Exploring Unique Features and Mediterranean Diet Patterns of Breast Cancer Patients in North Cyprus: Implications for recovery

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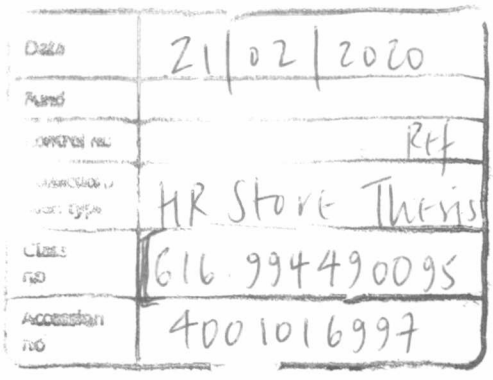
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Exploring Unique Features and Mediterranean Diet Patterns of Breast Cancer Patients in North Cyprus: Implications for recovery

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Abstract

Northern Cypriots follow a unique diet and little existing literature has documented this. One component, black olive oil (BOO) is distinct from other olive oils in terms of its taste, appearance and its manufacture. The aim of this research was to assess whether BOO, and other components of the diet can influence outcomes for breast cancer (BC) patients in Northern Cyprus, and to assess attitudes and dietary habits of BC patients during recovery.

The research was undertaken in North Cyprus and and investigated the dietary habits of a cohort of 140 BC patients with a view to assessing compliance to the Mediterranean diet and BOO consumption. The patients were assigned to two groups, those actively undergoing treatment for BC or those who had stopped being treated for three years or more. It was found that participants frequently consumed olive oil, which is a basic feature of the Mediterranean diet. An enhanced Mediterranean Diet Score (MDS) was significantly correlated with age, education, physical activity, total energy intake, protein, fat, carbohydrate, fatty acids and cholesterol. Similar to previous research, this study concluded that there is a relationship between improved breast cancer biomarkers and olive oil consumption, indicating that high olive oil consumption may aid recovery. A correlation was seen between monounsaturated fatty acid (MUFA) and oleic acid consumption with significantly reduced CEA BC biomarkers. There was

also a statistically significant reduction in CA15-3 CB biomarker associated with the consumption of BOO in the active treatment group, but not with in the treatment stopped group.

This study is the first to be conducted on the eating habits of BC patients, with a focus on the dietary awareness in relation to BC and the consumption of BOO, in North Cyprus. Interviews from a cross-section of 14 BC patients were analysed qualitatively using interpretative phenomenological analysis (IPA). Results indicated that participants were confused regarding what dietary advice to follow, and variety of sources was used. Widepsread uses of the internet to gain information as well as local opinion were noted. The views of patients on black olive oil fell into two groups: 1) that black olive oil was preferred because of its distinct taste and smell and 2) that black olive oil was not preferred because of its distinct taste and smell. The results indicate a need for clear professional advice on diet, as an aid to BC recovery.

The work ends with nutritional analysis of black olive oil and the effects of BOO polyphenols on BC and hepatoma cells and their growth rate. The level of polyphenols in BOO was found to be much lower than in extra virgin olive oil (EVOO) and they had less anti-oxidant activity. It was however found that BOO polyphenols inhibited growth of breast cancer cells at lower concentrations compared with polyphenols from traditional EVOO. Moreover polyphenols extracted from one source of BOO could selectively inhibit growth of BC cell lines compared with a non-cancerous control cell line. The results of this study has shed light on, and identified the need for, further studies on BOO to understand its relationship to human health.

Key Words: Breast Cancer, Black Olive Oil, Olive Oil, North Cyprus, Cyprus, Mediterranean diet, Nutrition, Food chemistry.

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Abbreviations

AHEI	Alternate Healthy Eating Index
AICR	American Institute for Cancer Research
aMED	Alternate Mediterranean Diet Score
B1	Thiamine
B2	Riboflavin
B6	Pyridoxal
BC	Breast Cancer
BMI	Body Mass Intake
BOO	Black Olive Oil
BP	Blood pressure
°C	Centigrade
CA	Cancer Antigen
Ca	Calcium
Ca	Cancer
CEA	Carcinoembryonic
CHO	Carbohydrate
cm	Centimetre
CVD	cardiovascular disease
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazy
Dr.	Doctor
EPA	eicosapentaenoic acid
EPIC	European Prospective Investigation into Cancer and Nutrition
et al.	and others
etc....	Etcetera
EVOO	Extra virgin olive oil
Fe	Iron
FFQ	Food frequency questionnaires
GI	Glycaemic Index
GL	Glycaemic load
GLOBOCAN	Global cancer
HDL	High Density Lipoprotein
HEI	Healthy Eating Index
HER-2/neu	Human epidermal growth factor receptor 2
K	Potassium
kg	Kilogram
LDL	Low Density Lipoprotein
LMU	London Metropolitan University
m^2	Metre squared
MCF-7	Michigan Cancer Foundation-7 (breast cancer cell line)
MD	Mediterranean Diet
MDA-MB-231	Metastatic human breast cancer cell line
MDAS	Mediterranean Diet Adherence Screener
MDS	Mediterranean Diet Score

MeOH	Methanol
mg	Milligram
Mg	Magnesium
min	Minute
mL	Millilitre
mRNA	Messenger Ribonucleic acid
MUFA	Monounsaturated fatty acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
Na	Sodium
NC	North Cyprus
NEU-H	Near East University Hospital
NHS	National Health Service
nm	Nanometre
OO	Olive Oil
p	Probability statistical significance testing
P	Phosphorus
PA	Physical activity
PIS	Patient Inform Sheet
Prot.	Protein
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TG	Tri Glyceride
TOBR	Specific Nutritional Guide of Turkey
UK	United Kingdom
USA	United States of America
v/v	Volume/volume
Vit.	Vitamin
VOO	Virgin olive oil
w-3	Omega-3
w-6	Omega-6
WHO	World Health Organization
y	Year
Zn	Zinc
µg	Microgram
µL	Microliter

Chapter I-General Introduction

1.1 Nutrition

Nutrition comes at the forefront of human needs (Baysal, 2009). The aim of nutrition is to make sure that an individual is receiving adequate and balanced amounts of energy and nutrient requirements according to the individual's age, gender, physical activity and physiological state. A balanced diet means, besides energy, that all the nutrients (carbohydrates, protein, fat, vitamins, minerals, and water) are sufficient to requirements (Samur *et al.,* 2002).

Optimal nutrition aims at "minimum risk of disease, the maximum good conduct / health, and hence a maximum healthy life" (Baysal, 2009). However, a diet must also provide pleasure and fitness for the consumer. Being fit is the feeling of optimal health, and feeling good about yourself. Therefore, in addition to the nutritional effects of one's diet, the beneficial physiological and psychological effects must also be taken into account (Yücecan, 2008).

In order to control chronic illnesses around the world, international public health priorities are beginning to concentrate on the development of preventive health care instead of medical treatments alone. This would be less costly and much more effective (Gibney *et al.,* 2006). High fat, saturated fat and sodium consumption, as well as low calcium and low pulp (inadequate consumption of fruits and vegetables and whole grains) intake increase the risk of certain chronic diseases. Many of the major life threatening diseases are diet related, including coronary heart disease, some cancers and diabetes. Modern nutrition and physical activity patterns can spread from one country to another, not unlike like viruses (European Health Report, 2009). A slight change in lifestyle factors, such as improved diet and increased physical activity, is thought to

lower the risk of Type 2 diabetes by 60% (Yücecan, 2008; Baysal, 2009). 80% of the risk factors of premature coronary heart disease are posed by an unhealthy diet, sedentary lifestyle and smoking (Mahan *et al.,* 2012). Between 1/5 and 1/3 cases of breast, colon, endometrium, kidney, and oesophagus cancer are found to be caused by unhealthy body weight and an inactive lifestyle (Yücecan, 2008). A third of all factors leading to cancer can be prevented through optimal nutrition, normal body weight and physical exercise. An inactive lifestyle leads to cardiovascular diseases, and doubles both the risk of death from a stroke, and developing type II diabetes, increasing the risk of obesity twofold (Yücecan, 2008). Inadequate and unbalanced nutrition has a direct link to the formation of some diseases (pellagra, beriberi, scurvy, anaemia, rickets, etc.), and an indirect link to the formation of infectious diseases, cardiovascular disease, diabetes, hypertension, liver disease, obesity, etc. (Pekcan, 2004).

1.2 Cancer

Cancer is essentially a disease whereby cells no longer have the normal checks on cell proliferation. Energy metabolism, in relation to mitochondrial functions, may contribute to the development of cancer cells. This theory is based on the tumour cells in question preferentially using glycolysis over mitochondrial oxidative phosphorylation for glucose-dependent ATP production even when there is enough oxygen to fuel mitochondrial respiration, a phenomenon which is known as the ''Warburg effect'' (Dang, 2012; Jones and Thompson, 2009; Munoz-Pinedo *et al.*, 2012). This distinguishes cancer tissue from 'normal' tissue (Dang, 2012). Alterations in cellular metabolism could likely contribute to the malignant phenotype (Jones and Thompson, 2009).

Can cancer be malignant or benign? A malignant tumour is caused by disturbances that include abnormal cell growth and has the potential to invade or spread to various other parts of the body. Benign tumours, however, do not spread to other parts of the body and for this reason are not as dangerous. A new lump, abnormal bleeding, a persistent cough, weight loss without adequate reason, and a change in stool patterns are the most common symptoms of tumours. However, these symptoms are not always indicators of cancer but can be caused by other problems. There are more than 100 cancer types that affect humans (WHO-cancer fact sheet, 2014; National Cancer Institute-Cancer, 2014).

Cancer is a growing burden. Especially in developing countries, there is an increase in cancer due to certain habits that cause cancer, such as smoking. Cancer is the most common cause of death in economically developed countries, and the second most common cause of death in developing countries (WHO, 2014). The impact of cancer is increasing in economically developing countries partly as a result of population growth and an aging population. However, these are not the only reasons for the increase in cancer. It is also due to certain habits such as smoking, decreased physical activity, and ''westernized'' diets (Jemal *et al.*, 2011). According to Cancer Incidence and Mortality Worldwide (GLOBOCAN, 2012), there were 14.1 million new cancer cases, 8.2 million deaths resulting from cancer and "32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide. 57% (8 million) of new cancer cases, 65% (5.3 million) of the cancer deaths and 48% (15.6 million) of the 5-year prevalent cancer cases were observed in less developed regions" (GLOBOCAN, 2012).

Among cancer types, lung cancer has the highest incidence worldwide (1.61 million, 12.7% of the total), followed by breast (1.38 million, 10.9%) and colorectal cancer (1.23 million, 9.7%). The most common causes of cancer deaths are lung cancer

(1.38 million, 18.2% of the total), stomach cancer (738,000 deaths, 9.7%) and liver cancer (696,000 deaths, 9.2%) (Ferlay *et al.,* 2010).

In females, breast cancer is the most common type, whereas in males it is lung cancer. Following lung cancer are stomach and liver cancer in males, and cervical and lung cancer in females in the developing world, and colorectal and lung cancer among females, and colorectal and prostate or lung cancer among males in the first world (Jemal *et al.,* 2011).

Cancer is one of the most important public health problems in many countries (WHO, 2014). In the United States, for instance, one in four deaths is caused by cancer (WHO, 2014). The American Cancer Society carries out a survey each year in order to record the numbers of new cancer cases and deaths expected in the United States in the current year. It presents updated information on cancer incidence, mortality, and survival.

According to these findings, 1,660,290 new cancer cases and 580,350 cancer deaths were estimated to occur in the United States in 2013. The results show that cancer death rates have declined by 20% from their peak in 1991 (215.1 per 100,000 population) to 2009 (173.1 per 100,000 population). Death rates are still in decline for all four major cancer sites (lung, colorectal, breast, and prostate) (Siegel *et al.,* 2014).

According to Hincal *et al.'s* (2008) cancer incidence research in North Cyprus from 1990 to 2004, breast cancer has the highest incidence among women at 30%. Worldwide incidence of breast cancer investigated by GLOBOCAN (2008) shows that breast cancer is the most common cancer in women (with an incidence of 22.9%) and leads to the highest death rates in women with cancer (13.7% of all cancers) (Ferlay, 2010). On the other hand, Hincal *et al.* (2008) show that the incidence of breast cancer

in North Cyprus is lower than in Southern and Northern Europe despite a lower average age incidence (AAI) in North Cyprus in comparison with other European countries.

According to North Cyprus government statistics, the number one cause of mortality in North Cyprus between 1994 and1997 was heart disease, and the second was cancer. However, from 1997 to 2002 cancer was the number one cause of mortality, and the second was heart disease (North Cyprus government statistics, 2002).

1.3 Cancer Metabolism

Dividing cells are reinforced by the convergence of both innate and external multiple molecular mechanisms that change the core cellular metabolism (Pavlova and Thompson, 2016). This results in the fast production of adenosine triphosphate in order to preserve energy and a heightened biosynthesis of macromolecules. Furthermore, cellular redox becomes tighter in order to sustain its condition. Cancer cells must change form in order to answer carbohydrate, protein, lipid and nucleic acid needs. This is quite similar to the changes which occur in rapidly proliferating 'normal' cells that respond to physical development in contrast to constitutive cells that adapt autonomously (Pavlova and Thompson, 2016; Munoz-Pinedo *et al.,* 2012).

1.4 Breast Cancer

Women's breasts are constituted of fat, connective tissue and a great many very small glands, known as lobules, which produce milk (Figure 1.1). If a woman bears a child, the milk exits the nipple through tiny tubes known as ducts, which allow her to breastfeed (NHS, 2014).

Human bodies are made up of billions of cells. Normally, cells grow and multiply in a controlled way. New cells are only made when and where they are needed.

In cancer, something goes wrong in this orderly process and cells start to grow and then multiply uncontrollably (NHS, 2014).

There are various symptoms of breast cancer but the most common one is a lump or thickening in the breast tissue (it should be noted here that most breast lumps are not cancerous). With early diagnosis, cancer can be treated before it spreads to other parts of the body (NHS, 2014).

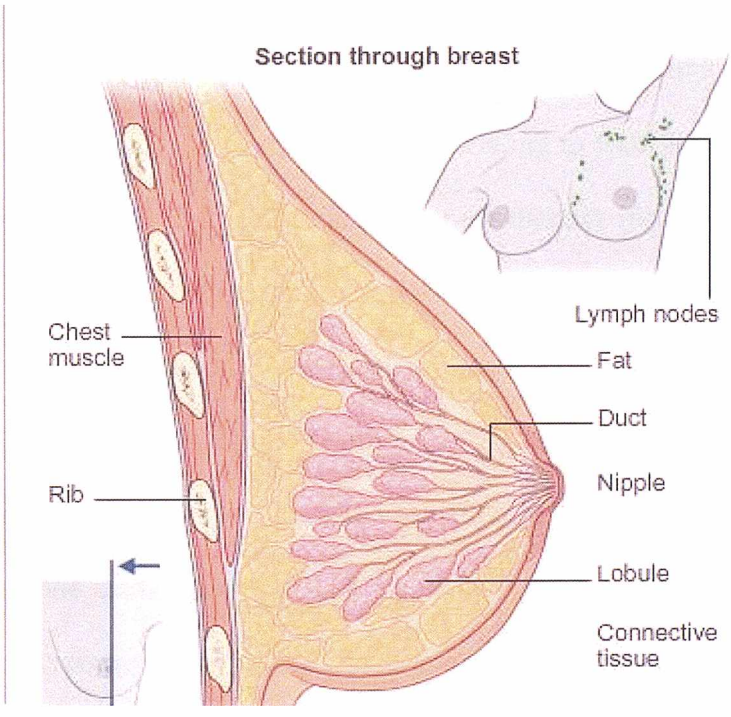


Figure 1.1 Section through breast (taken from; NHS-Breast cancer)

The factors which contribute to the worldwide variations in incidence rates of cancer result from differing patterns in reproductive and hormonal factors, and the availability of early detection possibilities. A long menstrual history, recent use of postmenopausal hormone therapy or oral contraceptives, the factor of giving birth later in life than the average, are among the reproductive factors that lead to cancer. Another significant factor is alcohol consumption. Early diagnosis through mammography increases the chance of treatment and saves lives. However, this is an expensive option

which is not viable in most developing countries. Recommended early detection strategies in such countries include the promotion of "awareness of early signs and symptoms, and screening by clinical breast examination" (Jemal *et al.*, 2011).

Cancer develops from a clone of cells that have escaped normal regulation of growth, proliferation, differentiation and intercellular relationships. These abnormalities of function derive from a disordered expression of key genes, resulting in altered cellular phenotypes. The disordered gene expression may result from genetic mutation or from epigenetic factors that may silence genes that should be active or switch on genes that should be silent. Factors relating to food and nutrition may directly influence both processes. Specific nutrients may directly influence the expression of key genes, for instance through abnormalities of methylation of the promoter regions of genes or of histone acetylation, which can influence DNA structure and accessibility of genes to mRNA for transcription (Jalili *et al.*, 2013; Choi and Friso, 2010). In studies conducted on rats, deficiency of zinc and selenium may inhibit methyl group utilization and cause histone and genomic DNA hypomethylation. Vitamin C deficiency leads to DNA hypomethylation in lung cancer. Folate, vitamin B12 and methionine are needed for DNA methylation reactions. DNA methylation can be changed by folate, methionine, choline, vitamin B12 and also omega-3 fatty acids (Friso and Choi, 2002; Jalili *et al.*, 2013).

1.4.1 Stages

The stages of cancer are crucial because awareness of these help you and your specialist to choose the best options for treatment. The stage of a cancer shows how large the cancer is, and whether it has spread. This can be determined through tests and

scans that lead to diagnosis. Breast cancer has three grades; low grade – grade 1 (slow growing), intermediate grade – grade 2, and high grade – grade 3 (faster growing).

Low grade cancers usually grow more slowly than high grade ones. High grade cancers are more likely to recur after initial treatment. But the grade can only give us guidance as to how an individual cancer will behave, and individual cancers may behave in different manners (Cancer Research UK, 2014).

Breast cancer which has been detected early will entail that the cancer has not spread beyond the breast or the lymph nodes in the armpit on the same side of the body. Thus, the cancer has not spread to any other part of the body. Local recurrence means that the cancer has returned to the area of the breast subsequent to treatment. Locally advanced breast cancer means that the cancer has not spread to any other part of the body, but may be bigger than 5 cm across or growing into the skin or muscle of the chest area, or exists in the lymph nodes in the armpit; and these lymph nodes are either bound to each other, or to other structures. Secondary breast cancer is also known as metastatic breast cancer or stage four breast cancer. It means that the cancer has spread to other parts of the body, for example the liver or bones (Cancer Research UK, 2014).

1.4.2 Types of Breast Cancer

There are several different types of breast cancer which can develop in different parts of the breast. Breast cancer is usually divided into two - non-invasive and invasive.

Non-invasive breast cancer

Also known as cancer or carcinoma *in situ*, non-invasive cancer occurs in the ducts of the breast and has not developed the capacity to spread outside the breast. This

form of cancer does not usually manifest itself as a lump, and is often detected through the use of a mammogram. The most common type of non-invasive cancer is ductal carcinoma *in situ* (DCIS).

Invasive breast cancer

Invasive breast cancer has the potential to spread outside the breast to other parts of the body; however, this does not mean it always behaves in this manner. The most prevalent type of breast cancer is invasive ductal breast cancer. It grows in the cells that line the breast ducts. Around 80% of all incidences of breast cancer are invasive ductal breast cancer (Cancer Research UK, 2014).

Other types of breast cancer

Less frequently seen breast cancer cases are invasive lobular breast cancer, which occurs in the cells that line the milk-producing lobules, inflammatory breast cancer and Paget's disease of the breast. Breast cancer has the potential to spread to other areas, usually through the lymph nodes (small glands that filter bacteria from the body) or the bloodstream. In this case, it is called "secondary" or "metastatic" breast cancer (Cancer Research UK, 2014).

1.4.3 Treatment

Surgery, chemotherapy and radiotherapy are methods used in the treatment of breast cancer. Some cases of breast cancer can also be treated with biological or hormone treatments (NHS, 2014).

Treatment for breast cancer cannot be generalised because it depends on various circumstances such as:

- The type of breast cancer
- The size of the breast tumour
- The stage of the breast cancer
- The grade of the cancer cells
- History of menopause
- Whether or not the cancer cells in question have receptors for certain cancer medication
- The general health of the patient

(Cancer Research UK-treatment for breast cancer, 2014; NHS-treatment, 2014)

1.4.4 Breast Cancer Biomarkers (antigens CEA & CA 15-3)

Breast cancer in women is the most common malignancy and the second most common cause of cancer-related mortality worldwide. Two clinically used breast cancer antigens, Cancer Antigen 15-3 (CA 15-3) and CA 27.29, are detectable in fewer than 10% of early-disease patients and in about 75% of advanced-disease patients (Dong *et al.,* 2013). Neither antigen is recommended for screening or diagnosis of the onset of breast cancer (Dong *et al.,* 2013; Zhong *et al.,* 2008). Insteaad, there are several advantages to using serum antibodies as markers for tumour development. First, tumour-associated autoantibodies circulate in the blood much earlier than serum antigens. Second, antibodies may be more abundant than antigens, especially at a low tumour burden. Thirty percent of patients with ductal carcinoma *in situ* in which the proto-oncogene HER-2/neu is over-expressed have serum antibodies specific to this protein (Zhong *et al.,* 2008).

The carcinoembryonic antigen (CEA) is a glycoprotein antigen (Mirabelli and Incoronato, 2013; Scott and Renner, 2001). CEA is expressed on a number of tumours of gastrointestinal origin, and in breast and lung cancer. Although it was first diagnosed as an onco-fetal antigen, CEA is in fact also expressed in non-malignant, non-fatal adult tissues such as normal colonic mucosa, lung and lactating breast tissue. CEA is shed from the surface of tumour cells, and raised serum levels of CEA have therefore been used to detect occult tumours. However, increased serum levels can also result from smoking and inflammatory bowel disease (Mirabelli and Incoronato, 2013).

CA 15-3 is the soluble form of MUC-1 protein, a large type I transmembrane glycoprotein (Mirabelli and Incoronato, 2013). The functional role of MUC-1is that it is supposed to be connected with the lubrication, hydration and protection of the external surfaces of epithelial tissue layers; also it is concerned with lining ducts and lumens in diverse areas of the body. Indeed, MUC-1 is strongly expressed by epithelia of glands and ducts - also as goblet and columnar cells of epithelial tissues where it performs a protective function by inhibiting the microbial access to the cell wall and preventing degradative enzyme activity (Mirabelli and Incoronato, 2013).

Use of biomarkers

These biomarkers are used in clinical practice, but are not useful for screening programs and diagnosis of BC (Mirabelli and Incoronato, 2013; Dong *et al.,* 2013; Harris *et al.,* 2007). CA 15-3 alone is not sensitive or specific enough for cancer screening. However, it is an indication of the patient's response to the treatment, and is helpful in monitoring for recurrence of cancer, especially in metastatic disease. CA 15-3 can be used for screening, diagnostic, or staging tests in primary breast cancer therapy, and CEA can be used for screening, diagnosis, staging, or routine surveillance after

primary therapy (Khatcheressian *et al.*, 2012; Harris *et al,* 2007). CA 15-3 can be used to get an idea of to what extent cancer might have developed (the tumour burden). CA 15-3 can only be used as a marker if the cancer is producing elevated amounts of it, so this test would not be appropriate for all with breast cancer (Duffy, 2006).

96% of patients with local and systemic recurrence have elevated CA15-3, which can be utilised to predict recurrence earlier than would be the case using radiological and clinical criteria. A 25% increase in the serum CA15-3 is linked with the progression of carcinoma tumours. A 50% decrease in serum CA15-3 is linked to a positive response to the treatment in question. CA15-3 is more effective than CEA in early detection of breast cancer recurrence (Mirabelli and Incoronato 2013; Duffy, 2006).

1.5 Cancer and Nutrition

There is little extant literature on either breast cancer nutrition in North Cyprus or black olive oil. This study is the first to include black olive oil. There is not enough literature to discuss outcomes of this research. Since the relavant literature spans a broad area and is very heterogeneous, a narrative review of the literature was conducted as it was more appropriate in this case instead of a systematic review. CINAL/Medline, Pubmed and Science Direct data bases were used, using search terms such as breast cancer, breast cancer and nutrition, olive oil, olive oil and breast cancer, BOO, MD, CEA, CA 15-3, cancer biomarkers and MD and breast cancer.

