was packed separately for ease of distribution. This also enable the subjects to consume each day's share easily. Snacks were packed in polythene covers and beverages in air tight bottles.

3.5 CONDUCT OF THE FEEDING EXPERIMENT

The efficiency of spirulina incorporated food supplements among the subjects with precancerous condition of the oral cavity was tested by supplementary feeding experiment of six months duration. The subjects in the EG were given spirulina incorporated food supplements and the subjects in the CG were given the same supplement prepared without the addition of spirulina powder.

Each selected food supplement was given for one week alternately to the subjects. This was distributed to the subjects on sundays. The subjects in both the groups were asked to gather at the panchayat community hall on every sundays at a particular time and the investigator personally handed over the food supplements to the subjects.

Attendance of the subjects were recorded on these days. Informal visits were made to the houses of the subjects at random to check the regularity in consumption of the supplemented products.



Fig. 8. Consumption of spirulina supplements by the respondents

3.6 EVALUATION OF THE FEEDING EXPERIMENT

The impact of spirulina incorporated food supplements on the subjects with pre-cancerous condition of the oral cavity was evaluated by carrying out clinical and biochemical investigations.

3.6.1 Clinical Evaluation

Clinical examination of the oral cavity of the subjects were carried out again after the six months period of feeding trial by the same experts from Regional Cancer Centre, Thiruvananthapuram, who functioned in the medical team for screening the subjects.

Specially designed check list (Appendix-1) which was used at the beginning of the experiment for carrying out the clinical examination of the oral cavity was used again in order to evaluate the impact of feeding trial on the precancerous condition of the oral cavity. Clinical examination was done for both the subjects in the EG and CG.

Presence or absence of clinical symptoms were assessed by giving score for each of the symptoms and by working out the total percentage scores.

3.6.2 Biochemical Evaluation

Blood samples were collected with the help of a qualified lab technician before and after the feeding trial for both the subjects in the EG and CG.

Biochemical parameters like haemoglobin and serum β -carotene levels were estimated in order to evaluate the effect of the feeding trial on haemoglobin and serum β -carotene levels. The same laboratory procedures applied earlier was followed.

3.7 STATISTICAL ANALYSIS

The data collected were subjected to statistical analysis and interpreted in terms of percentage analysis, mean, correlation analysis, chi-square and test of significance.

RESULTS

4.RESULTS

The present study entitled "Impact of spirulina (*Spirulina fusiformis*) food supplement on premalignant conditions in women" was undertaken to assess the impact of spirulina on the precancerous condition of the oral cavity. The results of the study are presented under the following heads.

4.1 Distribution of respondents based on socioeconomic and dietary pattern

4.2 Distribution of respondents based on clinical symptoms of oral cavity

4.3 Nutritional status of respondents

4.4 Distribution of respondents based on personal habits

4.5 Evaluation of the formulated spirulina food supplements

4.6 Impact of spirulina food supplementation on women showing oral precancerous symptoms

4.7 Effect of nutrition and health education

4.1 SOCIO-ECONOMIC AND DIETARY PATTERN OF THE RESPONDENTS

4.1.1 Socio-economic Status of Respondents

The socio-economic status of the subjects selected (both experimental and control group) was studied with reference to their personal characteristics, family profile and economic status.

Details on personal characteristics of subjects viz., age, education, occupation of the subjects are presented in Table 1.

Table 1. Personal characteristics of the respondents

Sl. No.	Characteristics	Category	Experimental group	Control group	Total
1.	Age (years)	40-50	14 (46.70)	13 (43.30)	27 (45.00)
		Above 50	16 (53.30)	17 (56.70)	33 (55.00)
		Total	30	30	60
2.	Education	Illiterate	12 (40.00)	18 (60.00)	30 (50.00)
		Primary	15 (50.00)	10 (33.30)	25 (41.70)
		Upper primary	3 (10.00)	2 (6.70)	5 (8.30)
		Total	30	30	60
3.	Occupation	Fish vending	17 (56.70)	15 (50.00)	32 (53.30)
		Coir work	4 (13.30)	5 (16.70)	9 (15.00)
		Casual labourer	2 (6.70)	-	2 (3.40)
		Unemployed	7 (23.30)	10 (33.30)	17 (28.30)
		Total	30	30	60

Figures in parenthesis denote percentage

The data revealed that 53.30 per cent of the subjects in the experimental group (EG) and 56.70 per cent subjects in the control group (CG) were above 50 years of age and the remaining 46.70 per cent subjects in the EG and 43.30 per cent subjects in the CG belonged to the age group of 40 to 50 years.

Assessment of educational status of the respondents is very important in any research attempt. When educational status of the subjects was analysed it was observed that 40.00 per cent subjects in the EG and 60.00 per cent subjects in the CG were illiterate. While 50.00 per cent in the EG and 33.30 per cent in the CG had attained primary level education, 10.00 per cent subjects in the EG and 6.70 per cent subjects in the CG were educated upto upper primary level.

The employment level gives an indication about income and economic status of the study group. On assessing the occupational status of the subjects, it was found that in addition to fish vending the subjects were engaged in other occupations like coir work and also as casual labourers. Details further revealed that 23.30 per cent of the subjects in the EG and 33.30 per cent subjects in the CG were unemployed. About 56.70 per cent subjects in the EG and 50.00 per cent in the CG had taken up fish vending as their occupation. While 13.30 per cent subjects in the EG and 16.70 in the

CG were engaged in coir work. The remaining 6.70 per cent in the EG were working as casual labourers to earn their livelihood.

Family characteristics of the respondents are presented in Table 2.

Table 2. Family characteristics of the respondents

Sl. No.	Characteristics	Category	Experimental group	Control group	Total
1.	Type of family	Nuclear	25 (83.30)	15 (50.00)	40 (66.70)
		Joint	5 (16.70)	15 (50.00)	20 (33.30)
		Total	30	30	60
2.	Family size	Upto 4	14 (46.70)	7 (23.30)	21 (35.00)
		5-6	11 (36.60)	12 (40.00)	23 (38.30)
		More than 6	5 (16.70)	11 (36.70)	16 (26.70)
		Total	30	30	60
3.	No. of adults in the family	1 - 2	9 (30.00)	6 (20.00)	15 (24.90)
		3 - 4	14 (46.70)	15 (50.00)	29 (48.40)
		More than 4	7 (23.30)	9 (30.00)	16 (26.70)
		Total	30	30	60
4.	No. of children in the family	0	11 (36.70)	5 (16.70)	16 (26.70)
		01	6 (20.00)	5 (16.70)	11 (18.30)
		02	7 (23.30)	7 (23.30)	14 (23.30)
		More than 2	6 (20.00)	13 (43.30)	19 (31.70)
		Total	30	30	60
5.	No. of employed persons	1 earning member	6 (20.00)	3 (10.00)	9 (15.00)
		2 earning member	13 (43.30)	11 (36.70)	24 (40.00)
		More than 2	11 (36.70)	16 (53.30)	27 (45.00)
		Total	30	30	60

Figures in parenthesis denote percentage

As summarized in Table 2, the distribution of the subjects in relation to type of family revealed that 83.30 per cent subjects in the EG and 50.00 per cent subjects in the CG were members of nuclear type family. The remaining 16.70 per cent subjects in the EG and 50.00 per cent in the CG belonged to joint families.

The family size of the subjects indicated that 46.70 per cent subjects in the EG and 23.30 per cent in the CG belonged to small sized families with members upto 4. Families of 36.6 per cent subjects in the EG and 40.00 per cent in the CG were medium sized having five to six members, while 16.70 per cent of the subjects in the EG and 36.70 per cent subjects in the CG belonged to large sized families having more than six members.

Details regarding the number of adult members in the family indicated that 30.00 per cent of the subjects in EG and 20.00 per cent of the subjects in CG belonged to families having either one to two adult members in their families while 46.70 per cent subjects in the EG and 50.00 per cent subjects in the CG were having three to four adults in their family, 23.30 per cent in the EG and 30.00 per cent in the CG had more than four adult members in their family.

It was also revealed from Table 2 that 20.00 per cent subjects in the EG and 43.30 per cent in the CG were having more than two children (below 15 years of age) and 23.30 per cent of the families both in the EG and CG were found to have two children. Twenty per cent families in the EG and 16.70 per cent in the CG were having only one child while 36.70 per cent in the EG and 16.70 per cent in the CG had no children in their family.

Details pertaining to the employment status of the family members showed that 36.70 per cent of the subjects in the EG and 53.30 per cent in the CG belonged to families with more than two employed persons. It was noted that 43.30 per cent subjects in the EG and 36.70 per cent subjects in the CG were categorised under families with two employed persons and 20.00 per cent in the EG and 10.00 per cent subjects in the CG belonged to families with only one employed person.

Economic status of the study subjects is depicted in Table 3.

Table 3. Monthly income of the families

Sl. No.	Income range (Rs.)	Experimental group	Control group	Total
1.	Family Income			
	<1000	2 (6.70)	-	2 (3.40)
	1000 - 3000	19 (63.30)	19 (63.30)	38 (63.30)
	> 3000	9 (30.00)	11 (36.70)	20 (33.30)
2.	Percapita income			
	< 500	6 (20.00)	10 (33.30)	16 (26.70)
	500 - 600	12 (40.00)	16 (53.30)	28 (46.60)
	> 600	12 (40.00)	4 (13.40)	16 (26.70)

Figures in parenthesis denote percentage

Distribution of the families based on monthly income presented in Table 3 revealed that 6.70 per cent families of the subjects in the EG were earning a monthly income of less than Rs. 1000/-. Majority (63.30 %) of families both in the EG and CG belonged to the monthly income group ranging from Rs. 1000 to Rs. 3000. Thirty per cent and 36.70 per cent families in the EG and CG respectively had acquired a monthly income above Rs. 3000.

The per capita income of the families of the subjects was also assessed and it was noticed that 20.00 per cent in the EG and 33.30 per cent in the CG had per capita income less than Rs.500 per month. Among 40.00 per cent of the families in the EG and 53.30 per cent among CG, the per capita income was only between Rs.500 to 600. Forty per cent families in the EG and 13.40 per cent in the CG had a monthly per capita income above Rs.600.

4.1.2 Dietary and food consumption pattern of the families

Dietary pattern and food consumption pattern of the subjects in the experimental group (EG) and control group (CG) were assessed with regard to monthly per capita expenditure on food, food habits, frequency of use of various food items and frequency of use of β -carotene rich foods.

Table 4. Distribution of families based on monthly per capita expenditure on food

Per capita expenditure on food / month (Rs.)	Experimental group	Control group	Total
< 300	21 (70.00)	25 (83.30)	46 (76.70)
300 - 400	6 (20.00)	4 (13.40)	10 (16.70)
> 400	3 (10.00)	1 (3.30)	4 (6.60)
Total	30	30	60

Figures in parenthesis denote percentage

It is noted from Table 4, that the monthly per capita food expenditure of 70.00 per cent subjects in the EG and 83.30 per cent in the CG were below Rs.300. Twenty per cent women in EG and 13.40 per

cent in the CG had per capita expense between Rs.300 to Rs.400 on food every month and only 10.00 per cent in the EG and 3.30 per cent in the CG were found to be spending more than Rs.400 per person on food per month.

An enquiry into the food habits of the subjects under study revealed that all the subjects (100 %) both in EG and in CG were non-vegetarians. Fish was the non vegetarian item mainly included in their diets.

4.1.2.1 Frequency of Use of Various Foods

Frequency of use of various food items among the subjects were assessed by assigning scores ranging from one to six depending upon frequency of use *viz.*, never, occasionally, once in a week, twice in a week, thrice in a week and daily. Data collected based on these scores to determine the frequency of use of different food items in the daily diet is presented in Table 5.

It was noticed that energy rich foods like cereals, nuts and oil seeds (coconut), fats and oils, sugar and jaggery were used daily by all the subjects in both the groups (EG and CG).

Considering the frequency of use of roots and tubers, 6.70 per cent and ten per cent in the EG and CG included this item daily in their diet. Thirty per cent and 43.30 per cent in the EG and CG respectively included roots and tubers thrice in a week, 40.00 per cent in EG and 33.30 per cent in CG used twice in a week. While 13.30 per cent of the subjects in the EG and 6.70 per cent in the CG included roots and tubers once in a week. An occasional use of this item was seen among ten per cent and 6.70 per cent respectively in the EG and CG.

Data regarding the frequency of use of protein rich foods revealed that fish was used daily by all the families in the EG and CG. It was found that ten per cent in the EG and 20.00 per cent in the CG included pulses thrice in a week in their diet, 13.30 per cent and 6.70 per cent

Table 5. Frequency of use of various food items by the respondents

Food item	Group	Daily	Thrice in a week	Twice in a week	Once in a week	Occasionally	Never
Cereals	EG	30(100.00)	-	-	-	-	-
	CG	30(100.00)	-	-	-	-	-
Pulses	EG	-	3(10)	4(13.30)	2(6.70)	21(70.00)	-
	CG	-	6(20)	2(6.70)	5(16.70)	17(56.60)	-
Root and tubers	EG	2(6.70)	9(30)	12(40.00)	4(13.30)	3(10.00)	-
	CG	3(10.00)	13(43.3)	10(33.30)	2(6.70)	2(6.70)	-
Green leafy vegetables	EG	-	-	-	-	14(46.70)	16 (53.30)
	CG	-	-	-	-	10(23.30)	20(66.70)
Vegetables	EG	-	3 (10)	5(16.70)	7 (23.30)	15(50.00)	-
	CG	2 (6.70)	1(3.3)	5(16.70)	12 (40.00)	10(33.30)	-
Fruits	EG	-	-	2 (6.70)	4(13.30)	24(80.00)	-
	CG	-	-	3 (10.00)	6(20.00)	21(70.00)	-
Nuts and oil seeds	EG	30(100.00)	-	-	-	-	-
	CG	30(100.00)	-	-	-	-	-
Milk and milk products	EG	-	-	7(23.30)	5(16.70)	18(60.00)	-
	CG	-	-	6(20.00)	11(36.70)	13(43.30)	-
Fats and oils	EG	30(100.00)	-	-	-	-	-
	CG	30(100.00)	-	-	-	-	-
Egg	EG	-	-	1(3.30)	4(13.40)	16(53.30)	9(30.00)
	CG	-	-	2(6.70)	3(10.00)	13(43.30)	12(40.00)
Fish	EG	30(100.00)	-	-	-	-	-
	CG	30(100.00)	-	-	-	-	-
Meat	EG	-	-	3(10.00)	20(66.70)	7(23.30)	-
	CG	-	-	3(10.00)	22 (73.30)	5(16.70)	-
Sugar and jaggery	EG	30(100.00)	-	-	-	-	-
	CG	30(100.00)	-	-	-	-	-

Figures in paranthesis denotes percentage

EG – Experimental group

CG – Control group

used this twice in a week, 6.70 per cent and 16.70 per cent in the EG and CG respectively used this item once in a week. Seventy per cent in the EG and 56.60 per cent in the CG included pulses only occasionally in their diet. It was again observed that 23.30 families in the EG and 20.00 per cent in the CG included milk and milk products twice in a week, 16.70 and 36.70 per cent respectively used once in a week and 60.00 per cent and 43.30 per cent included this only occasionally in their diet.

Regarding the frequency of use of egg it was found that 3.30 per cent in the EG and 6.70 per cent in the CG included egg twice in a week. While 13.40 per cent and ten per cent included egg once in a week in their diet, 53.30 per cent and 43.30 per cent of the families in the EG and CG used it occasionally in their diet. However 30.00 per cent and 40.00 per cent families in EG and CG never included egg in their diet. Ten per cent in both groups included meat twice in a week while majority of the subjects in the EG and CG *ie.* 66.70 per cent and 73.30 per cent respectively included meat once in a week in their diet. While 23.30 per cent and 16.70 per cent in the EG and CG included it occasionally.

Data regarding the frequency of use of protective foods, *viz.*, vegetables, green leafy vegetables and fruits evidenced that only ten per cent families in the EG and 3.30 per cent in the CG used vegetables thrice in a week in their diet. While 16.70 per cent in both the groups included twice in a week, 23.30 per cent in the EG and 40.00 per cent in the CG used vegetables once in a week in their diet. Majority of the families *ie.*, Fifty per cent in the EG and 33.30 per cent in the CG used vegetables only occasionally in their diet. Green leafy vegetables were occasionally used by 46.70 per cent and 23.30 per cent families among EG and CG respectively. Data indicated that 53.30 per cent families in the EG and 66.70 per cent in the CG never included green leafy vegetables in their diet.

It was revealed that 6.70 per cent families in the EG and 10.00 per cent in CG included fruits twice in a week in their diet. While 13.30 per

cent and 20.00 per cent respectively in EG and CG used once in a week and majority *ie.* 80.00 per cent families in the EG and 70.00 per cent families in the CG used fruits only occasionally.

Table 6. Frequency score of different food items

Food item	Frequency score		
	Experimental group (n=30)	Control group (n=30)	Total (n=60)
Cereals	100	100	100
Pulses	44	39	46
Roots and tubers	68	74	74
Green leafy vegetables	24	22	23
Vegetables	48	52	50
Fruits	38	40	39
Nuts and oil seeds	100	100	100
Milk and milk products	44	46	45
Fats and Oils	100	100	100
Egg	32	30	31
Fish	100	100	100
Meat	44	49	48
Sugar and jaggery	100	100	100

Table 6 gives the frequency score for different food items among the subjects calculated based on the scores obtained on use of different food item. It was revealed that food items *viz.* cereals, fish, nuts and oil seeds, fats and oils, sugar and jaggery obtained the maximum score (100 %) for both the EG and CG. This was followed by root and tubers with score of 68 and 74 respectively for EG and CG. The scores for vegetable was found to be 48 and 52 for EG and CG. Meat, milk and milk products, pulses obtained frequency score of 44 each by the EG, and the corresponding figures for CG were 49, 46 and 39 respectively. Score obtained for fruits was 38 by the EG and 40 by CG. Egg recorded a low frequency score of 32 and 30 by the EG and CG respectively. The least frequency score was for green leafy vegetables and it was 30 and 22 respectively for the EG and CG.

Table 7. Classification of food items based on food frequency scores

Frequency range	Experimental group	Control group
Most frequently used food item Score (100 – 80)	Cereals, fish, nuts and oil seeds, fats and oils, sugar and jaggery	Cereals, fish, nuts and oil seeds, fats and oils, sugar and jaggery
Moderately used food item Score (79 – 50)	Root and tubers	Roots and tubers, vegetables
Less frequently used food item Score (below 50)	Pulses, milk products, meat, fruits, vegetables, egg, green leafy vegetables	Pulses, milk products, meat, fruits, egg, green leafy vegetables

When the frequency score of using different food items were consolidated to three groups *ie.* the score ranging from 100 to 80 as most frequently used food items, 79 to 50 for moderately used and below 50 as less frequently used food items (Table 7), it was observed that the food items most frequently used by the EG included cereals, fish, nuts and oil seeds, fats and oils, sugars and jaggery. The moderate frequently used items were found to be roots and tubers. Meanwhile vegetables, pulses, meat, egg, fruits, milk and milk products and green leafy vegetables were categorized as less frequently used foods by the experimental groups.

The food items most frequently used by the CG included cereals, fish, nuts and oil seeds, fats and oils, sugar and jaggery. Moderate frequently used foods included roots, tubers and vegetables. The food items less frequently used were pulses, meat, egg, fruits, milk and milk products, green leafy vegetables.

4.1.2.2 Frequency of Use of β -carotene Rich Foods

Fruits and vegetables have a strong protective role to play and the protective effect of these is attributed to micronutrients especially vitamin A and β -carotene. Vitamin A and β -carotene appeared to be protective in the promotional aspects of cancer. Data regarding the frequency of use of β -carotene rich foods in the daily diet is presented in Table 8.

Focusing on the use of green leafy vegetables, it was observed that green leafy vegetables like amaranth was used occasionally by 23.30 per cent and 13.30 per cent subjects in the EG and CG while majority (76.70 and 86.70 %) never used amaranth in their diets.

Ten per cent and 16.70 per cent subjects in the EG and CG were using spinach occasionally in their diet. At the same time 90.00 per cent subjects in the EG and 83.30 per cent in the CG have never used spinach as a food item.

Data revealed that drumstick leaves was used more than twice in a week by 3.30 per cent subjects in the EG while non of the subjects in the CG included the item more than twice a week. However 83.40 per cent in the EG and 76.70 per cent in the CG included drumstick leaves occasionally while 13.30 per cent and 23.30 per cent in the EG and CG never used drumstick leaves as a food item.

Considering the frequency of use of cabbage, 63.40 per cent subjects in EG and 70.00 per cent subjects in the CG were found to included cabbage more than twice in a week in their meal while 30.00 per cent and 23.30 per cent in the EG and CG used the vegetable only occasionally. But 6.60 per cent subjects each in the EG and CG never used cabbage in their diets. Coriander leaves was used occasionally by 80.00 per cent subjects in the EG and 90.00 per cent in the CG, 20.00 and 10.00 per cent subjects in the EG and CG never used coriander leaves in their diet.

Regarding use of other vegetables it was noticed that only 6.70 per cent of the subjects in the EG included drumstick daily in their diet, 10.00 per cent in the EG and 16.70 per cent in the CG used it more than twice in a week. 80.00 per cent and 76.60 per cent subjects in the EG and CG used it occasionally. While 3.30 per cent in EG and 6.70 per cent in CG never included drumstick in their diet.

Beans was included more than twice in a week by 20.00 per cent subjects in the EG and 13.30 per cent subjects in the CG. Remaining

Table 8. Frequency of using β -carotene rich foods.

Food items	Group	Daily	More than twice a week	Occasionally	Never
Green leafy vegetables					
Amaranth	EG	-	-	7(23.30)	23(76.70)
	CG	-	-	4(13.30)	26(86.70)
Spinach	EG	-	-	3(10.00)	27(90.00)
	CG	-	-	5(16.70)	25(83.30)
Drumstick leaves	EG	-	1(3.30)	25(83.40)	4(13.30)
	CG	-	-	23(76.70)	7(23.30)
Cabbage	EG	-	19(63.40)	9(30.00)	2(6.60)
	CG	-	21(70.0)	7(23.30)	2(6.70)
Coriander leaves	EG	-	-	24(80.00)	6(20.00)
	CG	-	-	27(90.00)	3(10.00)
Other vegetables					
Drumstick	EG	2 (6.70)	3(10.00)	24(80.00)	1(3.30)
	CG	-	5(16.70)	23(76.60)	2(6.70)
Beans	EG	-	6(20.00)	24(80.00)	-
	CG	-	4(13.30)	25(83.40)	1(3.30)
Little gourd	EG	-	5(16.70)	24(80.00)	1(3.30)
	CG	-	9(30.00)	20(66.70)	1(3.30)
Ladies finger	EG	-	10(33.30)	20(66.70)	-
	CG	-	6(20.00)	22(73.30)	2(6.70)
Tomato (ripe)	EG	3(10.00)	22(73.30)	5(16.70)	-
	CG	4(13.30)	23(76.70)	3(10.00)	-
Roots and tubers					
Carrot	EG	-	-	30(100.00)	-
	CG	-	-	28(93.30)	2(6.70)
Potato	EG	-	3(10.00)	7(23.30)	20(66.70)
	CG	-	4(13.30)	5(16.70)	21(70.00)
Yam	EG	-	-	2(6.70)	28(93.30)
	CG	-	-	4(13.30)	26(86.70)
Fruits					
Banana	EG	-	3(10.00)	27(90.00)	-
	CG	-	4(13.30)	23(76.70)	3(10.00)
Jack fruit	EG	-	-	30(100.00)	-
	CG	-	-	30(100.00)	-
Mango (ripe)	EG	-	-	30(100.00)	-
	CG	-	-	30(100.00)	-
Orange	EG	-	-	2(6.70)	28(93.30)
	CG	-	-	-	30(100.00)
Papaya	EG	-	-	2(6.70)	28(93.30)
	CG	-	-	4(13.30)	26(86.70)

Figure in parenthesis denotes percentages

80.00 per cent and 83.40 per cent subjects in the EG and CG used it occasionally and 3.30 per cent subjects in the CG never used beans in their diet.

It was noticed that little gourd was included more than twice in a week by 16.70 per cent and 30.00 per cent of the subjects in the EG and CG respectively. Eighty per cent and 66.70 per cent subjects in the EG and CG used this vegetable occasionally while 3.30 per cent subjects in both the groups never used this in their diet.

Ladies finger was used more than twice in a week by 33.30 per cent and 20.00 per cent of the subjects in the EG and CG. About 66.70 per cent and 73.30 per cent subjects in the EG and CG used ladies finger occasionally while 6.70 per cent subjects in the CG never used this vegetable in their diet.

It was observed that tomato (ripe) was included daily by 10.00 per cent and 13.30 per cent subjects in the EG and CG. However 73.30 per cent subjects in the EG and 76.70 per cent in the CG used tomato more than twice in a week, while 16.70 per cent and 10.00 per cent subjects in the EG and CG included tomato occasionally in their diet.

Among the β -carotene rich foods categorized under roots and tubers, carrot was used occasionally by all the subjects (100 %) in the EG and 93.30 per cent in the CG. At the same time, 6.70 per cent subjects in the CG never used carrots in their diet.

Potato was used more than twice in a week by a group of 10.00 per cent and 13.30 per cent subjects in the EG and CG respectively. Where as 23.30 per cent and 16.70 per cent subjects in the EG and CG included potato occasionally while 66.70 per cent and 70.00 per cent in both the groups never used potato in their diets.

It was found that yam was included occasionally by 6.70 per cent and 13.30 per cent subjects in the EG and CG while 93.30 per cent subjects in EG and 86.70 per cent in the CG were not in the habit of using yam in their diet.

Data pertaining to the frequency of use of β -carotene rich fruits described that fruits like banana was used more than twice in a week by 10.00 per cent and 13.30 per cent subjects in the EG and CG respectively. Ninety per cent and 76.70 per cent subjects in the EG and CG included banana occasionally in their diets while 10.00 per cent subjects in the CG never included banana in their diets. Mango (ripe) and jackfruit were used occasionally in the diet by all the subjects in the EG and CG. It was noticed that 6.70 per cent subjects in the EG included orange occasionally in their diet, while 93.30 per cent subjects in the EG and 100.00 per cent subjects in the CG never used orange in their diet. Papaya (ripe) was used by 6.70 per cent and 13.30 per cent subjects in the EG and CG while it was never included by 93.30 per cent and 86.70 per cent subjects in the EG and CG.

Table 9. Frequency score of β -carotene rich foods

Food item	Frequency score	
	Experiment group	Control group
1. Green leafy vegetables		
Amaranth	31	28
Spinach	28	29
Drumstick leaves	47	44
Cabbage	64	66
Coriander leaves	45	48
2. Other vegetables		
Drumstick	55	52
Beans	55	53
Little gourd	53	57
Ladies finger	58	53
Tomato (ripe)	73	76
3. Roots and tubers		
Carrot	50	48
Potato	36	36
Yam	27	28
4. Fruits		
Banana	53	51
Jack fruit	50	50
Mango	50	50
Orange	27	25
Papaya	27	28

When the frequency score of different β -carotene rich food was analysed (Table 9) it was revealed that tomato and cabbage obtained maximum frequency scores 73 and 64 respectively by EG and 76, 66 by CG. Ladies finger and beans obtained frequency scores of 58, 55 respectively by EG while scores for the same items by CG were 53 and 53. Drumstick, banana and little gourd obtained frequency scores of 55, 53 and 53 by EG and the same were 52, 51, 57 by CG. Items like carrot, jackfruit and mango (ripe) obtained frequency score of 50 each by EG and 48, 50, 50 respectively by CG. Drumstick leaves, coriander leaves and potato recorded frequency scores of 47, 45 and 36 by EG and 44, 48, and 36 by CG. Frequency scores for amaranth, spinach and yam were 31, 28, 27 by EG and 28, 29, 28 by CG. Lowest frequency score in both groups were found for fruits like papaya and orange where the scores were 27 each by EG and 25 for papaya and 28 for orange by CG.

4.1.2.3 Actual Quantity of Food Consumed by the Respondents

The actual food intake of the subjects in the EG and CG was assessed by conducting one day recall method in order to determine the quality and quantity of food and also the nutrient content of the diet consumed by the women.

From the data collected by recall method, the raw equivalent of the foods consumed was computed. The nutrient value of foods consumed were calculated using the food composition tables of ICMR (1999). The quantity of each food item consumed was compared with quantity specified as per balanced diet (ICMR, 1999).

Table 10 represents the mean actual food intake of the subjects in the experimental group and control group.

Table 10. Actual food intake of the respondents

Food item	RDA*(g)	Average quantity of food consumed		% of RDA met	
		EG	CG	EG	CG
Cereals	350	325.40	327.20	92.97	93.48
Pulses	50	7.00	5.00	14.00	10.00
Roots and tubers	50	43.80	38.60	87.60	77.20
Vegetables	75	11.50	9.30	15.33	12.40
Fruits	60	5.00	4.30	8.33	7.16
Green leafy vegetables	100	3.60	3.00	3.60	3.00
Milk and milk products	250	106.30	109.00	42.52	43.60
Flesh food (mainly fish)	90	71.60	74.00	79.55	82.22
Fat and oils	35	15.00	12.60	42.85	36.00
Sugar and jaggery	30	11.20	13.50	37.33	45.00

* ICMR (1999)

Among the various food items consumed by the subjects, the intake of cereals met 92.97 per cent of RDA for the subjects in the EG and 93.48 per cent for subjects in the CG. The intake of pulses, fruits, green leafy and other vegetables were very poor for both the groups and the mean consumption rate was below 20.00 per cent of the RDA. The intake of milk and milk products, fats and oils, sugar and jaggery were also poor for both the groups and the consumption rate was below 45.00 per cent of the RDA. The intake of roots and tubers met 87.60 per cent of RDA for EG and 77.20 per cent of RDA for CG. Intake of flesh foods met 79.55 per cent of RDA for EG and 82.22 per cent of RDA for CG. However fish was the only flesh food consumed by the women.

4.1.2.3.1 Mean nutrient intake of the respondents

The mean nutrient intake of the subjects in the EG and CG per day is presented in Table 11.

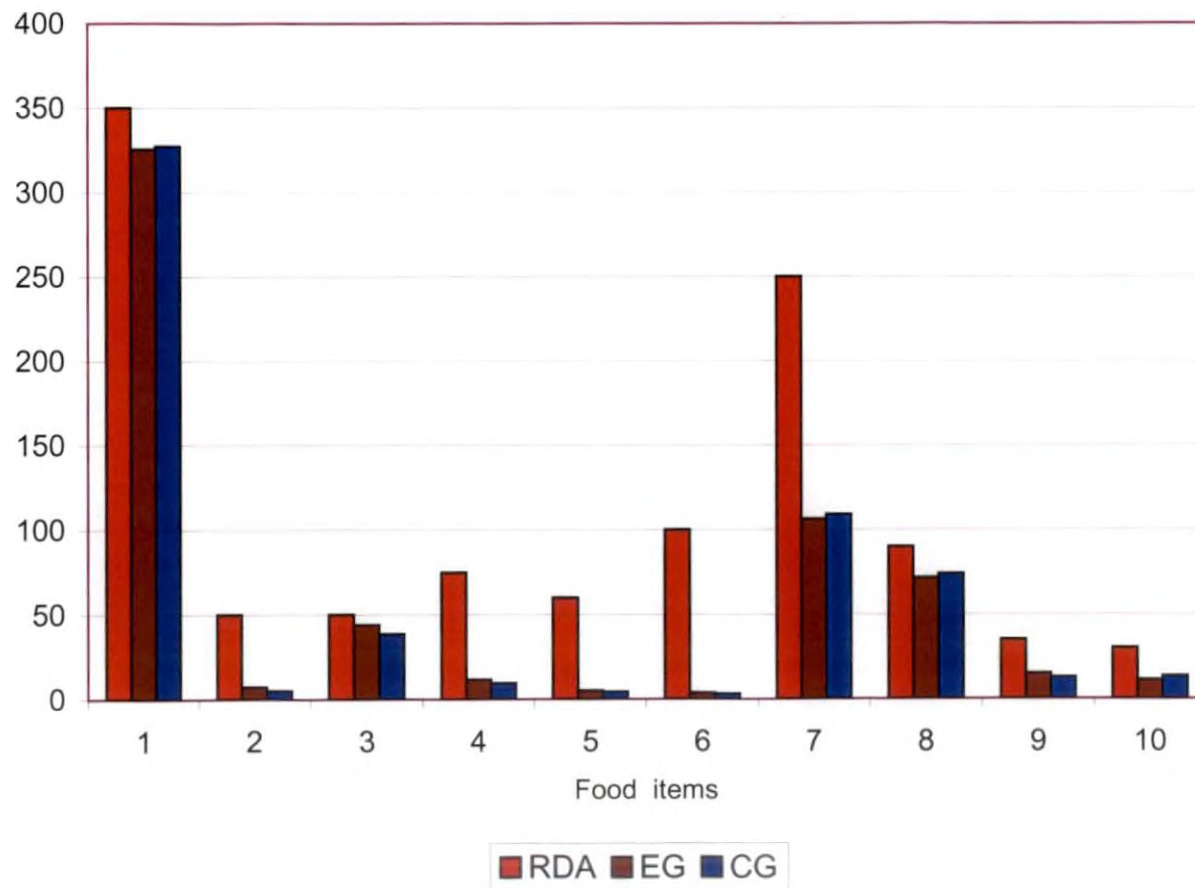
Table 11. Mean nutrient intake of respondents per day

Nutrients	**RDA (g)	Average intake of nutrient		't' value	% of RDA met	
		EG	CG		EG	CG
Calories (Kcal)	2255	1589.70	1591.10	0.029	70.49	70.55
Protein (g)	50	41.10	42.30	0.806	82.20	84.60
Calcium (mg)	400	456.40	512.20	2.258*	114.10	128.05
Iron (mg)	30	17.90	18.20	0.394	59.66	60.66
Vitamin A (μ g)	600	205.20	202.10	0.206	34.20	33.68
Vitamin C (mg)	40	20.80	19.40	0.279	52.00	48.50

*Significant at 1 % level

** ICMR, 1999

Fig. 1. Actual food intake



The intake of calories was below the RDA for both control and experimental groups. The percentage of RDA met by calories in their diet was 70.49 and 70.55 for the EG and CG. Protein intake was 82.20 per cent for EG and 84.60 per cent of RDA for CG. The intake of iron and vitamin C was low and the percentage intake noted was 59.66 per cent and 52.00 per cent respectively for EG and 60.66 per cent and 48.50 per cent for CG. Survey also revealed that dietary intake of vitamin A (β -Carotene) was very poor and was as low as 34.20 per cent among EG and 33.68 per cent for CG. However, calcium intake was found slightly higher than the RDA *ie.* 114.10 per cent for the EG and 128.05 per cent of RDA for CG.

When the average intake of nutrients by the EG and CG was subjected to statistical analysis, it was found that there was no significant difference in the intake of nutrients between the EG and CG. Except for a slight difference found in the intake of calcium. This shows that the dietary and food consumption pattern of the EG and CG was similar and found to be inadequate.

4.2 CLINICAL SYMPTOMS OF ORAL PRECANCEROUS CONDITION IN THE RESPONDENTS

Women habituated with betel chewing were screened for clinical symptoms of oral precancerous conditions through clinical examination of their mouth by conducting a medical camp at their locality. Symptomatic women were further investigated for details related to incidence of cancer in the family, type of oral precancerous condition (leukoplakia and sub-mucous fibrosis), type and size of leukoplakia, type and Inter Labial Diameter (ILD) of sub-mucous fibrosis, difficulty in swallowing and talking, intolerance to spices, problems related to taste perception and appetite. The results obtained are presented below.

Since the family history leads to a clue for the occurrence of certain cancers, details on such line was collected and is shown in Table 12.

Table 12. Distribution of respondents based on family history of cancer

Family history of cancer	EG	CG	Total
Yes	2 (6.70)	3 (10.00)	5 (8.30)
No	28 (93.30)	27 (90.00)	55 (91.70)
Total	30	30	60

Figures in parenthesis denote percentage

Table 12 revealed that 6.70 per cent subjects in the EG and 10.00 per cent in the CG revealed a family history of cancer while the remaining 93.30 per cent in the EG and 90.00 per cent in the CG did not show any history of cancer in their family.

Further details on the type of oral precancerous condition identified among the subjects were collected and is depicted in Table 13.

Table 13. Distribution of respondents based on the type of oral precancerous condition

Symptoms of oral precancerous condition	EG	CG	Total
Leukoplakia	26(86.70)	25(83.30)	51(85.00)
Submucous fibrosis (SMF)	4(13.30)	5(16.70)	9(15.00)
Total	30	30	60

Figures in parenthesis denote percentage

Table 13 revealed that in majority of the subjects both in EG (86.70 %) and CG (83.30 %) the precancerous symptom present was leukoplakia. The rest 13.30 per cent in the EG and 16.70 per cent in the CG were identified with oral submucous fibrosis (SMF) as the related symptom.

Details regarding the type and size of leukoplakia, type and Inter Labial Diameter (ILD) of submucous fibrosis and other characteristics related to precancerous condition of the oral cavity is presented in Table 14.

Table 14. Specific details on the precancerous condition of the respondents

Characteristics	EG	CG
1. Leukoplakia		
a. Type		
Homogenous	11 (36.70)	18 (60.00)
Ulcerated	15 (50.00)	7 (23.30)
b. Size		
< 2 cm	23 (76.70)	16 (53.30)
> 2 cm	3 (10.00)	9 (30.00)
2. Sub-mucous fibrosis (SMF)		
a. Type		
Localized	-	1 (3.30)
Generalized	4 (13.30)	4 (13.30)
b. Inter-labial diameter (ILD)		
> 3 cm (mild)	-	1 (3.30)
1-3 cm (moderate)	4 (13.30)	4 (13.30)
1 cm (severe)	-	-
3. Talking		
Clear	30 (100.00)	30 (100.00)
Nasal twang	-	-
4. Ability to swallow		
Not impaired	30 (100.00)	30 (100.00)
Impaired	-	-
5. Intolerance to spices		
Yes	22 (73.30)	25 (83.30)
No	8 (26.70)	5 (16.70)
6. Taste		
Yes	23 (76.67)	29 (96.70)
No	7 (23.33)	1 (3.30)
7. Appetite		
Yes	22 (73.30)	26 (86.70)
No.	8 (26.70)	4 (13.30)

Figure in parenthesis denote percentage

It was observed that, out of the 86.70 per cent leukoplakic subjects in the EG, 36.70 per cent was identified as homogenous type and 50.00 per cent ulcerated type. In the CG out of the 83.30 per cent leukoplakic subjects, 60.00 per cent belonged to homogenous type and 23.30 per cent was classified as ulcerated type.

Considering the size of leukoplakia, 76.70 per cent subjects in the EG and 53.30 per cent in the CG the size observed was less than 2 cm while 10.00 per cent subjects in the EG and 30.00 per cent in the CG the size of leukoplakia was more than 2 cm.

On assessment of the type of SMF and inter-labial diameter (ILD), it was observed that 13.30 per cent subjects each in the EG and CG was affected with the generalized type of SMF while 3.30 per cent of the subjects in the control group were symptomatic with localized type.

With regard to the ILD it was noticed that among 13.30 per cent subjects both in the EG and CG the ILD was between 1 to 3 cm (moderate). Further, among 3.30 per cent subjects in the CG, the ILD was more than 3 cm (mild). While none of the subjects in the EG and CG exhibited severe type (ILD, 1 cm) of SMF.

While assessing the presence of other characteristics associated with oral precancerous condition among the subjects, it was noticed that none of them both in EG and CG either had any difficulty to talk or an impairment in the ability to swallow.

On verifying the ability to consume spicy foods, it was found that 73.30 per cent in the EG and 83.30 per cent in the CG faced an intolerance to spices while 26.70 per cent in the EG and 16.70 per cent in the CG were able to tolerate spicy food.

Regarding their sense of taste perception, 76.67 per cent and 96.70 per cent respectively in the EG and CG faced no problem with the sensation of taste. Whereas among 23.33 per cent in the EG and 3.30 per cent in the CG, their taste buds had been affected due to the appearance of precancerous symptoms in their oral cavity.

Data regarding the level of appetite of the women, majority of the subjects in the EG and CG (73.30 per cent and 86.70 per cent) reported that they does not have the problem, however, 26.70 per cent in the EG and 13.30 per cent in the CG complained that they were undergoing a condition of poor appetite.

4.3 NUTRITIONAL STATUS OF THE RESPONDENTS

Assessment of nutritional status of the respondents in this study was done by recording anthropometric measurements and by estimating biochemical parameters.

Anthropometry is one of the most frequently used methods for assessing nutritional status. Parameters like height and weight of the subjects were measured and the data are presented in Table 15.

Table 15. Mean height and weight of the respondents

Age (years)	No. of subjects		*Standard		Mean height (cm)		Mean weight (kg)	
	EG	CG	Ht. (cm)	Wt (kg)	EG	CG	EG	CG
40 - 50	14. (46.70)	13 (43.30)	155	50	148.71	145.61	45.54	44.02
Above 50	16 (53.30)	17 (56.70)			151.32	148.44	44.68	44.73

Figures in parenthesis denote percentage

*ICMR, 1994

Mean height and weight of the subjects in the EG and CG were found to be below the ideal height (155 cm) and weight (50 kg) suggested for a reference women (ICMR, 1994). The mean height of the subjects belonging to the age group 40 to 50 years in the EG and CG was found to be 148.71 cm and 145.61 cm respectively. Subjects in the age group above 50 years recorded a mean height of 151.32 cm and 148.44 cm respectively by the EG and CG.

The mean weight of the subjects belonging to the age group 40 to 50 years in the EG and CG was found to be 45.54 kg and 44.02 kg and the corresponding data for subjects in the age group above 50 years were 44.68 kg and 44.73 kg respectively for each group.

Body Mass Index (BMI) of the subjects according to the ICMR classification was worked out and is presented in Table 16.

Table 16. Body Mass Index of respondents

Body Mass Index		EG	CG	Total (%)
<16.0	CED Grade I	-	-	-
16.1 – 17.0	CED Grade II (moderate)	1(3.30)	1(3.30)	3.30
17.1 – 18.5	CED Grade III (mild)	5(16.70)	7(23.40)	20.60
18.6 – 20.0	CED, Low weight (normal)	15(50.00)	12(40.00)	45.60
20.1 – 25.0	Normal	9(30.00)	10(33.30)	31.70

Figures in parenthesis denote percentage

Table values revealed that 20.00 per cent of the subjects in the EG and 27.00 per cent in the CG were either in the group of Chronic Energy Deficiency (CED) I or II. Fifty per cent of subjects in the EG and 40.00 per cent in the CG though classified as normal, were found to have lower body weight when compared to reference standards. None of the subjects in the EG and CG suffered severe energy deficiency while 30.00 per cent subjects in EG and 33.30 per cent in the CG were found to be healthy.

Biochemical assessment represents the most objective assessment of nutritional status of an individual, frequently providing pre or sub-clinical information. Assessment of blood haemoglobin and serum β -carotene levels were carried out on a sub sample and the details are given in Table 17.