Dietary factors (such as obesity) are thought to be the second biggest cause of cancer (30%) after tobacco in many countries (AICR-Cancer Risk Awareness Survey Report, 2015), whereas in developing countries the correlation between cancer and diet is estimated at twenty percent. Therefore, highlighting the influence of nutrition on the

risk of cancer is crucial for health studies. However, current research does not provide many insights into the effect of diet on cancer risk, and requires deeper investigation. According to research carried out more than four decades ago, there is a greater ratio of colorectal cancer as well as prostate cancer and breast cancer in relatively more developed countries where animal products, sugar and fat are consumed regularly (AICR-Cancer Risk Awareness Survey Report, 2015). In less developed and developing parts of the world where eating habits are more archetypal and include fewer animal products, sugar and fat, the types of cancers that can be found in Western countries are less common. In contrast, the number of cancer types such as cancer of the liver, oesophagus and stomach are higher in these places. As Key (2004) indicates, eating habits that might cause cancer are accompanied by other risk factors, for example, lack of physical activity and smoking.

A study which had a small sample size (72 BC women) assessed the importance of nutritional and physical status with cancer outcomes during recovery. They measured each patient's waist and mid-arm circumferences, body mass index, subjective global assessment, body composition via bio-electrical impedance analysis (BIA) and muscle thickness. Furthermore, they evaluated nutritional status and metabolic syndrome. They concluded that there was a significant relation between waist circumferences and BIA outcomes with metabolic syndrome. However, they could not relate nutritional status or metabolic syndrome with cancer stages or with various complications of cancer such as leucopenia, nausea and vomiting (Bering *et al.*, 2015).

A qualitative study tried to understand breast cancer sufferers' improvement patterns during treatment. The study population completed behaviour and activation/problem-solving interventions. Breast cancer survivors set 141 achievement goals from 17 women with breast cancer in their recovery stage. Over half of the goals

were to add powerful physical activity to their routine, and the other goals were, respectively, challenge in exercise, work, nutrition, playing an instrument, controlling stress levels and enhancing social life. Physical activity weighed in as the most helpful factor among survivors (Lyons *et al.,* 2015).

Moreover, it is a well-known fact that side effects of cancer therapy may lead to loss of appetite, including changes in taste and smell, constipation, diarrhoea, dry mouth, lactose intolerance, nausea, sore mouth or throat and trouble swallowing, vomiting, and weight gain or loss. All affect eating habits and nutritional status, and are symptoms that can cause malnutrition or a change in weight (Rock, 2012).

A prospective study investigated taste changes (such as salty, sour mouth, umami and appetite) during chemotherapy. They categorised timing during chemotherapy cycles as early, middle or late. Taste changes recovered at the late stage of the cycle of chemotherapy and showed more reaction, reduced taste function, in the early stage of chemotherapy, which led to lower calorie intake and reduced appetite (Boltong *et al.,* 2014).

By modulating food intake the metabolism of tumour cells in the body can be controlled. Indeed, for more than a century it has been known that low caloric consumption is considered as lessening the possibility of several human diseases including cancer, while excess caloric intake is associated with higher cancer risk and shortened lifespan. It is now clear that lowering caloric intake in general, or of specific macronutrients, can prolong life spans and improves health in a broad range of organisms when compared with unrestricted food intake (Rubio-Patino *et al.,* 2016). De Grey (2016) points out that illnesses increase metabolic aging by wearing out our vital organs and damaging our DNA.

Thus, body weight is a major risk factor in chronic diseases. A normal healthy body mass index is comprehended as 18.5-25 kg/m^2. Excessive weight in adults should be kept under control. A recent umbrella review outlined strong evidence that obesity, high body mass index, weight gain and high waist perimeter are linked to cancer, and being overweight might cause a higher risk of certain cancer types such as colorectal cancer, breast cancer or cancer of the oesophagus (Kyrgiou *et al.,* 2017). Furthermore, alcohol consumption above 2 units per day will increase the risk of certain cancer types such as the cancer of pharynx, liver, oesophagus in the oral cavity and potentially in the breast. Aflatoxin might result in a greater risk of liver cancer whereas high intake of salt in childhood as in salted fish or foods often increases the risk of nasopharyngeal and stomach cancer. Too much preserved food intake increases the risk for colorectal cancer. Eating and drinking excessively hot meals and drinks appeared to increase risk of oral cavity, pharynx and oesophagus cancer. The umbrella review article which compared 204 cohort systematic reviews and meta-analysis studies to investigate obesity and cancer risk concluded that eating colourful fruit and vegetables and increasing antioxidant intake and physical activity results in lower cancer risk (Key et al., 2004; Kyrgiou et al, 2017). These studies strongly support the relation between cancer and being overweight, and increased adiposity tissues in eleven out of thirty six cancer types, especially in women cancer patients. However, the statistical test that they used was not sensitive to 95% prediction intervals (Key *et al.,* 2004; Kyrgiou *et al,* 2017).

Colourful fruit and vegetables provide a high range of vitamins, minerals, and antioxidants known as phytochemicals which fight against free radicals (which can cause cell and deoxyribonucleic acid (DNA) damage) and can strengthen the body and reduce the risk of cancer as well as other potential illnesses. Thus, it can be assumed

that eating 5-9 portions of colourful fruit and vegetables results in lowering the risk of cancer (Poff *et. al.,* 2015).

The European Prospective Investigation into Cancer and Nutrition (EPIC)'s research on the German adult population investigated the association between four healthy lifestyle factors with cardiovascular disease, diabetes, and cancer (Ford *et al.*, 2004). These daily life routines were: not smoking, sustaining a body mass index that is below 30, exercising at least 3.5 hours every week, keeping your eating habits healthy (which means consuming a lot of fruit and vegetables, replacing white bread with whole-grain bread as well as reducing the intake of meat). After 7-8 years of following up, results showed a strong positive relationship between a healthy lifestyle and decreasing chronic diseases such as diabetes by 93 per cent, myocardial infarction by 81 per cent, stroke by 50 per cent, and cancer by 36 per cent (Ford *et al.,* 2004).

Maintaining a healthy body weight, engaging in more physical activity, and cutting back on alcohol intake, were seen to be the most effective strategies available when it comes to reducing the risk of developing breast cancer (Kushi *et al.,* 2012). Cancer research the UK has listed healthy eating choices aimed at preventing breast cancer (see Table 1.1.).

A high olive oil diet acts as a negative modulator of carcinogenesis (Moral *et al.,* 2008; Menendez *et al.,* 2010). Moral *et al.* (2008) conclude that high olive oil and high corn oil diets induce different changes in the expression of the differentiation-related genes. As a result, modifications in the mRNA and protein expression of these genes may be one of the mechanisms by which the lipids of the diet can be exerting modulatory effects. Solonas *et al.* (2010) investigated mechanisms of extra virgin olive oil and high corn oil modulatory actions on experimental mammary cancer in rats. They

found that EVOO and corn oil affected different cellular signalling pathways, but that tumours from rats fed with EVOO had a more benign phenotype compared with tumours from rats fed with corn oil. Regarding DNA damage, EVOO increase apoptosis and diminished the mono-ubiquitylated proliferating cell nuclear antigen levels, which may account for decreased tumour growth. EVOO is believed to have more antioxidant effects than vitamin E on lipid and DNA oxidation (Owen *et al.,* 2000)

In Greece a study carried out among 14,000 women showed that breast cancer can be related particularly to low consumption of vegetables and fruit and high consumption of meat (Details of the study are mentioned in Chapter II under the heading MD and BC) (Trichopoulo *et al.,* 2010). Fung *et al.* (2006) assessed the association between diet and the risk of breast cancer on postmenopausal women by using the Healthy Eating Index (HEI), Alternate Healthy Eating Index (AHEI), Diet Quality Index- Revised (DQI-R), Recommended Food Score (RFS), and the alternate Mediterranean Diet Score (aMed). Food frequency questionnaires (FFQ) were used five times during the period of the study to investigate the participants' dietary information. The study ran between 1984 and 1998, and 3580 women with breast cancer were examined. This study was the first to investigate five diet indices with prudence on chronic diseases such as cancer. Fung *et al.* (2006) found that women who had scored high in the Alternate Healthy Eating Index, Recommended Food Score, and the alternate Mediterranean Diet Score had a lower risk of oestrogen receptor negative (ER-) breast cancer.

Table 1.1 Advice given for breast cancer prevention

• Eat a colourful range of foods	• Maintain a healthy weight
• Choose foods rich in fibre	• Eat at least five portions of fruit and vegetables a day
• Limit saturated fat	• Limit sugar (in food and drinks)
• Lower your salt intake	• Moderate your alcohol intake
• Drink approximately two litres of water a day	

(Yildiz, 2012; Cancer Research UK- diet and cancer, 2014)

Demetriou *et al.* (2012) conducted the first study into breast cancer risk and MD in Greek Cypriots. The study was a large case control study which included 935 individuals and 817 individuals in the control group. The study deeply queried breast cancer in Greek Cypriot women in relation to their demographic, dietary habits, life quality, activities and body measurements. They used 32 items from a FFQ and Panagiotakos's 9 point Mediterranean diet scoring. They did not find any significant relation between the total score and reducing BC risk, however, yet they concluded that high consumption of vegetables, fish, legumes and olive oil may have a protective effect and reduce the risk of BC. This study is a model for future studies which will be implemented in cancer and MD, both in the northern and southern part of Cyprus.

The most common cancer in North Cyprus among women is breast cancer, as mentioned previously. Unfortunately, there is no research to this day into the relationship between diseases and the type of diet in North Cyprus. There is, however, unpublished research on the type of diet practiced in North Cyprus, but not on breast cancer and diet, for which there is a great need. The aim of this research is to shed light on the nutritional diet of breast cancer victims, and to build on the evidence base.

1.5.1 Dietary Carbohydrates/Proteins and Cancer

The main energy source of normal body tissues are carbohydrates. The human body needs to produce ATP for the transformation of chemical energy in foodstuffs to heat in order to continue functioning and living. ATP is produced via glycolysis and oxidative phosphorylation (Krebs cycle). "Cancer cells are more dependent on aerobic glycolysis, fatty acid synthesis and glutaminolysis for proliferation. This difference suggests that targeting metabolic dependence could be a selective approach to treat cancer patients" (Zhao *et al.,* 2013). Since the initial discovery of cancer cells it was believed that cancer cells require much more glucose (aerobic) than normal cells to develop and grow. Furthermore, during the glycolysis stage a basic environment develops in which normal body cells cannot sustain living but in which cancer cells continue multiplying and spreading (Ho *et al.,* 2011). This hypothesis has a great bearing on why most present day nutritional cancer research is based on ketogenic, low carbohydrate and high protein diets. No one has so far researched into the direct effect of protein diets on cancer or cancer cells. However, more and more assumptions and research are basing their hypothesis on the fact that high protein diets have a protective role in relation to cancer (Ho, 2011; Key and Spencer, 2007).

Moreover, as excessive carbohydrate intake is associated with obesity (the increase of fat cells in the body) which has been proven to be the basis of many chronic illnesses, research has shown that it also plays a role in cancer. Excessive fat cells in the body create an imbalance in the secretion of insulin which increases the insulin requirements of the body, creating another topic for research. In cases of obesity insulin resistance can increase and consumption of carbohydrate results in a higher level of plasma insulin. High glycaemic index and glycaemic load, leading to hyperinsulinemia, have been related to a higher risk of breast cancer (Silvera, 2005; Amadou, 2015). A

nine year follow up study conducted on 62,739 postmenopausal French women with breast cancer found a significant relation between the consumed carbohydrate and the risk of cancer in women who are overweight with a large waist perimeter. The same study also reported no significant relation between fibre intake and overall breast cancer (Lajous *et al.,* 2008).

The common belief is that that sugar "feeds" cancerous cells (Kushi *et al.,* 2012); however, sugar or glucose is the primary fuel source of all cells in the body, and feeds the brain via the bloodstream. Blood glucose is obtained from carbohydrates, vegetables, fruit, whole-grain products, as well as dairy produce that is low fat. Consumption of foods that contain protein also generates glucose in the system when there is not enough carbohydrate in the diet. The link between cancer and sugar is not straightforward because consuming food that contains a lot of sugar might result in excessive calories. This might cause weight gain and an increase in the amount of fat in your body, which is associated with a higher risk of certain cancer types (Kushi *et al.,* 2012).

The Warburg effect;

Initially, Warburg hypothesized that mitochondrial impairment that leads to irreversible defective respiration of cells could cause the development of cancer (Justus *et al.,* 2015).

Normal cellular processing as well as energy generation required during rapid cell division relies on ATP. Moreover, tumour cells must keep away from the abnormal micro environment that exists in stressful metabolic conditions in order to multiply, thus they re-programme their metabolism in order to adjust to the progress of the tumour (Martinez-Outschoorn *et al.,* 2016). The change that takes place due to the replacement

of oxidative phosphorylation with glycolysis in the production of adenosine triphosphate, also known as the Warburg effect, is considered the best kind of phenotype occurring in the metabolism of the tumour cells, even under normal oxygen concentrations. Thus, many changed cells obtain most of the energy that they need through aerobic glycolysis which transforms glucose to lactate as opposed to absorbing it in the mitochondria by oxidative phosphorylation (Pavlova and Thompson, 2016). Justus *el al,* (2015) noted that although this process seems to be quicker it does not produce the same amount of ATP in relation to the intake of glucose. This indicates a greater need for glucose in tumour cells in order to compensate for the higher level of energy, redox and biosynthesis. More recently the recognition that many oncogenes and tumour suppressor proteins also lead to cell metabolism regulation as well as controlling cell cycles and apoptosis (cell death) has become more apparent.

The higher production of energy from glucose that turns into pyruvate results from the total oxidation of a great amount of pyruvate into carbon dioxide through oxidative phosphorylation. Oxygen is present at the oxidation of pyruvate into carbon dioxide, whereby a majority of the regular tissues undergo oxidative phosphorylation. In environments without oxygen, regular cells separate glycolytic pyruvate away from mitochondrial oxidation and direct it to lactate formation. Warburg's studies contrastingly showast growth of ascites tumour when they are exposed to aerobic settings still causes the lactic oxidation of glucose. However, the Warburg effect does not generate production of adenosine triphosphate efficiently from glucose, and a higher rate of glucose uptake needed to maintain ATP through glycolysis alone, is characteristic of cancer cells (Cantor and Sabatini, 2012).

The availability of glucose from foods, the glycaemic index (GI), is an important nutritional parameter different from the blood glucose response that relies on different

food containing equal amounts of carbohydrates. The glycaemic index is tested during the two hours after eating food containing 50g of digestible carbohydrates (three hours for diabetic people), blood glucose increase is measured, and taking foods containing the same amount of carbohydrates as a reference percentage is calculated. In other words, the increase in blood glucose level is compared with reference to food with a glycaemic index of 100, and calculated. White bread or glucose syrup is used as a reference food.

Glycaemic load (GL) determines the level of insulin and glycaemic response to a food and is calculated as he carbohydrates consumed from a food based on its GI value and the amount consumed (Akbulut et al., 2013; Memis ve Sanlier, 2009).

In the five food groups carbohydrates with a diverse chemical structure are the main source of energy in a properly balanced diet. It is recommended to take 50% to 60% of energy from carbohydrates, including 10% to 20% of calories from sugars and not more than 10% from added sugars (Mahan et al., 2012). For adults an adequate intake of dietary fibre is 20 to 25 grams per day. From a nutritional point of view, a dominant effect on postprandial glycaemia is the glycaemic index (GI) of foods. The GI is defined as the measurement of the effect of 50 g of digestible carbohydrates of a test food on blood glucose level compared to the effect of 50g of glucose. GI of various food products can be rated as low (GI < 55), medium (55-70) or high (> 70). However, GI is not a precise measurement to estimate the nutritional value of daily food intake, because it is determined experimentally and does not include real portions of consumed carbohydrates. A better measurement to estimate the nutritional value of daily food intake is glycaemic load (GL), which is calculated based on the GI and the portion size of food eaten. As the results of numerous studies have shown, increased GI and/or high

GL diets are linked to a higher risk of diabetes (type 2) and cardiovascular problems as well as cancer (Rozanska et al., 2016).

The EPIC study included 519978 individuals aged between 25 to 70 years, and assessed the association between fibre intake and bowel cancer. They found an inverse relation between large bowel cancer and high fibre food intake (Bingham *et al.,* 2003).

A diet high in fat (ketogenic diet) that consists of reasonable amounts of protein and less carbohydrate might suggest a physiological condition which fasting or exercising can activate (Wu *et al.,* 2016). Protein is crucial for a healthy diet, and various sources of protein can affect cancer risk in diverse ways (Wu *et al.,* 2016). Processed meat and excessive intake of animal source proteins proved to be a higher risk factor for cancer. In contrast, plant sources of proteins like soya are suggested to be a protective type of protein for cancer along with increased fibre intake in a diet (Maskarinec *et al.,* 2016; Yang *et al.,* 2016).

According to research, certain health problems, for example diabetes (type 2) and certain cancer types, can be affected by increased glucose in the blood and a higher insulin level as well as chronic low-grade inflammation (Smyl, 2016). Being physically active and fasting might influence the increased glucose in blood and insulin level positively. What is more, it can cause a higher concentration of plasma ketone bodies. As a result of this layered process, there might be a decrease in inflammation. Animal model studies show that a ketogenic diet offers a practical alternative in assisting cancer treatment (Smyl, 2016).

1.5.2 Dietary Lipids and Cancer

Out of the huge number of cancer cases in the USA approximately 117,000 every year are associated with excessive body fat. The area covered by fat cells in the

body is important. An apple shaped body indicates increased fat around the abdominal area that surrounds vital organs. It is known that high body fat increases the risk of certain cancer types, among which are oesophageal cancer, cancer of the kidney, colorectal cancer, pancreatic cancer and postmenopausal breast cancer. According to research, visceral fat tissue causes the generation of inflammatory cytokines and hormones such as leptin, oestrogen and insulin. An increase in these elements is linked to an increased possibility of cancer (Hopkins, 2016). Excessive dietary fat intake increases the abundance of pro-inflammatory cytokines (e.g. leptin, resistin, etc.) and angiogenic factors (growth factors) (Sundarams and Yan, 2016).

Cancers like breast cancer, prostate, colon, ovary and the like are all believed to have a significant relationship with fat consumption especially with total saturated fat (Hu, 2013; Brasky, 2016). Total fats as well as saturated and omega 6 are also linked with several types of cancer (Xia, 2015; Hu, 2013). The type of lipid and its content is important in different types of cancer. For example, most MUFAs and omega-3 have a protective effect on cancer, and the ratio between fatty acids is important in healthy situations (Hu *et al.,* 2013; Santos and Schulze, 2012; Othman, 2007). Following a healthy lifestyle and choosing olive as the main fat source could lower cancer incidence. Mutations of cells in the human body caused by cancer are partly due to toxins from food that damage DNA. Free radicals generated from these toxins can attack DNA. To reduce free radicals, vitamins and antioxidants are needed such as those contained in olive oil. Polyphenols are the component in olive oil that shows up as pro-nutrients known as antioxidants. Polyphenol content in olive oil increases the more it is unfiltered during the olive oil pressing process. EVOO amongst olive oils has the highest polyphenol content, suggesting that EVOO has the highest potential protective effect against cancer (Foscolou *et al.,* 2018).

The consumption of olive oil in Mediterranean countries has been shown in many studies to lead to a lower incidence in cancer. Olive oil includes oleic acid which is a MUFA, and also phenolic compounds which are related to lowering cancer risk (Schwingshackl *et al.*, 2017).

After dietary lipids are digested, they are mostly stored in adipocytes or used in the production of energy by oxidation. Some fatty acids, such omega-3, modulate inflammatory responses, and may have anti-carcinogenic activities (Toren, 2013).

Moderate protein intake, high fat and low CHO are features of a ketogenic diet, leading to production of ketone bodies as a partial substitute for glucose as metabolic fuel. The reduced availability of glucose may limit the growth of cancer cells, which are known to consume large amounts of glucose. Some studies suggest that a ketogenic diet reverses the pathways of redox signalling which are linked to malignant tumours (Branco *et al.*, 2016). Several studies indicated that ketogenic diets may potentially limit growth of cancer cells by limiting or cutting down the CHO that limits cancer cells' ATP production (Branco *et al.*, 2016; Rubio-Patiño *et al.*, 2016; Freeman *et al.*, 2007).

A ketogenic diet, as mentioned earlier, consists of low levels of carbohydrate and higher fat. It was initially designed as a therapy for refractory epilepsy. Experiments on animals that receive the ketogenic diet for cancer treatment offer promising outcomes (Freeman *et al.*, 2007). Ketogenic diets produce a condition which resembles fasting with only water and excessive physical activity. This is a condition in which blood glucose decreases while blood ketone bodies are elevated (Rubio-Patiño *et al.*, 2016). Many tumour cells develop an addiction to glucose in order to survive and grow. Therefore, ketogenic diets might cause metabolic stress in tumour cells by reducing the

availability of glucose and increasing blood ketone bodies which depend on the function of mitochondria for metabolism (Meidenbauer *et al.,* 2015).

Tan-Shalaby's (2016) study investigated the effect of a ketogenic diet on seventeen cancer patients at an advanced stage who did not undergo chemotherapy, and most of them were overweight or obese. These patients absorbed between 20 and 40g of carbohydrate for a month, after which it was found that they had no unbalanced sign of serum glucose, ketones or lipids. Those who lost 10% of their weight proved to have a much better response to treatment. Due to variations in participants' compliance with the timing of blood draw and fasting, this study could not yield accurate glucose and ketone values. Yet, the study suggested patients' nutritional status may improve their lifestyle and lead to a positive response to chemotherapy.

1.5.3 Vitamins and Minerals

Free radicals are unstable molecules that lead to cell damage. Exposure to ingested toxins and environmental pollutants and excessive UV-A will increase free radicals in our tissue. As free radicals accumulate, the oxidative stress will damage DNA, proteins and lipids, which may cause proteins to alter cell structure. Excessive free radicals can lead to chronic disease such as Alzheimer's disease, Parkinson's syndrome and heart problems as well as cancer and diabetes (Lobo *et al.,* 2010).

Antioxidants are compounds that neutralize free radicals and restrict the oxidation of molecules. Thus, Larouche *et al.,* (2016) claim that consuming antioxidants helps reduce cell inflammation levels. Several foods include antioxidant-phytochemical compounds such as polyphenols, flavonoids, vitamin A as carotenoid, vitamin E as alpha-tocopherol, vitamin C as ascorbic acid and the mineral Selenium all are powerful antioxidants. In particular β-tocopherol, zinc, and selenium antioxidants act on the

breast tissue and have a protective effect, particularly on postmenopausal women (Larouche *et al.*, 2016).

1.6 Complementary/Alternative Medicine in Cancer Treatment

Alternative medicine is defined as treatments or health services that are used instead of conventional medical treatment and are sometimes not accepted by modern biomedicine. Complementary medicine is used as a supplement with medical treatment and care (*Guide for people with cancer - thinking about complementary and alternative medicine*, 2005). Complementary and alternative medicine (CAA) use is increasing rapidly. In research undertaken over a period of 10 years covering different illnesses (Rose, 2009), and especially on cancer patients, CAA frequency use increased from 17% to 85%. Furthermore, the most frequently used substitutes in these studies were plant-mixtures. Examples of alternative medicine use in this study are described in the qualitative interviews with participants Chapter III- heading 3.5-theme 7.

The dearest wish of all cancer patients, apart from wishing their cancer will not recur is to return to their state of health prior to contracting cancer. Only sometimes is this possible with modern medical treatments. However, and unfortunately, modern cancer treatment methods are far from perfect; in most cancer types the treatment's success rate is low and has temporary or permanent side effects. Some studies which base their foundations on previous scientific bases obtain that a healthy diet along with being physically active decrease the recurrence of certain types of cancer like breast, prostate and colon cancer (Isik and Akcay, 2014).

1.7 Breast Cancer and Obesity

Obesity can be defined as increased body fat, an increase in the body mass index (BMI), and it can be relevant to the development of breast cancer (BC) (Assiri *et. al.*,

2015). Cell-autonomous changes can cause a given cancer cell to proliferate and, in the course of time, contribute to tumour genesis. This has been observed in the study mentioned below, and these changes in whole-organism metabolism, for example, the onset of obesity, are associated with a significantly increased risks of contracting a variety of cancers (Dang, 2012). Obesity has an effect on many hormones that are known to be linked with BC. Adipose tissue in the body may contribute to the development of cancer by secreting adipokines, such as leptin, adiponectin, visfatin, and resistin. These could affect breast tissue through endocrine, paracrine or autocrine pathways (Yao-Borengasser *et. al.,* 2015). The anatomy of milk-secreting organs of females favours close interaction with breast tissue and breast adipose tissue. The major link between obesity and BC metastasis and the onset of cancer may be due to adipokines produced by breast adipose tissue that acts within the breast environment where tumour cells exist (Schaffler *et. al.,* 2007; Tworoger *et. al.,* 2007; Assiri, *et al.,* 2015).

A cohort follow up study investigated short term weight gain in BC women over 40 years from 1980 to 2006, and checked their BMI during and after menopause. They routinely followed up (updated every 2 years) risk factors of breast cancer via mail questionnaires. The study found a significant connection between short-term weight change and the risk of breast cancer (Rosner *et. al.,* 2015). A study was conducted with 82 participants who had been newly diagnosed with BC, among whom some were pre and some were post-menopausal. There was an investigation into the correlation of resistin, visfatin, adiponectin, and leptin. There were significantly higher levels of leptin, resistin, and visfatin in postmenopausal BC patients than their respective controls (Assiri *et. al.,* 2015). Tamaki *et al.* (2015) conducted a study in Okinawa on women who suffered from post and pre-menopausal breast cancer. This study demonstrated that

the Okinawan women who were above a healthy weight, and/or obese, were statistically more likely to develop breast cancer compared to others who were not overweight and/or obese. Another study found a statistically significant relation with higher auxiliary lymph node ratio in the obese women (Kaviani *et al.,* 2013). A review study concluded that obese patients were more likely to contract disease when compared with non-obese patients; the tumours of the patients who were obese were larger, were more likely to induce metastases in lymph nodes, and were less likely to be positive for Her2 (Haakinson *et al.,* 2012).

1.8 Breast Cancer and Physical Activity

Movement of any limbs that requires energy is known as physical activity and physical activity is an essential element in energy balance. Physical activity is associated with lowering the risk of certain cancers such as colon and breast cancer (National Cancer Institute-Physical Activity and Cancer). A sedentary lifestyle is one of a handful of proven breast cancer risk factors (Lynch *et al.,* 2011).

Studies show that physically active women face a lower risk of developing breast cancer than women who undertake little physical activity. Furthermore, the risk of breast cancer has been demonstrated through research to be reduced with physical activity in both pre-menopausal and post-menopausal women via the androgen signalling pathway (Zhang *et al.,* 2015; National Cancer Institute-Physical Activity and Cancer). However, The Fifth Korea National Health and Nutrition Examination Survey, which took place between 2008 and 2011, examined the associations with physical activity in women and survival of breast cancer and colorectal cancer. The mean physical activity levels were expressed as the metabolic equivalent of task-MET-minutes/week. Their findings showed no significant difference in breast cancer subjects

in all three groups (n= 10,167, mean age= 48.55± 16.27). The subjects were categorized as "never diagnosed with cancer" (Group 1), "0-4 years since cancer diagnosis" (Group 2), or "five or more years since cancer diagnosis" (Group 3). In groups 1 and 2, and groups 1 and 3 in colorectal cancer subjects, findings are statistically significant (Jung Kang, 2015). Exercise, especially in women who are approaching menopause, can assist/result in reducing hormone levels such as insulin and insulin-like growth factors I, enhancing the response of the immune system, as well as helping to reduce body fat and body mass through a balanced weight (National Cancer Institute-Physical Activity and Cancer). Also, studies show that physical activity, maintenance of social roles, relationships and responsibilities will increase breast cancer survival (Mackenzie, 2015).

At least 30 minutes per day of moderate physical activity has been recommended for adults by The Centre for Disease Control and Prevention (CDC) (National Cancer Institute-Physical Activity and Cancer).

Part of the study described in this thesis assesses the physical activity levels of the 140 women with breast cancer. A comparison has been made between physical activity, cancer biomarkers (CEA and CA 15-3) and the Mediterranean diet score to assess its influences on the treatment procedures in relation to breast cancer. In the study described in this thesis 140 women with breast cancer were investigated and their weight (before the diagnosis for breast cancer, during and after), height and body mass index were measured to analyse their obesity values. Their treatments and the relation between breast cancer processes have been compared. One of the aims of this study is to establish whether the weight or body mass index has any effect on the state of breast cancer. During the treatment, patients can gain or lose weight due to the side effects of treatment. The discussion of this status of the participants can be found in Chapter II.

1.9 The Overarching Hypothesis of the Study

Black olive oil is unique to Cyprus, and there is a lack of information and research on the nutritional ingredients, amount consumed, and choices of the public. Also, there is an ongoing popular debate about black olive oil; one side thinks black olive oil is healthy and the other side thinks black olive oil is unhealthy due to its special processing methods.

This research investigates the nutritional value of black olive oil, the effect of polyphenols derived from BOO on breast cancer cell lines, breast cancer patients' adherance of eating habits to MD, and the use of olive oil/BOO in North Cyprus. Recent research shows that olive oil polyphenols and Mediterranean diet have a protective effect on breast cancer. The aim of this research was to assess whether BOO, and other components of the diet, can influence outcomes for BC patients. The research was undertaken in Cyprus and examined the nutritional components of the local olive oil BOO, the frequency of consumption and its effects on breast cancer.

It is useful for the researcher to critique her own research stance when considering the studies embarked upon in order to answer the research question. The researcher's interest in black olive oil was pressured by a quest to provide answers to dietary conundrums from her country of origin, N Cyprus, on the question of black olive oil, to provide a starting point for further research.

As a Cypriot researcher, living in a small Mediterranean island has advantages and disadvantages; firstly living in a country with a small population, word of mouth can spread fast, across the whole country, changing habits based on correct and incorrect information. The researcher grew up in a garden full ofolive, orange and lemon trees. It was same in every householder in Cyprus and inhabitants would collect

olives oranges and lemons, and process them as juice and store them for all season. Collected olives were always sent to mills and produces olive oil from them. Originally it was requested from mill to produce black olive oil from our olives that we gave them. Then, as health awareness began to grow, word of mouth had it that black olive oil contained a high amount of aflatoxin and could cause cancer, perhaps as the number one cause of cancer in the country. On the other hand scientists announced that olive oil has a protective effect on cancer (Chp. IV, heading 4.2) and you should consume wisely, especially in breast cancer if you use EVOO. So after dramatically reducing BOO consumption, and feeling hurt by this loss of tradition, the population was left unsure where BOO protected from or promoted cancer. Nutritional science has started to increase in Cyprus in 2008 with Near East University opening Nutrition and Dietetics Department yet currently there are still no suitable food science laboratories present.

As a Cypriot Dietitian, the starting point of this researcher was to adopt an approach from that could be described as belonging to a pragmatist paridgm (Brierley, 2007) in order to address a number of pressing contemporary practical research questions. A mixed methods approach was deamed appropriate as is often the case to address a study with diverse aims and objectives (Tashakkori and Creswell, 2007). Combining questionnaires and interviews in a single research study brings together the advantages of breadth and depth associated with these two respective methods (Teddie and Tashakkori, 2009). The combined methodologies can present a more complete picture of a research topic that can address a range of research questions and therefore provide further insight that can enhance theory development and practice.

The starting point of determining the consumption of black olive oil among the population under study, and then further exploring the meaning behind peoples' dietary

choices and understanding, was addressed. It was then warrented to explore the composition of the oil and the effect of black olive oil on cancer cells.

The purpose of bringing BOO to a scientific platform may lighten up, or strengthen, the concerns about it, but at least will bring about some evidence to base decisions upon.

Black olive oil has a huge social value in Cyprus, which is why it is very important to evaluate it. The purpose of this research was not only to introduce black olive oil to the scientific world, but also to address N. Cypriot national concerns (whether it is healthy or not) about black olive oil. The design of this study started with questioning breast cancer patients in North Cyprus. The relationship of olive oil and a Mediterranean diet with breast cancer is discussed in chapter II. Breast cancer incidence is high in North Cyprus. (The statistics of cancer and breast cancer in North Cyprus are also discussed in chapter II.) A Mediterranean diet scoring tool was used to evaluate participants' eating style because Cyprus is a Mediterranean island and olive oil is the main source of this lifestyle (discussed in Chapter II as well). Chapter II was designed to get answers on: how often participants use olive oil; whether they prefer black olive oil; what their eating habits are; whether they are eating things approximate to a Mediterranean diet; and whether there are any relationships between black olive oil and breast cancer recovery.

To gather more information about participant's knowledge on the relation between olive oil and BC recovery these broad questions arose: do they have concerns about black olive oil? Which olive oil type do they prefer? Are they aware of the link between nutrition and BC? A qualitative methodology was chosen in order to explore cancer patients' thoughts and feelings. This detailed exploration enables a different

understanding of the factors that influence cancer patient's beliefs about lifestyle, food choices and what drives these beliefs, as well as an understanding of why and where they seek out information.

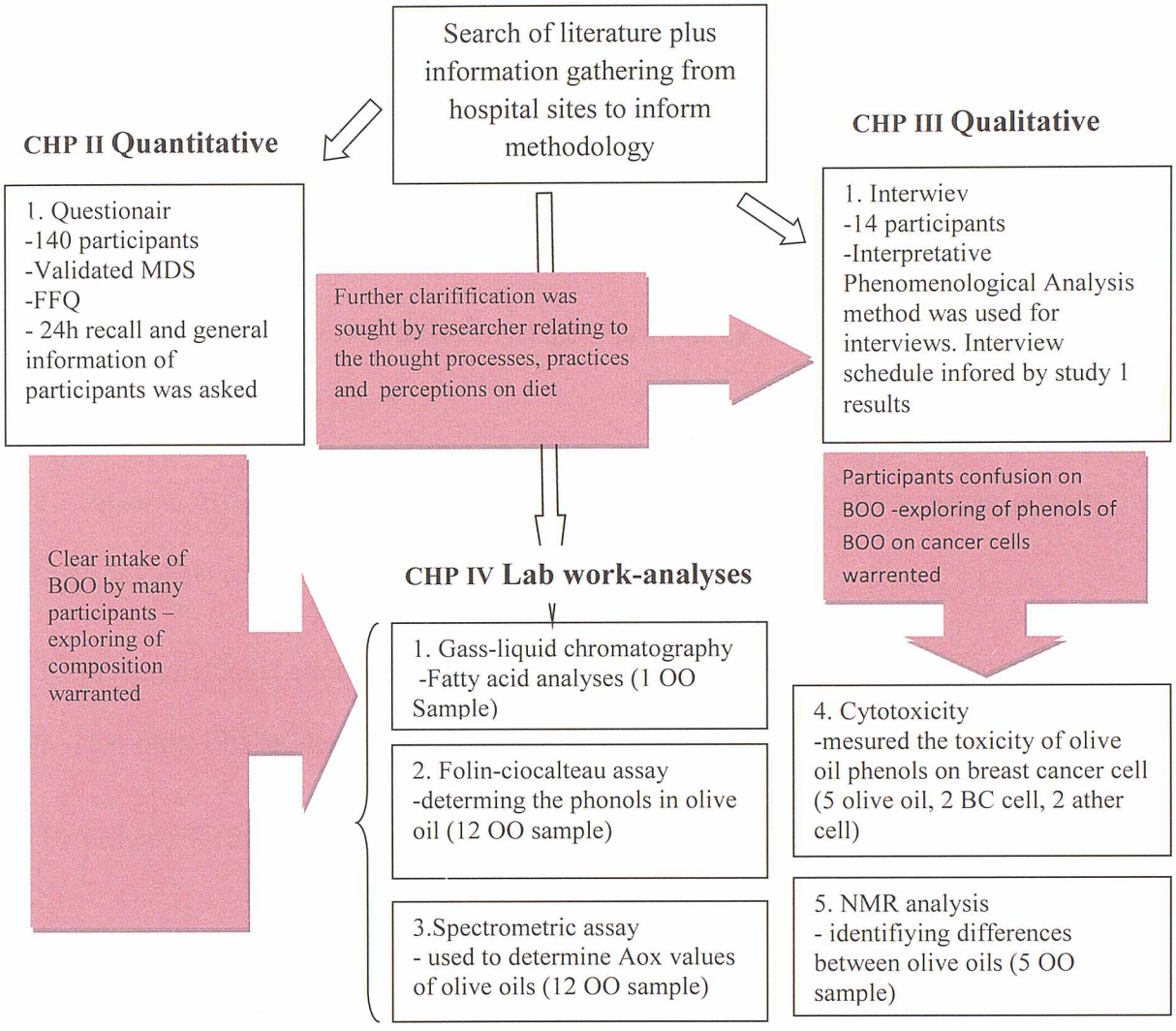
Unlike quantitative research, which is positioned in within a positivist epistemological perspective, often aiming to test or verify a hypothesis, qualitative research is positioned more within the contructivist epistemological perspective, concerned with exploring experiences and social factors that can underpin and shape the hypotheses of qualitative research (Dezin and Lincoln, 2000). Interpretative Phenomenological Analyses has been used in Chapter III. A few volunteers from Chapter II and a few new patients were combined and interviewed as a pilot group to verify information collected on the relationship of participant's cancer awareness and lifestyle. Quantitative research gives an indication of cancer patients' foods choices, lifestle and eating habits at that current time of life. It does not provide a detailed understanding of these beliefs or the reasons behind them, nor does it explain their motivations behind these changes. Gaining a more detailed understanding of cancer patients' beliefs about lifestyle, change in food choices and their changes in lifestyle, may help explain any gaps in knowledge.

Furthermore, the study explored the nutritional values of black olive oil in order to understand the differences between EVOO and BOO, including the effect on breast cancer cell lines in a laboratory environment. This was to address rumours surrounding black olive oil, and this can be found in chapter IV. Methodology of the study can be seen in figure 1.2.

1.10 Approaches to Reviewing the Literature

Since the study spans a broad area, exploring the dietary patterns of the population of focus for the first time, perceptions of diet and breast cancer recovery, impact of dietary items on BC and BOO the relevant literature base is very broad and heterogeneous. As previously mentioned, there is also very little literature on either breast cancer patients' nutrition in North Cyprus or black olive oil. Therefore, a narrative review of the literature was conducted as it was considered more appropriate in this case to do so rather than conducting a systematic review on one specific topic. The literature in this chapter is relevant throughout the thesis. In each subsequent study chapter, topic-specific literature relevant to the findings is reviewed in greater detail. The literature is presented in this way to provide structure to the findings of each study and enable the reader to consider the relevant literature directly with the study's findings. The literature reviewed was conducted through a thorough search on the databases Science Direct and Pub Med Central, using the following MeSH terms 'Breast Cancer, cancer nutrition, BC and nutrition, Mediterranean diet, MDS, breast cancer and MD, black olive oil, olive oil and BC, olive oil and MD, breast cancer cell lines, BC cell lines and olive oil, IPA'. The search was first conducted in 2011, and repeated periodically until 2018. The references listed in retrieved articles were scrutinised to identify any additional missed studies. Since there were no published articles relating to BOO the literature related to this was extracted using books from the Rustem book store (located in Nicosia Cyprus) detailing the specific olive trees and quality of olives, and details on olive processing were obtained directly from the manufacturers of BOO who were based in the region.

Figure1.2 Studies methodology flow chart



1.11 Research Aims and Objectives

The overall aim of this study is to gain insight into the impact of diet of North Cyprus Breast Cancer patients on their recovery, and specifically;

1. To assess the dietary intake of cancer patients in North Cyprus in relation to a traditional Mediterranean diet;

2. To explore perceptions and dietary changes reported by cancer patients undergoing treatment;

3. To examine the effects of dietary and olive oil consumption on breast cancer tumour markers in breast cancer (BC) patients;

4. To determine the chemical characterization of black olive oil (BOO) and compare it with other olive oils to identify any special characteristics of BOO;

5. To provide further insight into the impact that BOO polyphenols may have on BC cells.

2. Chapter II- Study I; Mediterranean Diet

This chapter focuses on Mediterranean Diet (MD), breast cancer and North Cyprus dietary consumption.

The aim of the work described in this chapter is to determine the relation between breast cancer and Mediterranean diet and to see the effectiveness of Mediterranean Diet on breast cancer biomarkers.

2.1 Mediterranean Diet

Little is known about the effect on the current NC diet on BC risk and recovery. However, earlier studies do exist that relate MD and breast cancer. NC is a Mediterranean country with a diet that has some shared features of what is known as the MD; therefore the literature on MD and BC was reviewed.

The Mediterranean Diet has been asserted to be a model of healthy eating (Sofi *et al.,* 2008) and became popular in the 1990s. Ancel Keys was the first to mention the Mediterranean Diet in the 1960s, pointing out evident health benefits. Variations of the Mediterranean diet have paved the way for all studies to be evaluated for the relationship between the suitability of this diet and several outcomes such as overall mortality, incidence of mortality, as well as frequency of, or death the ratio from, cancer - overall or from specific cancer sites (Trichopoulou *et al.,* 2000). The key aspects of this diet are high consumption of olive oil, legumes, unrefined cereals (complex carbohydrates), fruits, and vegetables, moderate to high consumption of fish, moderate consumption of dairy products (mostly cheese and yogurt), moderate wine consumption (once or twice a week), and a low level of processed food consumption in relation to meat and meat products (Noah and Truswell, 2001; Ferro-Luzzi, 1989).

Olive oil is often considered characteristic of the Mediterranean diet. Olive oil is the principal source of fat. Total fat in this diet is 25% to 35% of calories, with saturated fat at 8% or less of calories. The Mediterranean diet is rich in fibre (fruit, vegetables and legumes) and mono saturated fatty acid (olive oil) and poor in saturated fatty acids (Martınez-Gonzalez *et al.,* 2008).

2.2 Mediterranean Diet and Diseases

Many studies have shown that the Mediterranean style of eating has a role in lowering mortality and morbidity of chronic disease (cardiovascular disease, diabetes mellitus, obesity, cancer…etc.). Lipid profiles and glycaemic control will be improved in people with diabetes and cardiovascular disease with high consumption of monounsaturated fatty acids, and also high intake has been said to improve insulin sensitivity (Paniagua *et al.,* 2007).

Martinez-Gonzalez *et al.,* (2008) worked with 13,380 university graduate students without diabetes, and followed up the study for around four years to investigate MD and the incidence of diabetes. They used FFQ and 9 point MD scoring concerning dietary habits. Those who had followed MD had a reduced incidence of diabetes. The result was not surprising, because the study populations were from Mediterranean countries and they already had similar nutritional behaviours to those of the MD. Also, the population was highly educated; leading to the assumption they had an awareness of healthy eating and took care to implement this.

A meta-analysis of 50 studies found that MD reduced the risk of metabolic syndrome (MS) (Kastorini *et al.,* 2011). Also, they found a positive relationship between waist circumference, high-density lipoprotein cholesterol, triglycerides, blood pressure, and glucose with MD. In another meta-analysis it was found that low-

carbohydrate, low-GI, Mediterranean, and high protein diets cause improved biochemical markers of cardiovascular risk in people with diabetes and overall diabetes stages (Ajala *et al.* 2013).

In another study the relationship between obesity and nephrolithiasis was investigated, involving 478 individuals with BMI over 25 kg/m^2. Study results showed no difference between stone formers and non-stone formers to low adherence to the Mediterranean Diet concerning nephrolithiasis (Soldati *et al.*, 2014).

2.2.1 Cardiovascular Disease

In 1999 Lorgeril *et al.* found that the MD did not reduce the major risk factors (high blood pressure and cholesterol) of cardiovascular disease. To reduce cardiovascular mortality and morbidity one should use a cardio protective diet rather than MD. A study of 2,650 persons was conducted; BMI levels between 29 to 35 kg/cm^2 were compared between a low fat diet and MD to see weight changes and CVD. The Mediterranean diet appeared to be having more favourable changes than low-fat diets in weight changes, clinical changes in cardiovascular risk factors and inflammatory markers (Nordmann *et al.*, 2011). The Mediterranean diet contains a very high level of monounsaturated fats (MUSF), (mostly oleic acid) which studies insist are linked to a lower risk of coronary heart disease (Estruch *et al.*, 2013). There is also evidence that the antioxidants in olive oil improve cholesterol regulation and LDL cholesterol reduction, and that it has other anti-inflammatory and anti-hypertensive effects (Trichopoulos, 2012; Barberger-Gateau et al., 2011). Estruch *et al.* (2013), compared MD with olive oil and MD with nuts to see which MD would improve CVD to a greater extent. It was found that persons with a high risk of CVD can protect themselves with both MD high in olive oil or nuts more than a control group (advised to

reduce dietary fat). In the group randomized to a Mediterranean diet supplemented with extra virgin olive oil a combination of stroke, myocardial infarction, and cardiovascular deaths, was reduced by 30% and in the Mediterranean diet with mixed nuts compared with controls lower 28% (Estruch *et al.,* 2013). In four meta-analysis studies (Trichopoulou *et al.,* 2003, Knoops *et al.,* 2004; Mitrou *et al.,* 2007) it was concluded that the Mediterranean diet significantly reduced the risk of mortality from cardiovascular diseases (Sofi *et al.,* 2008).

2.2.2 Mediterranean Diet and Neurological Disorders

Researchers thought the Mediterranean diet had efficacy in reducing inflammation, and so it could lower the risk of depression. Sanchez-Villegas *et al.* (2009) investigated depression levels with Trichopoulou's Mediterranean diet pattern;

(1) High ratio of MUFAs to saturated fatty acids (SFAs),

(2) Moderate alcohol intake,

(3) High intake of legumes,

(4) High intake of cereal (such as bread),

(5) High intake of fruit and nuts,

(6) High intake of vegetables,

(7) Low intake of meat and meat products, and

(8) Moderate intake of milk and dairy products. Later, the same authors added another component:

(9) High fish intake. Except fruit and nuts, the monounsaturated- to saturated-fatty acids ratio and legumes, there was a statistically significant (95% confidence intervals) relation.

Two studies conducted in America (USA) Scarmeas et al., (2006) and Gao et al., (2007) discovered that consumption high in legumes, wholegrain products, fruits, vegetables, fish, MUFA:SFA ratio, moderate alcohol and low red and processed meat (Mediterranean diet style eating) diminish Parkinson's and Alzheimer disease.

2.2.3 Mediterranean Diet and Cancer / Mortality

In six meta-analysis studies (Knoops *et al.,* 2004; Lagiou *et al.,* 2006; Fung *et al.,* 2006; Mitrou *et al.,* 2007, Benetou *et al.,* 2008) it was concluded that the Mediterranean diet significantly lowers the risk of mortality from cancer (Sofi *et al.,* 2008).

Trichopoula *et al.,* in 2009 investigated each component of the Mediterranean diet and overall mortality. They found a positive relationship between moderate consumption of ethanol, low consumption of meat and meat products, and high consumption of vegetables, fruits and nuts, olive oil, and legumes with mortality, and a minimum relationship with other components of diet with mortality. Overall, the Mediterranean diet was associated with a significant reduction in total mortality.

2.3 Mediterranean Diet and Breast Cancer

There is a complication in the assertion that MD is associated with a lower risk of BC. Some researchers found that this is the case, and some found to the contrary. Further research is needed to investigate the relationship between MD and BC to understand the specific characteristic pattern of MD that affects BC cells. In a study

with one to one control groups the association between each separate component of the MD and BC women was investigated. It was found that a one unit increase in the Mediterranean Diet score is associated with a 9% lower risk of contracting breast cancer (Mourouti *et al.*, 2014).

Recent studies suggest that olive oil has a protective effect against BC cells *in vitro, in vivo* as well as being part of the MD (Menendez *et al.*, 2005; Moral *et al.*, 2008; Trichopoulo *et al.*, 2010). It would be fair to assume that Mediterranean countries are protected from BC due to their diet, and therefore have a lower cancer incidence (Bosetti *et al.*, 2009). However BC in NC is still the number one cause of morbidity; a cohort study showed that BC was the most common cancer in NC women, with 30% of the population being diagnosed with it (Hinçal *et al.*, 2008).