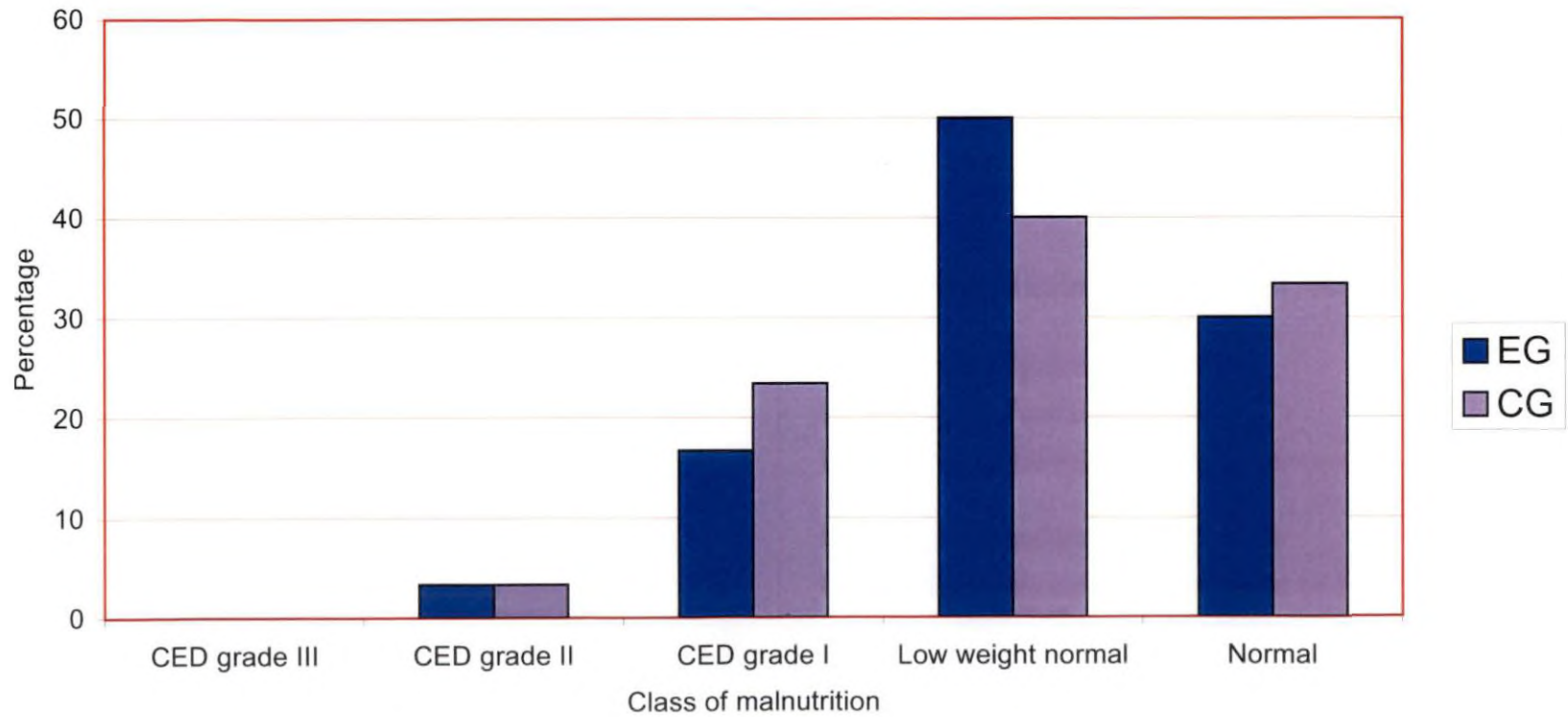
Table 17. Distribution of respondents according to blood haemoglobin and serum β -carotene level

Constituents	Normal range	Details of subjects		Total %
		EG	CG	
Haemoglobin (g/dl)				
Below 10	*11.00g/100ml	25(83.30)	27(90.00)	86.70
10 – 11		5(16.70)	3(10.00)	13.30
Total		30	30	100.00
Serum β-carotene (μg / dl)				
Below 50	**50-250 μ g/100ml	4(40.00)	4(40.00)	40.00
50 – 60		2(20.00)	4(40.00)	30.00
61 – 70		2(20.00)	1(10.00)	15.00
71 – 80		2(20.00)	1(10.00)	15.00
Total		10	10	100

Figures in parenthesis denote percentage

*Swaminathan (1993) **Usharani *et al.* (2001)

Fig. 2. BMI of fisher women



In the present study the iron status of the respondents were assessed with regard to their haemoglobin level. Data presented in Table 18 revealed that for all the subjects both in EG and CG haemoglobin content present in the blood were below the normal level of 11.0 g / 100 ml. Haemoglobin level of 83.30 per cent women in the EG and 90.00 per cent women in CG was found to be below 10 g / 100 ml. Among 16.70 per cent subjects belonging to EG and 10.00 per cent subjects belonging to CG, the haemoglobin level was between 10 to 11 g / 100 ml blood.

Serum β -carotene level of the subjects were assessed. As evident from the Table 18, 40.00 per cent of the respondents both in the EG and CG were found to be having serum β -carotene level below 50 μ g / dl.

Twenty per cent and 40.00 per cent women in the EG and CG showed a level between 50 – 60 μ g / dl, 20.00 per cent in the EG and ten per cent in the CG the β -carotene level was found to be between 61 to 70 μ g / dl. The remaining 20.00 per cent and 10.00 per cent subjects in EG and CG showed a serum β -carotene level between 71 – 80 μ g / dl.

4.4 DISTRIBUTION OF RESPONDENTS BASED ON THEIR PERSONAL HABITS

An individual's health depend on his habits and the way of living. Accordingly details on the personal habits of the respondents *viz.*, chewing, smoking and alcoholism, were enquired and details are depicted in Table 18.

Table 18. Details on the personal habits of respondents

Sl. No.	Characteristics		EG	CG
1.	Habits	Chewing	30(100.00)	30(100.00)
		Smoking	-	-
		Alcohol	-	-
2.	Years of chewing	< 20 yrs	6(20.00)	1(3.30)
		20-30 yrs	15(50.00)	14(46.70)
		> 30 yrs	9(30.0)	15(50.00)
3.	Frequency of chewing (per day)	< 5	11(36.70)	8(26.70)
		5-9	16(53.30)	20(66.70)
		10+	3(10.00)	2(6.60)
4.	Duration of chewing (min)	< 5	2(6.70)	1(3.30)
		5-10	22(73.30)	20(70.00)
		>10	6(20.00)	8(26.70)

Figure in parenthesis denote percentage

Table data revealed that all the subjects (100 %) in the EG and CG had practiced only chewing and were not used to either smoking or alcoholism. Further details collected on the chewing habits indicated that 30.00 per cent women in the EG and 50.00 per cent women in the CG had been accustomed to this habits for more than a period of 30 years. Practice of chewing habits for a period between 20 – 30 years had been followed by 50.00 per cent women in the EG and 46.70 per cent in the CG. However 20.00 per cent in EG and 3.30 per cent in CG were found to be used to this habit since below 20 years.

Considering the daily frequency of chewing betel pan it was found that 36.70 per cent subjects in the EG and 26.70 per cent in CG chewed less than five times a day. Majority of the subjects in the EG and CG *ie.*, 53.30 per cent and 66.70 per cent reported the frequency to be between five to nine times per day. Ten per cent subjects in the EG and 6.60 per cent in the CG were found to be chewing even more than ten times a day.

With respect to the duration of chewing majority of the subject in the EG and CG (73.30 per cent and 70.00 per cent) reported that the time taken for chewing was 5 to 10 minutes. However 20.00 per cent in the EG and 26.70 per cent in the CG took more than ten minutes while 6.70 per cent women in the EG and 3.30 per cent in the CG took less than five minutes for each betel quid chewing.

4.5 EVALUATION OF THE FORMULATED SPIRULINA FOOD SUPPLEMENTS

Spirulina incorporated food supplements standardized at the laboratory were evaluated with respect to nutritive value, organoleptic qualities and preference level of the recipes at the field level. Data pertaining to the above parameters are presented below.

4.5.1 Nutritive Value

The nutritive value *viz.*, calorie, protein, calcium, iron, β -carotene and vitamin C content for per portion of the six supplements were

worked out using the food composition table (ICMR, 1999). It may be noted that the six spirulina incorporated recipes contained the same amount of spray dried spirulina powder per portion. The details are presented in Table 19.

Table 19. Nutrient composition of spirulina incorporated food supplements, per 100 g

Spirulina food supplements	Quantity / portion	Calories (kcal)	Protein (g)	Calcium (mg)	Iron (mg)	β -carotene (μ g)	Vitamin C (mg)
Lemon-ginger squash	30 ml	100.70	1.00	13.70	0.80	2088.00	3.30
Rice ball	60 g	216.70	3.30	35.90	3.60	2062.00	0.70
Coconut chutney powder	67 g	235.9	5.80	48.30	2.90	2088.00	2.60
Rava Laddu	72 g	275.30	4.80	32.90	1.17	2071.70	1.60
Bengal gram ball	57 g	260.40	6.90	24.80	3.10	2195.00	0.59
Green-gram chutney powder	32 g	102.50	7.40	64.70	3.20	2129.00	0.59

Considering the calorific value of the six recipes rava laddu had the highest calorie content (275.30) followed by bengal gram balls (260.40), coconut chutney powder (235.90), rice balls (216.70), green gram chutney powder (102.50) and lemon-ginger squash (100.70).

Protein content was maximum in green gram chutney powder (7.40) followed by bengal gram balls (6.90), coconut chutney powder (5.80), rava laddu (4.80), rice balls (3.20) and lemon ginger squash (1.00).

The levels of calcium indicated that green gram chutney powder had the maximum content (64.70 mg). Coconut chutney powder, rice balls, rava laddu, bengal gram balls and lemon-ginger squash gave calcium values of 48.30, 35.90, 32.90, 24.80 and 13.70 mg respectively.

With regard to iron content rice balls was found to have the highest level (3.60 mg) followed by green gram chutney powder (3.20).

bengal gram balls (3.10), coconut chutney powder (2.90), rava laddu (1.10) and lemon-ginger squash (0.80 mg).

The vitamin C level per portion of lemon-ginger squash was 3.3 mg and coconut chutney powder, 2.60 mg. Rava laddu, rice balls, bengal gram balls and green gram chutney powder contained 1.60 mg, 0.70 mg, 0.59 mg and 0.59 mg of vitamin C respectively.

4.5.2 Acceptability of the Food Supplements (Laboratory Level)

Assessment of organoleptic qualities is carried out mainly to draw conclusion about a particular food from a large population through selection of a limited number of panel members.

The spirulina incorporated food supplements standardized were presented before a panel of 15 technical experts. Organoleptic quality parameters such as appearance, colour, flavour, taste and overall acceptability were assessed on a five point scale and the results are presented in Table 20.

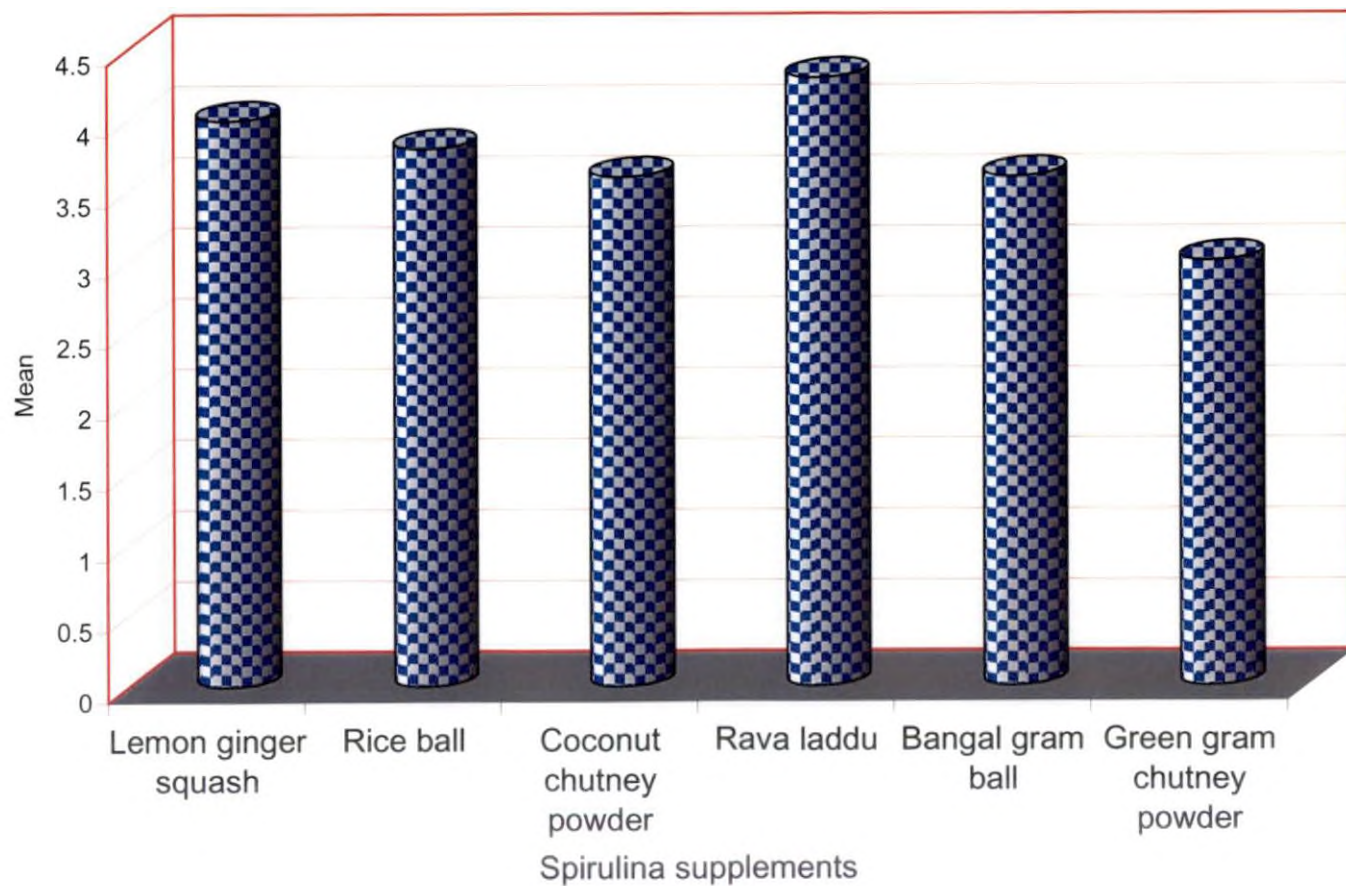
Table 20. Acceptability of spirulina incorporated food supplements (mean scores)

Spirulina food supplements	Appearance	Colour	Flavour	Taste	Overall acceptability
Lemon-ginger squash	3.6	3.5	3.7	4.1	4.0
Rice ball	3.6	3.5	3.6	4.0	3.8
Coconut chutney powder	3.1	3.3	3.0	3.2	3.6
Rava laddu	3.7	3.6	3.6	4.1	4.3
Bengal gram ball	3.4	3.2	3.5	3.8	3.6
Green gram chutney powder	3.0	2.9	2.4	2.8	3.0

Perusal of the data showed that the mean scores for appearance for the spirulina incorporated food supplements ranged from 3.7 to 3.0. It was observed that the maximum score was for rava laddu (3.7) followed by lemon-ginger squash (3.6), rice balls (3.6), bengal gram balls (3.4), coconut chutney powder (3.1) and green gram chutney powder (3.0).

The mean score for colour for the spirulina recipes ranged from 3.6 to 2.9. Rava laddu scored a maximum of 3.6 while lemon ginger squash

Fig. 3. Overall acceptability of the spirulina supplements (laboratory level)



and rice balls scored 3.5 each, coconut-chutney powder (3.3), bengal-gram balls (3.2) and green gram chutney powder (2.9) respectively.

Regarding the quality parameter flavour the mean scores ranged from 3.7 to 2.4. It was observed that the maximum score was for lemon-ginger squash (3.7) followed by rice balls and rava laddu scoring 3.6 each, bengal gram balls (3.5), coconut chutney powder (3.0) and green-gram chutney powder (2.4).

The variation in senses for the quality parameter taste was from 4.1 to 2.8. Both rava laddu and lemon-ginger squash scored as high as 4.1 followed by rice balls (4.0), bengal-gram ball (3.8), coconut-chutney powder (3.2) and green gram chutney powder (2.8).

Scores for overall acceptability of the recipes ranged from 4.3 to 3.0. Rava laddu scored the maximum of 4.3 followed by lemon-ginger squash (4.0), rice balls (3.8), bengal gram balls (3.6), coconut chutney powder (3.6) and green gram chutney powder (3.0).

4.5.3 Preference Level of the Food Supplements (Field Level)

Acceptance of any food supplement will be influenced by the likes and dislikes of the particular group participating in the experiment. Hence a field test was conducted among 15 subjects selected randomly to arrive at conclusions on the preference of the subjects and to select the more acceptable recipes for the experiment. The results are presented in Table 21.

Table 21. Preference ranking of spirulina incorporated food supplements

Spirulina food supplement	Preference order					
	1 st (%)	2 nd (%)	3 rd (%)	4 th (%)	5 th (%)	6 th (%)
Lemon-ginger squash	66.70(10)	33.30 (5)	-	-	-	-
Rice ball	13.30 (2)	33.30 (5)	46.70 (7)	6.70 (1)	-	-
Coconut chutney powder	6.70 (1)	13.30 (2)	26.70 (4)	40.00(6)	13.30(2)	-
Rava laddu	20.00 (3)	60.00 (9)	20.00 (3)	-	-	-
Bengal gram ball	-	-	13.30 (2)	26.70(4)	53.30(8)	6.70 (1)
Green gram chutney powder	-	6.70 (1)	6.70 (1)	20.00(3)	26.60(4)	40.00(6)

Figures in parenthesis denote number of respondents

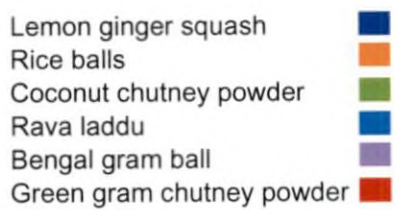
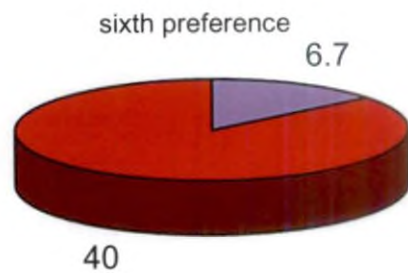
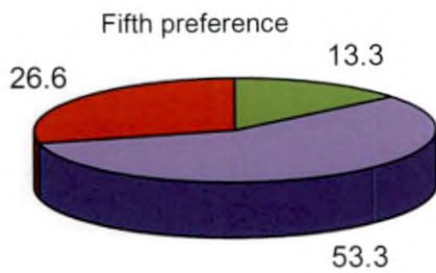
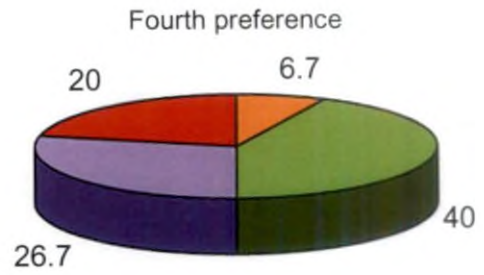
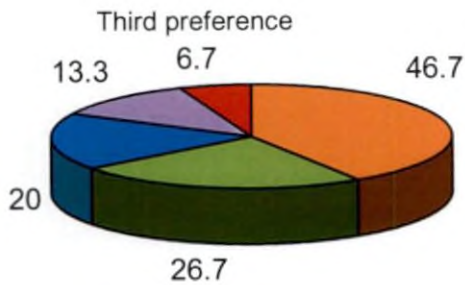
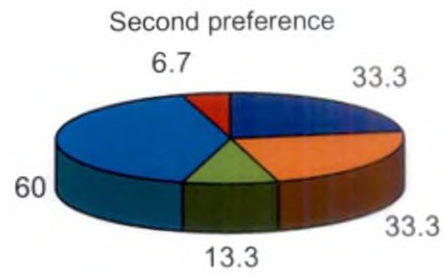
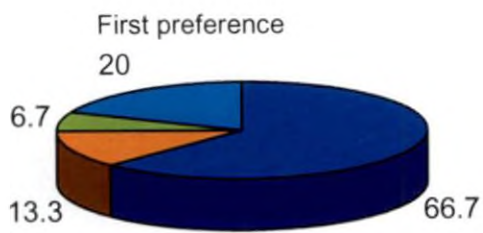


Fig. 4 Preference status of spirulina food supplements (Field level)

When the first preference was noted it was clear that the highest percentage (66.70) of subjects recorded lemon ginger squash as their most preferred item. Twenty per cent of the subjects preferred rava laddu as their first choice. While 13.30 per cent gave preference to rice balls as the best item and 6.70 per cent preferred coconut chutney powder as their first choice. At the same time none of the consumers ranked bengal gram balls and green gram chutney powder as the first preferred item.

While taking an account of the second preference by the subjects it was observed that majority *i.e.* 60.00 per cent preferred rava laddu as their second choice. However 33.30 per cent of the subjects gave preference to lemon ginger squash and rice balls as their second choice, 13.30 per cent subjects preferred coconut chutney powder as their second choice. While none of the subjects preferred bengal gram laddu and green gram chutney powder as their second choice.

When the third preference was taken into consideration, it was found that 46.70 per cent subjects pointed out rice balls as third choice, while 26.70 per cent preferred coconut chutney powder. 20.00 per cent of the subjects preferred rava laddu as their third choice while 13.30 per cent and 6.70 per cent gave preference to bengal gram balls and green gram chutney powder respectively as their third choice. None of the subjects graded lemon-ginger squash for their third choice.

Regarding next preference 40.00 per cent of subjects ranked coconut-chutney powder as their fourth choice. Bengal gram balls, green gram chutney powder and rice balls were ranked by 26.70, 20.00, and 6.70 per cent respectively as their fourth preference. None of the women graded lemon-ginger squash as their fourth preference.

As the fifth preference, 53.30 per cent subjects listed bengal-gram balls. While 26.60 per cent and 13.30 per cent of the subjects ranked green-gram powder as their fifth choice. None of them preferred recipes

like lemon-ginger squash, rice balls and rava laddu to be graded as fifth choice.

Forty per cent subjects ranked green gram chutney powder to be their sixth choice. While 6.70 per cent gave their sixth choice to bengal gram balls. It was also noted that none of the subjects ranked lemon-ginger squash, rice balls, rava laddu and coconut chutney powder to be considered as their sixth choice.

Results of preference ranking by the subjects revealed that highest percentage of the subjects preferred lemon-ginger squash, rava laddu and rice balls as their first, second and third choices.

Nutritional composition of the selected recipes for supplementation are given in Table 22.

Table 22. Nutritional composition of the selected recipes

Spirulina incorporated food supplements	Qty per portion	Nutritive value / 100 g						Overall acceptability	Preference rank
		Calories (kcal)	Protein (g)	Calcium (mg)	Iron (mg)	β -carotene (μ g)	Vitamin C (mg)		
1. Lemon ginger squash	30 ml	100.70	1.00	13.70	0.80	2088.00	3.30	4.00	I
2. Rava laddu	72 g	275.30	4.80	32.90	1.17	2071.70	1.60	4.30	II
3. Rice balls	60 g	216.70	3.30	35.90	3.60	2062.00	0.70	3.80	III

4.6 IMPACT OF SPIRULINA FOOD SUPPLEMENTATION ON THE RESPONDENTS

The impact of food supplements with spirulina at the level of one gram sprayed dried powder per day for a period of six months was evaluated with respect to assessing the changes in the precancerous symptom of the oral cavity, changes in serum β -carotene level and changes in general health profile.

4.6.1 Changes in precancerous symptoms of the oral cavity

Observational studies have found that persons who ingest more β -carotene or β -carotene rich food supplements exhibit a reduced risk of several chronic diseases including cancer.