Castello *et al.* 2014 compared the effects of diets classified by the Alternate Healthy Index (AHEI) and the Alternate Mediterranean Diet Score (aMED) and the risk of BC. They included 1,017 BC cases and a 1,017 strong healthy control group at a similar age. AHEI diets had a higher risk of BC, especially in pre-menopausal women. The aMED diet lowered the BC risk. The study provides novel results regarding the harm arising from Western eating styles, with regard to cancer and the potential protective effect of MD against aggressive type cancer tumours. Also, the MED had a stronger protective effect on triple-negative breast cancer tumours. Mixed consumption of high fibre, high n-3/n-6 ratio, high polyphenols and low-GI food (olive oil, non-refined wheat bread and wine) characteristic of the Mediterranean Diet is associated with reducing BC risk (Lorgeril and Salenp, 2014)

In a meta-analysis, 26 studies were assessed looking at the relationship between dietary patterns and the risk of BC. They found that the Mediterranean style of eating

(composed largely of vegetables, fruit, fish, and soy) decreased the risk of BC. They said that there were no significant relationships between traditional patterns and BC; only the Mediterranean Diet had a significant effect in lowering BC risk. The study suggested that eating fish, fibre, vegetables, and fruit instead of alcoholic beverages, red meat, and processed food will protect against breast cancer (Albuquerque *et al.,* 2014). Contrary to these studies, in another meta-analysis (twenty-one cohort studies included) no significant relationship between breast cancer and MD was found. The MD was associated with lower overall cancer mortality and incidence by 10% (Schwingshackl and Hoffmann, 2014). Cade *et al.* (2011), compared MD and the World Health Organization Healthy Diet Index (WHO HDI) with the risk of BC. 828 breast cancer cases with a 9 year follow up. They also could not find statistically significant signs that the MD had effects in lowering BC risk. There was a relationship between post-menopausal women with BC and MD. There was no strong association between the two diets and breast cancer risk. Trichopoulou *et al.* (2010) in their study could not find a significant relationship between overall BC risk and the Mediterranean Diet. However, there was a statistically significant association with a dencreasing BC risk in post-menopausal women. From 19 observational studies conducted in Greece, Spain, Italy and France it was concluded that the Mediterranean diet was not associated with reducing breast cancer risk in the all cohort studies; however, there was a statistically significant association in relation to post-menopausal women. In addition, high consumption of olive oil was associated with reduce breast cancer risk in all cohort studies (Psaltopoulou *et al.* 2011).

A single blind PREDIMED study evaluated MD with supplements (EVOO, nuts and control group) on 4,282 women for 4-5 years. MD with extra virgin olive oil had the highest preventative effect on breast cancer, and MD with nuts was also positive

(Berrino, 2016). Hirko *et al.* (2016) investigated dietary patterns Alternative Healthy Eating Index (AHEI), alternate Mediterranean diet (aMED), and Dietary Approaches to Stop Hypertension and the risk of breast cancer subtypes. However, they were unsuccessful in finding any significant associations between the AHEI or aMED dietary patterns and risk of breast cancer subtypes. A review article concluded that MD has a potential for lowering the risk of chronic diseases. In two important large studies MD patterns were found to have a protective effect against breast cancer (Martinez-Gonzalez and Martin-Calvo 2016). A study conducted on Greek Cypriots evaluated interactions between MD and geneson, the levels of specific metabolites that could be linked to diet, in women with breast cancer. Participants were previously genotyped for nine polymorphisms of genes involved in one-carbon metabolism, oxidative stress, and xenobiotic metabolism. The serum levels of 14 key metabolites were measured. Levels of flavin mononucleotide and 5-methyltetrahydrofolate were linked to MD and to a genetic variant in glutathione transferase (GSTM1), demonstrating that variation in dietary intermediates can be modulated by genetics as well as by diet (Kakkoura *et al.*, 2016). In this study genetic analysis is not included, and future work could look at the interplay between genes and diet.

2.4 Aims and Objectives

The aim of the work described in this chapter is to determine the relationship between breast cancer and the Mediterranean Diet and to assess the effectiveness of the Mediterranean Diet on breast cancer biomarkers by:

1- Identification of features of the NC diet that may influence BC recovery;

2-Investigation of adherence to MD among BC suffers in NC;

3- Assessment of the BMI, biomarkers and nutritional intake of BC patients in NC;

4- Assessment of the frequency of consumption of BOO in BC patients;

5- Investigation of the effect of BOO and EVOO consumption on BC biomarker changes.

2.5 Methodology

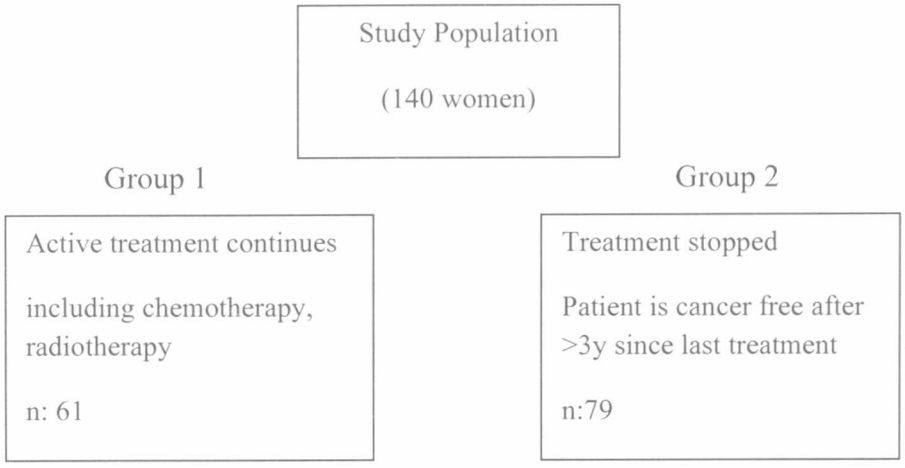
2.5.0 Study Design

This was a cross sectional study on breast cancer patients attending the two main hospitals in NC; Dr. Burhan Nalbantoglu Hospital and the Near East University Hospital.

2.5.1 Participants

The study was conducted among women aged 19 - 65 who were diagnosed with BC and still had BC, and those who had BC and had been cancer-free for three years after treatment. The age range was limited to 65, since those over the age of 65 are classified as older adults by the WHO, and aging may cause bias. There was only one man who had breast cancer at that time in North Cyprus. A small minority of men are BC patients; therefore, to avoid gender bias and heterogeneity of findings, the man was left out of the study population. Breast cancer patients who were aged between 19 to 65 and had visited Burhan Nalbantoglu and Near East University hospitals during 2011-2013 and consented to take part in the study, were included in the study population. 140 subjects were included in the study based on national statistics of North Cyprus BC incidence. This is a cross-sectional study that gives current information about participant's diet, food choices, physical activity and adaption to MD. A breakdown of the study population groups can be seen in Figure 2.1 below.

Figure 2.1 Distribution of study population



Due to the small population of NC a power calculation was not used to estimate an exploratory sample size; this was a convenient sample size. In 2011 there were 800 breast cancer consultations (newly diagnosed and post-treatment) in the Oncology outpatient clinic of Burhan Nalbantoglu Hospital of the TRNC. There are only two hospitals that treat oncology patients present in the TRNC, Burhan Nalbantoglu and Near East University hospital, which follow cancer patient's consultations together. The study cohort was selected from local patients at a single point in time. The information that has been taken from participants reflects their current knowledge, and the study assumes participants' past knowledge.

Inclusion and Exclusion Criteria:

Inclusion: - Females,

-Aged between 18-65,

-Attending Burhan Nalbantoglu or Near East University hospitals

- 1, 2 or 3 stage of cancer

Exclusion: -Males,

-Over aged 65,

-Terminal cancer

- 4th stage of cancer

The groups and characteristics of the breast cancer patients who participated in this study are given in the above table. Based on these criteria the population of the study was designed to include 61 women who were still receiving cancer treatment (chemotherapy and/or radiotherapy and/or medicine) placed in first group. In the second group, 79 breast cancer patients were included who had been diagnosed with BC, had received their cancer treatment, and had been cancer-free for at least three years after their cancer treatment had stopped. Patients are considered at risk in their first two years after successful BC treatment ends, when they are required to attend check-ups every 2-3 months. At the end of the 3rd year after treatment, if there is no concern about the illness the patient is considered healthy (National Cancer Institute, 2010). The second group in this study included those who had completed their treatment and did not have cancer after three years' post-treatment. Patients in their final stage of life were not included to the study. The efficiency of nutritional intakes (nutrients and MDS items) was compared between the groups during their treatments.

2.5.2 Procedures

Patients were recruited from the two biggest hospitals of NC; Dr. Burhan Nalbantoglu Government Hospital and Near East University Hospital (NEU-H). In both of these hospitals Dr. Özlem Gürkut is responsible for the Oncology department, and the study was conducted under her charge. Before the study commenced ethical

approval from the London Metropolitan University Ethics Committee, and permission from Near East University Hospital was sought and granted (Annex 6).

2.5.3 Methods

Every individual who met the inclusion and exclusion criteria for the study was given an information sheet (Annex 2-Patient Inform Sheet [PIS]) regarding the study. If a patient was willing to participate in the study, they were asked to sign a consent form.

In Chapter 1, section 1.9 there is a short explanation as to why MDS was chosen for this study. Patients were initially interviewed for 25-35 minutes. Their personal information, nutritional habits, physical activities and anthropometric measurements (height, weight and waist circumferences) were obtained directly from the participants. Participants were then invited for in-depth interviews, conducted by the researcher who conducted face to face interviews, using a semi-structured interview schedule (Annex 1-Questionnaire). In the government hospital, patients were interviewed in the chemotherapy room, and in the NEU-H patients were interviewed in the clinic room next to Dr. Özlem Gürkut's clinic. Dr. Özlem Gürkut oriented the patients to the researcher before and after her meeting with them. A questionnaire was specifically designed for the study and can be found in Annex 1. The following information was obtained from the patient's medical records:

Tumour biomarkers – CEA and CA 15-3

Personal information – address, telephone, age

Weight (kg)

Height (cm)

To evaluate their nutritional status, a triple pass 24-hour recall (Wiehl, 1942) and food frequency questionnaire (FFQ) was taken and analysed. The interviewer asked the respondents to recall all food and drink consumed during the previous 24 hrs. Familiar food habits of the population were inquired into in their native language. A household measure, photographs and food models were used to describe portion size. A validated FFQ was used (Fernandez-Ballart *et al.,* 2010). The previously used validated tool, a MDAS 9-item index, has now been replaced by a 14-item MD scoring tool, and many recent studies use this tool (Georgoulis *et al.,* 2014; Maderuelo-Fernández *et al.,* 2014; Trichopoulou *et al.,* 2014; Psaltoupolou *et al.,* 2013; Leaon-Munoz *et al.,* 2012; Martinez-Gonzalez *et al.,* 2012; Schroder *et al.,* 2011). By adding five more questions to the MDAS 9-item tool the present 14-item MD tool has become more suitable in measuring the Mediterranean Diet. Two questions were added concerning nutritional intake. The other three concentrate on the frequency of intake. Accordingly, 12 questions are based on food consumption and two questions on food frequency (Figure 2.2). This tool is deemed the most appropriate and the most preferred in PREDIMED studies (Estruch *et al.,* 2013; Sanchez-Villegas *et al.,* 2013; Martinez-Gonzalez *et al.,* 2012). Also, the Mediterranean diet was measured using the validated Mediterranean Diet Adherence Screener (MDAS), which includes 14 questions (Schroder *et al.,* 2011), shown in Fig. 2.2. Each variable can be scored 1 or 0 (see Table 3.1). The total Mediterranean score could range between 0 and 14. If the Mediterranean diet score is 5 and under it is classified as low MDS, if it is between 6 and 9 it is classified as moderate and if it is 10 or over is classified as high MDS (Martinez-Gonzalez *et al.,* 2012). Portion sizes were estimated using a food photograph catalogue (Rakicioglu *et al.*, 2006); the usual portion size for this population was derived from the Nutritional Guide of Turkey. Energy and nutrition consumption was evaluated within the framework

recommended by the Specific Nutritional Guide of Turkey (TOBR) (General Directorate of Ministry of Health for Primary Health care Services of Turkey [TCSBTSHGM], 2004) guidelines. From the patients' 24-hour recalls, their monounsaturated fatty acid intake was analysed with BeBIS software and from this information they were divided into three groups (inadequate, normal and excessive) according to the consumption of monounsaturated fatty acids as a percentage of total energy intake. The percentage of monounsaturated fatty acids in the first group - under 12%; in the second group - between 12-15%; and in the third group - over 15% (Krauss et al., 1996; Baysal et al., 2002). The patients' tumour biomarkers (carcinoembryonic antigen (CEA) and CA 15-3) were recorded and correlated with the consumption of monounsaturated fatty acid and MDAS.

Figure 2.2 Validated 14-item Questionnaire of Mediterranean diet Adherences

Questions	Criteria for 1 point
Do you use olive oil as the principal source of fat for cooking?	Yes
How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)?	≥4 tbsp
How many servings of vegetables do you consume per day? Count garnish and side servings as 1/2 point; a full serving is 200 g.	≥2 (≥1 portion raw or as salad)
How many pieces of fruit (including fresh-squeezed juice) do you consume per day?	≥3
How many servings of red meat, hamburger, or sausages do you consume per day? A full serving is 100–150 g.	<1
How many servings (12 g) of butter, margarine, or cream do you consume per day?	<1
How many carbonated and/or sugar-sweetened beverages do you consume per day?	<1
Do you drink wine? How much do you consume per week?	≥7 glasses
How many servings (150 g) of pulses do you consume per week?	≥3
How many servings of fish/seafood do you consume per week? (100–150 g of fish, 4–5 pieces or 200 g of seafood)	≥3
How many times do you consume commercial (not homemade) pastry such as cookies or cake per week?	<3
How many times do you consume nuts per week? (1 serving = 30 g)	≥3
Do you prefer to eat chicken, turkey or rabbit instead of beef, pork, hamburgers, or sausages?	Yes
How many times per week do you consume boiled vegetables, pasta, rice, or other dishes with a sauce of tomato, garlic, onion, or leeks sauted in olive oil?	≥2

2.5.3.1 Statistical analyses

The aim is to test each different parameter against the BOO, MDS and biomarkers.

Correlation of variables shown in Tables 2.23 to 2.31 was assessed by point bi serial correlation for categorical and continuous variables and by pearson correlation for association between two continuous variables.

In tables 2.23, 2.24, 2.34 and 2.35 a chi squared test was used to analyse association between categorical variables and one way ANOVA for continuous variables. Also given are standard deviation, median and percentage of MDS total score levels. One way ANOVA test is used to statistically analyse the effect of different parameters on BOO, MDS and Biomarkers. The main assumptions read as; independent of cases, the data has normal distribution and variances are homogeneous.

Standard deviation and median values have been given according to age groups in Tables 2.25 to 2.30.

Frequency and percentages values of general and nutritional information are given in Tables 2.1 to 2.14 and 2.16 to 2.18.

Average, minimum and maximum values of general and nutritional information are given in Tables 2.19 to 2.22.

In Table 2.17 anthropometric measurements are given according to age groups.

Missing values were removed when estimating percentage in analyses.

2.6 Results

This study was conducted between 2011 and 2013, and in this period 140 women were involved in the study.

General information

Table 2.1 Educational status of the individuals who participated in the study

	Treated		Active treatment		Total	
	n	%	n	%	n	%
Primary school	20	25.3	18	29.5	38	27.1
Secondary school	22	27.8	16	26.2	38	27.1
High school	27	34.2	22	36.1	49	35.0
University	7	8.9	4	6.6	11	7.9
Postgraduate	3	3.8	1	1.6	4	2.9
Total	79	100.0	61	100.0	140	100.0

27.1% of participants indicated that they were graduates of primary school, 27.1% of participants were graduates of secondary school, 35.0% of participants were graduates of high school, and 7.9% of participants were graduates of university and 2.9% of them were postgraduates. Most participants indicated that they were graduates of high school.

Table 2.2 Smoking status of the individuals who participated in the study

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
No, never smoked	41	51.9	40	65.6	81	57.9
Smoked and quit	24	30.4	18	29.5	42	30.0
Still smoking	14	17.7	3	4.9	17	12.1
Total	79	100.0	61	100.0	140	100.0

More than half (57.9%) of the participants indicated that they had never smoked in their life. 30.0% of them had smoked and quit after their diagnosis, and 12.1% of the participants were still smoking.

Table 2.3 Main cancer status of the individuals who participated in the study

	n	%
BC is main cancer	117	83.6
BC is metastasis from other Ca	8	5.7
BC is main cancer and spread	15	10.7
Total	140	100.0

5.7% of the individuals who participated in the study had BC metastasis from other cancers, 10.7% of the individuals had BC as a main cancer, but it had spread, and the rest of the individuals (83.6%) just had BC without spreading, and metastasis from other cancers.

Table 2.4 Status of the individuals participating in the study who received chemotherapy and radiotherapy treatment

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
Chemotherapy						
Yes	79	100.0	61	100.0	140	100.0
Total					140	100.0
Radiotherapy						
Yes	72	91.1	31	50.8	103	73.6
No	7	8.9	30	49.2	37	26.4
Total	79	100.0	61	100.0	140	100.0

All of the participants had chemotherapy treatment, and 103 individuals out of 140 also had radiotherapy treatment.

Table 2.5 Status of the individuals participating in the study that had side effects from the treatment

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
Sore mouth	5	6.3	4	6.6	9	6.4
Sore stomach	16	20.3	17	27.9	33	23.6
Amnesia	2	2.5	3	4.9	5	3.6
Pain	1	1.3	1	1.6	2	1.4
None	20	25.3	16	26.2	36	25.7
All	35	44.3	20	32.8	55	39.3
Total	79	100.0	61	100.0	140	100.0

39.3% of participants indicated that they had experienced all the symptoms that cancer treatment causes. Only 6.4% of them had just a sour mouth, 23.6% of them had

just a sore stomach, 3.6% of them had just amnesia, 1.4% of them had just pain, and only 25.7% of them reported that they had no such symptoms during their treatment.

Table 2.6 CEA cancer biomarker ranges status of the individuals who participated in the study

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
0.21-2.5(normal)	48	60.8	31	50.8	79	56.4
Over 2.5	31	39.2	30	49.2	61	43.6
Total	79	100.0	61	100.0	140	100.0

More than half (56.4%) of the participant had a normal range of CEA biomarkers, and 43.6% of them were over the limit.

Table 2.7 CA153 breast cancer biomarker status of the individuals who participated in the study

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
Under 33 (normal)	60	75.9	40	65.6	100	71.4
Over 33	19	24.1	21	34.4	40	28.6
Total	79	100.0	61	100.0	140	100.0

More than half (71.4%) of the participants had a normal range of CA 153 biomarkers, and 28.6% of them were over the limit.

Table 2.8 Cancer survival of breast cancer patients during treatment

	Treated		Active Treatment		Total	
Survival	n	%	n	%	n	%
Yes	60	75.9	42	68.9	102	72.9
No	7	8.9	14	23.0	21	15.0
Total	67	84.8	56	91.8	123	87.9
Missing	12	15.2	5	8.2	17	12.1
Total	79	100.0	61	100.0	140	100.0

21 breast cancer patients who participated in the research did not survive cancer, and 102 of them are still alive.

Table 2.9 Menopause status of individuals who participated in the study

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
Yes	53	67.1	50	82.0	103	73.6
No	26	32.9	11	18.0	37	26.4
Total	79	100.0	61	100.0	140	100.0

73.6% of the individuals indicated that they were in menopause.

Table 2.10 Diet status of individuals who participated in the study

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
Diet changed	35	44.3	36	60.0	71	50.7
Diet unchanged	44	55.6	25	40.0	69	49.3
Total	79	100.0	61	100.0	140	100.0

Half of the individuals who participated in the study indicated that they had changed their diet after their diagnosis of breast cancer.

Table 2.11 Changes in patients' diet

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
Reduced fat	5	6.3	5	8.2	10	7.1
High protein	8	12.7	6	11.5	14	12.1
I went to a dietician	7	8.9	6	9.8	13	9.3
High vegetable and fruit intake	9	11.4	7	11.5	16	11.4
Gave up eating tinned products	5	6.3	5	8.2	10	7.1
Low protein diet	1	1.3	1	1.6	2	1.4
Gave up eating grapefruit and pomegranate	0	0	4	6.6	4	2.9
Gave up eating packaged foods	0	0	2	3.3	2	1.4
Total	35	46.8	36	60.7	71	52.9
Missing	44	53.2	25	39.3	69	47.1
Total	79	100.0	61	100.0	140	100.0

7.1% of individuals who participated in the study indicated that they had cut down on eating fat, 12.1% of individuals increased their protein consumption, 9.3% of individuals went to a dietician to learn healthy eating and get medical nutritional treatment, 11.4% of individuals started to increase vegetable and fruit consumption,

7.1% of individuals had given up eating tinned products, 1.4% of individuals had reduced their protein consumption, 2.9% of individuals had given up eating grapefruit and pomegranate because of the side effects of their treatment, 1.4% of individuals had given up eating packaged foods.

Table 2.12 Use of BOO status of individuals who participated in the study

	Treated		Active treatment		Total	
	n	%	n	%	n	%
Yes	40	50.6	25	41.0	65	46.4
No	39	49.4	36	59.0	75	53.6
Total	79	100.0	31	100.0	140	100.0

More than half (53.6%) of the participants indicated that they did not use black olive oil.

Table 2.13 Status of individuals who participated in the study concerning frequency of BOO use

	Treated		Active Treatment		Total	
	n	%	n	%	n	%
Every day or 5 days in a	26	32.9	16	26.2	40	28.6
week	13	16.5	6	9.8	16	11.5
2-3 days in a week	3	3.8	6	9.8	9	6.4
Once a week	42	53.2	28	45.5	65	46.4
Total						
Missing	37	46.6	33	54.1	75	53.6
Total	79	100.0	61	100.0	140	100.0

28.6% of individuals who participated in the study indicated that they used BOO almost every day, 11.5% of them used it 2-3 days in a week, and 6.4% of them used it just once a week.

Table 2.14 Frequency and percentage of the 14-item MDS*

	Yes		No	
Questions	**n**	**%**	**n**	**%**
1. Do you use olive oil as the principal source of fat for cooking?	66	47.1	74	52.9
2. How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)?	60	42.9	80	57.1
3. How many servings of vegetables do you consume per day? Count garnish and side servings as 1/2 point; a full serving is 200 g.	105	75.0	35	25.0
4. How many pieces of fruit (including fresh-squeezed juice) do you consume per day?	89	63.6	51	36.4
5. How many servings of red meat, hamburger, or sausages do you consume per day? A full serving is 100–150 g.	41	29.3	99	70.7
6. How many servings (12 g) of butter, margarine, or cream do you consume per day?	86	61.4	54	38.6
7. How many carbonated and/or sugar-sweetened beverages do you consume per day?	81	57.9	59	42.1
8. Do you drink wine? How much do you consume per week?	22	15.7	118	84.3
9. How many servings (150 g) of pulses do you consume per week?	74	52.9	66	47.1
10. How many servings of fish/seafood do you consume per week? (100–150 g of fish, 4–5 pieces or 200 g of seafood)	46	32.9	94	67.1
11. How many times do you consume commercial (not homemade) pastry such as cookies or cake per week?	74	52.9	66	47.1
12. How many times do you consume nuts per week? (1 serving = 30 g)	54	38.6	86	61.4
13. Do you prefer to eat chicken, turkey or rabbit instead of beef, pork, hamburgers, or sausages?	101	72.1	39	27.9
14. How many times per week do you consume boiled vegetables, pasta, rice, or other dishes with a sauce of tomato, garlic, onion, or leeks in olive oil?	126	90.0	14	10.0

As can be seen in Table 2.14, more than half of the participants answered item 1, item 2, item 5, item 8, item 10, and item 12 as *No*. It means that participants got values less than critical values for these items. On the other hand, more than half of the participants answered item 3, item 4, item 6, item 7, item 9, item 11, item 13, and item 14 as *Yes*. This means that participants got values over than critical values for these items.

Nutritional and Anthropometric Information

Table 2.15 Mean and standard deviation of age, weight, height and BMI of individuals who participated in the study

	n	Minimum	Maximum	Mean	Std. Deviation
Age	140	26.0	65.0	51.6	9.3
Weight	140	42.3	140.0	70.6	14.0
Height	140	17.0	193.0	161.6	14.6
BMI	140	14.6	52.9	26.8	5.9
Valid N	140				

Average of age individuals who participated in the study was 51.6 ±9.3y. Mean weight of individuals was 70.6±14.0, mean height was 161.6±14.6, and mean Body Mass Index (BMI) was 26.8±5.9.

Table 2.16 Body mass index of the individuals who participated in the study

	Active treatment group		Treated group		Total	
BMI	**n**	**%**	**n**	**%**	**n**	**%**
<18.50 (underweight)	2	3.3	7	9.1	9	6.5
18.50-24.99 (normal)	17	27.9	23	29.9	40	29.0
25.00-29.99 (overweight)	24	39.3	34	44.2	58	42.0
30.00-34.99 (obese classI)	7	11.5	12	15.6	19	13.8
35.00-39.99 (obese classII)	8	13.1	1	1.3	9	6.5
≥40 (obese classII)	3	4.9	---	---	3	2.2
Total	61	100.0	77	100.0	138	100.0

As can be seen above, the body mass index of the participants were as follows: 6.5% were underweight, 29% were normal, 42% were overweight, 13.8% were obese class I, 6.5% were obese class II, 2.2% were obese class III. That is, more than half of the participants were overweight than normal.

Table 2.17 Comparison of Anthropometric measures of individuals according to age groups

	Age Groups							
	19-30 age (n=3)		**31-50 age (n=60)**		**Over 50 age (n=77)**		**Total**	
Antropometric measures	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
Weight (kg)	47.0	3.5	70.5	14.0	71.6	13.6	70.6	14.0
Height (cm)	165.0	0.0	164.0	6.5	159.6	18.6	161.6	14.6
BMI (kg/m^2)	17.3	1.3	26.3	5.5	27.7	6.1	26.9	6.0

As can be seen in Table 2.17, the mean score of weight was found 70.59 with standard deviation of 14.04, the mean score of height was found 161.60 with standard deviation of 14.57, and the mean score of BMI was found 26.88 with standard deviation of 5.97. According to the age groups, weight and BMI increased, but height decreased with ages.

Table 2.18 Physical activity status of individuals who participated in this study

	n	**%**
Every day or 5 days a week	17	12.1
2-3 days in a week	48	34.3
Once a week	10	7.1
Rare or non	64	45.7
Total	140	100.0

As can be seen in table 2.18 12.1% individuals who have conducted to study stated that they do physicals activity every day or 5 days a week, 34.3% of individuals do 2-3 days in a week, 7.1% of individuals do once a week and 45.7% of individuals doesn't do PA or goes rare.

Table 2.19 From 24h recall mean and standard deviation of fatty acid values of individuals who participated in the study

	n	Minimum	Maximum	Mean	Std. Deviation
SFA	140	2.0	53.2	21.2	9.3
PUFA	140	1.1	71.1	15.3	11.2
MUFA	140	4.8	78.0	24.1	11.9
MUFA perc	140	1.0	3.0	1.7	.8
Oleic acid	140	2.8	77.0	21.1	11.5
Cholesterol	140	.0	1220.3	194.7	149.9
Valid N	140				

Average of fatty acid distribution of the individuals is as shown in table 2.19.

Table 2.20 From 24h recall mean and standard deviation of Nutritional ingredient values of individuals who participated in the study

	n	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Energy (kcal)	140	199.7	3895.6	1675.6	562.8
Prot (g)	140	8.0	30.0	17.6	4.5
Fat	140	12.0	53.0	36.7	7.8
CHO	140	25.0	72.0	45.2	8.9
Fiber	140	3.1	53.9	21.2	9.2
Linoleic	140	.9	67.5	12.6	10.5
Linolenic	140	.2	5.5	1.1	.8
Colestrol	140	.0	1220.3	194.7	149.9
Vit A	140	113.9	37397.5	1849.9	4788.8
Karoten	140	.0	14.2	3.1	2.6
Vit D	140	1.2	59.1	15.1	9.5
B1	140	.1	1.7	.8	.3
B2	140	.2	7.9	1.3	.9
B6	140	.1	2.8	1.2	.5
Vit E	140	1.0	58.0	13.2	9.6
Vit C	140	.0	345.2	114.9	80.5
Na	140	474.5	6212.5	2969.0	1242.4
K	140	164.6	5282.7	2357.2	907.0
Ca	140	53.6	1640.7	745.8	320.9
Mg	140	28.7	621.1	255.5	104.7
P	140	158.3	2558.8	1113.7	390.9
Folic Acid	140	47.4	1141.6	262.1	143.7
Fe	140	1.7	19.8	10.1	3.7
Zn	140	1.4	17.9	8.4	3.2
Valid N	140				

Average of nutritional ingredient distribution of the individuals is as shown in table 2.20.

Table 2.21 Mean and standard deviation of cancer biomarker values of individuals who participated in the study

	n	Minimum	Maximum	Mean	Std. Deviation
CEA	140	.3	76.9	5.4	11.4
CA153	140	4.3	776.3	48.3	93.9

Average of CEA and CA15-3 cancer biomarkers of individuals are respectively 5.4±11.4 and 48.3±93.9.

Table 2. 22 Mean and standard deviation of MDS of individuals who participated in the study

	n	Minimum	Maximum	Mean	Std. Deviation
Total MDS	140	2.0	13.0	7.7	2.4
Treatment Stopped	79	2.0	13.0	7.5	2.4
Active treatment	61	2.0	12.0	8.1	2.4

Average of MDS for treatment stopped and active treatment group are respectively 7.5 ±2.4 and 8.1 ±2.4 and overall average is 7.7±2.4.

Table 2.23 Adherence to the Mediterranean diet (14-item score) by characteristics of participants

	MDS levels			
Adherence to MDS	≤ 5	6-9	≥ 10	P*
n	28	72	40	
Smoking				
Non-smokers, %	46.4	58.3	65.0	0.001
Former smokers, %	17.9	33.3	32.5	
Current smokers, %	35.7	8.3	2.5	
Education				
Primary or less, %	10.7	29.2	35.0	.168
Secondary, %	28.6	25.0	30.0	
Higher, %	60.7	45.8	35.0	
BOO				
Yes, %	53.6	48.6	37.5	.369
No, %	46.4	51.4	62.5	
BOOuse				
Every day or 5 days in a week, %	46.7	51.4	94.4	.004
Less than 5 days in a week, %	53.3	48.6	5.6	
Physical Activity				
At least once a week, %	42.9	54.2	62.5	.278
Rare or None, %	57.1	45.8	37.5	

Table 2.23 Continue…

Survive/Deceased				
Yes, % No, %	88.5 11.5	79.4 20.6	85.3 14.7	.532
MUFA	24.46 ± 12.33	23.37 ± 9.17	25.31 ± 15.61	.706
Oleicacid	21.71 ± 12.09	20.49 ± 8.77	22.06 ± 15.23	.762
Weight	66.25 ± 13.34	69.34 ± 10.68	75.88 ± 18.09	.011
CEA	4.06±3.017	5.71±12.39	5.87±13.29	.779
CA15-3	67.23±95.1	38.36±68.40	52.95±127.23	.363

Means±SD unless otherwise stated.
*One-way ANOVA tests (continuous variables) or chi-squared tests (categorical variables).

As shown in table 2.23, 28 participants got less than 5 points, 72 participants got between 6 and 9 points, 40 participants got above 10 points in the 14-item score of MEDAS. No differences in education level, BBO, physical activity, survive, MUFA, CEA, CA15-3 and oleic acid were found across categories of adherence to the Mediterranean diet. Adherence to the Mediterranean diet was directly associated with smoking, BOO use and weight. Non-smokers participants showed a greater adherence to the MDS than did participants in other categories of smoking. Participants, who use BOO more frequently than others, showed a greater adherence to the MDS. MDS levels increases with gaining participants' weight.

Table 2.24 Means (95% confidence intervals) for indexes of general obesity and abdominal obesity by adherence to the Mediterranean diet in all patients

Adherence to MDS	≤ 5	6-9	≥ 10	r	P*
n	28	72	40		
Body mass index (kg/m^2)	25.18 (12.20-38.15)	26,35 (17.18-35.53)	29.00 (14.51-43.50)	0.230	.006**
Body mass index (kg/m^2)	-	-	-		.250
Underweight or normal, %	40.7	38	27.5		
Overweight, %	33.3	46.5	40.0		
Obese classes,%	25.9	15.5	32.5		

** Correlation is significant at the 0.01 level (2-tailed)

As shown in table 2.24, there is a statistically significant relationship between BMI and MDS level. The correlation was found to be positive, low relationship and statistically significant (r=0.230, p<0.01). And also there were no significant differences in body mass index levels across categories of adherence to the Mediterranean diet (p=.250).

Table 2.25 Means (95% confidence intervals) for indexes of general obesity and abdominal obesity by adherence to the Mediterranean diet in the active treatment group

Adherence to MDS	≤ 5	6-9	≥ 10	r	P*
n	11	29	21	.332	.009**
Body mass index (kg/m^2)	24.7(11.7-37.7)	27.8(17.7-37.9	31.2(14.5-48.7)		
Body mass index (kg/m^2)					.470
Underweight or normal, %	45.5%	31.0%	23.8%		
Overweight, %	36.4%	44.8%	33.3%		
Obese classes,%	18.2%	24.1%	42.9%		

** Correlation is significant at the 0.01 level (2-tailed)

As shown above (Table 2.25), in patients undergoing active treatment there is a statistically significant relationship between BMI and MDS level. The correlation was found positive, low relationship and statistically significant (r=0.332, p<0.01). And also there were no differences in body mass index levels across categories of adherence to the Mediterranean diet (p=.470).

Table 2.26 Means (95% confidence intervals) for indexes of general obesity and abdominal obesity by adherence to the Mediterranean diet in the treated group

Adherence to MDS	≤ 5	6-9	≥ 10	r	p*
n	17	43	19	.083	.467
Body mass index (kg/m^2)	25.5(12.1-38.9)	25.4(17.3-33.5)	26.6(17.4-35.8)		
Body mass index (kg/m^2)					.320
Underweight or normal, %	37.5%	42.9%	31.6%		
Overweight, %	31.3%	47.6%	47.4%		
Obese classes,%	31.3%	9.5%	21.1%		

** Correlation is significant at the 0.01 level (2-tailed)

As shown above (Table 2.26), among treated patients there is a statistically significant relationship between BMI and MDS level. The correlation was found positive, a moderate relationship and statistically significant ($r=0.467$, $p<0.01$). Also there were no significant differences in body mass index levels across categories of adherence to the Mediterranean diet ($p=.320$).

Table 2.27 Mean and standard deviation of macronutrients consumptions according to the age groups

Age		**19-30**	**31-50**	**Over 50**
Energy, kcal	$\bar{X}$	910.7	1742.4	1653.5
	S	768.9	557.1	543.8
Water, mL	$\bar{X}$	487.0	1206.1	1140.4
	S	300.3	488.4	471.6
PROT, g	$\bar{X}$	48.4	72.7	70.3
	S	35.9	28.0	26.6
FAT, g	$\bar{X}$	44.7	70.1	69.7
	S	31.6	29.3	29.2
CHO, g	$\bar{X}$	78.2	195.7	179.5
	S	85.7	64.7	70.6
Fiber, g	$\bar{X}$	10.7	19.3	23.1
	S	10.1	7.8	9.9
Alcohol, g	$\bar{X}$	0.0	1.1	1.0
	S	0.0	6.4	7.1

As can be seen in Table 2.27, macronutrient consumption, except fibre, increased with age until 50. But over age 50, macronutrient consumption was starting to decrease. On the other hand fibre consumption increases with age.

Table 2.28 Mean and standard deviation of fatty acid consumptions according to age

Age	Age	19-30	31-50	Over 50
SFA, g	$\bar{X}$	17.7	22.8	20.1
	S	3.6	10.0	8.9
PUFA, g	$\bar{X}$	4.5	15.6	15.5
	S	5.5	12.1	10.6
MUFA, g	$\bar{X}$	12.8	23.9	24.8
	S	8.0	9.4	13.5
MUFA, %	$\bar{X}$	1.7	1.6	1.8
	S	0.6	0.8	0.9
Oleic acid, g	$\bar{X}$	11.0	20.4	22.2
	S	8.0	8.5	13.4
Linoleic acid, g	$\bar{X}$	3.9	12.6	13.0
	S	5.2	11.1	10.3
Linolenic acid, g	$\bar{X}$	0.6	1.2	1.2
	S	0.3	0.9	0.9
Cholesterol, g	$\bar{X}$	93.8	195.9	197.8
	S	26.0	138.8	160.4

As can be seen in Table 2.28, fatty acid consumptions, except SFA, PUFA and linolenic, increased with age. SFA, PUFA and linolenic consumption increased with age until 50, but over age 50, these macronutrients consumptions were starting to decrease.

Table 2.29 Mean and standard deviation of vitamin consumptions according to the age

Age		19-30	31-50	Over 50
VitA	$\bar{X}$	788.6	1732.1	1983.1
	S	713.4	4723.3	4953.5
Carotene	$\bar{X}$	1.4	2.9	3.3
	S	1.3	2.3	2.9
Vit E	$\bar{X}$	5.1	14.8	15.9
	S	5.8	9.4	9.6
B1	$\bar{X}$	0.4	0.8	0.8
	S	0.4	0.3	0.3
B2	$\bar{X}$	0.7	1.3	1.4
	S	0.4	1.0	1.0

Table 2.29 Continue…

B6	$\bar{X}$	0.6	1.3	1.3
	S	0.6	0.6	0.5
Folic acid	$\bar{X}$	161.5	238.4	284.6
	S	144.0	99.9	167.8
VitE	$\bar{X}$	4.4	12.6	14.2
	S	5.1	9.6	9.6
VitC	$\bar{X}$	85.5	102.5	125.8
	S	114.7	76.6	81.9

As can be seen in Table 2.29, vitamin consumptions increased with age.

Table 2.30 Mean and standard deviation of mineral consumption according to age

Age		**19-30**	**31-50**	**Over 50**
Na	$\bar{X}$	2155.4	3323.7	2724.5
	S	2380.1	1309.2	1075.8
K	$\bar{X}$	1159.4	2171.4	2548.7
	S	1031.2	733.2	968.7
Ca	$\bar{X}$	869.7	743.1	743.3
	S	353.0	279.4	352.1
Mg	$\bar{X}$	124.0	257.2	259.4
	S	70.0	97.2	109.1
P	$\bar{X}$	792.1	1113.9	1126.1
	S	482.5	352.4	415.8
Fe	$\bar{X}$	4.5	10.1	10.5
	S	3.6	3.7	3.7
Zn	$\bar{X}$	5.0	9.0	8.2
	S	1.2	3.4	3.2

As can be seen in Table 2.30, mineral consumption, except Vit K, and Zn, increased with ages. On the other hand Vit K, and Zn increased with age until 50, but over age 50, consumption of these minerals were starting to decrease.

Correlation

Table 2.31. Correlation between age, education, smoking, BMI, weight, macro-nutrients, alcohol, fruits, vegetables and MDS in active treatment and treatment stopped groups and across the total cohort.

MDS	Active treatment	Treatment Stopped	Total
Age	.000	.000	.000
Education	.005	.198	.007
Menopause	.195	.017	.079
Smoking	.208	.080	.006
BMI	.016	.002	.000
Weight	.031	.004	.000
Physical activity	.006	.570	.124
Black olive oil	.386	.104	.096
Energy	.000	.001	.000
Protein	.000	.001	.000
Fat	.000	.002	.000
CHO	.000	.001	.000
Alcohol	.026	.955	.725
SFA	.000	.000	.000
MUFA	.000	.000	.000
Oleic Acid	.000	.000	.000
PUFA	.006	.001	.000
Cholesterol	.003	.001	.000
Vegetables	.057	.150	.038
Fruits	.077	.020	.002

Correlation between age, education, physical activity, total energy intake, protein, fat, carbohydrate, SFA, MUFA, PUFA and oleic acid, cholesterol with MDS was found statistically significant in the active treatment group (Table 2.31). In the treatment stopped group there are similar results; there was a significant correlation between MDS and age, BMI, weight, total energy intake, protein, fat, carbohydrate, SFA, MUFA, PUFA and oleic acid, cholesterol. There were no statistically significant differences between menopause, physical activity, black olive oil, alcohol and vegetables with total MDS.

Table 2.32 Correlation between oleic acid, total fat consumption, total energy intake, MUFA and BC biomarkers across the total group

CA153 group		n	Mean	Std. Deviation	Std. Error Mean	p (sig)
Oleic acid	under 33 (normal)	100	20.3	8.9	0.9	.002
	over 33	40	23.3	16.2	2.6	
Fat	under 33 (normal)	100	37.1	7.7	0.8	.826
	over 33	40	35.6	7.8	1.2	
Energy	under 33 (normal)	100	1650	530	53.8	.112
	over 33	40	1750	620	97.9	
MUFA	under 33 (normal)	100	23.3	9.4	0.9	.002
	over 33	40	26.1	16.4	2.6	

A correlation between oleic acid, mono unsaturated fatty acid (MUFA) consumption with BC biomarker has been found statistically significant despite total fat consumption and energy intake with BC biomarker of participants showing no relation statistically.

Table 2.33 Correlation between weight, BMI, MDS, SFA, fibre, fruits, vegetables and BC biomarkers across the total group

CA153 group		n	Mean	Std. Deviation	Std. Error Mean	p (sig)
Weight	under 33 (normal)	100	70.2	13.7	1.4	.429
	over 33	40	71.7	15.1	2.4	
BMI	under 33 (normal)	100	54.3	277.7	27.8	.244
	over 33	40	27.6	6.6	1.0	
MDS	under 33 (normal)	100	7.8	2.4	0.2	.517
	over 33	40	7.6	2.4	0.4	
Fruits	under 33 (normal)	100	6.8	1.2	0.1	.952
	over 33	40	6.9	1.2	0.2	
Vegetables	under 33 (normal)	100	6.8	.6	0.1	.463
	over 33	40	6.7	.6	0.1	
Fibre	under 33 (normal)	100	20.1	7.8	0.8	.006
	over 33	40	23.9	11.9	1.9	
SFA	under 33 (normal)	100	21.2	8.9	0.9	.277
	over 33	40	21.1	10.5	1.7	

A correlation between fibre consumption with BC biomarker has been found statistically significant despite individuals weight, BMI, fruit, vegetables, saturated fatty acid (SFA) consumption and Mediterranean diet score (MDS) with BC biomarker of participants had statistically no relation.

Table 2.34 Correlations between biomarkers (CEA, CA15-3) and smoking, survivals, status, physical activity, education, BOO frequency, BOO use, oleic acid, MUFA, weight, BMI, total fat consumption, total energy intake in the active treatment group

	CEA	**CA15-3**
n	61	61
Smoking	.184	.375(**)
Survival status	.283(*)	.022
Physical activity	-.036	-.070
Education	-.080	.057
BOO freq	-.029	-.186
BOO use	-.159	.090
Oleic acid	.185	.167
MUFA	.198	.159
Weight	.110	-.073
BMI	.121	-.097
Energy	.182	.169
FATgr	.147	.118

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

As can be seen in table 2.31, there are only two statistically significant relationships in the active treatment group. These correlations are between smoking and CA15-3 and also between survival status and CEA. The correlation between smoking and CA15-3 was found positive, moderate relationship and statistically significant ($r=0.375$, $p<0.01$). And the correlation between survival status and CEA was positive and statistically significant as $p<0.05$. But the correlation coefficient was found to 0.283, indicating a low relationship.

On the other hand, correlations between biomarkers and the other variables were not found statistically significant as $p>0.05$.

Table 2.35 Correlations between biomarkers (CEA, CA15-3) and smoking, survival status, physical activity, education, BOO freq, BOO use, oleic acid, MUFA, weight, total fat consumption, total energy intake in the treated (treatment stopped) group

	CEA	CA15-3
n	79	79
Smoking	-.072	-.009
Survival status	.232	-.024
Physical activity	-.019	.050
Education	.071	.064
BOO freq	.002	.054
BOO use	-.044	.098
Oleic acid	.207	-.076
MUFA	.191	-.059
Weight	-.048	.185
BMI	-.097	.114
Energy	.199	-0.045
FATgr	.172	-0.041

It is seen in table 2.35, all correlations between biomarkers and the other variables were not found statistically significant as $p>0.05$.

28 participants got less than 5 points, 72 participants got between 6 and 9 points, 40 participants got above 10 points in the 14-item score of MEDAS. The present work shows that a considerable proportion of participants are overweight and obese (42.0% and 21.5% respectively). There is a statistically significant relationship between BMI and MDS levels. The correlation was found positive, low relationship and statistically significant ($r=0.230$, $p<0.01$). Also there was no significant difference in the body mass index levels across categories of adherence to the Mediterranean diet ($p=.250$). There were no differences with education, physical activity, survival, MUFA, CEA, CA15-3 and oleic acid found across categories of adherence to the Mediterranean diet. Adherence to the Mediterranean diet was directly associated with smoking, BOO use and weight. Non-smoking participants showed a greater adherence to the MDS than did smoking participants in other categories. Participants, who use BOO more frequently than others, showed a greater adherence to the MDS where MDS levels increased in

participants' who gained weight. The overall mean of MDS was 7.7±2.4, mean of treatment stopped group was 7.5±2.4 and active treatment groups were 8.1±2.4.

2.7 Discussion

This study was conducted between 2011 and 2013, and in this period 140 women were involved in the study. 79 participants had finished their treatment and 61 participants were in the active treatment group. Average age, weight and BMI of participants were respectively 51.6±9.3 years, 70.6±14.0kg and 26.8±5.9kg/m². Worldwide most breast cancer patients are approximately around 50 years old (40-70y) (Benz, 2008). 83.6% (n=117) of participants had breast cancer as a main cancer; 5.7% (n=8) of participant had breast cancer metastasis from other cancers; and 10.7% (n=15) of participants had breast cancer which had spread out to other body organs. All of the participants had chemotherapy, and 73.6% of participants had radiotherapy. General cancer biomarker CEA was in the normal range in 56.4% of patients (AT n=31 and TS n=48) and 43.6% had higher than normal levels, over 2.5ng/mL. Breast cancer biomarker CA 15-3 was in the normal range in 71.4% patients (AT n=40 and TS n=60) and 28.6% of patients had CA higher levels, the range being over 33U/mL.

In the study's population, 57.9% of participants had never smoked cigarettes, 30.0% of them had smoked before and given up, and 12.1% of participants were still smoking cigarettes. A cohort study conducted in Norway followed up 302,865 women of whom 1106 died of breast cancer. They examined women who were smokers before first childbirth, and mortality from BC. They found a significant relation between increasing breast cancer mortality and lifetime smoking (Bjerkaas *et al.,* 2014). With similar results Dossus *et al.,* (2014) worked on 9,822 women with breast cancer, and in a study of a 322,988 population concluded that active or passive smoking will increase

the risk of breast cancer. Also Panis *et al.,* (2012) in a review article concluded that smoking constitutes a risk of breast cancer. However, Lee and Hamling (2016) in a review article found a weak association with smoking and breast cancer. In this study a strong positive association was found between smoking and MDs levels (Table 2.2). MDS levels were higher in non-smokers then current smokers. Also, there was a strong relation between BOO use and MDS levels. MDS levels were higher in participants who consumed BOO every day or five days a week (Table 2.31).

In active treatment group, 82.0% (n=50) were in menopause, and in the group whose treatment had stopped 67.1% (n=53) of individuals were in menopause. Cordina-Duverger (2016), found increasing weight while ageing in post-menopausal women will increase the risk of breast cancer.

7.1% of individuals who participated in the study indicated that they had cut down on eating fat, 12.1% of individuals increased their protein consumption, only 9.3% of individuals went to a dietician to learn healthy eating and get medical nutritional treatment, 11.4% of individuals started to increase vegetable and fruit consumption and 1.4% of individuals had reduced their protein consumption. A systematic review of breast cancer and dietary patterns suggest an association of MD with decreased risk of BC, and only a single study indicated an increased risk, associated with a western diet (Albuquerque *et al.,* 2014). A Cypriot-based study found a correlation between a decreased risk of BC and a diet based on legumes, fish, fruit and vegetables (Demetriou *et al.,* 2012). Again in the same review article two American and Europe-based studies, in contrast to other studies, found a significant inverse relation with breast cancer and chicken, fish, and raw and cooked vegetables; however they found this relation only in post-menopausal BC patients (Albuquerque et al., 2014). In a control case, EpiGEICAM study which worked with 1,017 breast cancer patients it

was found that there was a significantly high risk of breast cancer associated with a western diet pattern, and conversely found a low risk with a Mediterranean diet (Castello *et.al.,* 2014). A study which used 126 component food frequency questionnaire to investigate the relation between post- diagnosis eating factors and survival after invasive breast cancer found no relation between survival of invasive breast cancer and energy, carbohydrate and protein. There was only a significant relationship between saturated fat and trans-fat (Beasley *et al.,* 2011). Wakai *et al.,* (2000) found similar results to Beasley's. With a similar result, Murtaugh *et al.,* (2011) found a significant relationship between fat in invasive breast cancer and carbohydrates with direct breast cancer, more strongly in post-menopausal obese breast cancer-suffering women.