Data showing the impact of spirulina food supplements on the precancerous conditions of the oral cavity viz., leukoplakia and submucous fibrosis (SMF) is depicted in Table 23.

Table 23. Impact on precancerous conditions of the oral cavity

Oral precancerous condition	Experimental group	Control group
Leukoplakia		
Complete remission	4 (13.40)	-
Regression	12 (40.00)	-
No change	10 (33.30)	23 (76.60)
New lesions	-	2 (6.70)
Submucous fibrosis (SMF)		
Complete remission	-	-
SMF regressed	3 (10.00)	-
No change	1 (3.30)	5 (16.70)
Total	30	30

Figures in parenthesis denote percentage

Post clinical examination of the subjects indicated that out of the 86.70 per cent leukoplakic subjects in the experimental group (EG), 13.40 per cent occupied a status of complete remission from the symptoms and a regression in leukoplakia was recorded among 40.00 per cent of the subjects. However, the symptoms remained unchanged among 33.00 per cent women and observed no evidence of advancement in any case among the experimental subjects.

Results of spirulina supplementation on 13.30 per cent women belonging to EG identified with submucous fibrosis (SMF) indicated that the symptoms were found to regress in 10.00 per cent subjects. Clinical observations also indicated that there was no further aggravation of the situation among the rest 3.30 per cent in the EG.

Clinical observations made among the control group (CG) who were given supplements without the addition of spirulina indicated that out of the 83.30 per cent leukoplakic subjects, the symptoms remained unchanged in 76.60 per cent women where as among 6.70 per cent women new lesions appeared. Regression or remission was not seen either in leukoplakia or in SMF.

Data regarding the effect on type and size of leukoplakia and the type and Inter Labial Diameter (ILD) of SMF due to supplementation of spirulina food is shown in Table 24.

Table 24. Distribution of respondents based on changes in the precancerous symptoms due to spirulina supplementation

Characteristics	EG		CG	
	Before	After	Before	After
1. Leukoplakia				
Type				
Complete remission	-	4 (13.30)	-	-
Homogenous	11 (36.70)	9 (30.00)	18 (60.00)	16 (53.30)
Ulcerated	15 (50.00)	13 (43.40)	7 (23.30)	9 (30.00)
Size				
≤ 2	23 (76.70)	20 (66.70)	16 (53.30)	15 (50.00)
> 2	3 (10.00)	2 (6.70)	9 (30.00)	10 (33.30)
2. Submucous fibrosis (SMF)				
Type				
Localized	-	-	1 (3.30)	1 (3.30)
Generalized	4 (13.30)	4 (13.30)	4 (13.30)	4 (13.30)
Inter labial diameter (ILD)				
> 3 cm (mild)	-	3 (10.00)	1 (3.30)	-
1-3 cm (moderate)	4 (13.30)	1 (3.30)	4 (13.30)	5 (16.70)
1 cm (severe)	-	-	-	-
Other characteristics				
3. Talking				
Clear	30 (100.00)	30 (100.00)	30 (100.00)	30 (100.00)
Nasal twang	-	-	-	-
4. Ability to swallow				
Not impaired	30 (100.00)	30 (100.00)	30 (100.00)	30 (100.00)
Impaired	-	-	-	-
5. Intolerance to spices				
Yes	22 (73.30)	1 (3.30)	25 (83.30)	28 (93.30)
No	8 (26.70)	29 (96.70)	5 (16.70)	2 (6.70)

Figures in parenthesis denote percentage

Detailed verification of the data regarding changes occurred with the various precancerous symptoms over a period of six months spirulina supplementation was made with respect to symptoms viz., type and size of leukoplakia, type and Inter labial diameter (ILD) of submucous fibrosis (SMF), difficulty in talking, swallowing and tolerance to spices.

Discussing on the type of leukoplakia it was found that among the leukoplakia subjects (86.7 %), 50.00 per cent were grouped under ulcerated type and 36.70 per cent were categorized as homogenous type of leukoplakia prior to supplementation. After six months of spirulina

supplementation there observed a complete remission of the symptoms among 13.30 per cent women. The percentage of ulcerated (moderate type) had been reduced to 43.40 per cent from 50.00 per cent and homogenous (mild type) to 30.00 percent from 36.70 per cent. While observing the situation among control group (leukoplakia 83.30 %), the percent of ulcerated type had been found increased from 28.30 per cent to 30 per cent due to conversion of few mild homogenous types to the ulcerated type. Thus the existing homogenous (mild) type had been reduced from 60.00 per cent to 53.30 per cent. Moreover regression or remission had not occurred even to the smallest extent.

Regarding change in the size of leukoplakia among the experimental women as per Table 24 showed that the number of subjects with leukoplakia size more than 2 cm. had been reduced to 6.70 per cent from 10 per cent and the percentage of smaller sized lesions (less than 2 cm) had improved from 76.70 per cent to 66.70 per cent. At the same time an increase in the lesion size had been observed in control group as the number of subjects with lesion size more than 2 cm had increased from 30.00 per cent to 33.30 per cent thus making a reduction in women with the smaller sized lesions (53.30 % to 50.00 %).

Regarding type of submucous fibrosis (SMF), no noticeable change had been observed in both localized and generalized type of SMF and was static both in EG and CG.

On comparing the Inter Labial Diameter (ILD) of SMF before and after supplementation, results indicate that there was an improvement in the ILD among the experimental women. The percentage of women with moderate ILD (between 1 to 3 cm) had been reduced to 3.30 per cent from a position of 13.30 per cent as a result of six months supplementation with 1 g spray dried spirulina thus making the moderate type ILD 3.30 per cent and shifted the remaining 10.00 per cent to the milder form (> 3 cm). Whereas in the control group the data revealed that 3.30 per cent of mild ILD (>3 cm) has been elevated to the moderate

type (between 1 to 3 cm), thus increasing the total percentage of moderate type of 16.70 per cent from the initial level of 13.30 per cent.

There was no noticeable change both among the EG and CG subjects related to talking and swallowing difficulties over a period of 6 months observation.

Regarding the tolerance to spices by symptomatic women due to the existing precancerous lesions 73.30 per cent of the subjects in the EG were not able to tolerate spices and only 26.70 per cent were free of this problem before the feeding experiment. Due to spirulina supplementation almost all the subjects (96.70 %) regained their tolerance to spices at normal levels in their food. By the end of 6 months the problem existed only with 3.30 per cent women. Whereas among the CG by the end of study period intolerance to spices was developed among more women (93.30 %) when compared to existing situation (83.30 %) at the onset of the study.

4.6.2 Change in Serum β -carotene Level

Carotenoids, the proven antioxidant, which is present abundantly in Spirulina, helps in general preservation of health as well as in chemoprevention of degenerative diseases. To generate data on this, serum β -carotene levels (on a sub sample of 10) were estimated before starting the feeding experiment and the same was done after 6 months of feeding 1 g spirulina per day.

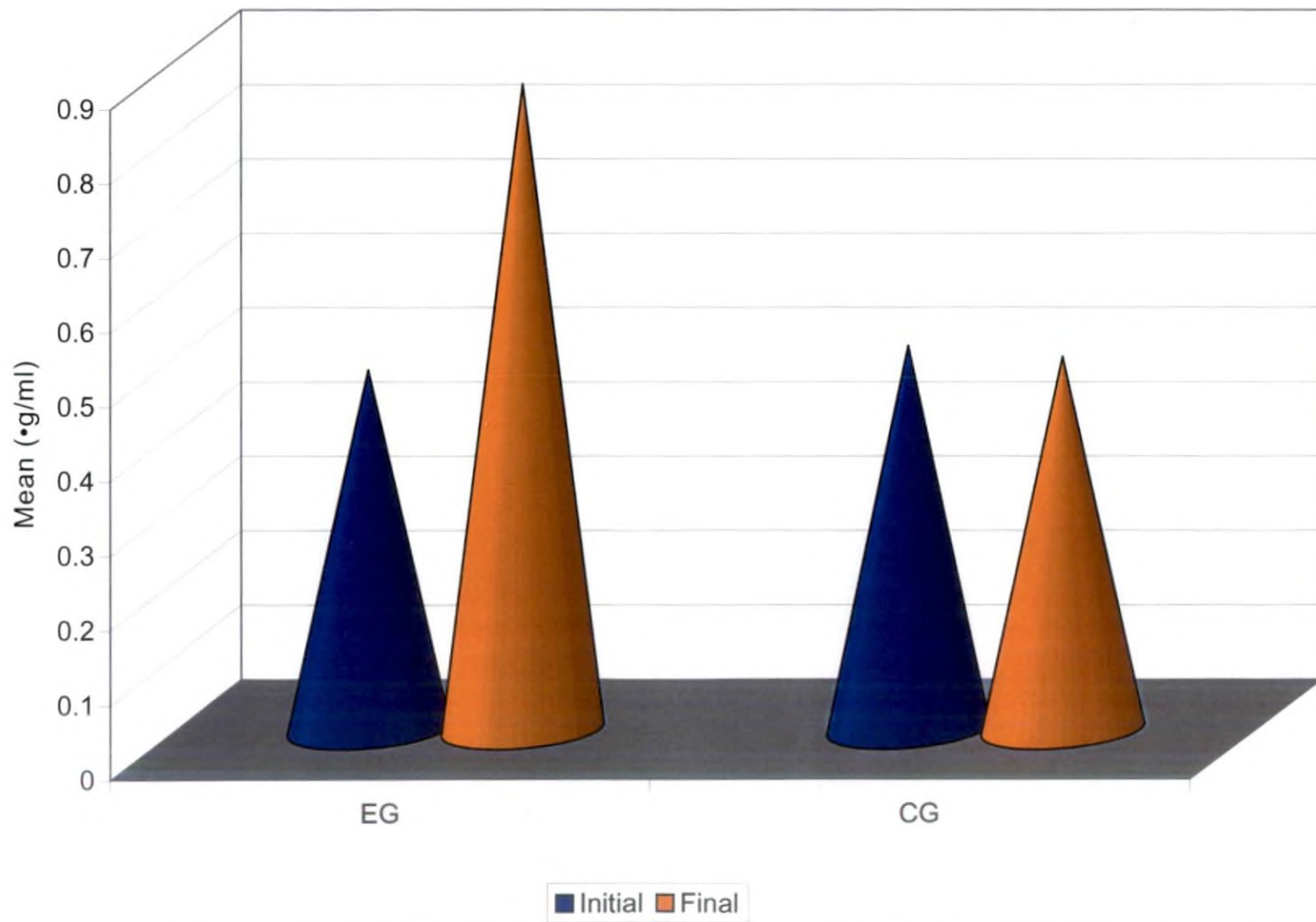
Table 25. Mean initial and final serum β -carotene level of respondents

Study group	Serum β -carotene levels ($\mu\text{g}/\text{ml}$)			
	Initial	Final	Variation	't' value
Experimental (with spirulina)	0.483	0.868	0.385	2.9*
Control (without spirulina)	0.516	0.501	-0.015	0.2

*Significant at 5 % level

Data depicted in Table 25 revealed that there was an increase in the serum β -carotene level of women belonging to the EG. The mean serum β -carotene value was increased to 0.868 $\mu\text{g}/\text{ml}$ from the initial value of 0.483 $\mu\text{g}/\text{ml}$. Whereas β -carotene level of control women was

Fig. 5. Initial and Final serum β -carotene level of the respondents



found to decrease as indicated by initial and final values i.e. 0.516 and 0.501 respectively. Statistical analysis showed that the increase in the serum β -carotene level in experimental group was significant at five per cent level. There was a significant positive correlation (0.6388*) observed between the initial and final serum β -carotene levels of the experimental group.

4.6.3 Changes in General Health Profile

The changes in general health of the subjects were monitored before and after supplementation of spirulina and the results noted are detailed in Table 26 and 27. Changes were mainly noted in their Hb level, taste perception and appetite.

Table 26. Mean initial and final haemoglobin level of respondents

Study group	Haemoglobin (g / dl)			
	Initial	Final	Variation	't' value
Experimental (with spirulina)	9.26	10.43	1.17	6.7*
Control (without spirulina)	8.96	8.33	-0.63	0.2

*Significant at 1 % level

Table values clearly indicate an increase in the haemoglobin level of women in the experimental group after six months supplementation. The mean initial value of 9.26 g/dl was increased to 10.43 g/dl after spirulina supplementation. There was a remarkable increase of 1.17 g in the blood Hb content of the experimental women. At the same time the haemoglobin level of women in the control group was found to decrease over this period as the initial and final values were 8.96 g/dl and 8.33 g/dl respectively.

The increase in the Hb level of the women in the experimental group was found to be statistically significant. There was a significant positive correlation (0.8882**) observed between the initial and final haemoglobin levels of the experimental group.

Fig. 6. Initial and Final blood Hb level of respondents

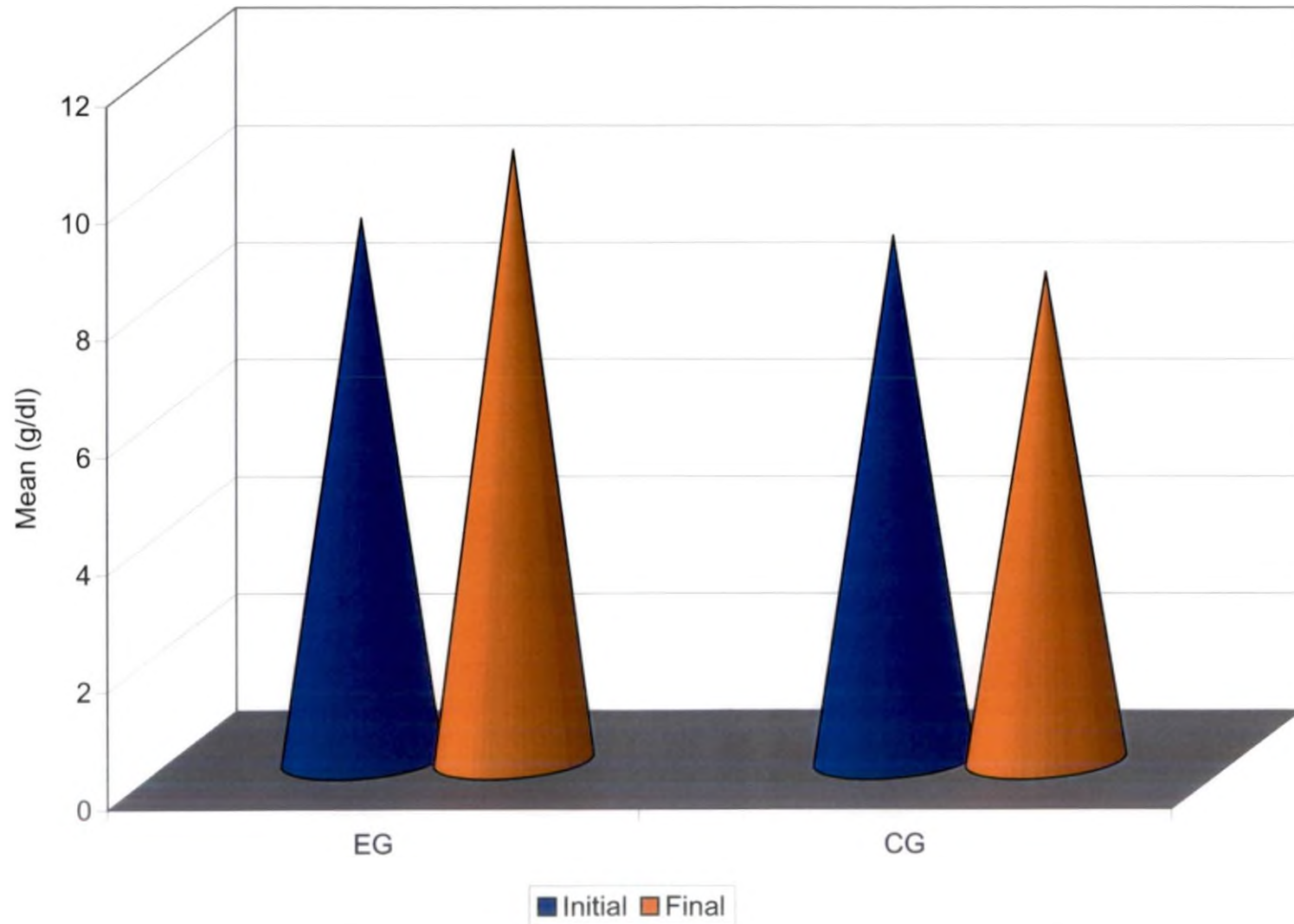


Table 27. Changes in general appetite and taste perception

Particulars	Experimental group		Control group	
	Before	After	Before	After
General Appetite Improved	-	8 (26.70)	-	-
Yes	22 (73.30)	22 (73.30)	26 (86.70)	23 (76.70)
No	8 (26.70)	-	4 (23.30)	7 (23.30)
Taste perception Improved	-	6 (20.00)	-	-
Yes	23 (76.70)	24 (80.00)	29 (96.70)	29 (96.70)
No	7 (23.30)	-	1 (3.30)	1 (3.30)

Regarding changes in the general appetite, 26.70 per cent subjects in the experimental group (EG) and 13.3 per cent in the control group (CG) complained of poor appetite before the experiment. However after six months of feeding experiment with spirulina, it was observed that 26.70 per cent in the experimental women recovered from this problem and regained good appetite. But there was totally no improvement of this situation among the control women.

Among the 30 subjects in the EG, 20.00 per cent reported an improvement in the sensation of taste perception after the feeding experiment. However, in the CG there was no improvement in their sensation of taste among those who reported the problem (3.30 %) before the experiment.

4.7 EFFECT OF NUTRITION AND HEALTH EDUCATION

Education is one of the major means of bringing about the transformation of the society. Nutrition and health education induces desirable behavioural changes for the ultimate improvement in the nutritional and health status of individuals. Unlike the impact of other nutritional interventions like supplementary feeding or prophylaxis with vitamin and mineral supplements, nutrition education has long term effects. Thus the effect of nutrition and health education on the women were evaluated by assessing the alteration in their personal habits oral hygiene, alteration in food habits, and gain in knowledge.

4.7.1 Alteration in Personal Habits and Oral Hygiene

Data on the effect of nutrition and health education on the habits and oral hygiene of the respondents are presented in Table 28.

Table 28. Changes in personal habits and oral hygiene of the respondents

Particulars	Experimental group		Control group		χ^2
	Before	After	Before	After	
Betelquid chewing					
Stopped	-	7(23.30)	-	-	36.74
Continuing with tobacco	30(100.00)	6(20.00)	30(100.00)	30(100.00)	
Continuing without tobacco	-	17(56.70)	-	-	
Wash mouth after chewing					
Yes	13 (43.30)	30(100.00)	9 (30.00)	9 (30.00)	21.02
No	17 (56.70)	-	21 (70.00)	21 (70.00)	
Oral Hygiene					
Good	-	5 (16.60)	-	-	22.94
Better	-	13(43.40)	-	-	
Poor	30 (100.00)	12(40.00)	30(100.00)	30(100.00)	

As an effort of the education programme, 23.30 per cent of the women in the EG could be persuaded to stop completely the habit of chewing. Moreover, another 56.70 per cent of the subjects could be made to switch over to chewing without including tobacco which is a less harmful practice compared to inclusion of tobacco in betel quid while chewing. Only 20.00 per cent women continued the habit of chewing with the inclusion of tobacco. At the same time all the subjects in the CG continued chewing including tobacco as the major ingredient since these control women were not given any education regarding its consequences and no effort was made to stop the habit.

Data regarding changes in the oral hygiene practice was also assessed and it revealed that all the women (100.00 %) followed the practice of washing mouth immediately after chewing while washing habit was not existing among most of the subjects (56.70 %) in the EG prior to the education programme. Notable improvement was found in their general oral hygiene habit also as the same existed in a poor condition in the pre-education period. It was noted that 16.60 per cent women had acquired a 'good' status in oral hygiene and quite a high

number of them (43.40 %) had improved to a 'better' status in oral hygiene, as a result of nutrition and health education. However among 40.00 per cent no change was observed. Meanwhile, oral hygiene of all the subject in the control group remained poor.

4.7.2 Alteration in Food Habits

The diet of fisherwomen is simple and relatively uniform, mainly consisting of rice and fish. In the present study there was not much alterations recorded in the food habits of the subjects after the nutrition and health education programme eventhough the basic knowledge regarding balanced diet, nutrients and role of diet in the prevention of cancer had been enhanced considerably.

4.7.3 Gain in Knowledge

An education effort is generally assessed on the basis of gain in knowledge of the participants. Accordingly a pre test to measure the existing knowledge and post test to quantify the knowledge gain using a common evaluation schedule was conducted and the details are depicted in Table 29.

There were altogether 25 statements and they were grouped, into four categories *viz.*, diet and cancer, importance of antioxidant vitamins,, significance of spirulina and personal habit and cancer. The education programme was conducted only for the subjects in the experimental group.

4.7.3.1 Diet and Cancer

Diet is considered as a major environmental risk factor for cancer. Six statements related to diet and cancer were included to test the knowledge gained on this aspect by the women.

As a result of the nutrition education programme, 70.00 per cent participants became aware of the relationship between diet and cancer.

There was a knowledge gain of 60.00 per cent in the post test, since only 10.00 per cent women had this information before.

After the education session 76.67 per cent subjects were equipped with the information that, high intake of refined carbohydrate and fats, beverages like coffee and tea may cause cancer while only 33.33 per cent had some presumption on this before. The statement on the importance to avoid burnt and smoked food as well as reheated oils as a preventive measure against cancer was answered correctly by 30.00 per cent women during pretest and this figure was increased to 83.33 per cent as an effort of education.

As high as 96.66 per cent women could positively respond to the fact that low intake of fruits and vegetables is associated with increased risk of cancer during the post test session, where as only a small percentage (33.33 %) were found to have this knowledge before the lecture class. As an effort of the class 66.66 per cent subjects were able to learn the role of Vitamin A in the prevention of cancer, while the same was known to merely 16.66 per cent women before the education session.

The information on the particular role of vitamin A among all the vitamins in the prevention of cancer had been known to merely 23.30 per cent women before the class and this knowledge was acquired by as much as 83.33 per cent after the nutrition education classes.

4.7.3.2 Importance of Antioxidant Vitamin

Consistent observation across wide population groups in different countries strongly support the key role played by vitamin A and β -carotene in chemoprevention. Knowledge gain of the respondents regarding eight statements on vitamins and its antioxidant property, revealed that merely 13.33 per cent subjects were aware of the fact that vitamins provide, immunity and protect our body from infections, while after the education programme as high as 63.33 per cent were able to gain this knowledge.