One of the aims of this study was to examine the effects of diet and olive oil intake of breast cancer tumour markers in breast cancer (BC) patients. In the active treatment group and the treated group there was no statistically significant relationship between oleic acid, energy consumption, BOO use and BC biomarkers (Table 2.31). However, the total study population showed a statistically significant relationship between oleic acid, mono unsaturated fatty acid (MUFA) consumption, and BC biomarkers (Table 2.32). Despite this, total fat consumption and energy intake with BC biomarker of participants showed no relation statistically.

The other aim of this study was to assess dietary intake in relation to a traditional Mediterranean diet among cancer patients who live in North Cyprus. Half of the study population had a 14-item score of MEDAS between 6 and 9 points (moderate). Average MDS of the study population was found to be 7.7±2.4 (min. 2.0, ma. 13.0). Average MDS in active treatment and post-treatment groups are respectively 8.1±2.4 and 7.5±2.4. A 14-Item Mediterranean diet assessment tool was used in the PREDIMED

trial study. 7,447 participants were included in the study in which the mean of MDS was 8.66±2.0 (Martinez-Gonzalez et al., 2012). In contrast to this study there were no differences in average age, prevalence of hypertension or prevalence of dyslipidaemia across the categories of adherence to the Mediterranean diet. In this study almost half of the 45.7% of participants who were investigated in the study stated that they had a fairly sedentary lifestyle. Only 12.1% of the participants stated that they were doing planned physical activity five days or more in a week, and 34.3% of the participants stated that they were active for 2-3 days in a week. Adherence to the Mediterranean diet was directly associated with physical activity, total energy intake, alcohol consumption, and educational level (Martinez-Gonzalez *et al.,* 2012). In a Greece-based study it was found that there was a lower cancer incidence among those who had a higher score in relation to a traditional Mediterranean diet, and those who had a lower Mediterranean diet score had a higher incidence of breast cancer (Benetou *et al.,* 2008). In another PREDIMED study, Hu *et al.* (2013), it was found that the mean score of the 14-item questionnaire was 8.6±1.9 (7,305 participants). Hu *et al.* found significant associations with gender, smoking, BMI, waist circumference, waist-to-height ratio, physical activity, educational level, marital status, and diabetes to MEDAS. In this study there was significant association between PA and MDS exclusively in the active treatment group (Table 2.31). 12.8% of individuals stated that they were physically active every day or five days a week; 34.3% of individuals stated that they were active 2-3 days a week; 7.3% of individual were active just once a week; and a higher percentage of individuals (45.7%) stated that they were not active. A significant association was found between education and MDS in the active treatment and in the total study population (Table 2.31). In contrast to Martinez-Gonzalez *et al.* (2012) this study did not find a significant association with alcohol consumption and MDS in all groups. A

significant relationship between smoking and MDS in the total study population was found. However, there was no significant relation within groups (AT and TS) with smoking and MDS. There was a statistically significant association between age, total energy intake, protein, fat, carbohydrates, SFA, MUFA, PUFA, oleic acid and cholesterol with MDS in all groups (Table 2.31).

In this study a statistically significant relationship was observed between BMI and MDS levels. The correlation found was positive, low relationship and statistically significant ($r=0.230$, $p<0.01$). However there were no differences in body mass index levels across categories of adherence to the Mediterranean diet ($p=.250$). In a Martinez-Gonzalez *et al.* (2012) study, an inverse association was found between the 14-items of the Mediterranean diet score and obesity. There was also an inverse association between MEDAS and both general obesity and abdominal obesity. Vitolins *et al.* (2014) made breast cancer cases lose 7.5% of their total weight, and researched the relation between waist circumference, percent fat mass, total cholesterol, and triglycerides in ER/PR-negative breast cancer patients.

The first study conducted on post-menopausal breast cancer in Greek Cypriot women, looked for the degree of adherence to a Mediterranean diet pattern that modifies breast cancer risk (Demetriou *et al.,* 2012). The study included 935 cases and 817 controls. They compared two different Adherences to the Mediterranean Diet pattern; one was created by Panagiotakos and the other by Martinez-Gonzalez. In both diet scores there was no association with BC risk. However, risk of BC decreased in association with high intake of vegetables and salads, fish and olive oil (Demetriou *et al.,* 2012). Couto *et al.* (2013) has also found the same result, in that, the Adherence to a Mediterranean dietary pattern was not statistically significantly associated with reduced risk of overall breast cancer.

Dietary intake recall questionnaires have limitations that can lead to inaccuracies. Single recall may questionnaires provide a poor measure of an individual's usual intake, and a minimum of three days of recorded dietary intake must be made to minimize errors (Jackson *et al.,* 2008). Dietary recall studies often include extra sub-sections which make participants tired, especially when working with participants who have chronic diseases like cancer. At some point while questioning the participants you can feel that they were prone to give an incorrect answer or could not remember food names. It is very hard to assume that the answers given are exact, in either the true amount, or type of food that they mostly eat. To address this issue a comparison was made by Holmes *et al.,* (2008) of four different dietary questionnaires. They concluded that the 24-hour recall method was the most preferred by interviewers for reporting diet, as it is more likely to have significantly more items of food reported, and a 24-hour recall provides the most consistent results across all age and sex groups compared with other methods (Holmes *et al.,* 2008).

In a review article which analysed 22 studies (10 cohort studies and 12 case control studies) that evaluated the association of dietary patterns comprised of fruit, vegetables, fish, and soy and its derivatives, with BC, 10 of the studies found significant inverse association with breast cancer with increased consumption of these food groups. In nine studies, there were no statistically significant differences between the evaluated dietary patterns and breast cancer (Albuquerque *et al.,* 2014). Of the BC patients who participated in this study, 75% indicated that they were consuming over 200g of vegetables every day, 63.6% indicated that they were consuming 3-5 portions of fruit every day, and 32.9% indicated that they were consuming 100-150g of fish per week. Bessaoud *et al.* (2012) examined the relationship between breast cancer risk and the consumption of fruit, raw and cooked vegetables, fish, and olive oil. They found no

association between breast cancer and high consumption of these foods. A 16.5 year follow up study found a positive relation between lower breast cancer incidence and riboflavin and folate intake in ER+ and PR+ cancers. Also a high riboflavin intake is associated with lower risk of HER2+ and HER2- cancers, but they did not find a positive relation with other micro-nutrients (Cancarini, 2015).

Another aim of this chapter is to investigate the relation between nutrition (nutrients) and biomarkers. A correlation can be seen between monounsaturated fatty acid (MUFA) and oleic acid consumption only with CEA ($p<0.05$) (Table 2.34). There was a statistically significant association between MUFA, oleic acid and consumption of BOO with CA15-3 levels in the active treatment group, whereas no such correlation was seen in the post-treatment group (tables 2.32 to 2.34).

A comparison between those with normal levels of biomarkers versus those with higher levels of cancer biomarkers CEA revealed differences in total energy intake, carbohydrates, MUFA, oleic acid, vitamin A, vitamin B2, Na, and folic acid. Consumption regarding protein, fat, CHO, PUFA, SFA, vitamin B1, B6, vitamin E, Mg, K, Ca, Fe, P and Zn intake with BC biomarker CEA of participants showing no relation statistically.

A comparison between those with normal levels of biomarkers versus those with higher levels of cancer biomarkers CA 15-3 revealed differences in protein, fibre, MUFA, oleic acid, vitamin B6, vitamin C, vitamin B1, K, Mg and P. Consumption regarding total energy intake, CHO, fat, PUFA, SFA, vitamin A, vitamin B2, vitamin E, folic acid, Na, Ca, Fe, P, Zn consumption and energy intake with BC biomarker CEA-15-3 of participants show no relation statistically. Results suggest that the levels of oleic acid and MUFA in Cypriot BC patients' diet may influence their recovery.

2.8 Limitations

When 24-hour recall is recorded, patients can suffer from forgetfulness or be tried because of the illness or chemotherapy, and therefore may not provide accurate results concerning food intake and estimating the portion size of their intake. This method may not represent the long-term dietary habits of the patient, and this will affect the outcome of the study, the relation between the diet and disease. Twenty-four hour recall is basically a retrospective method of diet assessment, where an individual is interviewed about their food and beverage consumption during the previous day or the preceding 24 hours (Black, 2000; National Diet and Nutrition Survay, 2016). However, a single 24-hour recall is not considered to be representative of habitual diet on an individual level. One single 24h recall does not estimate the usual intake of participants. Planning of two or more 24h diet recalls complicates field work. Single observation provides a little measure of individuals' intake, and is prone to underestimation due to omissions. While questioning the participants they may want to give wrong answers - bias in recording "good/bad" foods (Black, 2000).

Because of a disastrous flood in 2010 some of the patients' medical histories were lost. In addition, there is no computer-based system available to keep the old patients' records, which was problematic.

The North Cyprus Government hospital reported that 800 BC patients had applied to the hospital in 2011. The study population was considerably lower than expected. One year was spent collecting data from the study population, and because of time constraints it was possible to visit the hospital only two or three days a week during the year. Also an announcement by the Greek Cypriot Government was the biggest limitation to being able to access patients. The government announced that all

treatment expenses in Greek Cypriot Hospitals would be free for Northern Cypriots in their hospitals. That made many patients choose to move their treatments to Greek hospitals, which is why the study could not reach the population that had been expected. The groups in the study (active treatment group and treated group) had a small sample size; this affected the study's statistical findings, meaning that only extremely strong findings produced statistically significant results.

It is difficult to determine whether the outcome followed exposure in time or exposure resulted from the outcome, in cross sectional studies. Incidence and associations identified may be difficult to interpret. Due to low response and misclassification, cross sectional studies are susceptible to bias. It is not feasible to establish accurate cause and effect relationships without longitudinal data. Some variables that are statistically different on the impacted side may be a result of the impaction, and not necessarily a cause. Therefore, it is difficult to draw predictive conclusions based on these differences (Setia, 2016).

There has been no study conducted on BC and nutrition in NC before this study. There is no data available to compare the study's results nationwide. Follow-up qualitative research should be continued in order to avoid limitations and to assure the quality of the data that been taken at a point and analysed.

2.9 Conclusion

The study population's overall mean MEDAS score was 7.7±2.4, and in the treatment stopped groups the mean score was 7.5±2.4 and in the active treatment group was 8.1±2.4.

Olive oil intake is high in this population, and 46% participants report consumption of the distinct Cypriot BOO. Research comparing BOO to standard EVOO

could identify the type of OO consumption which could impact on dietary oleic acid and MUFA. Further investigation into potential links between diet and BC biomarkers is warranted in North Cypriot BC recovery.

This study is the first study to determine the relation between BC and nutritional status for women living in North Cyprus. It would be the first data that investigates populations of BC patients and their general information in NC. It will help future studies and encourage future research in NC. There is still a need to further investigate the effect of diet on BC recovery in the NC population.

3. Chapter III-Study II; Qualitative exploration of perceptions and expressions of participants on diet and cancer, using Interpretative Phenomenological Analysis

3.1 Introduction

In this chapter the psychological and social factors that surround the issue of cancer, and in particular breast cancer, have been explored and provide insight into the way patients lead their lives and were affected by the experience and, hopefully, overcoming, of cancer. Due to the exploratory nature of this study it was decided to use qualitative methodology, as justified below. The researcher finds the subjective perspectives of the persons concerned compelling for the following reasons. Firstly, the experience of living with cancer is something to be investigated subjectively (Smith 2011). The mental state of a cancer sufferer is hard to quantify or subject to objective critique. Secondly, the interaction between the subject and the researcher is an interesting dynamic, which has been explored below. Given the nature of this part of the research, qualitative analysis presents itself as the chosen pathway "inevitably" (Smith, 2008).

A key part of the research undertaken is the issue of interpretation. Any given subject can relate experiences, emotions and fears and such other subjective factors as they may wish to share with the researcher, yet for every two people concerned there are two different perspectives and interpretations. Add to that the impact of cancer on the family of the sufferer, and their perspectives and input, levels of support, and there is a very complex matrix to try to understand (Douglas and Davis 2014).

A qualitative approach to the psychological factors affecting those with cancer is a fairly recent thing. It arises from an understanding that qualitative analysis can be a useful tool in addressing the issue of individuals' comprehension of the world they live in. This is a move away from Logical Positivism as first posited by the Vienna School in the 1920s and 1930s. The idea that metaphysics can be discarded and only empirical evidence be seen as valid (the evidence of one's sense) gives way to a view that a given person's 'reality' stance about their world arises from their linguistic denotation of their surroundings, society's role in shaping a person's perceptions of themselves in the world, and the ready-made roles and situations that are implicit in, for example, gender and social class – and religious belief. This means that the focus is on, as mentioned above, the issue of interpretation. On the face of it, this may seem like thinking of an even greater vantage than Logical Positivism – Structuralism, even Determinism, whereby existentialist preoccupations with 'self' and choices are replaced by preoccupations with structures and relationships (Piaget, 1971). However, the approach to qualitative analysis in psychology propounded by, among others, Jonathan Smith (1995), is not Structuralist or Determinist insofar as proscriptive outcomes are not assumed or sought, and the line of inquiry is open-ended.

As previously mentioned, the interaction between the researcher and the subject is an important factor, and, a positive factor. A sense that someone cares and empathises and wants to know about one's concerns can be helpful in the emotional well-being of cancer sufferers. What Craig Earle calls "surveillance" of patients following their primary therapy is not only monitoring the patient medically, but also emotionally and psychologically (Ganz, 2007). This also translates into better rehabilitation/convalescence for the patient concerned (Ganz, 2007). For it is not true at all that after even the most successful treatment of cancer the persons concerned simply

resume their lives and 'forget all about it.' Just "relief, peace and celebrations" (Goodhard, 2011) – no. The repercussions are great, and the individual's needs are great in coping with the aftermath.

Many may feel pressurised into pretending to be relaxed and happy, maybe they may feel ashamed of their anxieties, seeing them as weakness and/or fearing that others will see them as weak (Goodhard, 2011). Being invited to voice their concerns in a formal but relaxed situation under conditions of anonymity can help. Therefore, for this study the role of the researcher should not be underestimated and in this research is (as touched on above) not merely that of a data collector, but also as a facilitator.

There are, of course, other qualitative research methods from the one this study employs, and they are discused briefly and there is an explanation why they were declined. Hypothetico-deductivism, posited by Karl Popper, suggests that, in conducting qualitative research, rather than induction leading to verification, deduction leading to falsification would be preferable. Through this process, false theories can be discarded in a process of elimination, and the truth can thus be distilled, or approached slowly (Willig, 2013). The present writer declines to follow this path, regarding it as too time consuming. Also, this approach seems too reliant on existing theories, thus precluding the generation of new theories. This is not the case with researchers chosen method. Moreover, as Kuhn points out, the experimenter might well not discard a theory if 'proven' false, but conclude that the experiment had gone wrong (Willing, 2013).

Auto-ethnography is a branch of qualitative research that was also rejected in this study. The contention that the current researcher, however much she may empathise with cancer sufferers, can help produce research outcomes of value through self-

reflection (Mcleod, 2011) seems unlikely. The field is too specific; and the researcher has never suffered from cancer. Likewise, a paradigmatic approach is seen as inappropriate to studies purposes, as seen this way as more useful in medicine and the scientific aspects of therapy, along with investigations into the physical causes of a participant's bodily and mental state (Mcleod, 2011) – and this is not the main thrust of this work. All in all, the researcher is satisfied adhering to the methodology advanced by Jonathan Smith.

In a similar vein, some therapeutic activities that cancer survivors can engage in can enhance their quality of life. The phenomenon of cancer in a person's life can be a very distressing thing. This is not only due to the debilitating side effects of treatment such as loss of appetite, difficulty in holding food down, tiredness and so on, but also high levels of anxiety and depression. There is also the issue of fear – the fear of the unknown, the fear of death. One example which can suffice to illustrate this is that of a distinguished scientist called Jane Plant who relates how she discovered she had breast cancer. "As I was walking around topless, hunting for my underwear, I suddenly saw in the low angle, late afternoon sunlight, a lump about the size of a large pea just under the skin of my left breast. I felt it and was immediately overwhelmed with a sense of fear and panic; my mouth went dry and I felt sick" (Plant, 2007). Plant goes on to document a kind of crash that her life took upon this revelation, and the sense of paralysis that she felt. Yet she picked herself up after a while by using various strategies, and by living an active life in spite of the terror that had been visited on her.

The main therapeutic activity associated with most ailments, and the main preventative measure is regular physical exercise – and so it is with cancer (Harvard health newsletter-Why is exercise protective against cancer?)

Yet more sedentary life enhancing activities are also very effective in helping cancer survivors to achieve a sense of normality, of wellbeing and of escape from the feeling of living through this terrible affliction with cancer intruding into every aspect of their lives. Positive experiences, creativity and, if possible, happiness increase one's level of health.

The main preoccupation of this thesis is diet in relation to breast cancer. As the research is concerned with the question of whether or not a Mediterranean diet is a significant factor in inhibiting breast cancer, the next step is to look at dietary matters in relation to this research subject. It is often asserted that highly industrialised food production is a factor in the incidence of cancer. " hormone disrupting and hormone-mimicking substances in our food, drink and environment ... are responsible for the dramatic increase in breast cancer and ovarian cancer throughout the West," writes Jane Plant (2007).

A detailed study into whether this is the case or not is outside the scope of this study, yet the study would contend that the Mediterranean diet is a healthy alternative to the modern Western diet – particularly in relation to processed foods, treated meats and what is known (not always accurately) as fast food. Processed meat, particularly, has become a big news item recently, with the WHO reported as linking it to a significant increase in the likelihood of bowel cancer (Plant, 2007). While the Mediterranean diet does include some processed meat, these are consumed in very small quantities, and the overall level of artificially processed foods (those with chemicals, those smoked or cured, and those preserved either chemically or with very high levels of salt) is low. One of the aims of this study is to explore awareness and understanding of dietary links to breast cancer. While many people understand the link between burnt meat, such as

often comes off a barbecue, and health concerns, not many understand the reason why this is so. There are also those who have little desire to forgo traditional pastimes such as the Cypriot barbecue party in order to pursue a healthier lifestyle.

The role of the researcher is very positive here too, as the very questioning in this respect raises awareness and perhaps prompts beneficial changes in diet.

This study wishes to gain insight into the periphrasis of the Mediterranean diet (MD), and, more challengingly, how food interfaces with cancer avoidance and survival. It is widely seen that the MD is one of the most beneficial, health-wise – in the world, to the extent that UNESCO has designated it an "intangible heritage of humanity" (Delgado, 2016). The MD is characterised by a large intake of fresh vegetables, a fairly low intake of meat and dairy products, pulses providing protein rather than meat, and the copious use of olive oil. As Delgado *et al.* (2016) put it, "the analysis of nutritional epidemiology studies, complemented with information provided by studies cellular and/or molecular levels, and enables the discussion of the multiple associations between the MD, health, well-being and longevity".

A very important aspect of the Mediterranean diet that this study focuses on here is black olive oil. The olive has been a part of the fare in the region possibly from Stone Age times. It is absolutely central to the MD, and as to black olive oil, the study will try to establish whether this particular type of olive oil is more beneficial than other types to health, and more beneficial specifically in relation to cancer avoidance and survival. This is a field little investigated, and the current writer can find no comprehensive study into the matter.

3.2 Interpretative phenomenological analysis

Interpretative phenomenological analysis (IPA) has been specifically developed by Jonathan A. Smith in 1995 to allow rigorous exploration of idiographic subjective experiences and, more specifically, social cognitions. IPA explores participants' views of a variety of issues. This analytical method aims to explore participants' perspectives on an issue rather than to describe the issue objectively (Downs *et al.*, 2014). IPA is concerned with the detailed examination of personal lived experience, the meaning of experience to participants and how participants make sense of that experience (Smith, 2011). In addition, it acknowledges a role for the researcher's own perspectives and experiences. Initially, a few transcripts were read several times and 'interesting or significant' parts of what the interviewee said were underlined and comments about these put in the left-hand margin of the transcript (Epstein and Ogden, 2005).

The method is dynamic, facilitating its adaptation to data from different sources, including interviews and diaries. It also enables exploration of shared themes in conjunction with individuals' own accounts (Richardson, 2006). IPA studies are usually conducted on relatively small sample sizes which are sufficient for the potential of IPA to be realised (Smith, 2011).

Reynolds and Lim (2007) interviewed eleven women who had cancer and who use art in the context of living with their illnesses. The study convincingly demonstrates the powerful positive effect of doing art (painting, pottery, and textile art) for these women. It offers a normalising experience. It is important to understand and approach BC patients' perceptions and managing their lifestyle and dietary changes due to their conditions.

3.3 Aims and objectives

The aim of this study was to explore breast cancer patients' perceptions and knowledge around the relationship between breast cancer and nutrition.

1-To gain insight into the level of knowledge that BC patients have around dietary management of their condition;

2-To explore the experiences of BC patients in being diagnosed and treated for BC;

3-To identify how participant's attitudes towards food have been influenced by their BC diagnosis and treatment;

4-To evaluate any changes they made with their diet since they have been diagnosed with cancer.

3.4 Methodology

3.4.1 Recruitment

Participants from Phase 2 (those who were undergoing active treatment and those who had completed treatment for BC within the last three years at two hospitals in North Cyprus) of this study were invited to attend a 1:1 interview. All participants were informed of the intention to conduct in depth interviews. All of those who expressed interest in participating in these interviews were contacted and an interview was arranged.

Inclusion and Exclusion criteria: Age range is up to a limit of 65 years, due to the increased likelihood of comorbidities that may influence diet, and also since dementia is more common in people over 65 years old. This enabled us to hold appropriate discussions with participants. Furthermore, critically ill BC patients, for the same reason as with over 65 year age participants, had not been included.

A semi-structured interview schedule explored the participants' experiences of cancer diagnosis and treatment, eating habits and changes in their eating behaviour, and the impact of illness on everyday life. Following each interview field notes were taken. An iPhone 4 was used to record the interviews. The interviews in most cases lasted for about 25 to 35 minutes and were fully conducted in Turkish. The interview schedule as seen in Annex-3 was used as a guide to take the conversation towards the areas of the study, but topics were explored as they arose. All were audiotaped and fully transcribed.

The transcribed interviews were analysed using Interpretive Phenomenological Analysis. The first transcript was translated into English, and the researcher and another researcher (Research Supervisor, Anne), coded this, deducting common and unique

messages from the transcript. The two researchers then compared their coding to ensure that similar labels were identified. The researcher then continued to code subsequent transcripts and the main and specific themes were created from the initial and targeted coding. The transcripts were then read independently, and coding allowed the identification of an initial list of themes and categories, which were then discussed and reorganised to create a coherent picture. From this key themes were created. Themes were deducted from each interviews transcript, unique feelings or key message that patient's imparted during the interviews.

Ethical approval was obtained from the appropriate ethics committee at the hospitals and from London Metropolitan University.

The interviews were generally conducted in hospitals (Burhan Nalbantoglu or Near East Hospital), but some preferred to have their interviews in their homes. The interview was developed with the participants and they were asked if they would give their consent for the study, and for the session to be audio recorded.

The interviews initially explored the participants' experiences of cancer diagnosis and treatment, eating habits and changes in their eating orders and the impact of illness on everyday life.

3.4.2 Data Analysis

Fourteen patients were interviewed who were diagnosed with breast cancer, each patient being given a different code. During the study over 25 patients were invited to take the interview, but due to personal reasons, or emotional issues some refused to participate in the study. The participants were divided into two groups, as in the previous section 3.4.1 and coded accordingly. The participants in the Active treatment group were coded as AT while those in the post-treatment group were named TS. There

were nine participants from the TS group and five from the AT group. For example, the first participant from the AT group (Table 3.1) is represented as "AT-1." This is explained further in the results section.

Before the interview the topic and the aim of the study was explained to the participants and a consent form (can be found in Annex-4) was filled by each participant. Each participant was subjected to ten questions prepared prior to the interview, on participants' thoughts on breast cancer, changes to their nutritional habits and lifestyles before and after being diagnosed with breast cancer. The interview took between 25-45 minutes. During the interview at some points additional questions were included. Due to the additional questions and conversation between interviewer and patient, the duration of the interview was often prolonged. The interviews took place in the hospital in the private rooms of doctors face to face, or some patients preferred to be at home for the interview.

The interviews were recorded on two different mobile phones (iPhone 4) and significant information was recorded by the interviewer in a note book. The interview was conducted in Turkish. The recordings of each participant and interviewer were carefully listened to individually and transcribed. Transcripts were first written in Turkish then translated into English by Olga Okan, who has a PhD in English language and Literature. All transcripts were listed twice and checked for similarity in both languages. The transcripts of each participant were read carefully and thoughts which were stressed were noted under themes. After all the transcripts were examined ideas were organised into fewer than seven main themes under which were many sub-themes. Initially the themes were firstly sketched on the transcripts then combined in a folder collectively. Finally, a schema was created via evaluating the themes and deciding which themes were main ones, and which themes were sub-themes. The main and sub

themes are given in schematized form in the below sections. Each main theme affects the other and each is evaluated individually.