Table 29. Knowledge gain of respondents

Sl. No.	Statements	Pre test score	Post test score
	Diet and Cancer		
1	There is no relation b/w dietary habits and cancer occurrence	3 (10.00)	21 (70.00)
2	High intake of refined carbohydrate, fats, coffee and tea may cause cancer	10 (33.33)	23(76.67)
3	It is better to avoid fried, burnt, smoked foods and use of reheated oils to prevent cancer	9(30.00)	25(83.33)
4	Low intake of fruits and vegetables is associated with an increased risk of cancer	10(33.33)	29(96.66)
5.	Vitamin A (β -carotene) has no role in the prevention of cancer	5(16.66)	20(66.66)
6.	The role of vitamin A (β -carotene) in the prevention of cancer is the same as other vitamins	7(23.33)	25(83.33)
	Antioxidant vitamins		
7.	Vitamins provide immunity and protect our body from infections	4 (13.33)	19(63.33)
8.	Vegetable and fruits are not rich in vitamins	3 (10.00)	27(90.00)
9.	Leafy, yellow and orange coloured vegetables and fruits have the same amount of β -carotene as other vegetables and fruits	2 (6.66)	22(73.33)
10	Carrot, ripe papaya, tomato and egg yolk are not particularly important in β -carotene	6 (20.00)	18 (60.00)
11	Leafy vegetables being low cost foods does not contain good amount of β -carotene	3 (10.00)	21(70.00)
12	β -carotene (vitamin A) is not an important micronutrient	9 (30.00)	27 (90.00)
13	β -carotene (vitamin A) neutralize free radicals before they attack cells	0	20 (66.66)
14	β -carotene (vitamin A) is good for eye sight, healthy skin and prevention of diseases	6 (20.00)	28 (93.33)
	Significance of spirulina		
15	Spirulina is an edible green coloured algae	0	25 (83.33)
16	Spirulina is not used as food	0	20 (66.66)
17	Spirulina is not rich in any nutrient	0	27 (90.00)
18	Spirulina contains equal amount of β -carotene as in carrot	0	19(63.33)
19	Spirulina can be consumed both as a food as well as medicine	0	30 (100.00)
20	Spirulina is a protective food against cancer and many degenerative diseases	0	30 (100.00)
	Personal habits and cancer		
21	Chewing, smoking and use of alcohol does not have any relation to cancer	8 (26.66)	28(93.33)
22.	Chewing may lead to oral cancer	4(13.33)	30(100.00)
23.	Washing the mouth after chewing and retaining the quid in the mouth, both has the same effect on the epithelial tissue	9 (30.00)	27(90.00)
24	Cessation of tobacco and alcohol habits will not help in reversal of precancerous symptoms in oral cavity	3 (10.00)	30(100.00)
25	Chewing with tobacco and chewing without tobacco have the same role to play in the occurrence of cancer	0	30(100.00)
	't' value		17.39*

*Significant at 1 % level

While 10.00 per cent of the women answered positively during pretest, that vegetables and fruits are rich sources of vitamins. This matter was learnt by majority of the women (90.00 %) during the post test period. However 73.33 per cent of the participants became aware that leafy, yellow and orange coloured vegetables and fruits have higher amount of β -carotene than other vegetables, which was known to as few as 6.66 per cent before the lecture class. The important food sources of β - carotene was familiar to 60.00 per cent subjects by the end of the nutrition class where as only 20.00 per cent had and idea on this before. At the time of pretest only 10.00 per cent women knew that low cost food items viz., green leafy vegetables contain good amounts of β - carotene. As a result of the lecture class on nutrition, 70.00 per cent subjects became familiar to this aspect.

Before imparting nutrition education only 30.00 per cent subjects could answer correctly the statement regarding the importance of β - carotene as a micronutrient. However as high as 90.00 per cent of the subjects gave positive answer to this during the post-test. None of the subjects were aware of the role of β -carotene in neutralizing free radicals. As a result of the education effort 66.66 per cent could acquire this information. While only 20.00 per cent of the subjects answered positively that β -carotene is good for eye sight, skin and for prevention of diseases during pre-test, the same was answered positively by majority (93.33 %) in the post test period.

4.7.3.4 Significance of Spirulina

Spirulina algae have higher content of β -carotene and other carotenoids than any other plant source. Six statements were imposed to evaluate the knowledge attained on spirulina and its importance. None of the participants could attempt any of the statements positively as they had not heard about spirulina earlier. There was surprising knowledge gain among the participants pertaining to the significance of spirulina.

Hundred per cent of the participants became aware and identified spirulina as a protective food against cancer. They also learnt that spirulina could be consumed both as food as well as medicine. During the post evaluation 90.00 per cent of the participants were able to realise that spirulina is rich in many nutrients. After the class 83.33 per cent were exposed to the fact that spirulina is an edible green coloured algae. About 66.66 per cent became aware that spirulina is used as food in many parts of the world and 63.33 per cent learned that spirulina contains much higher amount of β -carotene when compared to carrot. The knowledge on all the above points were negative before the attempt made by the investigator.

4.7.3.5 Personal Habits and Cancer

Several studies have indicated that personal habits, like tobacco, chewing, smoking and alcohol consumption are the major risk factor associated with cancer especially cancer of the oral cavity. Five statements were formulated to evaluate the knowledge gain on the relationship between personal habits and cancer occurrence. During post test a higher percentage (93.33) of women were able to state that chewing, smoking and use of alcohol have a positive relationship to cancer occurrence. Whereas this fact was agreed by only 26.66 per cent subjects prior to the class. All the participants became aware of the fact that betel chewing can lead to the occurrence of oral cancer while this was known only to 13.33 per cent before the class. Ninety per cent subjects could be persuaded and learn the beneficial effect of washing mouth after chewing betel quid. Before the education session only 30.00 per cent agreed that not washing mouth after chewing and retaining the quid in the mouth have adverse effects on the epithelial tissue. After listening to the nutrition and health education class imparted by the investigator all the subjects, readily framed their opinion that betel chewing by adding tobacco is worse than chewing without tobacco with

respect to the occurrence of cancer. Whereas none of the subjects could agree to this statement earlier. Only very few participants (10.00 %) knew that cessation of tobacco and alcohol habits would help in the reversal of precancerous symptoms that appear in the oral cavity, however after the lecture class all the participants were able to acquire this knowledge

Statistical analysis showed that the pre and post scores obtained by the experimental women were significant at one per cent level.

DISCUSSION

5. DISCUSSION

Spirulina, one of the blue-green microalgae, is considered as the food of the future, because of its amazing ability to synthesis high quality concentrated food. Carotenoids proven antioxidants which are present in spirulina helps in general preservation of health (Muratte, 1993).

The present study entitled "Impact of spirulina (*Spirulina fusiformis*) food supplement on premalignant conditions in women" was undertaken with a view to assess the effect of spirulina on the precancerous condition of the oral cavity in women.

The results of the study are discussed under the following heads

5.1 Socio-economic status

5.2 Dietary and food consumption pattern

5.3 Clinical symptoms

5.4 Nutritional status

5.5 Personal habits

5.6 Organoleptic evaluation of spirulina food supplements

5.7 Impact of spirulina supplementation

5.8 Effect of nutrition and health education

5.1 SOCIO-ECONOMIC STATUS OF THE RESPONDENTS

People living in the rural area were not able to lead a life worthy of human beings due to poverty and their health conditions was the result of the pernicious combination of several socio-economic factors like unemployment, lack of material advancement, poor housing, poor sanitation, malnutrition, social apathy, absence of will power and initiative to change for the better (Rao, 1991). A clear-cut conceptualization is a pre-requisite for notional estimation of the incidence of socio-economic situation of the population and to formulate and implement appropriate programmes for the alleviation and

eradication of the problems identified. With this aim, socio-economic profile of the women subjects in the fishermen community were ascertained.

5.1.1 Personal Characteristics of the Subjects

Age, education and occupation

The present study revealed that 45.00 per cent of the subjects with oral precancerous condition belonged to the age group of 40 to 50 years and 55.00 per cent of the subjects belonged to age group above 50 years. It has been reported that malignant transformation of leukoplakia was not observed in the age group of less than 35 years, and it was three and a half times higher for the age group of 55 years and older than for the age group of 35 to 54 years (Mehta *et al.*, 1981). Oral cancer is a disease of older age and it was reported by Nair *et al.* (1988) that 95.00 per cent of cancer occurred in individuals over 40 years of age. Reports by Sankaranarayanan and Duffy (1989) and Sankaranarayanan (1996) on oral cancer subjects also revealed that majority belonged to the age group 50 to 59 years.

Educational status and literacy rate have been proved to be powerful determinants of nutritional status as it may influence the awareness about importance of good nutrition, which can affect food choice. According to the KSI (2000) the literacy rate of Thiruvananthapuram district is as much as 89.22 per cent. But the present study revealed that majority of the subjects (50.00 %) were illiterate, 41.70 per cent had primary level of education and only 8.30 per cent had an education upto upper primary level. Kanwar and Koranne (1989) reported that 45.30 per cent of working females were uneducated and 34.60 per cent took education upto primary school level.

The people in the fishermen community in Thiruvananthapuram coastal areas were conscious about education. However they were ill-equipped for higher education and not capable to compete with others.

Financial inadequacy could be the major reason, lack of motivation and diseases were also other major causes of illiteracy.

The occupational status directly or indirectly influences the health condition of an individual. In the present study, 28.30 per cent were unemployed, 53.30 per cent were engaged in fish vending, 15.00 per cent were coir workers and a negligible number (3.40 %) were casual labourers. It was found that majority of the women were engaged in fish vending.

Karuna (1993) revealed that 75.30 per cent of the families in the fishermen community were mainly engaged in fishing and fish vending. Government of Kerala (1990) reported that in the marine sector of Kerala, 61.94 per cent of the fishermen were engaged in fishing and the rest in fish marketing and other related activities or in agriculture. It was also noticed in the present study that one third of the women were unemployed and this accounts to the reason for poverty and low health status among this community. Being a coastal area and presence of small coir factories few women were able to earn a livelihood working in this section.

5.1.2 Family Characteristics

Type of family and family size have an influence on the dietary habits of the subjects. The family system in India is changing at a fast rate. Most of them are following nuclear family system instead of traditional joint family system. The present study clearly indicate that most of the women belonged to nuclear family (66.70 %) and only 33.30 per cent belonged to joint family. It might be attributed to the fact that modern concept of nuclear family has gained much momentum in rural areas. Pre-dominance of nuclear type families among those residing in Thiruvananthapuram has been reported by Ranganathan (1996). The same trend is reflected in the present study also.

Family size had an important bearing on the nutrition of its members. The present study indicated that most of the subjects belonged to families with four members (35.00 %) and between 5 – 6 members (38.30 %). According to Park (1997) the average family size in India is four. The above fact in the present study is in accordance with the findings of Boora *et al.* (1999).

On assessing the number of adults and children in the families of the subjects it was found that in majority of the families there were three to four adults (48.40 %) and more than two children (31.70 %). Park (1997) had reported that the demographic profile of India is fast changing and it is characterized by adult population forming 60 per cent and young population (between the age of 15 years) forming 40 per cent.

Regarding the employment status of the subjects, it was found that majority of the family had two (40.00 %) or more than two (45.00 %) employed persons. According to Reddy *et al.* (1993) the employment status of the population is an important determining factor with respect to health and nutritional status. Dandekar and Rath (1971) had reported that due to unemployment and underemployment, at least 30.00 per cent of the population in India were living below the poverty line, which had directly affected their health status. In the present study though most of the families were having two or more than two earning members, due to lack of steady income and low earning capacity they were living below the poverty line.

5.1.3 Economic Status

Household income has to be taken into consideration because it is the family income which determines the socio-economic strata to which a person belongs. In the present study economic status of the families indicated that majority of them (63.30 %) had the monthly income in the range of Rs.1000 – 3000. Karuna (1993) also reported similar data with regard to fishermen families.

As per the survey conducted by KAU (1989), 38.80 per cent of the fishermen families were in the income range of Rs. 500 to 1000 and 38.60 per cent were in the range of Rs.1000 to 2000. According to Giriappa (1990) low average income and high expenditure levels are common features among labourers engaged in fishing, cultivation of vegetables and in plantation.

The present study indicated that most of the families had a per capita income of Rs.500 to 600.

Several studies has highlighted the association between oral cancer and deprivation, by indicating a poorer profile of morbidity and mortality in areas with low levels of socio-economic indicators.

The current investigation thus establishes the fact that the fisherwomen suffering from oral precancerous condition are from low socio-economic strata. Mc Furlane (1996) observed non-favourable trends in mortality for cancers of the mouth, tongue and pharynx and attributed it to the fact that the largest increase in incidence had occurred in socially deprived areas, the same ones in which survival was lower. Foster and Lowry (1997) also observed oral cancer incidence and mortality as linked to material deprivation. They suggested that material deprivation perse could increase cancer risk and would be more prevalent amongst persons subjected to poor living and working condition.

5.2 DIETARY AND FOOD CONSUMPTION PATTERN

Food is the major vehicle for improving the nutrition of people and hence assessment of food consumption and dietary habits of the people is very important. Dietary intake is markedly influenced by income level.

The present study revealed that majority (61.70 %) of the families spent Rs.1001 to 1500 on food. It is an established fact that poor households will spent higher proportion of their income on food. Lipton (1989) in his study on under nutrition and poverty had reported similar

results. Report by Kamal (2000) is also in line with the above result. However factors like food preference, availability of food items in the locality, knowledge of nutritional values of certain food items, relative prices of food articles and urgency of non-food expences were also found to determine the food expenditure pattern.

Most of the subjects (76.70 %) spent below Rs.300 as per capita expenditure on food in the present study. A survey conducted by National Nutrition Monitoring Bureau (1996) revealed that the dietary intake was found to be markedly influenced by income level.

As revealed in earlier studies conducted in Thiruvananthapuram district by Suja (1989), Felsy (1989), Sujatha (1990) and Karuna (1993) the food consumption pattern of the fishermen families was observed to be habitual non-vegetarian type.

5.2.1 Frequency of use of Food Items

Food frequency study indicates the frequency of use of different food items by the family and is usually influenced by dietary pattern of the family.

The present study indicated that in all the families the daily diet mainly comprised of energy rich foods especially rice. This dietary regim of low income families is supported by Johnson *et al.* (1994) who reported that the diet of the population comprised of food articles like cereals especially rice. This is also in accordance with the findings of Srinivasan *et al.* (1991) and Prema (1988).

Majority of the families included roots and tubers thrice in a week and it was noted that tapioca is the commonly used tuber crop. According to NNMB (1994) tapioca is the most commonly consumed tuber by the common population of Thiruvananthapuram. The reason is its easy availability and low cost compared to other tubers. Due to the same advantage it is used as the staple food among the economically

lower sections. This trend among the fisher folk was reported by Prema (1988).

Regarding the use of protein rich foods it was noted that most of the families included fish daily in their diets. This feature was supported by Karuna (1993) and Nayak (1993) in their studies on fishermen community. According to Nayak (1993) diet of fishing community of southwest coast of India consisted of rice and fish as primary item. Since fish vending is the major occupation of the women, the days left over fish after sales was taken for their household use. This has accounted for an almost regular use of fish in the diet. At the same time, there was only an occasional use of other protein foods, viz., pulses, egg, milk and milk products by most of the families except meat. Meat was included once in a week by a good number of the families. The same pattern in frequency of use of these food items was observed by Kung *et al.* (1996) and Vahab (1997). The comparatively higher cost of protein foods can be pointed out as the reason for the less frequency in use of meat, egg, milk and pulses.

Consumption of vitamin and mineral rich foods revealed an occasional use of vegetables and fruits by majority of the families. Use of green leafy vegetables was found to be very rare by the families. Sujatha (1990) reported on the negligible use of leafy vegetables by the coastal population. Survey among fishermen families by Krishna (1988) and Karuna (1993) observed that foods like vegetable and fruits, which are the major source of vitamins and iron, are rarely included in the diet. The low frequency of use of green leafy vegetables and other vegetables indicate their ignorance on the importance of this food group and is reflected in their health status assessed by biochemical tests. The rare use of these food items could also be related to their habitual pattern of diet consisting of rice and fish.

Even though all the families included nuts and oil seeds, fats and oils, sugar and jaggery daily in their diets, the quantity used was meager.

Thus the findings of the study points out that, rice and fish were the main items in the daily diets of the fisherwomen with occasional or rare use of protein and vitamin rich foods *viz.*, pulses, milk and milk products, egg, meat, green leafy vegetables, other vegetables and fruits. Even though majority of the respondents were employed, their income level was very low. Moreover household purchase of most of the items was limited to once in a week and was inadequate in quantity. They were forced to manage the whole week's meal with whatever being purchased except fish. Vegetables were purchased only on sundays since they do not go for work or sell fish on this day. They usually cook during the evening and the left over rice was taken for the next day along with fish curry and rarely included other protective food items. It would be apt to mention that this has become their habitual dietary pattern.

5.2.2 Frequency of Use of β -carotene Rich Foods

According to Ottensen *et al.* (1989) nutritional status pertains to the condition of health of the individual, affected by the intake of foods and the utilization of nutrients. Epidemiological evidences point to an inverse relationship between the intake of beta-carotene containing foods and the incidence of cancers at various sites. Beta-carotene has been reported to be a free radical trap and quencher of singlet oxygen. Vegetables, fruits and other beta-carotene foods are potent sources of antioxidant nutrients which may protect our cells from damage, halting the earlier process that lead to cancer. Results of the study show that β -carotene rich green-leafy vegetables like amaranth, spinach, drumstick leaves, cabbage and coriander leaves were used only occasionally by most of the respondents. Use of other vegetables rich in β -carotene like drumstick, beans, little gourd, tomato (ripe) and ladies finger were only occasional by most of the women. However very few subjects were including these items more than twice in a week and vegetables like drumstick and tomato (ripe) were used daily by a small group (6.70 and

10.00 per cent) of the women. Study by Ryan *et al.* (1984) covering six villages in South India revealed that these people consumed low amounts of vegetables and the quantity consumed was subject to seasonal variations.

Roots and tubers like carrot, potato and yam, which are good sources of β -carotene were consumed occasionally, or never by majority of the subjects. Other vegetables like drumstick, beans, little gourd and ladies finger were also occasional by majority of the families. Ripe tomato was the only item used more than twice in a week by most women. Fruits like papaya, mango (ripe), jackfruit, banana and orange were consumed occasionally or never by most of the women. Gopalan and Kaur (1989) pointed out that this trend of low intake of protective foods like vegetables and fruits is the common feature of the diets of low-income groups. Ryan *et al.* (1984) observed that the consumption of fruits were invariably absent in the diets of people living in villages of South India. Srinivasan *et al.* (1991) and Karuna (1993) also reported this feature in the diets of fishermen community.

Usha and Sujatha (1989) reported that the frequency of consumption of green leafy vegetables, yellow vegetables, fresh fruits and carrots was rare. Consumption of protective food was relatively low especially among the oral cancer patients. Such findings were reported by Prasad *et al.* (1995).

Tesoriere *et al.* (1993) has pointed out that dietary intake of carotenoids has been reported to have an inverse relationship with cancer occurrence. Whereas the results of the study indicated that in the food pattern of all women the frequency of use of β -carotene rich foods *viz.*, green leafy vegetables (amaranth, spinach, drumstick leaves, cabbage, coriander leaves), roots and tubers (carrot, potato, yam), other vegetables (drumstick, beans, little gourd, ladies finger, tomato) and fruits (banana, jackfruit, mango, orange, papaya) were occasional to never. Stahelin *et al.* (1991) observed that low plasma carotene which is known to reflect

carotene intake is associated with increased cancer risk. It may be concluded that added to the adverse effect of betel chewing, the significantly low intake of antioxidant nutrients especially β -carotene appears to have influence in the promotional aspects of oral precancerous symptoms in fisherwomen.

5.2.3 Actual Food and Nutrient Intake

Food recall survey revealed that the intake of various food was not balanced or adequate. An adequate diet or balanced diet which provides all essential nutrients in sufficient quantities and proportions is essential to meet the needs of the body. In the present study the intake of cereals, met more than 90 per cent of the RDA. The intake of roots and tubers were marginally adequate. But the intake of food items like vegetables (< 12 g), green leafy vegetables (<5 g), fruits (5 g), pulses (< 50 g) milk and milk products (< 110 g), fats and oils (< 15 g), sugar and jaggery (< 15 g) and flesh foods (15 g) other than fish were very low compared to RDA. It may be seen that all the subjects had haemoglobin level below the normal (11 g/dl) and serum β -carotene level was below the normal values of 50 – 250 μ g/dl among 40.00 per cent subjects. The reduced consumption of green leafy vegetables, other vegetables and fruits might be the reason accountable to such low levels of serum β -carotene and haemoglobin.

Chadha *et al.* (1995) in their study found that there was a higher intake of cereals and lower intake of pulses, vegetables, green leafy vegetables and flesh foods by the rural population. The food intake of people in the coastal areas of Thiruvananthapuram was also observed in tune with the above finding on rural population.

The intake of fish was met by nearly 80.00 per cent of the RDA. Fish being an item having easy accessibility and available at cheaper rate to the fishermen community, its consumption rate was almost satisfactory next to cereals. A study conducted among fishermen

community by Karuna (1993) revealed that their diet consisted mainly of rice and fish. The author also reported that fruits and vegetables were found rarely included in their diets.

The low intake of protective foods along with the regular use of tobacco in betel quid could have enrolled the development of precancerous signs in the oral cavity among the fisherwomen. Findings of Usha and Harshala (1989) on oral cancer patients revealed that the consumption of protective foods like pulses, milk, fresh fruits, vegetables specially green leafy vegetables were in meagre quantities. Thus the actual quantity of food consumed by the fisherwomen with oral precancerous symptoms were found to be low in general with respect to all the food groups. This could be attributed to their low economic status, type of job they are engaged and also food aversions and related difficulties viz., intolerance to spices due to the presence of the lesions in the oral cavity. The kind and amount of food consumed affects the physical well being of an individual.

Food is a prime necessity of life and the presence of essential nutrients in the food determines growth, health and efficiency of a population (Maxine and Sumathi, 1973).

Nutrient intake by the fisherwomen revealed that calorie intake was below 75.00 per cent of the RDA. Other nutrients such as vitamin A (nearly 35.00 % of RDA), iron (nearly 60.00 % of RDA), vitamin C (nearly 50.00 % of RDA) were very low compared to RDA. Study by Karuna (1993) on fisherwomen revealed that their diet was inadequate in calories, vitamins and in minerals especially iron. The same results were reported among the women engaged in coir work in Thiruvananthapuram by Ranganathan (1996). According to Chādha *et al.* (1995) the intake of total calories, iron, retinol, riboflavin and vitamin C were less than RDA in low income populations.

It should be emphasized that vitamin A (retinol / carotene) was the most deficient nutrient found in the diets of these women. This could be

clearly attributed to the poor consumption of meat, egg, green leafy or other vegetables and fruits.

Protein intake could be met more than 80.00 per cent of RDA. Intake of calcium (nearly 115.00 % of RDA) was found slightly higher than the RDA. This could be related to the regular consumption of fish. Similar observations have been reported by NNMB (1994).

Studies conducted on women under unorganized sectors also revealed such a poor nutrient intake (Mitra, 1983; Sujatha, 1990).

Prasad *et al.* (1995) stated that high risk of oral cancer is associated with low intakes of carotene, iron, thiamin, riboflavin, folic acid and vitamin C. Usha and Harshala (1989) reported that in oral cancer patients, the intake of all nutrients were below the recommended allowance of ICMR, specially the low intake of carotene, riboflavin and iron was notable. The precancerous condition prevalent among the fisherwomen in the present study could be attributed to an interplay between two sets of factors, food and long established chewing habits.

Assessment on the nutrient intake by the fisherwomen with precancerous condition revealed that the diet was inadequate in calories, protein, iron, vitamin A and vitamin C while the intake of calcium was found to be adequate and it was found to be 114.10 per cent for experimental group and 128.05 per cent for control group above that of RDA contributed mainly by fish.

The results strongly recommend for a higher intake of dietary carotenoids and other carotene containing supplements as a protective measure against cancer.

5.3 CLINICAL SYMPTOMS OF THE ORAL CAVITY OF THE RESPONDENTS

Clinical examination is one of the essential parts of all nutritional survey, since the ultimate objective is to assess levels of health of

individual and population groups as influenced by the diet and life style (Swaminathan, 1990).

Sixty symptomatic women in the age group of 40 to 55 years were selected through clinical examination conducted among 210 betel chewers.

Informations related to incidence of cancer in the family, clinical symptoms regarding type of oral precancerous condition (leukoplakia and submucous fibrosis), type and size of leukoplakia, type and interlabial diameter of submucous fibrosis, difficulty in swallowing and talking, intolerance to spices, problems related to taste perception and appetite are discussed.

In the investigation a total of 8.30 per cent women have reported a family history of cancer. Study by Sharada and Sivaparvathi (2002) on cancer patients reported similar findings where seven per cent patients were found to have a family history of cancer. There could be even chances of unreported family incidences due to lack of awareness about such diseases by the respondents.

The natural history of development of the disease shows that over 75.00 per cent of oral cancers arise from pre-existing precancerous lesions. Gupta *et al.* (1989) reported that leukoplakia and submucous fibrosis (SMF) are well established precancerous lesions.

The present study revealed that in majority of the fisherwomen (86.70 % in EG and 83.30 % in CG) leukoplakia was the precancerous symptom and the remaining subjects were identified with oral submucous fibrosis. According to Mehta *et al.* (1981) the average annual incidence of oral leukoplakia in Kerala is reported to be $2.1 / 10^3$ males and $1.5 / 10^3$ females. Reports on prevalence rate of submucous fibrosis varied from 0.2 per cent to 1.2 per cent.

Occurrence of such precancerous lesions gave an indication to identify a subset of population at highest risk for developing oral cancer among tobacco habitues who belong to a high risk group..

Regarding the type of leukoplakia it was noted that one third of the fisherwomen in the experimental group were identified with homogenous type and majority (50.00 %) with ulcerated type. In the control women majority belonged to homogenous type and one fourth suffered from ulcerated type of leukoplakia. Pandey *et al.* (2001) also reported 66 per cent oral cancerous cases with ulcerated type of leukoplakia. According to Silverman (1984) the non homogenous lesion *viz.*, ulcerated leukoplakia are at particularly high risk for occurrence of oral cancer. In this study only ulcerated and homogenous type of leukoplakia could be identified among the women belonging to fisherman community and habituated with betel chewing.

On the type of Sub Mucous Fibrosis (SMF) it was observed that 13.30 per cent fisherwomen each in experimental and control group were affected with generalized type of SMF and none was found to be having symptoms of localized type. Pindborg (1980) observed that a rough calculation reveals the presence of not fewer than 2,50,000 cases of SMF in India.

According to Maher *et al.* (1997) oral submucous fibrosis (SMF) is an oral precancerous condition characterized by symptoms such as intolerance to spicy food, altered salivation, progressive difficulty in opening the mouth, and signs like rigidity and stiffening of the oral mucosa and depapillation and altered mobility of the tongue. Thus Inter Labial Diameter or ILD gives an indication to the extent to which an individual can open his/her mouth. In the current investigation among the SMF cases the inter labial diameter was between 1 to 3 cm (moderate) in most of the women (13.30 % in EG and CG). Among few control women the ILD was more than 3 cm (mild) and none of the women in both the groups exhibited severe type (ILD, 1 cm) of SMF. Sankaranarayanan (1996) also reported that the condition of SMF is characterized by stiffness of oral mucosa, restricted mobility, stricturing

of angles of the mouth, inability to protrude the tongue, altered pronunciation and intolerance to spicy hot food.

Padmanabhan *et al.* (1988) reported that in oral precancerous conditions the mucosa feels woody with intolerance to chillies. In the current study majority of the experimental and control women experienced an intolerance to spices, while the remaining subjects could tolerate spicy food.

The study also revealed that the ability to get the real taste of food is also found altered with the existence of precancerous lesions eventhough majority of the women faced no problem regarding taste perception, few among them were prone to problems with taste perception.

The intolerance to spicy food and problems related to taste perception could be attributed to the changes in the oral mucosa due to continuous exposure to betel quid.

Complaints on poor appetite was also reported by the women in both the groups. The low appetite may be due to their irregular meal pattern and general ill health resulted by poor nutrition and characteristic clinical symptoms seen in them.

Thus from the present investigation details regarding clinical symptoms of the oral cavity reveals that there were incidence of family history of cancer among the families. Majority of the subjects suffered from ulcerated and homogenous type of leukoplakia and generalized type of SMF. The ability to open the mouth (ILD) in most of the women were of moderate severity. Majority of the fisherwomen experienced intolerance to spicy food and few had problems related to even taste perception and appetite.

5.4 ASSESSMENT OF NUTRITIONAL STATUS

Nutritional status is one of the critical indicators of health, therefore regular nutritional assessment is important to maintain the health of the respondents (Mourya and Jaya, 1997).

5.4.1 Anthropometry

Anthropometry is the measurement of human body at various ages and levels of nutritional status which is helpful in assessing sub-clinical stages of malnutrition (Rao and Vijayaraghava, 1998).

In the study the mean height of the subjects belonging to the age group 40 to 50 years in the EG and CG was found to be 148.71 cm and 145.61 cm respectively. Subjects in the age group above 50 years recorded a mean height of 151.32 cm and 148.44 cm respectively by the EG and CG.

The mean weight of the subjects belonging to the age group 40 to 50 years in the EG and CG was found to be 45.54 kg and 44.02 kg and the same for subjects in the age group above 50 years was 44.68 kg and 44.73 kg respectively. The data reveals that the mean height and weight of the subjects in the EG and CG were below the ideal height (155 cm) and weight (50 kg) suggested for a reference woman (ICMR, 1994). This reveals that the subjects selected were having poor nutritional status based on the above two parameters viz., height and weight.

Prasad *et al.* (1995) in their study on oral cancer patients who were either tobacco smokers or tobacco chewers reported a mean body weight of 45.2 kg which is below the ideal body weight suggested for a reference woman.

Studies on women in unorganized sectors also showed similar results. Anjula (1989) found that ten per cent of the women had their body weight below 38 kg and height less than 145 cm. According to Karuna (1993) 70.00 per cent of the fisherwomen in Thiruvananthapuram

were below the ideal height and 88.67 per cent were below ideal weight when compared with the Indian reference woman.

Body Mass Index (BMI) is of value in distinguishing the nutritional status of different groups monitoring the adequacy of food and in specifying the proportion of malnutrition in a population.

As indicated in a report of NIN (1991b), body mass index values between 18.5 and 25 was considered to be compatible with health for both men and women. While in the present study 20.00 per cent of the subjects in the EG and 26.70 per cent in the CG were either in the group of Chronic Energy Deficiency (CED) I or II ie., BMI between 16.1 and 18.5. The remaining 50.00 per cent in EG and 40.00 per cent in the CG had BMI with low weight, CED. Only 30.00 per cent in the EG and 33.30 per cent in the CG belonged to normal range of BMI. In a study by Prasad *et al.* (1995) the mean body mass index of oral cancer patients was reported to be 17.

The low body weight, height and body mass index is a reflection of poor nutritional status due to poor dietary habits and low purchasing power of the subjects. It has been documented that protein energy malnourished individuals were at greater risk for developing cancer as they have a higher metabolic susceptibility to carcinogens.

5.4.2 Biochemical Assessment

In the present study the haemoglobin level of all the subjects was below the normal level of 11g / 100 ml. Forty per cent of the subjects both in the EG and CG were found to have deficient serum β -carotene levels. Eventhough the serum β -carotene levels of the remaining 60.00 per cent subjects both in the experimental and control group are with in the prescribed normal levels, it touched only the lower limits (50 – 80 μ g / dl) of the normal range (50 - 250 μ g / dl).

Usha and Sujatha (1989) reported that cancer patients had deficient serum levels of vitamin A, iron, zinc and protein. According to

Stahelin *et al.* (1991) low plasma carotene is associated with increased cancer risk. Prasad *et al.* (1995) also reported low serum vitamin A and low haemoglobin levels in oral cancer patients.

Low blood haemoglobin and serum β -carotene levels of the subjects is an indication of low dietary intake of foods containing vitamins and minerals and the relatively low consumption of green leafy vegetables, fresh fruits and other iron rich foods

5.5 PERSONAL HABITS OF THE RESPONDENTS

The life style pattern of a patient reflects his / her physiological as well as the psychological health.

Among the risk factors associated with personal habits, tobacco consumption seems to be the most important and widely reported (Thomas and Kearsley, 1993; Gupta *et al.*, 1996). According to Franseschi *et al.* (1992) and Mc Farlane *et al.* (1995) tobacco smoking, alcohol consumption and betel quid chewing habits are known risk factors for oral cancer. The incidence of oral cancer for individuals who smoke, drink alcohol and chew betel quid is 123-fold higher than for abstainers (Ko *et al.* 1995).

Gupta *et al.* (1992) reported that smokeless tobacco poses a major risk to women who generally use smokeless forms. In India smokeless tobacco use is shown to cause a variety of periodontal diseases such as recession of gums and oral mucosal lesions, many of which are precancerous. Sanakaranarayanan (1988) reported, in the South Indian states of Kerala and Tamil Nadu 40 per cent to 70 per cent of adult men and 20 per cent to 40 per cent of adult women chew pan.

In the current investigation all the women in the experimental and control group were heavily habituated with betel chewing. Betel quid chewing is a well known habit practiced among women belonging to fishermen community in the coastal areas of Thiruvananthapuram.

The reason narrated by the women is that when they take up fish vending at their younger age, they are forced to get into these habits in order to overcome the smell of fish during vending hours.

According to Hashibe *et al.* (2000) tobacco chewing was a stronger risk factor for oral leukoplakia among women than for men. Study by Mathew *et al.* (1993) emphasise the fact that tobacco chewing has highest effect as a risk factor for cancers of oral cavity. Habits *viz.*, smoking and alcoholism were not observed among the women under study.

The number of years since the women were used to the habit of chewing has an important role in the development of oral precancerous symptoms.

It was observed that majority of the women subjects in the experimental and control group were accustomed to this habit for more than 30 years and between 20 to 30 years. The remaining few recorded chewing history for less than 20 years. Study by Sankaranarayanan (1989) in relation to habit of chewing and duration in years indicated similar trends. The author also pointed out that the risk rate was higher with women who were chewers for a period between 31 to 40 years.

The fact that these fisherwomen start chewing as early as the beginning of their teenage and continued the habit throughout their life can be attributed to the longer duration in years of chewing. Reports by Stich *et al.* (1991) and George *et al.* (1994) reveal similar reasons for the existence of chewing habit for longer years.

The daily frequency seems to be the major predictor of risk with chewing. Present investigation revealed that majority of the women in the experimental and control group (53.30 % and 66.70 %) chewed between five to nine times a day. Few women (10.00 % and 6.60 %) chewed more than ten times a day and the remaining (36.70 % and 26.70 %) chewed less than five times a day. Similar trend was seen in the study carried out by Sankaranarayanan (1989) where majority of the

females chewed five to nine times a day. In another study by Stich *et al.* (1988) the trial participants had chewed an average of 13.1 ± 9.1 quids per day. Sankaranarayanan (1996) suggested that daily frequency and early age of starting tobacco chewing are the major predictors of risk.

The mucosa of betel quid chewers has been reported to demonstrate diverse histopathological changes. Such variations are due to variability in chewing habits, quid consistency, duration of exposure and individual susceptibility to disease (Canniff *et al.*, 1986). The investigation revealed that majority of the women (73.30 % and 70.00 %) in the study group chewed for five to ten minutes. One fourth of the women chewed for more than ten minutes while the remaining chewed for less than five minutes. Stich *et al.* (1988) reported that on an average each quid was kept in the mouth for 26.1 ± 25.4 minutes.

In the present study it was noticed that most of the women never washed their mouth after chewing and their oral cavity was exposed to the quid ingredients which are proved to be carcinogenic in nature.

The features of the study on personal habits of the subjects threw light to the fact that all the fisherwomen in the study were chronic tobacco habitués. The number of years, frequency and duration of chewing were high among majority of the fisher women.

5.6 EVALUATION OF SPIRULINA FOOD SUPPLEMENTS

5.6.1 Acceptability at Laboratory Level

Beyond satisfying the nutritional needs, the foods chosen by people and the quantity consumed depends upto its acceptability. Sensory quality is one of the criteria for the acceptability of any food product by consumers (Herrington, 1991).

Thirumaran (1993) opines that product development and acceptability by the people depend on the overall acceptability. Assessment of organoleptic qualities is carried out mainly to draw conclusion about a particular food from a large population through

selection by the limited number of panel members. Acceptability of food supplements formulated incorporating spray dried spirulina powder carrying an algal flavour which is normally unfamiliar to the traditional culinary taste was scrutinized before feeding.

All the developed supplements were assessed for organoleptic qualities by technical experts (N = 15). The information on the specific sensory characteristics of a food must be obtained by product oriented tests and this information was obtained in the laboratory from the selected trained technical experts (Watts *et al.*, 1989). Quality parameters such as appearance, colour, flavour, taste and overall acceptability were assessed by them.

The first impression of food is usually visual and major part of our willingness to accept a food depends on its appearance. It is a composite of all information about the product and its environment which reaches the eye (Birch *et al.*, 1988). The appearance and colour of the spirulina products were assessed by the panel members and the scores proved that rava laddu was liked best in most of the characters by the judges. Lemon ginger squash and rice ball showed some what similar scores as that of rava laddu. Score values for appearance and colour of bengal gram ball, coconut chutney powder and green gram chutney powder were also satisfactory but lower than that of the other products.

Spray dried spirulina powder originally posses a dark green colour and this parameter had influenced the panel members while scoring the recipes. However inspite of the green colour of the product contributed by spray dried spirulina algae, the scores proved that the eye appeal of the food supplements were highly acceptable to the judges. It was obvious that the green colour of the recipes could not marr their acceptability. The right colour combination created by ingredients used in rava laddu, lemon-ginger squash, rice balls and coconut chutney powder have helped in its colour acceptability. These ingredients when mixed together along with spirulina powder gave a pleasing appearance

than the other two recipes viz., bengal gram ball and green gram chutney powder where the ingredients induced a dull appearance slightly reducing their acceptability.

Ranganna (1991) has stated that flavour is an important factor which enriches the consumer preference of a particular product. It was observed that among the six spirulina incorporated food supplements formulated the flavour attribute of lemon-ginger squash was liked best by the judges. The familiar flavour of lemon and ginger was easily detected by the judges and this must have increased the acceptance of the product. More or less similar scores on flavour preference was observed for rava laddu, rice ball, bengal gram ball and coconut chutney powder. The roasted flavour of the main ingredients in the recipes viz., rava, rice, bengal gram and coconut might have contributed to the good aroma since roasting enhances the flavour of any product. The flavour score for green gram chutney powder was less as the ingredients used were not able to mask the slight off flavour originally present in spirulina.

According to Rolls *et al.* (1981) in the various quality parameter tests, the first preference goes to taste. Taste of the Spirulina recipes were similar to that of flavour choice *i.e.*, lemon-ginger squash and rava laddu were proved to be the most acceptable items in taste by the members. A more or less parallel taste acceptance was obtained for rice balls also. Taste is the major attribute which determines the acceptability of a food material. The combined taste of lemon and ginger ultimately gave a pleasing mouth feel which turned to be preferable to the judges. The addition of ghee, milk and cardamom in rava laddu and like wise coconut, jaggery and cardamom in rice balls enhanced the taste of these products. Bengal gram balls and coconut chutney powder also attained a rather good taste score even though the preference was lower to that of rava laddu, lemon-ginger squash and rice balls. Cashew nut powder in bengal gram balls, roasted coconut and spices in coconut chutney powder have contributed to its taste. Green gram chutney

powder was not found much acceptable and the reason may be the presence of typical spirulina taste which could not be masked well by the ingredients in green gram chutney powder.

The range of overall acceptability scores of these spirulina supplements when judged by the panel members was between 4.0 and 3.0 showing a well desirable and top acceptance to rava laddu. Rava laddu remained as the best spirulina supplement with highest preference in attributes like appearance, colour and taste giving way to its overall acceptability.

Lemon-ginger squash was also highly acceptable closer to rava laddu in overall acceptability range. This beverage with its cordial taste of lemon and ginger could completely hide the spirulina odour. Acceptability of rice balls was also good as per the judges particularly for its good taste, flavour and eye appeal. More or less similar acceptance level was obtained by coconut chutney powder and bengal gram balls. Panel acceptability for green gram chutney powder was found satisfactory with an average grading in most of its attributes.

A glance to the organoleptic quality assessment of the spirulina product evidenced that rava laddu bagged wide acceptability followed by lemon ginger squash and rice balls. The score values of these supplements were found to be high in most of the sensory parameters. The other supplements *viz.*, coconut chutney powder and bengal gram balls also obtained good acceptability as these recipes also received an acceptable level above 70.00 per cent. The acceptability for green gram chutney powder was slightly lower when compared to other supplements.

5.6.2 Preference of the Respondents

For the development of new food products by the use of new ingredients, their acceptability should be assessed by conducting preference test on the consumers (Watt *et al.*, 1989). Therefore the subjective reactions of the fisherwomen (N = 15) in the order of their

preference to the six spirulina food supplements was helpful to select the most preferred three recipes for supplementary feeding.

Preference study on the spirulina food supplements conducted among the respondents clearly indicated that lemon-ginger squash was the best preferred item ranking first position (66.67 %). Kaur and Khurdiya (1993) pointed out that fruit based beverages are becoming increasingly popular. The flavour and taste of spirulina incorporated lemon ginger squash was proved excellent in this study.

Rava laddu was the next product choiced by majority (60.00 %). These type of snacks are quite familiar to the present respondents. Moreover the addition of spirulina had not altered its sensory attributes and the overall acceptability was proved to be high in the organoleptic evaluation.

Rice ball was identified as the third preferred product among the women out of the six spirulina recipes to which they were exposed. The retention of the original taste of this traditional snack inspite of the incorporation of spirulina powder as an additional ingredient, has been evidenced from this field testing. The taste, flavour and colour of rice balls was found to be acceptable in this study.

The fourth preferred item was coconut chutney powder which is again a traditional item. The ingredients mainly roasted coconut and spices in the recipe easily enabled to cover up any conspicuous trace of spirulina in it. Bengal gram ball was ranked fifth in the preference by most women. The taste of spirulina could be better identified in this recipe compared to the other four recipes and hence its preference was ranked next to the above items. Most of the women ranked green gram chutney powder as their sixth preference. In this product there was a visible taste of spirulina and the basic ingredient in green gram along with spirulina might not have caught the fancy of these fisherwomen.

In a nut shell, preference study conducted for spirulina supplements clearly indicated that lemon ginger squash was

overwhelmed by rava laddu and rice balls to take up the first position. Rava laddu and rice balls were already found more or less equal in all the quality parameters and hence in preference ranking these items were identified as the second and third preferred products.

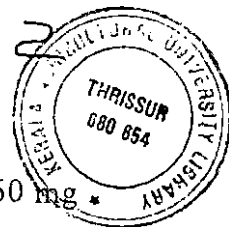
5.6.3 Nutrient Composition

The nutritional composition of the six spirulina food supplements formulated indicated that all the supplements provided higher amount of beta carotene and satisfactory levels of calories, proteins, calcium, iron and vitamin C per 100 g.

The calorie content of the six supplements ranged between 275.30 Kcal to 100.70 Kcal. Calorie content of rava laddu, coconut chutney powder, bengal gram balls and rice balls were more or less comparable. The calorie content of lemon ginger squash and green gram chutney powder was slightly lower than the other four recipes. Squash being a beverage containing water as an ingredient and the inclusion of only few ingredients in green gram chutney powder have resulted in comparatively low calorie content of these items.

The range of protein content varied between 7.40 g and 1.00 g. Green gram chutney powder acquired the highest protein value followed by bengal gram ball being pulse based preparations. The protein content of coconut chutney powder, rava laddu and rice balls were also satisfactory. Lemon-ginger squash had the lowest protein content.

Calcium content of the supplements ranged from 64.70 mg to 13.70 mg per 100 g. Like protein, calcium content was also maximum in green gram chutney powder due to the high contribution of calcium from green gram and pepper in the recipe. This is followed by coconut chutney powder, rice balls, rava laddu and bengal gram balls. Water being the major component, calcium content of lemon-ginger squash was low.



In the spirulina supplements iron content was in the range 3.60 mg to 0.80 mg. Rice balls stood superior in iron content due to the additional contribution of jaggery. Green gram chutney powder, bengal gram ball and coconut chutney powder were having more or less similar values. Lemon ginger squash consisted the lowest iron content.