The themes, along with participant supported quotes, are presented and explained in the results section. The quotes of the participants explain and highlight the importance, as well as giving the actual meaning of the themes. Some sub-themes are very individual while others depict feelings and thoughts that are common to many participants.

3.5 Results

N=14 participants were interviewed.

Figure 3.1 Distribution of study population

14 Breast Cancer (30-60y age)	
Treatment stopped (TS) N: 9	Active Treatment (AT) N: 5

Results in this section show the themes that emerged from the IPA analyses of the participants' responses. Fourteen people participated in the study of which nine had completed their treatment, and five of which were still undergoing treatment. Where quotes from the transcripts are used, the participant's code is stated and this is preceded by AT for active treatment and TS for treatment stopped. For example; AT-1 is "active treatment participant 1". A very wide variety of areas have been obtained from the transcripts. Eight key themes were identified, these are: side effects, source of information, issues relating to self-awareness, physical impact of eating, impact of emotional wellbeing, general emotions impact, perceptions of BOO and eating (Figure 3.1).

3.5.1 Emergent Themes

Theme 1. Quality of Life

Symptoms and side effects of treatment were cited as having a major impact on quality of life by most participants. Four out of fourteen of the sample expressed concerns about the side effects of vomiting and over-sensitivity. One individual (TS-3) said, "When I experience extreme nausea, I go directly to bed." Six out of the sample said they experienced sleepiness. One subject (TS-1) said, "After radiotherapy within half an hour I feel that I can't stand up. I feel so tired that I sleep for about five to six hours." Another subject (AT-5) said, "I want to be lazy; I feel really bad about myself." These effects will lead patients to lose their quality of life.

Some participants (TS-1, TS-7 and AT-3) stated they suffered from stomach-ache; all said they experienced pain. Pain is a side effect of treatment that can affect their appetite. Several mentioned that they lost their appetite, indeed felt aversion to food; others wanted to eat all day as they did not feel that they were replete after a meal.

As can be seen, in general the cancer treatment; radiotherapy, chemotherapy and pills side effects made participants feel exhausted.

Theme 2. Believes in Dr.

In all cases, doctors were a source of information available to the patients. One subject (TS-8) remarked, "I have not made any changes to my diet because I have had no advice to that effect. I have faith in my doctors."

Only two of the (TS-2, TS-5) fourteen subjects said that they received information from dieticians, and all said they received information from the internet. Six out of the fourteen said they found friends a source of information on nutrition in

relation to cancer. One remarked that "The internet and friends say 'try this, try that.' This tea or this food will make you feel better."

Six out of the fourteen participants (TS-1, TS-2, TS-5, TS-8, AT-1, AT-4) said they were influenced by friends. TS-1 said, "I was influenced by the people around me and by the internet. Everyone suggested 'drink this tea, eat this food etc.'"

AT-1 said, "I haven't really tried much in the way of dietary suggestions, but everyone says try this and that. And I felt that I had to try these things. The best thing I came across was kefir, and that's what I used the most."

Theme 3. Dietary awareness

Awareness concerning the link between diet and breast cancer was a subject of importance to the participants. Most of the subjects recognised a link between eating habits and cooking methods and cancer. AT-2 stated "Most importantly, I cut down on sugar and reduced any given portion. I cut down on carbohydrates, but of course I didn't stop eating such things totally. I know that's not good. I replaced it with brown bread." It should be noted here that this was an illogical switch, as brown bread is as high in carbohydrates as white bread.

Eight participants expressed an awareness of a possible link between burnt food and cancer. TS-9 said, "I'm aware that burnt food causes cancer too. So I stopped having burnt food a long time ago".

TS-3 pointed out, "When we have a barbecue, I never eat burnt meat. I also started to eat a lot of vegetables." Fibre and antioxidants in vegetables removes free radicals from body. On the subject of the health benefits of olive oil, six out of the fourteen participants (TS-1, TS-3, TS-6, AT-2, AT-3 AT-5) expressed a knowledge of

the subject, and stated that they acted on it, using olive oil in preference to other types of oil.

AT-2, "I always used olive oil with meals. We use olive oil a lot; we have a lot of salads". Olive oil is very important and commonly used in the Mediterranean diet.

Eight out of the fourteen participants (TS-1, TS-3, TS-4, TS-6, TS-9, AT-1, AT-2, AT-4) had looked into the subject of cooking methods, and had concluded that they may have been using inappropriate preparation and cooking methods in relation to health issues. TS-6, "when I make green beans with meat I never fry the meat or the tomatoes because I know that you're not supposed to fry olive oil. I never heat or fry it. I only add it to the water after the dish is cooked." When people cook with olive oil they think that they are cooking healthy foods but they don't realize that olive oil's beneficial effects are destroyed in this way. Scientists should focus on this issue and try to clarify this opinion.

Theme 4. Perceptions concerning BOO

Seven of the participants (AT-2, AT-3, AT-4, TS-3, TS-5, TS-6 and TS-8) thought BOO had a strong taste, but this was not always seen in a positive light. TS-3 said, "I don't like its smell." AT-4 pointed out, "I mostly prefer lighter olive oil; black oil is a bit heavy. I don't prefer it to other olive oils."

Three participants (AT2, AT-4 and TS-5), in contrast, mentioned that they actually preferred BOO due to its strong taste and smell. Because of BOO being exposed to heat twice, as discussed in chapter IV, its nutritional ingredients and flavour is different from classic olive oils. To admire this aromatic flavour of BOO depends on individual's taste perceptions. In a large zone of Cyprus BOO is consumed excessively. Some portions of the population even grow their own olives and produce BOO at home,

or send their olives to a mill prepare it. Participants were also confused about the health effects of BOO and do not know whether to believe if it is good or bad for health.

TS-3 "So now you can tell us at the end of your study whether or not if we can use BOO".

Theme 5. Emotional influences

-Body image

The aesthetic impact of surgery on participants' self-esteem was apparent in many participants and appeared to have far reaching consequences. Ten out of the fourteen expressed aesthetic concerns in relation to their breasts.

TS-5, "I was shy and didn't want to go out. Even I didn't wanted my husband to see me after my surgery"

AT-3"I felt that my femininity gone"

One participant stated that this caused them to make a decision about treatment. TS-3 said, "I refused radiotherapy because I care about my physical appearance, I always did." The researcher noted a lack of logic here. It is possible that she thought that turning down radiotherapy would result in losing one of her breasts.

-Weight changes

During the treatment of cancer, side effect weight changes (gain or loss) could be a problem. Some participants had concerns and give a reaction for this situation.

AT-2, "I knew that these would cause me to put on weight so I went on a diet. I didn't see a dietician, though. Actually, I've got a lot of questions to ask you, too."

General emotions

-Initial reactions (all)

When participants first found out that they were ill or that they were a breast cancer patient they had very individual responses to learning about their serious disease. Some participants went into shock after they learnt they had breast cancer; however, some could not accept this idea (illnesses) and ignored it altogether.

TS-7, "I didn't know what to do but I didn't collapse psychologically. I didn't really accept it as an illness."

TS-2, "But of course when I first found out about it I felt horrible. But that lasted only for a day, and then I pulled myself together."

-Hope after surgery (3/14)

Some of the participants felt psychologically stronger after their operation and thought that they had been given a second chance in life. They thought that the operation was their salvation. AT-3 said "As soon as I got out of the operation, I pulled myself together and went out shopping and had fun."

-Astonishment

Being diagnosed as a cancer patient for most participants was a shock and led to acting in astonishment and not knowing what to do.

-Thinks of cancer as part of life (1/14)

One of the participants (TS-1) thought that whatever we are confronted with in life happens because we were meant to experience it, and that cancer was one of the

things that she was meant to experience in her life process. Thus she did not take cancer as a shock. She stated it thus;

"When I first found out I thought that this is not something extra ordinary and that this exists in this world. I wasn't filled with fright or anything else and said to myself that I have cancer and that some get over it and others don't and I will live as long as God permits."

-Taking a risk

One of the participants diagnosed as having breast cancer took a big risk and refused to have chemotherapy, her refusal being based on research she did herself, and she wanted to direct the treatment process herself. She took this risk due to both the side effects of chemotherapy and due to thinking of the aesthetic affects it would have on her appearance.

TS-4, "In America, they do genome testing for these illnesses. The test results were at borderline. So it was up to me to decide if I wanted chemotherapy and I decided against it. I did lots of research on chemotherapy. Lots! I didn't like the idea."

Theme 6. Emotional Wellbeing

-Initial shock *(all)*

All the participants described their experiences in the learning process attendant on finding that they had cancer, experiencing shocks with different emotions and forms of expressions. One of them stated it as follows;

TS-6, "I felt like I was drunk."

-Fear of death

Some of the participants still live with the deep shock of their illness, with a heavy burden, and when explaining their feelings to the interviewer broke into tears. AT-2, "We took a PET scan, and it appeared in the bones. That's where I lost it."

"It wrote that it was in my bones….At that moment my screams could probably be heard from below…that means that I can go I said."

-Fear of consequences of mastectomy (almost all)

Nearly all the participants after having the mastectomy felt that they had lost their feminine appearance and their womanhood, and thought that they were being scrutinized constantly by others.

-Only happens to other people

As a community we all know that something called cancer exists in the outside world and think that it would not come near us and that everything we do is healthy and that this will protect us. AT-5 - "my doctor used to say to me that he could nearly say that I was in the zero risk groups that are how clearly he used to say. I never thought that this could happen to me."

-Thinking positive

One participant had very positive thoughts. She saw cancer as a basic natural disease and that if she could not get over it that situation had to be lived. She did not think it was a big problem. TS-1, "Alright I'm going get rid of all the bad things inside my body".

-Anxiety

During the whole process from the time that they found out about their illness and during the treatment process they explained how from time to time they thought about what was going to happen to them, their families and death, and how they panicked. They told of how fear was ever present, if only sometimes for an instant.

Theme 7. Eating

All participants had tried different nutritional approaches, some of which were appropriate while others were not backed up by evidence or guidelines. Reduction in sugar and portions of carbohydrate was a common theme following diagnosis

-Confusion regarding diet

Many participants clearly experienced confusion regarding the advice that they were given:

Another said (TS-1), "Everyone has an idea to give me advice on nutrition."

TS-9, "I found out that fructose also turns into sugar in the body and raises glycaemic index. These are things I learnt through the internet, newspapers and magazines. I cut down on fruit. I don't eat fruit much, maybe one portion of fruit a day only."

Six out of the fourteen (TS-2, TS-4, TS-5, TS-7, AT-3, AT-4) participants said they were influenced by friends. AT-4 said, "I was influenced by the people around me and by the internet. Everyone suggested 'drink this tea, eat this food etc." TS-4 said, "I haven't really tried much in the way of dietary suggestions, but everyone says try this and that. And I felt that I had to try these things. The best thing I came across was kefir, and that's what I used the most."

-Changes from side effects

The vomiting, nausea, dizziness and burning sensations created by radiotherapy or chemotherapy caused patients to make short term changes in their eating habits.

-Diet changes

To combat cancer some of the participants stated that they undertook very serious and strict dietary changes. The most common of these as stated was to totally remove sugar and carbohydrates from their diet. The changes patients revealed to the interviewer are as shown below:

-Sugar and cancer (12/14)

TS-9 - "I learned that sugar and sweet things trigger cancer; it feeds the tumour. I even found out that in PET scans; they inject some liquid which is radioactive glucose."

TS-5 - "I also found out that sugar is bad for tumours."

AT-1 -"I used to eat a lot of sugar before I got breast cancer; I couldn't cut down on it. My diet was based on sugar."

-High protein diet

AT-3 - "I followed the Karatay (high protein diet and avoiding bread and fruit) diet. I still do at times. I bought her books, I really liked her diet, and I think it makes sense. Of course I don't know how accurate it is."

-Buying organic food (3/14)

They feel safer when they buy organic foods. TS-2 - "I try to buy organic honey"

-Cut down fruit (sugar)

TS-6 - "I like all kinds of fruit but only one portion a day. For instance, I found out that watermelon has a lot of sugar in it and that sweet fruit are not good for you. That's what I heard, maybe it's not true, and we should talk about this in detail later. I cut down on fruit.'

-Cut white carbohydrates

Again through media searches they found information about white carbohydrates related with health. AT-2 "I cut down on carbohydrates. Especially white rice and bread"

-cut fries (all)

TS-7 - "Of course I cut down a lot on fries - that was the first thing I reduced. Then I cut down on sweets."

-Cut fast foods

There is a link between fast food and cancer. Even the cooking method or chemical substances used, or the nutritional ingredients of fast food. AT-4, "Until one year ago I used to have a lot of fast food. Two or three times a week."

-Wash fruit & vegetables with vinegar water

They use vinegar to wash their fruits and vegetables to remove pesticides or insects on them.

-Avoid canned food

TS-1, "I used to have canned beef and ham in the fridge all the time but now I never buy them. I learnt about how unhealthy they are. No canned foods, no fast foods, or very little; same for fried food."

-Increase vegetable and fruit (all)

TS-3, "I always consumed plenty of fruits and vegetables."

-Increase herbal drinks and kefir

Kefir is a probiotic drink like Ayran with a stronger taste. AT-1 - "No, I consumed my normal meals but made some changes; like I started drinking kefir and herbal teas."

-Avoid grapefruit

Grapefruit has a food and drug interaction with cancer chemotherapy agents. AT-3, "They banned drinking grapefruit because it could affect the medication I was taking."

-Avoid yogurt and ayran (gives inflammation)

Ayran is a traditional Turkish drink which is made of yoghurt. It is made by mixing yoghurt with water, salt and, discretionally, dry mint. AT-5 - "I couldn't consume yoghurt or ayran because it caused bloating in me."

-Use of wood fire for barbeque

Barbeque is a common cooking method in Cypriot culture. TS-1 - "....we had to burn wood instead of charcoal. Before we never took any notice of this. Now my brother cooks everything on wood for us. You should not eat the fat ever."

-Avoid smoke

Smoking cigarettes is always related with cancer. TS-8 - "I found out that smoking triggers breast cancer. I had smoked for 20 years but I quit 7-8 years ago. But apparently, it had already caused some damage."

TS-4, "Keep away from cigarettes. The kids have all started smoking outside."

-Remove skin from fruit

There is a widespread belief that fruit skin absorbs pesticides which are carcinogenic particles. A participant (TS-4) - "I always peel fruits' skin before I eat, I don't believe the hormones or pesticides the farmers use will be removed however much you wash them."

-Eat healthily

"I'm just trying to eat healthily," said AT-1.

-Calcium and cancer (4/14)

AT-3, AT-5, AT-6 and TS-2 believe that dairy products will increase the risk of cancer. That is why they cut all calcium-rich food products from their diets.

-Avoid burned food

TS-7 - "If the bread is burnt slightly we used to scrape it and eat it. That is, we used to like it crispy. Now you shouldn't eat burnt bread or meat or anything else ever."

-Alternative medicine

Alternative medicine choices always give people hope. They love to believe that they have different opportunities thereby. TS-2 - "These are not really medications but dietary supplements. It strengthens the immune system and everything....on it it says it's an extract of olive leaf."

AT-2 - "It has certain times, which is if you take it at the precise times indicated it is more effective" he said.

TS-5 - "I went to a place once that they took my blood and sent it overseas to find out what I should eat and what I shouldn't; they did a metabolic balance test."

3.6 Discussion

Breast cancer had made a large impact on participants' lives. Some participants (TS-1, TS-7 and AT-3) stated they suffered from stomach-ache; all said they experienced pain. Pain is a side effect of treatment that can affect their appetite. Several mentioned that they lost their appetite, indeed felt aversion to food; others wanted to eat all day as they did not feel that they were replete after a meal. These issues create imbalanced nutritional orders in breast cancer patients. In all cases, doctors were a source of information available to the patients. Doctors advising patients about nutrition is a very important element. Doctors should give their patients correct

guidance and should be well equipped and have a strong knowledge of nutrition medication, nutrition illnesses relationships. The internet is a powerful source of information for cancer as it is for other topics. Thus, it is the easiest and fastest means of obtaining information for many patients so doctors and other health professionals need to make their patients aware of how to obtain the correct information by screening the available sites

Health Professional Communication and Patient Trust

Confusion regarding conflicting advice had emerged as a theme. In this type of treatment the doctor/dietician communication should be strong and all health personnel should work together as a team towards the needs of their patients so that they are in accordance with each other. Thus, the patient will feel safer, and the period of treatment will be both quicker and more successful. In finding solutions teamwork provides a wider vision, provides a more comprehensive and detailed treatment for specific patients and diseases, leads to increased trust between patients and work peers, enables better communication that leads to faster recovery of patients, reduces hospital expenses and time spent during hospitalization. Recently, work has been undertaken for team research/education to be added to the process of education.

Research indicates that increase in trust between patients and health personnel leads to decreases in time in hospital and faster recovery. Mutual trust lies in the foundation of teamwork and multidisciplinary teamwork – as it leads to shorter hospitalization and faster recovery. Multidisciplinary teamwork leads to better protocol preparation of health personnel, better follow-up of patients during and after treatment. Furthermore, in hospitals that apply team work, personnel acquire better skills

(Tomizawa *et al.,* 2017; Lazzara *et al.,* 2016; Mayo and Wolley, 2016; Eddy *et al.,* 2016; Schönfelder and Nilsen, 2016; McGonagle *et al.,* 2016).

Views on Specific Foods

Many participants mentioned concerns with consuming dairy products. The data on calcium consumption and cancer is not clear. In a large cohort study (273,152 women) followed for 15 years (Azoulay *et al* 2016), no association between calcium channel blockers and increased risk of breast cancer was found. In contrast, a meta-analysis which matched nine cohort and eight case-control studies found a significant relation between long term use of calcium channel blockers and risk of breast cancer (Li, 2014). Another study worked on sisters of breast cancer sufferers who had not been diagnosed with BC (50,757, aged between 35 and 74) but had used calcium channel blockers for about ten years to treat heart disease. Wilson *et al.,* (2016) could not find any link between the uses of calcium channel blockers with risk of breast cancer.

Tao *et al.* (2015), investigated the ratio of intake of magnesium and calcium with breast cancer mortality. While they did not find any significant relationship between calcium intake and breast cancer survival, they did find a relationship between dietary magnesium intake and breast cancer mortality ($p=0.09$). Researchers concluded mortality might increase with those who have a high calcium/magnesium intake ratio. Another study found a significant risk with increasing intake of bread and cereal dishes, pork and processed meats, and sugar and candies. The contrary was observed for high intake of milk, poultry, fish, raw vegetables, potatoes and coffee and tea, which prevents and lowers the development of breast cancer (Franceschi, 2006). Weight changes in cancer patients cause changes in the progress of the illness. As weight increases this increases the chances of obesity, which results in negative effects or cancer progression.

Although these weight changes may stem from the progress of the disease or the treatment process, this is directly related to the eating behaviour of the individual (Welti *et al.,* 2017; Nasrah *et al.,* 2016; Terranova *et al.,* 2016). Improved diet and exercise after diagnosis of breast cancer has shown to improve prognosis (Pegington *et al.,* 2017). Zick *et al.* (2017), showed that high intake of antioxidant rich foods will improve inflammation and fatigue. This shows that changes in food choices (e.g changing animal sources to plant sources) will enhance the prevention of cancer. The study which Saetang and Sangkhathat (2017) presented showed that the content of diet underlies the link between metabolic syndrome and colorectal cancer. A diet high in fat and low in fibre increases pro-inflammatory cytokines and adipokine levels which will affect bacterial function in the gut in colorectal cancers. A randomised controlled trial tested the effect of 'Healthy Habits for Life' on a composite health behaviour risk index (CHBRI) over breast cancer patients in London and Essex. They observed changes in three to six month periods of patients' lifestyles, eating habits, food choices, physical activity, alcohol and smoking status. Twenty patients participated in a qualitative interview.

The findings of blind research methods are fed back to participants and the organisations concerned, (Beeken *et al.,* 2016).

Participants pointed out that they increased eating fruit and vegetables for health reasons, while a few participants wanted to cut fruit consumption due to its sugar content. A study showed that high fruit consumption in adolescence was associated with lowering the risk of breast cancer even in oestrogen and progesterone receptor negative cancer than oestrogen and progesterone receptor positive cancer (p: 0.02.). Especially carotene rich fruits and vegetables have a higher association with lowering breast cancer risk (Farvid, 2016). The mean carotene level of participants was 3.1± 0-14.2. Sugar

consumption is related with insulin. Sugar rich foods which include high glycaemic index will effect insulin secretion which might increase the risk of breast cancer (mammary carcinomas) in menopausal status (p=0.01) (Seely and Horrobin, 1983; Silvera *et al.,* 2005). Having the right diet is important in the healing and protection processes of disease. Depending on the dose, what is consumed can be toxic. "Every time you eat and drink; you are either feeding disease or fighting it". (Heather Morgan).

Modes of Cooking

A few participants loved to eat burned foods like bread, potatoes… etc. and they had to learn to live without them after they were diagnosed with breast cancer. BBQ cooking is a traditional common cooking method in Cyprus. BBQ is the way of socialising with friends and family in Cyprus. This is when high temperatures are applied to carbohydrate based foods such as bread, potatoes, cereals, biscuits… etc. Every single piece of information on the internet about nutrition is not necessarily true; surprisingly, information about burnt meat and bread is definitely right on track. "Acrylamide" will form. Acrylamide is known as a carcinogen for the human body. The International Agency for Research on Cancer (IARC) grouped acrylamide and high temperature frying as group 2A - probably carcinogenic to humans (Capei, 2015; Lachenmeier, 2009). Due to BBQ employing high temperatures to cook meat and other foods formation of carcinogenic compounds such as; N-nitroso-compounds and polycyclic aromatic hydrocarbons (PAH) increases (Bernstein *et al.,* 2015; International Agency for Research on Cancer, 2015). In North West China research was undertaken on the formation of polyurethane foam sampler and polycyclic aromatic hydrocarbons in outdoor barbeques and their effects on human health. The study looked at those who consumed meat cooked on the BBQ and others who had direct exposure to the gasses that occur during dermal contact. Customers who consumed meat cooked on the

charcoal grill showed PAH limits at a high 95% while those who were exposed to the gasses had low levels of cancer risk PAH. The results of the study showed that PAH levels of meat cooked especially on the charcoal grill triggered serious health problems (Wu *et al.,* 2015). Another study conducted in Sweden analysed the polycyclic aromatic hydrocarbons (PAH), benzo(a) pyrene (B(a)P) and PAH4 (B(a)P, chrysene, benzo(b)fluoranthene, and benz(a)anthracene) content of products most frequently purchased from supermarkets that could be barbequed at home. It was observed that red meat, pork, sausages, sugar and cereal consumption were preferred, most of which can generated PAH on barbecues. The above mentioned matters consist mainly in products that can be barbequed. However, it was noticed that there has been a 2% decrease in the consumption of BBQ products by the Swedish people in the last ten years, and the same decrease was noticed in cancer incidence. The results point to a direct link between BBQ cooking methods and cancer risk (Abramsson-Zetterberg *et al.,* 2014). The results of another study conducted in Spain on colorectal tumours and consumption of especially white meat, red meat, giblets and smoked foods showed a positive correlation between colorectal cancer and the method of cooking (De Batlle *et al.,* 2016).

This evidence may suggest that the participants in this study who restricted barbequed food intake were correct to do so for health reasons, and the impact of this on their social life and biopsychosocial wellbeing should be recognised by the health professionals involved in their care.

Olive Oil

Olive oil has a protective effect on breast cancer, and knowledge of this is very common. However, at the same time the right information could be used in the wrong way or it could be exaggerated (Othman, 2007). As olive oil has a protective effect on

breast cancer too much consumption of olive oil can increase total fat consumption. Too much oil stored as fat in the body tissues can help cancer cells move in and around in the body (Santos and Schulze, 2012). Besides this, olive oil has a protective effect when it is consumed raw and in small amounts (Othman, 2007). Many people use olive oil as frying oil because they think it is a healthy. Again, high temperature frying could cause formation of carcinogenic components inside foods (International Agency for Research on Cancer, 2015). While people are trying to use olive oil because of its health benefits, it is possible to use it in a wrong way and turn it to a bad fat in relation to overall health. The traditional olive oil of North Cyprus, Black Olive Oil, has a narrative about it in which they say that in some areas they leave olives to dry in sunlight as it gets mouldy. Mould can produce aflatoxin which is a known carcinogen. However, BOO's aflatoxin analysis has not been studied before, so there is no evidence to support or oppose this potential hazard. Although there are no published studies relating to this, anecdotal evidence from the manufacturer suggests that many N. Cypriots believe in this narrative and refuse to use black olive oil because of its aflatoxin content.