Beta-carotene content of all the formulated recipes were very high and is fully accountable to the high β -carotene content of spray dried spirulina powder (2038 $\mu\text{g/g}$) included in the recipes. The β -carotene content of the different supplements varied between 2195 μg and 2062 μg .

The vitamin C content of the supplements varied between 3.30 mg to 0.59 mg. Vitamin C content of lemon-ginger squash could be found superior as lemon is rich source of vitamin C added to the contribution from spirulina. The vitamin C content of coconut chutney powder and rava laddu remained almost closer to lemon-ginger squash. This was followed by rice balls, bengal gram balls and green gram chutney powder.

In a nutshell, nutrient composition of spirulina supplements indicated that rava laddu had the highest calorie content, protein and calcium level was highest in green gram chutney powder, iron in rice balls, β -carotene in bengal gram ball and vitamin C in lemon-ginger squash. There was no wide variation among different spirulina supplements in nutrient content except for lemon-ginger squash. Since water constitutes the major portion in squash, it can be rightly stated that the calories, proteins and iron provided per share of the drink seems to be adequate and similarly the vitamin C and calcium contents were also satisfactory.

5.7 IMPACT OF SPIRULINA SUPPLEMENTATION ON WOMEN WITH PRECANCEROUS CONDITION OF ORAL CAVITY

The blue-green micro algae (spirulina) used in the daily diets of natives of Africa and America have been found to be a rich natural source of proteins, carotenoids (β -carotene/retinol) and other micronutrients. Being an effective provitamin, β -carotene shows important degree of antioxidant characteristics in low oxygen pressure (Yamuchi *et al.*, 1993). It has been shown that β -carotene which is wide spread in nature as plant food has a preventive effect against cancer due to its ability to react with free radicals (Block and Langseth, 1994; Singh *et al.*, 1997).

Experimental studies in animal models and humans have demonstrated spirulina to be effective against several diseases. According to Annapurna *et al.* (1991a) spirulina is one of the richest sources of natural β -carotene, the provitamin A. The effects of spirulina include antioxidant activity because of high content of superoxide dimutase and β -carotene (Sheshadri and Dai, 1992). Micronutrients such as β -carotene are thought to protect against oral cancers through an antioxidant effect. Mathew *et al.* (1995) evaluated the chemopreventive activity of *Spirulina fusiformis* in pan tobacco chewers in Kerala. Complete regression of lesions was observed in 20 of 44 evaluated subjects supplemented with spirulina. It has been reported that spirulina has rich chlorophyll which has the ability to heal wounds (Arora, 1996).

Many health effects of spirulina has been reviewed. Annapurna (1991b) examined the bioavailability of total carotenes and β -carotenes from spirulina and the author concluded that spirulina can be used as a source of vitamin A, in the diet and also stated that it has higher β -carotene than any other plant source. Sharma (1992) opines that spirulina is a rich source of natural bio-chelated vitamins containing all the vitamins in highly bio-available form.

Fica (1984) proved the effect of spirulina in combating various nutritional deficiencies viz., weight loss in conjunction with gastric resection, tubercular infection, chronic pancreatitis and gastritis, rheumatoid arthritis, anaemia and diabetes mellitus. Studies carried out by Ramamoorthy and Kumari (1996) indicated that spirulina plays a key role in weight reduction, lowering blood cholesterol levels and improving the lipid profile of patients.

Spirulina has a hypoglycemic effect on non-insulin dependent diabetic patients (Anuradha and Vidhya, 2001). Spirulina is favourable for normalizing the adaptive potential of children's bodies in conditions of long lived low dose radiation (Loseva and Dardynskaya, 1993).

Current investigation also puts light on the positive impact of *Spirulina fusiformis* in combating the precancerous condition of oral cavity and in improving general health.

5.7.1 Changes in Precancerous Condition of the Oral Cavity

Food items like the blue-green algae *Spirulina fusiformis* which are rich in several micronutrients especially β -carotene exhibit an ability to suppress carcinogenesis. With this point of view the effect of spirulina supplementation for a period of six months on precancerous condition of the oral cavity (leukoplakia and submucous fibrosis) in women heavily habituated with betel chewing was analysed. The results revealed that in 13.40 per cent experimental women complete remission of the precancerous condition viz., leukoplakia was observed. Among 40.00 per cent women there was regression in the condition. In the case of submucous fibrosis eventhough complete remission was not observed within the study period, regression of the condition was recorded in ten per cent experimental women. Clinical observations on control women who were supplemented with the same food item without the addition of spirulina revealed that there was a slight aggravation in precancerous (leukoplakia) symptoms observed earlier. The condition was found to be

slightly aggravated in nearly seven per cent women after six months period and in the remaining (76.70) the condition remained static.

Study by Mathew *et al.* (1995) in reversing oral leukoplakia by administering spirulina (1 g/d for 12 months) showed complete regression of lesions in 20 of 44 evaluated subjects. This is an indication that with continuous supplementation of spirulina there will be great improvement in the remission of precancerous symptoms in the oral cavity. The effect of spirulina could be attributed to the high content of the micronutrient β -carotene (2038 $\mu\text{g} / \text{g}$) as several studies have proved vitamin A and β -carotene to be effective chemopreventive agents. In previous trials it was observed that the formation of new leukoplakia was prevented in individuals who continued to chew during the entire period of the trial and whose mucosa was thus exposed daily to tobacco and arecanut related carcinogens and promoters. The maintenance of a protective effect through the continuous administration of lower doses of β -carotene or vitamin A suggests the possibility that a beneficial effect could be considerably prolonged by the periodic administration of heavy and low doses of chemopreventive agents.

Study by Stich *et al.* (1991) on tobacco chewers showed that administration of vitamin A (60 mg/ wk) for six months resulted in complete remission of leukoplakia in 57 per cent, β - carotene (2.2 mmol / wk) induced remission of leukoplakia in 14.8 per cent. Similar results were observed by Krishnaswamy *et al.* (1995) and Flagg *et al.* (1995).

Further analysis on the impact of spirulina on changes in factors related to precancerous signs of the oral cavity in experimental women, it could be highlighted that there was promising improvement recorded in the type of severity of the symptoms. The ulcerated type of leukoplakia was reduced to a milder homogenous type in nearly seven per cent subjects. Among the 13.30 per cent suffering from submucous fibrosis, the Inter Labial Diameter which indicate the extent to which one can open the mouth, improved in nearly three per cent subjects from a

moderate level to milder level. There was also improvement seen in tolerance of spicy food by about 23.40 per cent women in the experimental group.

An encouraging and positive impact on the disease characteristic was noted. The changes observed may be due to the combined effect of all the antioxidant vitamins and minerals present in spirulina. The spirulina supplied for the present investigation contained protein (64.84 %), minerals (8.51 %), total carotene (4801 $\mu\text{g} / \text{g}$), β -carotene (2038 $\mu\text{g} / \text{g}$), calcium (500 mg/kg), iron (400 mg / kg), chlorophyll (1.20 %) and moisture (4.20 %). A combination of micronutrients (Vitamin A, B complex, C, D and E) and minerals (Fe, Ca, Cu, Zn, Mg) was evaluated for its efficacy in controlling oral precancerous condition by Maher *et al.* (1997). Significant improvement in symptoms notably intolerance to spicy food, burning sensation and mouth opening was observed at exit. Also significant proportion of leukoplakia regressed at exit. The above findings corroborates with that of Stich *et al.* (1990, 1991).

The present study thus stresses the positive effect of spirulina in regressing leukoplakia conditions of the oral cavity due to its concentrated nutritional profile.

5.7.2 Changes in Serum β – carotene Levels

It has been stressed by Comstock (1991) that for all cancers the combination of low carotene and low vitamin A was associated with a significantly elevated risk and that among different cancer cases oral cancer has strong and significant association with low levels of serum β -carotene. Nomura *et al.* (1997) also reported low serum β -carotene in upper aero digestive tract cancer patients.

Analysis of the data in the present investigation revealed low levels of serum β -carotene in majority of the women screened with oral cavity precancerous symptoms. Depressed serum levels of β -carotene assessed prior to supplementation may be due to a low daily ingestion of

foods containing β -carotene by the women who are in turn heavy tobacco chewers. Stich *et al.* (1990) stated that low plasma carotene is known to reflect carotene intake. Moreover depressed serum levels of β -carotene were also observed among chronic tobacco chewers.

It has been rightly pointed out by Ciferri (1983) that spirulina algae have a higher content of β -carotene and other carotenoids than any other plant sources. Venkataraman (1992) reported that spirulina has 20 times as much β -carotene as carrot.

The spray dried spirulina used for the present supplementation study contained 2038 μg of β -carotene per gram.

On analysing the effect of supplementation of spirulina food supplements at a rate of one gram spirulina per day for six months revealed a comparatively positive impact on serum β -carotene levels. The mean initial serum β -carotene level were found to be 0.483 μg / ml which was low compared to the standard level of 0.50-2.50 μg / ml. The serum β -carotene levels of experimental women significantly increased by a level of 0.385 μg / ml after six months supplementary feeding with one gram of dried spirulina. Decrease in the β -carotene level of women belonging to control group over six months period gives a clear indication to the beneficial effect of spirulina in improving β -carotene level.

The significant increase in β -carotene level could be attributed to the high β -carotene content of spirulina as well as its bioavailability. It has been reported that carotenes present in spirulina is completely available (NIN, 1990).

Controlled studies carried out at NIN (1990) in adults and children to find out bioavailability of spirulina as a source of vitamin A showed an increase in plasma vitamin A over the basal value in spirulina fed groups when compared with control group. Annapurna *et al.* (1991b) evaluated the effect of daily supplementation of vitamin A (300 μg for

one month) in preschool children which showed significant improvement in the serum retinol level from the initial level.

The serum β -carotene level of the experimental women in the present study significantly increased because of the relatively high availability of β -carotene from spirulina compared to that from green leafy vegetables. β -carotene in spirulina serves as an antioxidant as well as a potent quencher of highly reactive singlet oxygen thus preventing the tissue damage. So it is suggested that spirulina can be used as a dietary source of vitamin A. Thus the experiment evidenced that spirulina could be a low cost alternative to ensure adequate intake of vitamin A.

5.7.3 Changes in General Health Profile

The improvement in general health of an individual reflects the effect of supplementation. The current investigation evaluates the impact of spirulina supplementation on the general health of the fisherwomen participating in the trial. The significant improvement in the blood haemoglobin level, general appetite and taste perception could be recorded among the experimental women. Sheshadri and Dai (1992) reported that dietary supplementation of *Spirulina fusiformis* to preschool children revealed general improvement in health.

Haemoglobin level gives an indication of an individual's general health. In the current investigation supplementation with spirulina contributed an average increase of 1.17 g / dl in blood haemoglobin level. This results is in tune with observations of Seshadri and Valliamma (1992) among malnourished children where there was an increase of 1.33 g/ dl in blood haemoglobin level with the supplementation of two grams of spirulina per day for 36 days. This increase in serum haemoglobin level has been explained to the high bio availability of iron content from spirulina.

The significant increase in haemoglobin level among the experimental women in the present study also can be attributed primarily to the highly available form of iron content present in spirulina (400 mg / kg). More over spirulina is considered to be the richest source of β -carotene which inturn have accounted for the absorption of iron at a higher rate. It is established that vitamin A supplementation improved the haemoglobin level by facilitating a better absorption or by promoting better utilization of iron by the bone marrow. There are several possible sites where vitamin A may exert its action. Of these, the most important ones are uptake and release of iron in the liver, uptake of iron by the bone marrow (which is the site of haemoglobin synthesis) and in haemoglobin synthesis itself (Wolde *et al.*, 1993).

Study conducted by Takuchi (1988) on women supplemented with 4 g spirulina for 30 days showed increase in their average blood haemoglobin content by 21 per cent. Another study by Mani *et al.* (2000) on young anemic girls supplemented with five gram of spray dried spirulina powder for 30 days also revealed an increase in blood haemoglobin levels. Thus it is appropriate to highlight the use of spirulina as an effective means to combat iron deficiency anaemia.

Appetite in most people is a pleasant sensation that causes a person to desire and anticipate food. The sense of taste refers to the ability of the taste organs to perceive and recognize the four basic tastes- sweet, sour, salty and bitter (Maney and Swamy, 2001).

There was improvement in general appetite level among 26.70 per cent experimental women after the intake of spirulina. More over 20.00 per cent women expressed that they could experience an improvement in taste sensation also. This could be linked primarily to the changes that were observed in the precancerous condition of oral cavity in these women. Secondly, the general restoration of health which is a well defined function of vitamins and minerals present in spirulina might have indirectly influenced in improving appetite and taste. The combined

effect of all the micronutrients supplemented by spirulina would have contributed to this positive change.

Miao (1987) reported that children belonging to the age of 2-6 years recovered in a short period from bad appetite, diarrhoea and constipation as a beneficial effect of a baby nourishing formula containing 1.5 g spirulina, 12 g baked sprout, vitamin B₁ and zinc. According to Fica (1984) spirulina tablets given to patients with nutritional deficiencies improved their proteinogram.

On personal interaction with the experimental women during the later days of supplementation, most of them expressed a state of well being in the course of spirulina intake. Improvement in their vitamin A status could be responsible for this positive feeling with their general well being. Vitamins are "accessory nutrients" that are required for the proper utilization of the bulk food of the diet – carbohydrates, fats and proteins and for the maintenance of good health thus creating a feeling of vitality and vigour (Manay and Swamy, 2001).

5.8 EFFECT OF NUTRITION AND HEALTH EDUCATION

Education is a learning process through which knowledge is acquired and attributes are developed resulting in intelligent and skillful behaviour.

According to Neelakantan (1991) the village people are illiterate and ignorant particularly on matters of health and they should be given an awareness on the importance of right food for health and healthful living.

George (1987) reported that 51.00 per cent of the adult population in the coastal areas of Thiruvananthapuram are found to be illiterate. To the same tune about 50 per cent of the fisherwomen in the current study were found to be illiterate which would have been responsible for their ignorance and lack of awareness in the field of health and nutrition. Therefore there is a need for nutrition education to create awareness and

improve knowledge of these women who belong to the socially and economically backward community.

Moreover, it was observed that the population living in the coastal areas of Thiruvananthapuram were the most socially deprived and less exposed group to health information. This again have lead to their poor health and living condition which might have played a role in the occurrence of the premalignant oral cancerous symptoms in these women. Mc Farlane *et al.* (1996) observed non-favourable trends in mortality for cancers of mouth, tongue and pharynx and attributed it to the fact that the largest increase in incidence had occurred in socially deprived areas. According to Forster and Loury (1997) different risk factor behaviours, such as smoking, alcohol consumption and poor diet could be more prevalent amongst persons subjected to poor living and working conditions. They also reported that besides having great risk of developing diseases, under privileged subjects are less exposed to health information, may be less capable of changing behaviour, have lower access to early diagnosis and fewer therapeutic resources.

Thus it can be rightly said that nutrition and health education is an educational measure for inducing desirable behavioural changes for the ultimate improvement in the nutritional and health status of individuals. Unlike the impact of other nutritional interventions like supplementary feeding or prophylaxis with vitamin and mineral supplements, nutrition education has long-term effects.

Current investigation indeed put forward a welcoming result that the nutrition and health education imparted through lectures and demonstrations could make a positive impact on the fisherwomen in the experimental group as the effect was evaluated on the basis of alteration in personal habits/oral hygiene, alteration in food habits and in knowledge gain.

5.8.1 Alteration in Food Habits

Nutrition deals with all that makes a man healthy, functioning and creative human being through a well chosen diet (Merchant, 1999). Bosley (1986) says that the fundamental objectives of education in nutrition is to help individuals to establish food habits and practices that are consistent with nutritional needs of the body and adapted to the cultural pattern and food resources of the area they live in.

The dietary pattern of fisherwomen revealed that the diet consisted of a relatively uniform food pattern comprising of rice and fish. It has been noted that nutrition and health education had a highly positive impact in creating awareness and knowledge among these women. But the adoption level of this under privileged group was comparatively meagre. Their work pattern, low income, socioeconomic and other underprivileged situation are drawbacks to their poor ability to bring alteration in dietary habits.

Rao (1991) reported that people living in the rural areas were not able to lead a life worthy of human beings due to poverty. The author added that their health condition was the result of the pernicious combination of several socioeconomic factors like unemployment, lack of material advancement, poor housing, poor sanitation, malnutrition, social apathy, absence of will power and initiative to change for the better.

The same factors were responsible even in the case of these people living in the coastal areas of Thiruvananthapuram for the poor adoption rate inspite of their willingness to implement changes advocated to improve their life status. Due to the same barrier there was no significant alteration found in the food habits of the experimental fisherwomen in the present study.

5.8.2 Alteration in Personal Habits/Oral Hygiene

Inter individual variations among the subjects in their characteristics depends primarily on two factors one is genetic, and the other is environmental. The personal characteristics, which have acquired may have varying influences on the health of an individual. Impact of nutrition and health education in bringing about changes in the personal habits *viz.*, betel chewing, practice of washing mouth after chewing and on oral hygiene practices were the yardsticks assessed in this aspect.

On evaluation as much as 23.30 per cent fisherwomen were able to stop the habit of chewing and majority (56.70 %) of them could be persuaded to exclude tobacco in the quid. As a result of the education session majority of the women became aware that tobacco consumption is the major risk factor responsible for development of the pre-cancerous condition in their oral cavity and that later it could transform into oral cancer.

According to Vanderwaal (1997) the cessation of tobacco habits, being the most common known aetiological factor of oral leukoplakia, has been shown to be an effective measure with regard to the incidence of leukoplakia and thereby the incidence of oral cancer as well. Gupta *et al.* (1986) demonstrated that in general, changes in the tobacco habits of the population can be achieved through education efforts. It revealed that 37 per cent of the intervention group either stopped or reduced their tobacco habits. As indicated in a report of KMIO (1992), health education when under trial settings could result in a significant proportion of subjects giving up tobacco habits. An earlier study revealed that many of the precancerous lesions regressed when tobacco use was stopped or substantially reduced (Banoczy, 1984).

Studies by Varghese (2001) revealed that there is a change in leading sites of cancer in Kerala in comparison to Mumbai during the last two decades. Of these the gradual decrease in the frequency of oral cancer in

both sexes requires a special consideration. Public awareness and considerable decrease in tobacco habits account for the decrease in oral cancer. This shows that the public education campaign, which was in full swing during 70's and 80's in Kerala, brought about a healthy impact in the occurrence of cancer.

On analysis it could also be noticed that almost all the subjects in the experimental group switched over to washing mouth after chewing which was only 43.30 per cent before the education programme. Thus the oral hygiene status with majority of the subjects (60.00 %) could be improved to 'Good' or 'better' level as a result of the education sessions. It could be right to say that all the subjects became aware that, oral hygiene also plays a role in the incidence of oral cancer along with tobacco consumption which the fisherfolk were not aware of before the nutrition and health educational sessions.

According to Graham *et al.* (1977) poor dental hygiene accompanied by tooth loss is another suspected risk factor for oral cancer. Many epidemiological studies have revealed that indicators of poor dental hygiene such as loss, poor dentition and infrequent practice of oral hygiene habits are independent but only weak risk factors for oral cavity cancer (Bumdgard *et al.*, 1995).

Homann *et al.* (2001) support evidence that poor dental status increase oral cavity cancer risk. It has been suggested that bacterial over growth in patients with poor dental hygiene may lead to an increased rate of microbial metabolites with possible carcinogenic potentials. Lopez *et al.* (2000) reported on the influence of oral hygiene level on the appearance of tumors and it was found that daily brushing is a protective factor.

It could be concluded that nutrition and health education sessions could make a healthy impact on the views of the fisherwomen on habits of chewing and oral hygiene practices. This is obvious from the alterations observed in these aspects.

5.8.3 Knowledge Gain

The unfortunate state of nutrition in India is attributed to several factors, poor dietary intake caused by poverty or ignorance about proper feeding is the main causative factor. Lack of awareness regarding nutritional needs and paucity of information also aggravated the problem.

To combat all these problems nutrition education is a good measure (Krishnaswami and Vijayaraghava, 2000).

Assessment of the nutrition and health education given to the experimental subjects on the basis of pre and post tests on aspects like diet and cancer, importance of antioxidant vitamin, significance of spirulina, personal habits and cancer showed a remarkable increase in the scores as a result of the education sessions. Before the education programme (pre test) eventhough few of the subjects were aware of some of these aspects but were ignorant as there was no one to motivate them for adoption. None of the women had heard about spirulina earlier and therefore no body could give a positive response in the pre test session. To the contrary the post test response from the women revealed their knowledge gain in all the aspects including information on spirulina. This proved the positive impact of the nutrition and health education session extended to them as part of this investigation and also the success in creating awareness on nutrition and health practices.

Devdas *et al.* (1994) stated that many studies demonstrated the positive impact of education in improving nutritional knowledge.

Jaya and Sivaraj (1996) in their study pointed out education programme as most effective method of creating awareness in the aspects of nutritional and health practices. Similar opinion was given by Hemalatha and Prakash (2002).

SUMMARY

6. SUMMARY

The study entitled 'Impact of spirulina (*Spirulina fusiformis*) food supplement on premalignant conditions in women' was undertaken with an objective to formulate food supplements incorporating spirulina and to assess its impact on premalignant conditions of the oral cavity of selected subjects.

Two coastal villages viz., Puthukuruchy and Maryanad in Thiruvananthapuram district were selected for the study. Through medical camps organized with the help of doctors from Regional Cancer Centre, Thiruvananthapuram, sixty fisherwomen in the age group of 40-55 years with pre cancerous symptoms of the oral cavity and habituated with betel quid chewing were identified and selected for the study. The sixty fisherwomen were divided equally into experimental and control groups of 30 members each.

The socio-economic and dietary profile of the respondents were enumerated. Evaluation of the personal profile of the fisherwomen indicated that majority (55.00 %) of the respondents were above 50 years of age and most (50.00 %) of them were illiterate and were engaged in fish vending.

The socio-economic background of the selected families revealed that 66.70 per cent of the respondents belonged to nuclear families with an average family size of five, with three adult members and two or more than two children. Most (45.00 %) of the families had more than two employed adults.

An enquiry of the personal habits revealed that all the respondents were tobacco chewers and 50.00 per cent of them were accustomed to the habit for more than 30 years. Almost 66.70 per cent respondents chewed 5 to 9 quids per day and 70.00 per cent chewed for a duration of five to ten minutes each time.

On assessing the economic status, majority (63.30%) of the families had a monthly income that ranged between Rs.1000 to 3000 and with a percapita monthly income of Rs.500 to 600.

Data collected on dietary and food consumption pattern of the respondents revealed that 76.70 per cent spent below Rs.300 as expenditure on food per month. Dietary survey also revealed that all the respondents were habitual non-vegetarians. On assessing frequency of use of different food items, it was revealed that rice and fish were the main items of their daily diet. Most of them used pulses, milk and milk products, egg and meat rarely. The use of green leafy vegetables and fruits were occasional or never. Though all the families used nuts and oil seeds, fats and oils, sugar and jaggery daily the quantity consumed by individuals per day was found to be meager.

Data on the frequency of use of β -carotene rich foods, viz., green leafy vegetables, roots and tubers, other vegetables and fruits in the daily diet by the respondents were occasional or never.

The adequacy of diets consumed by the respondents were assessed by 24 hour recall method. The data obtained revealed that the diets were ill balanced and inadequate. It was noticed that cereal intake met more than 90.00 per cent of RDA and the intake of fish was sufficient to meet nearly 80.00 per cent of RDA. The intake of other food items were below the RDA. The data also revealed that the diets of the women were deficient in all the nutrients except calcium when compared to RDA.

Family history leads to a general clue for the occurrence of certain cancers and the data collected on such line evidenced that about 8.30 per cent of the respondents reported a family history of cancer. Detailed clinical examination of the oral cavity indicated that the type of pre cancerous symptom identified with 85.00 per cent respondents was leukoplakia. The remaining 15.00 per cent were showing submucous fibrosis (SMF) as the symptom.

Examination of the type and size of leukoplakia, type and interlabial diameter (ILD) of SMF and other characteristics exhibited by the respondents revealed that 60.00 per cent of them suffered from homogenous type of leukoplakia with size less than 2 cm. Most (13.30 %) of the respondents had generalized type of SMF with ILD between 1 to 3 cm (moderate). In spite of the above oral symptoms, all the respondents were able to talk clearly and their swallowing ability was also not impaired. Majority (83.30 %) of them complained intolerance to spices. Few were prone to problems associated with taste perception and appetite.

The nutritional status of all the respondents were poor as revealed by the height and weight, which was below the ideal height and weight suggested for a reference women. Majority (45.00 %) of the respondents belonged to the category with BMI between 18.6 to 20.0. Biochemical assessment indicated a low haemoglobin and serum β -carotene levels among the respondents. In 86.70% respondents the Hb level was below 10 g/dl and in 40.00 per cent serum β -carotene level was below 50 μ g/dl which are below the acceptable values.

As the objective of the study was to assess the impact of spirulina supplementation on premalignant condition of the oral cavity, spirulina containing supplements had to be given to the experimental group to study the effect of the same. Hence six spirulina incorporated food supplements (1g dried spirulina / portion) viz., lemon-ginger squash, rava laddu, rice ball, bengal gram ball, coconut chutney powder and green gram chutney powder were standardized in the laboratory. The nutritional composition of the supplements revealed that rava laddu had the highest calorie and iron content. Protein and calcium content was maximum in green gram chutney powder while lemon ginger squash had considerable amount of vitamin C. β -carotene content was more or less same in all the supplements.

Organoleptic evaluation of the supplements at laboratory level using a taste panel identified rava laddu as the most acceptable

supplement containing spirulina followed by lemon ginger squash, rice balls and coconut chutney powder.

Preference ranking of the spirulina supplements at the field level by the selected fisherwomen revealed that highest percentage of the women preferred lemon ginger squash, while rava laddu and rice ball were rated as second and third choices respectively. These three highly preferred supplements were selected for the supplementary feeding trial. Three supplements were utilized for the study to avoid monotony since the feeding trial was of a long duration.

The selected three spirulina supplements were administered alternately for six months on a daily basis to the experimental group under study which supplied one gram spirulina per day. The control groups were given the same snacks prepared without the addition of spirulina.

Impact of the spirulina supplement on the pre-cancerous condition of the oral cavity was evaluated through a thorough clinical examination of the subjects by the same medical experts who were involved in screening them initially. Examination of the oral cavity of the experimental women revealed that among the 86.70 percent suffering from leukoplakia, there was complete remission among 13.40 per cent and regression was seen in 40.00 per cent while no perceivable change was seen among the remaining 33.30 per cent leukoplakic women. Meanwhile in the control group the condition was found to aggravate in 6.70 per cent of respondents. It was also noted that in the experimental group the number of subjects having ulcerated type (moderate) of leukoplakia had been reduced as they were shifted to the mild form (homogenous type). While in the control group the percentage of ulcerated type increased from 28.30 per cent to 30.00 per cent. The size of the leukoplakia lesions were also found reduced among the experimental women whereas in the control women lesions size was further increased. With regard to submucous fibrosis (SMF) among the 13.30 per cent cases there was regression in 10.00 per cent and the rest remained without change. There was an

improvement in the interlabial diameter (ILD) (the extent to which the person can open his/her mouth) among the experimental group where as in the control group the mild type of ILD has been elevated to the moderate type. Almost all the subjects in the experimental group regained their tolerance to spices which can be considered as a positive impact of feeding spirulina over a period of six months.

Changes in serum β -carotene levels of the respondents in the experimental group before and after the feeding trial revealed that there was an average increase of 0.385 $\mu\text{g/ml}$ after six months of supplementation. At the same time a decrease was noted in the β -carotene level of control women (-0.015 $\mu\text{g/ml}$). There was an average increase of 1.17 g/dl on the haemoglobin level of the experimental group as a result of spirulina supplementation.

Supplementary feeding with spirulina also indicated improvement in general appetite and taste perception among the experimental women. However in the control women there was no improvement in the situation.

An intensive nutrition and health education programme was also imparted to the fisherwomen in the experimental group in order to create awareness among them on health and nutritional aspects. The effect of nutrition and health education was evaluated on the basis of gain in knowledge, alteration in their personal habits and food habits.

Prior to the education session a pre test was carried out to assess the extend of awareness of the respondents regarding "diet and cancer", "importance of antioxidant vitamins in cancer prevention", "significance of spirulina" and "relationship between personal habits and incidence of cancer". After the education session a post test was conducted with the same set of statements (used for pre-test) to evaluate gain in knowledge. Statistical analysis showed that the pre and post scores obtained by the experimental women were significant at one per cent level.

Due to the education programme 23.30 per cent respondents stopped the habit of chewing and 56.70 per cent excluded tobacco while

chewing. All the experimental subjects followed the practice of washing mouth after chewing and their oral hygiene had been improved to a 'good' status which existed at a poor level before the education session.

Alteration in food habits was not obvious as the diet of fisherwomen was relatively simple consisting of rice and fish.

The salient findings of the study can be summarized as :

1. The fisherwomen having oral precancerous symptoms were consuming inadequate amount of β -carotene rich foods especially vegetables and fruits.
2. Serum β -carotene and blood haemoglobin levels of the respondents confirmed a low status compared to the normal values.
3. Spirulina food supplementation could improve the serum β -carotene and blood haemoglobin level of the women and offered a positive impact on the existing precancerous symptoms of the oral cavity.
4. The formulated food supplements incorporating spray dried spirulina powder were found to be acceptable and hence can be used as therapeutic agents for treating selected precancerous symptoms.

Spirulina is a low cost alternative to alleviate precancerous condition of the oral cavity and to ensure adequate intake of vitamin A and iron in order to boost general health. Not only treatment but improving diet, personal habits and oral hygiene are essential to improve the health status of the subjects who suffer from the dual burden of social oppression and ill health being prone to development of a debilitating disease like cancer. Realization of the preventive potential role of micronutrients should be achieved by modulation of diet rather than pharmacological supplements and spirulina algae is one such food item which could be easily and acceptably incorporated into daily diet.

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7. REFERENCES

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*-Originals not seen

**IMPACT OF SPIRULINA (*Spirulina fusiformis*) FOOD SUPPLEMENT
ON PREMALIGNANT CONDITIONS IN WOMEN**

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8. ABSTRACT

The study entitled 'Impact of spirulina (*Spirulina fusiformis*) food supplement on premalignant conditions in women' was undertaken with an objective to formulate food supplements incorporating spirulina and to assess its impact on premalignant conditions of the oral cavity of selected subjects.

The study was undertaken among two fishermen communities viz., Puthukuruchy and Maryanad in Thiruvananthapuram district.

Clinical assistance from Regional Cancer Centre aided in conducting medical camp to screen 60 fisherwomen in the age group of 40-55 years with oral precancerous symptoms who were habituated with betel quid chewing. The 60 women were divided into experimental and control groups of 30 members each.

Personal profile of the respondents indicated that majority were above 50 years of age, illiterate and engaged in fish vending.

Most of the families were of nuclear type and their monthly income ranged between Rs.1000 to 3000.

Dietary surveys revealed an absolutely ill balanced but uniform pattern comprising rice and fish. All the respondents were non-vegetarians and fish was the main non-vegetarian food in their diet. However consumption of vegetables, green leafy vegetables, pulses, egg and milk and milk products was less. The diet of the women were deficient in all the nutrients except calcium when compared to RDA.

An enquiry on the personal habits revealed that all the women were tobacco chewers with 50.00 per cent chewing for more than 30 years. Majority of them chewed 5 to 9 quids a day for a duration of 5 to 10 minutes each time.

Clinical examination of the oral cavity of the respondents indicated that 85.00 per cent suffered from leukoplakia and the remaining showed submucous fibrosis (SMF) as the precancerous condition.

Majority of the respondents suffered from homogenous type of leukoplakia with size less than 2 cm and generalized type of SMF with an interlabial diameter (ILD) between 1 to 3 cm. Most of them complained an intolerance to spices and a few were prone to problem associated with taste perception and appetite.

Nutritional status revealed that all the women were below the normal height and weight standards. Body mass Index of the women showed that 45.00 per cent suffered from energy deficiency. Among 86.70 per cent the blood haemoglobin and among 40.00 per cent the serum β -carotene levels were below the acceptable values.

For the feeding trial six spirulina based supplements incorporating one gram spray dried spirulina per portion were standardized in the laboratory. The six supplements were lemon-ginger squash, rava laddu, rice balls, bengal gram balls, coconut chutney powder and green gram chutney powder. Three highly preferred supplements (lemon-ginger squash, rava laddu and rice balls) were selected for the feeding trial of six months duration.

Assessment of the impact of spirulina supplementation on the precancerous condition of the oral cavity revealed that among the 86.70 percent suffering from leukoplakia, there was complete remission among 13.40 per cent and regression was seen in 40.00 per cent. Mean while in the control group the condition was found to aggravate. It was also noted that in the experimental group the number of subjects having ulcerated type (moderate) of leukoplakia had been reduced as they were shifted to the mild form (homogenous type). With regard to submucous fibrosis (SMF) among the 13.30 per cent cases there was regression in 10.00 per cent. Almost all the subjects in the experimental group regained their

tolerance to spices which can be considered as a positive impact of feeding spirulina over a period of six months.

Changes in serum β -carotene levels of the respondents in the experimental group revealed that there was an average increase of 0.385 $\mu\text{g/ml}$ after six months of supplementation. At the same time a decrease was noted in the β -carotene level of the control women. There was an average increase of 1.17 g/dl on the haemoglobin level of the experimental group as a result of spirulina supplementation.

Supplementary feeding with spirulina also indicated improvement in general appetite and taste perception among the experimental women.

Nutrition and health education imparted to the experimental women revealed an excellent gain in knowledge from the post-test scores.

Due to the education programme 23.30 per cent respondents stopped the habit of chewing and 56.70 per cent excluded tobacco while chewing. Their oral hygiene could also be improved.

Acceptable spirulina food supplements could be formulated and the present trial evidenced that these supplements imposed a positive role in reversing the precancerous symptoms of the oral cavity in fisherwomen.

APPENDICES

APPENDIX I

KERALA AGRICULTURAL UNIVERSITY
College of Agriculture, Vellayani
Department of Home Science

**SCHEDULE USED TO ELICIT INFORMATION ON CLINICAL
EXAMINATION OF THE ORAL CAVITY**

General information

1. Name of the respondent :
2. Age :
3. Address :

Clinical Examinations

4. Family history of cancer
5. Leukoplakia
- a. Maximum dimension in cm
- b. Type
- (i. Homogenous ii. Nodular iii. Ulcerated
iv. Verucous v. Erythro-leukoplakia)
6. SMF (Sub mucosal fibrosis)
- (1. Localized 2. Generalised)
7. Inter Labial Diameter
- (i. mild (3 cm) ii. Moderate (1 – 3 cm), iii. Severe (1 cm)
8. Intolerance to spices
- (i. Yes, ii. No)
9. Talking
- (i. Clear, ii. Nasal twang)
10. Ability to swallow
- (i. Not impaired, ii. Impaired)

11. Taste

(i. Yes, ii. No)

12. Appetite

(i. Yes, ii. No)

13. Anthropometry

(i. Weight, ii. Height, iii. BMI)

14. Biochemical Examination

(i. Haemoglobin ii. Serum β -carotene)

APPENDIX - II

KERALA AGRICULTURAL UNIVERSITY
College of Agriculture, Vellayani
Department of Home Science

**SCHEDULE USED FOR ASSESSING SOCIO-ECONOMIC AND DIETARY
 PROFILE OF THE RESPONDENTS**

A. Socio-economic profile

1. Educational status of the respondents
2. Whether the respondent is an earning member
3. If yes, specify the job
4. Type of family
5. No. of family members Male Female
Adults
Children
6. No. of employed persons in the family
7. Economic status (Monthly income in Rs.)
8. Per capita income of the family
9. Monthly expenditure on food

B. Dietary pattern

10. Food habit
11. Frequency of use of various food items by the respondents

Food items	Frequency					
	Daily	Thrice in a week	Twice in a week	Once in a week	Occasional	Never
Cereals						
Pulses						
Root and tubers						
Green leafy vegetables						
Other vegetables						
Fruits						
Milk and milk products						
Egg						
Fish						
Meat						
Nuts and oilseeds						
Fats and oils						
Sugar and Jaggery						

12. Frequency of using β -carotene rich foods

Foot item	Frequency			
	Daily	More than twice in a week	Occasional	Never
Green leafy vegetables				
a. Amaranth				
b. Cabbage				
c. Coriander leaves				
d. Drumstick leaves				
e. Spinach (Palak)				
Roots and tubers				
a. Carrot				
b. Potato				
c. Yam				
Other vegetables				
a. Drumstick				
b. Beans				
c. Little gourd				
d. Tomato (ripe)				
e. Mango (green)				
f. Ladies finger				
Fruits				
a. Banana				
b. Jack fruit				
c. Mango (ripe)				
d. Orange				
e. Papaya (ripe)				

APPENDIX III

Formula for making food use frequency table

$$\text{Score} = \frac{R_1S_1 + R_2S_2 \dots \dots \dots + R_nS_n}{N}$$

S_n = Scale of rating

R_n = Percentage of respondents selecting a rating

N = Maximum scale rating

APPENDIX - IV
KERALA AGRICULTURAL UNIVERSITY
College of Agriculture, Vellayani
Department of Home Science

Schedule used to ascertain the actual food intake of the respondents (24 hour recall method)

Type of food preparation	Raw quality of each ingredient (g)	Total cooked amount (g)	Individual intake (cooked volume) (g)

APPENDIX -V

Education schedule used to evaluate gain in knowledge of the respondents

Sl. No.	Statements	YES	NO
	Diet and Cancer		
1	There is no relation b/w dietary habits and cancer occurrence		
2	High intake of refined carbohydrate, fats, coffee and tea may cause cancer		
3	It is better to avoid fried, burnt, smoked foods and use of reheated oils to prevent cancer		
4	Low intake of fruits and vegetables is associated with an increased risk of cancer		
5.	Vitamin A (β -carotene) has no role in the prevention of cancer		
6.	The role of vitamin A (β -carotene) in the prevention of cancer is the same as other vitamins		
	Antioxidant vitamins		
7.	Vitamins provide immunity and protect our body from infections		
8.	Vegetable and fruits are not rich in vitamins		
9.	Leafy, yellow and orange coloured vegetables and fruits have the same amount of β -carotene as other vegetables and fruits		
10	Carrot, ripe papaya, tomato and egg yolk are not particularly important in β -carotene		
11	Leafy vegetables being low cost foods does not contain good amount of β -carotene		
12	β -carotene (vitamin A) is not an important micronutrient		

13	β -carotene (vitamin A) neutralize free radicals before they attack cells		
14	β -carotene (vitamin A) is good for eye sight, healthy skin and prevention of diseases		
	Significance of spirulina		
15	Spirulina is an edible green coloured algae		
16	Spirulina is not used as food		
17	Spirulina is not rich in any nutrient		
18	Spirulina contains equal amount of β -carotene as in carrot		
19	Spirulina can be consumed both as a food as well as medicine		
20	Spirulina is a protective food against cancer and many degenerative diseases		
	Personal habits and cancer		
21	Chewing, smoking and use of alcohol does not have any relation to cancer		
22.	Chewing may lead to oral cancer		
23.	Washing the mouth after chewing and retaining the quid in the mouth, both has the same effect on the epithelial tissue		
24	Cessation of tobacco and alcohol habits will not help in reversal of precancerous symptoms in oral cavity		
25	Chewing with tobacco and chewing without tobacco have the same role to play in the occurrence of cancer		

APPENDIX VI
KERALA AGRICULTURAL UNIVERSITY
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Department of Home Science

**SCHEDULE USED TO ELICIT INFORMATION ON PERSONAL HABITS AND
 ORAL HYGIENE OF THE RESPONDENTS**

1. Do you have the habit of

Smoking Chewing Alcohol

(i. Yes ii. No)

2. Chewing

		From (Age)	To	No./day	Duration (days)
With tobacco	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Without tobacco	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3. Smoking

		From (Age)	To	No. of times /day
Cigarettes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Beedi	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cigar	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4. Alcohol

		From (Age)	To	ml/ day
Beer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Toddy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Country Liquor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

5. Do you wash your mouth after chewing tobacco ?

(i. Yes, ii. No)

6. Do you always wash your mouth after taking food ?

(i. Yes, ii. No)

7. Oral hygiene

(i. Good, ii. Poor)

APPENDIX – VII
KERALA AGRICULTURAL UNIVERSITY
College of Agriculture, Vellayani
Department of Home Science

EVALUATION CARD FOR TRIANGLE TEST

Name of the participant :

Sugar solution :

Note: Two of the 3 samples are identical

Sl. No	Code no. of samples	Code no. of the identical samples	Code no. of the odd sample
1	XYZ		
2	ABC		

APPENDIX - VIII

KERALA AGRICULTURAL UNIVERSITY
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Department of Home Science

**SCORE CARD FOR ASSESSING THE ORGANOLEPTIC QUALITIES OF
 THE SPIRULINA SUPPLEMENTS**

Product

Tested by

Date

Age

Sl. No.	Property	Score	A	B	C	D	E	F
1.	Appearance Very good Good Fair Poor Very poor	5 4 3 2 1						
2.	Colour Very acceptable Acceptable Slightly acceptable Moderately acceptable Unacceptable	5 4 3 2 1						
3.	Flavour Very pleasant Pleasant Moderately pleasant Unpleasant Not at all pleasant	5 4 3 2 1						
4.	Taste Excellent Very good Good Fair Poor	5 4 3 2 1						
5.	Overall acceptability Excellent Very good Good Fair Poor	5 4 3 2 1						

APPENDIX – IX

KERALA AGRICULTURAL UNIVERSITY
College of Agriculture, Vellayani
Department of Home Science

SCORE CARD FOR PREFERENCE TEST

Product	Like extremely	Like very much	Like moderately	Dislike slightly	Dislike moderately	Dislike extremely

APPENDIX - X

SPIRULINA INCORPORATED FOOD SUPPLEMENTS

1. Rava laddu

Ingredients	Amount
Rava	200 g
Milk	½ cup
Ghee	2 tablespoon
Sugar	150 g
Dry coconut (grated)	100 g
Cardamom powder	1 tsp
Cashew nut and kismis	10 g (each)
Spirulina	8 g

Method: Fry cashew nut and kismis in ghee. Slightly fry the grated coconut in ghee. Fry the rava in the ghee till brown. Along with rava add all the ingredients except milk and mix well. Divide into 8 equal parts. Add ½ cup water in the milk and boil. Add this milk little by little to each part of the rava and make out into laddu.

2. Rice balls

Ingredients	Amount
Rice flour	200 g
Coconut	100 g
Jaggery	100 g
Cardamon	1 tsp
Spirulina	7 g

Method : Wash the rice and dry it in the sun. Fry it and powder in a mixi and keep aside. Mix the coconut and jaggery in the mixi. Add the powered rice, spirulina and cardamon with the coconut and jaggery mixture and mix thoroughly. Divide into 7 equal parts and make out into small laddu.

3. Lemon-ginger squash

Ingredients	Amount
Lemon juice	100 ml
Ginger juice	50 ml
Sugar	450 g
Water	300 ml
Spirulina	20 g
Sodium benzoate	1 tsp

Method : Boil water and mix the sugar in it. Add ginger juice to this and keep aside. Mix lemon juice, spirulina and sodium benzoate and stir well. Allow it to cool and store in bottles.

4. Bengal gram balls

Ingredients	Amount
Roasted bengal-gram flour	200 g
Sugar (Powdered)	150 g
Cashewnut (Powdered)	50 g
Spirulina	8 g
Ghee	½ cup

Method : Mix 1, 2, 3 and 4 ingredients and keep aside. Divide this into eight equal parts. Add ghee in each and make out into balls.

5. Coconut chutney powder

Ingredients	Amount
Coconut	200 g
Red chilli (dry)	10 g
Coriander powder	5 g
Moong dhal	50 g
Pepper	15 g

Ginger	15 g
Curry leaves	2 stems
Tamarind	30 g
Salt	As required
Spirulina	5 g

Method: Scrape coconut and add chilli, coriander powder, pepper, ginger, curry leaves. Fry the ingredients in low flame. When brown in colour powder it in a mixer by adding tamarind and salt to taste. Along with this add spirulina and mix well. Divide into five equal parts and store in packets.

6. Green gram chutney powder

Ingredients	Amount
Roasted green gram powder	200 g
Pepper	50 g
Salt	to taste
Spirulina	8 g

Method: Mix 1, 2, 3 and 4 ingredients together. Divide into eight equal parts and store in packets.

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