In 2011 the total incidence of cancer rate per 100,000 was calculated to be 201 and the prevalence rate at 460. The TRNC population obtained from the National Planning Bureau was used in the incidence and prevalence and calculated by standardized rate per 100,000. The five most common cancer types among all patients were breast (19.8%), prostate (12.6%), colorectal (9.7%), thyroid (6.4%), and lung (5.6%) (Gokyigit and Demirdamar, 2016). This study pointed out that chemicals used in farming and processing methods of foods have a link with cancer. The results of Gokyigit and Demirdamar's study (2016) showed that there were 592 new cancer cases in 2011.

Getz (2010) and Talwar *et al.* (2016) noted that weight loss, loss of appetite, loss of taste and swallowing difficulties were some common side effects of cancer treatment which lead to patients changing their food choices and quantities. In other words, patients either limited their food choices or eliminated some foods or even reduced their intake during treatment. A participant TS-4 stated, "I always peel fruits' skin before I eat; I don't believe the hormones or pesticides the farmers use will be removed no matter how much you wash them". It is at this stage that the role of a dietician becomes more prevalent as they play a key role in supporting these people living with and beyond cancer by assessing and managing the effects of cancer treatment and reducing the risk of malnutrition through appropriate nutritional counselling and support (BDEA-specialist dietician in oncology). The dietician must provide recommendations that will make up for the loss in nutritional value, energy and taste that cancer patients experience during treatment (Beck *et al.,* 2015). All health professionals, doctors, dieticians, nurses, physiotherapists, phycologists, biochemists and the like must all work as a group, in unison (Lucius and Trukova, 2015).

Unfortunately, cancer patients in the TRNC are not advised by hospitals or by doctors to consult with a dietician or to follow a given diet. Patients obtain their nutritional knowledge from the doctor or the nurse, and thus most cancer patients are unaware of the relationship between cancer and nutrition. It is a well-known fact these days that in chronic illnesses, malnutrition and weight loss play a vital role in the life span, treatment duration, and even the cause of death in cancer patients (Lucius and Trukova, 2015).

3.7 Limitations of the study

During the study over 25 patients were invited to take the interview. However, due to a personal reasons/emotional problems, they refused to participate in the study. It was hard to convince patients to share their experiences and speak out about their path during that stage of their life. One of the biggest limitations in the use of the IPA was that once the participants began talking it was very easy to deviate from the planned sequence. Once the interview began it was not easy to progress in the planned manner as participants very easily deviated either into concerns with their illness or their lives in general. It became difficult to return to the sequence in the desired manner after this break-off. The location of the interview was also a problematic factor for some participants. There were too many distracting aspects leading to the topics under discussion being sidetracked. Because the topic was very sensitive, progression became difficult as the emotional level of feelings increased. Sometimes we came to a full stop. In such situations the interviewer must act professionally, and return to the interview without hurting the participant's feelings.

Some of the themes identified may be relevant to breast cancer patients in neighbouring Mediterranean countries and breast cancer patients as a whole, but due to unique aspects of the NC diet, some are only relevant to this population group.

3.8 Conclusion

Many participants clearly experienced confusion about nutrition regarding the advice that they were given. To combat cancer some of the participants stated that they undertook very serious and strict dietary changes.

All participants had tried different nutritional options, some of which were in line with clinical guidelines and others not. The biggest nutritionally focussed theme that emerged was a perceived relationship between sugar intake and cancer.

Participants were very concerned about diet, and keen to try anything to keep the cancer at bay. To combat cancer some of the participants stated that they undertook very serious and strict dietary changes. Many participants clearly experienced confusion about the nutritional management of their condition, and would benefit from further advice. In particular, uncertainty regarding whether Cypriot Black Olive Oil was beneficial or harmful to their health was an issue, and further research into this matter is warranted.

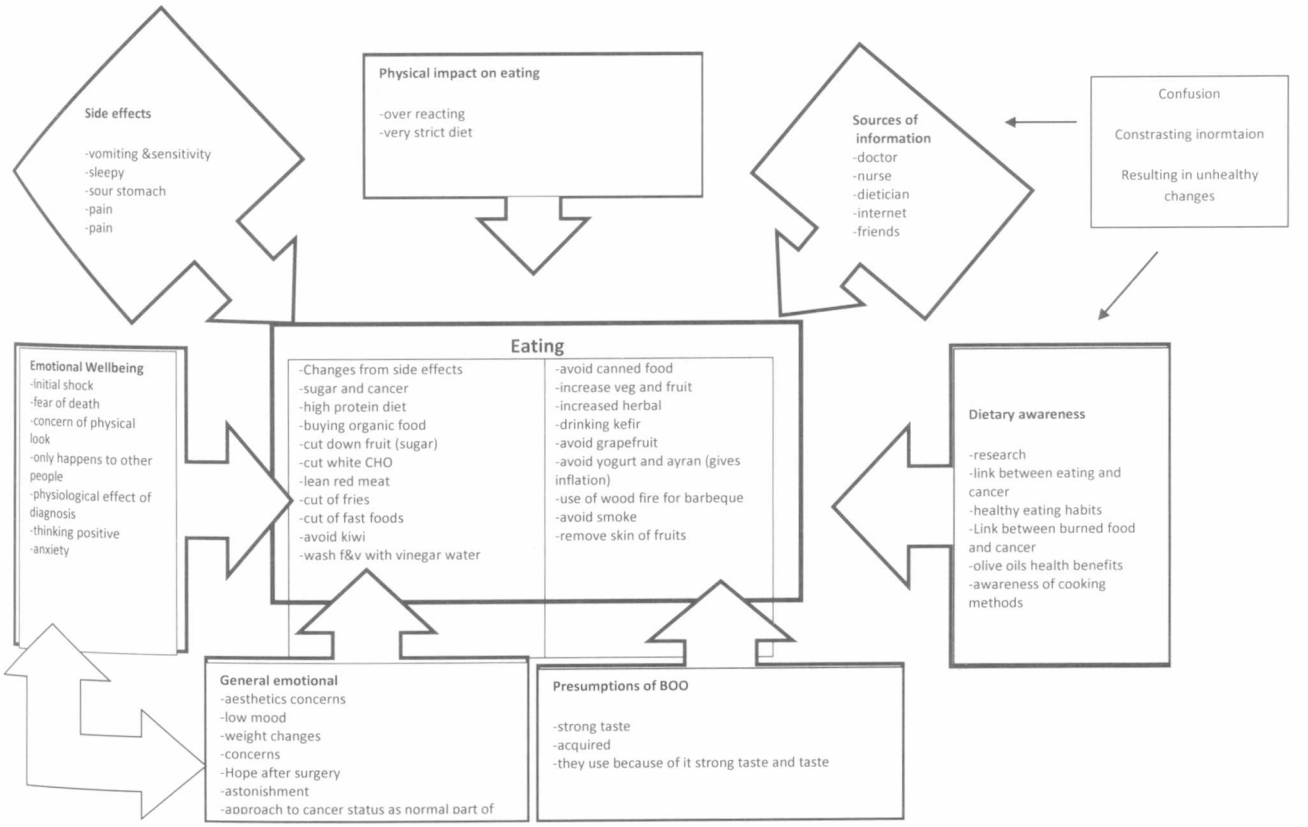
-The literature base and the current study, suggest that diet can influence BC recovery (Chapter II- Study I; Mediterranean Diet).

-People need to stay well-nourished to stay strong, fight infections, keep a lean body mass, and fight cancer. Therefore, dietary confusion impacting on overall nutritional status needs to be addressed.

-Therefore clarity of information from health professionals and referrals to a dietician when appropriate is essential.

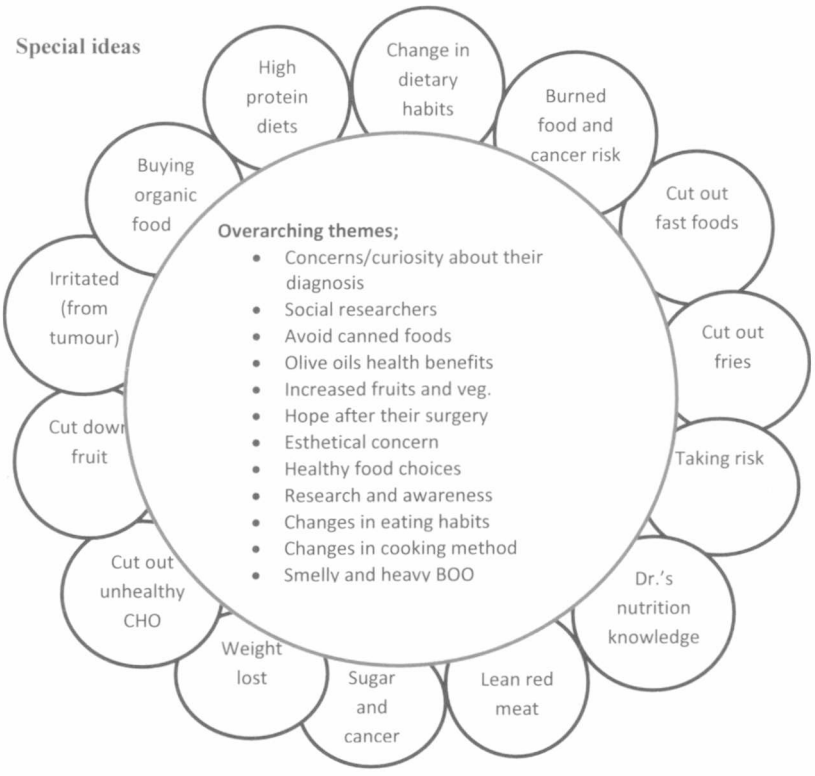
-These findings can be used to improve care pathways for NC BC patients.

Figure 3.2 Key themes



The following are the range of themes on the experiences of participants from this study;

Figure 3.3 Qualitative themes



4. Chapter IV-Study I; Analyses of Black Olive Oil Composition

4.1 Health and Olive oil

The olive tree, *Oleaeuropea L.*, has multiple origins and grows in a subtropical climate, common in Mediterranean countries (Wikipedia-Oleaoleaster). The oil derived from the fruit of the tree, the olive, is a main component of the traditional Mediterranean diet, which has been linked with a high quality, long life in good health. The unique, healthy qualities of olive oil compared with other vegetable oils are attributed to the high content of monounsaturated fatty acids, especially oleic acid, and phenolic compounds (Bosetti *et al.,* 2009; Kiritsakis and Markasis, 1984; Visioli and Galli, 1998).

A number of studies have investigated olive oil's benefits to health, and a selection are mentioned here. Epidemiological studies have suggested a link between consumption of olive oil, as part of a Mediterranean diet, to a reduced risk of chronic diseases, such as cardiovascular disease, some types of cancer, diabetes, metabolic syndrome, arthritis, Alzheimer's disease and even obesity (Trichopoulou *et al.,* 2010; Cade *et al.,* 2011). Intervention studies confirmed the beneficial effects of a Mediterranean diet, supplemented with olive oil or nuts, for managing metabolic syndrome (Mayneris-Perxachs *et al.*, 2014). An increasing number of studies have focussed on the content of phenols (Rohman and Man, 2011; Perez *et al.,* 2014).

Epidemiological evidence has led to advice that individuals with cardiovascular disease (CVD) should follow a Mediterranean style diet, which is low in saturated and trans fats, cholesterol, sodium, and simple sugars, and high in vegetables, fish, olive oil, whole grains and an increased intake of fruit (Trichopoulou *et al.*, 2010; Cade *et al.*,

2011; Mayneris-Perxachs *et al.*, 2014). Studies have shown that the pattern of fatty acids consumed will affect CVD. A high consumption of olive oil will lower rates of morbidity and mortality (Mayneris-Perxachs *et al.*, 2014). Also, a better lipid profile will reduce blood pressure (BP) levels (Fito *et al.*, 2005; Mayneris-Perxachs *et al.*, 2014). Dietary saturated fat increases the risk of coronary atherosclerosis and coronary heart disease, and countries which consume high amounts of saturated fat had higher cholesterol levels (Dalen and Devries, 2014). According to the American Heart Association (AHA) dietary recommendations in 1957, "diet may play an important role in the pathogenesis of atherosclerosis and the fat content and the total calories in the diet are probably important factors. The type of fat, rather than the total or the ratio or balance between the saturated and certain unsaturated fats maybe the determinant."(Fito *et al.,* 2005; Mayneris-Perxachs *et al.,* 2014; Ravera *et al.,* 2016). *In vitro* and *in vivo* studies have shown oleic acid and phenolic compounds contain the antioxidant activity of olive oil (Simopoulos, 2001; Konstantinidou *et al.,* 2010; Armutcu *et al.*, 2013). Studies show that dietary oleic acid could decrease LDL cholesterol and TG levels without decreasing HDL (Guisado *et al.,* 2008; Miller *et al.,* 2011). An olive oil antioxidant compound is accepted as a cleaner of free radicals, and it has been reported that its strong antioxidants inhibit LDL oxidation (Armutcu *et al.*, 2013). Research on the Mediterranean diet has shown cardio protective effects due to its specific components (food groups) like intake of olive oil (mono saturated fat) and other dietary patterns (nuts, legumes, fish, fibre, poultry, fruits), even though they do not decrease total serum cholesterol or low-density lipoprotein cholesterol (Dalen and Devries, 2014).

4.2 Breast Cancer and Olive oil

Bray *et al.* (2012) summarised evidence that the consumption of high amounts of animal products such as meat and dairy products increases daily total fat consumption. In studies on animal mammary tumour models, Willet *et al.* (1997), conclude that the type of fat and lifestyle (like reducing physical activity level) have a major effect on BC growth, that the high consumption of animal fat was positively associated with the risk of BC and colon cancer ($p<0.01$). Dietary lipids have been linked as a risk factor for BC (Binukumar and Mathew, 2005) and hence some types of fat may be important in prevention of BC (Solanas *et al.,* 2010). The prevention of diseases relies greatly on the overall pattern of all dietary factors in the diet. Based on the high total fat content of the Mediterranean diet, it contains mainly olive oil-monounsaturated fatty acids. A case control study conducted in Greece concluded that consuming olive oil more than once a day may lower the risk of BC in postmenopausal women by replacing other fat sources (Tritipucula *et al.,* 1995). In a controlled *in vivo* study, Solanas *et al.* (2010) investigated chemo preventive mechanisms of olive oil and corn oil in rats. The rats in the study were put into three groups; high corn oil content diet, high olive oil content diet, and a control diet group. The extra virgin olive oil had greater chemo preventative effect on BC compared with the other groups (Solanas *et al.,* 2010).

Sieri *et al.* (2008) investigated the fat consumption and breast cancer risk on 319,826 women. After 8.8 y of follow-up 7119 women developed breast cancer. Those women who had high intakes of fat and specific fatty acids, including total, animal, saturated, polyunsaturated, and trans-unsaturated fats had increased risk of breast cancer ($p=0.038$). No significant association of breast cancer with total, monounsaturated, or

polyunsaturated fat was found. Yee *et al.* (2010) looked at the effect of ω-3 fatty acids on breast adipose tissue fatty acid profiles in women who had a high breast cancer risk. According to that study ω-3 fatty acid supplements in women at high risk of breast cancer showed dose-dependent effects on breast adipose tissue fatty acid composition. However Larsson *et al.* (2009) have found no protective effect of Conjugated Linoleic Acid (CLA) against breast cancer development in Swedish women. Cottet *et al.* (2009) analysed diet and breast cancer risk, and in a 9.7-year follow-up period 65,374 women were studied. Two dietary patterns were used, ''alcohol/Western'' (essentially meat products, French fries, appetizers, rice/pasta, potatoes, pulses, pizza/pies, canned fish, eggs, alcoholic beverages, cakes, mayonnaise, and butter/cream), and ''healthy/Mediterranean'' (essentially vegetables, fruits, seafood, olive oil, and sunflower oil). The first pattern resulted in increased BC risk and second had negative association with BC risk ($p = 0.007$ and $p = 0.003$). Shannon *et al.* (2007) identified an association between erythrocyte fatty acid concentrations and breast cancer risk among women in Shanghai, China and found that ω-3 and eicosapentaenoic acid (EPA) lowers the breast cancer risk.

Olive oil is an important feature of Mediterranean countries and it is characteristic of their diet to consume high amounts of olive oil (Buckland *et al.,* 2012). Buckland *et al.* (2012) studied the relation between mortality and olive oil consumption on 40,622 participants. Compared to low consumption of olive oil the mortality rate was reduced 26% in those with high consumption.

Age, educational level, BMI appear to be factors that can contribute to the risk of BC incidence. In a prospective EPIC study conducted in Greece, a comprehensive

study examined a range of risk factors of breast cancer including a range of factors of the Mediterranean diet. The study included a large population and the findings were strengthened by repeating the assessments within a year. They found a significant relationship between high consumption of olive oil and reduced risk of BC in post-menopausal women (Trichopoulou *et al.,* 2010). Studies on Greek Cypriots have shown that higher socio-economic status and education were related with healthier diet (Katsarou *et al.,*; 2010 Tyrovolas *et al.,* 2011) but there is still little known about the diet of Turkish Cypriots who predominantly reside in Northern Cyprus (NC).

4.3 Olive oil in Cyprus

As a Mediterranean population Cypriots consume large amounts of olive oil. Cypriots grow olive trees (*Olea europea* L.), sell olives and produce a special kind of extra virgin olive oil. The colour of the oil that is obtained from these olives is a very dark green, which is why it is called Black Olive Oil (BOO). To produce BOO fully grown black olives are collected and boiled. The olives are filtered after the boil and left under the sun to be dried until they are about to get mouldy (Tatar, 2008; Yorgancioglu, 1999). When the olives get 'greasy' under the skin they are collected and sent to the mill, and crushed to obtain oil. The colour of the oil that is obtained from these olives is a very dark green and that is why it is called black olive oil (BOO). Even though the preparation of BOO is more complicated compared with normal types of olive oil, the distinct, aromatic taste of BOO is popular and manufacturers are prepared to devote the resources to supply the demand. Despite the popularity of BOO there is a perception among northern Cypriots that BOO is unhealthy. It is assumed that the vitamins are wasted through the boiling process and that black olive oil is not rich in vitamins. Besides, it is assumed that it might be unhealthy because the olives are left to get musty

(Tatar, 2008). These assumptions are not based on research, and therefore may not be accurate. It has been reported that the degree of acidity of BOO is low and that it has a sharp smell (Yorgancioglu, 1999). Consumption of olive oil in North Cyprus is high, similar to other Mediterranean countries (Kurucuoglu, 2010; Nuri, 2010; Okan, 2010), but BOO appears to be unique to northern Cyprus and the proportion of consumers preferring black olive oil in this region has not been studied. Until now, the properties of BOO have not been investigated and in particular a chemical analysis of fatty acid and polyphenol content has not been undertaken.

4.4 Aims and Objectives

The aim of the research described in this chapter is to characterise the chemical, biochemical and cytotoxic properties of BOO and compare them with more common forms of olive oil such as extra virgin olive oil. In order to achieve this aim the following objectives were attempted:

1- Identification of the key chemical components of BOO;

2- Assessment of the total polyphenol content and antioxidant capacity of BOO compared with other OOs;

3- Comparison of the cytotoxic potential of BOO extracts towards breast cancer cells compared with other OO extracts;

4- Comparison of the NMR spectrum of BOO with that of other OOs.

4.5 Methods

All laboratory work was carried out by the author, at LMU, unless otherwise stated.

4.5.1 Chemicals

Methanol, hexane, propyl gallate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were of analytical grade and purchased from Sigma-Aldrich (Poole, UK).

4.5.2 Olive Oils

BOOs were compared with EVOOs and VOOs. EVOOs were obtained from green olives, sourced from a local supermarket. BOOs are not commercially available in the UK and were obtained from green olive suppliers in Cyprus. Twelve different samples [there were four basic types] of olive oil were compared, four of BOO, two samples of virgin olive oil, one sample of golden drop olive oil, two sample of EVOO, and two samples of normal supermarket oil made from green olives. As stated in section 4.5.3 Sample 1 is fermented BOO obtained from a village in Cyprus. Olives are first boiled and then left to ferment before oil is obtained. Sample 2 is homemade BOO in which olives are boiled but not left to ferment. Samples 3 and 6 are a sample of BOO that can be found in the supermarkets and is manufactured in Cyprus, and which is also boiled before obtaining the oil. Samples 4 and 5 were obtained from supermarkets in London. Sample 4 is Italian EVOO. Sample 7 is Golden Drop olive oil produced from distillation of olives without using a mechanical process. Samples 8 to 11 are EVOO and virgin olive oil (VOO) that can be found in the supermarkets, and are manufactured in North Cyprus. Samples 8 and 10 are EVOO and VOO obtained using a hot press, and

samples 9 and 11 are EVOO and VOO obtained using a cold press. Sample 12 is BOO obtained cold pressed.

The 12 samples of olive oils made from the four different processes used in this study listed in Table 4.1. EVOO and VOO are named grades of olive oil defined by the *Codex Alimentarius*, the European Commission and the International Olive Council (Codex Alimentarius-Olive Oil, 2001). VOOs are produced only by physical processes, which means no chemicals or solvents are used, except water to wash the olives after harvesting. After harvesting olives are washed, pressed and bottled. EVOO is defined as having content of free oleic acid of no more than 0.8% (w/w) and VOO no more than 2.0% (w/w) (Codex Alimentarius-Olive Oil, 2011). To manufacture Golden Drop, olives are collected, sent to the mill and then the olives immediately go to filtration. Unlike other olive oils, golden drop olive oil processing omits the pressing process.

Preparation of BOO is described in section 4.3. The main differences between BOO and EVOO are that olives are boiled before they produce olive oil in BOO. Olives are exposed to heat twice in BOO manufacture.

Table 4.1 Olive oils used in the study

Sample	**Description**	**Date**
1 "Fermented" BOO	Olives washed, boiled, left to dry; allowed to ferment in the sun, but enclosed. Then pressed. Homemade.	Prepared 2010; stored cool RT for two years then 4 °C for one year
2 Normal BOO	No fermentation; boiled, sun-dried, then pressed. Home-made.	Prepared 2012; stored cool RT for two years then stored 4 °C for one year
3 Karpaz BOO	No fermentation; commercial brand; Karpaz [name of the village where it is produced] olive oil. Haspolat (zone) zeytin isleme tesisleri Lefkosa/KKTC tel.no; 03922335798	30.11.2011

Table 4.1 Continue …

4 Napolina EVOO	Normal brand; Obtained directly from olives by mechanical means. www.napolina.com	Bought 01.09.13; use before Jan 2015
5 FilippoBerio EVOO	Normal brand; blend; Obtained directly from olives by mechanical means. www.filippoberio.com	Bought 01.09.13; use before 28.02.2015
6 Evtad BOO	Cypriot brand. Olives washed, boiled, left to dry; allowed to ferment in the sun, but enclosed. Then pressed.	Prepared 11/2013 stored cool RT
7 Evtad Golden Drop	Cypriot brand. Olives are collected sent to the mill and without press immediately filtered.	Prepared 11/2013 stored cool RT
8 Evtad Hot press EVOO	Cypriot brand. Olives are collected sent to the mill and get pressed (hot), then filtered.	Prepared 11/2013 stored cool RT
9 Evtad Cold press EVOO	Cypriot brand. Olives are collected sent to the mill and get pressed (cold), then filtered.	Prepared 11/2013 stored cool RT
10 Evtad Hot press VOO	Cypriot brand. Olives are collected sent to the mill and get pressed (hot), then filtered.	Prepared 11/2013 stored cool RT
11 Evtad Cold press VOO	Cypriot brand. Olives are collected sent to the mill and get pressed (cold), then filtered.	Prepared 11/2013 stored cool RT
12 Cold press BOO	Cypriot brand. Olives washed, boiled, left to dry; allowed to ferment in the sun, but enclosed. Then cold pressed.	Prepared 01/2016 Stored cool RT

Bosque-Sendra's *et al.,* (2011) study showed that olive oil stored at 0-8°C up to 24 month showed no change on peroxide value, acidity, waxes and delta-K values. The phenolic content was also un-altered as well (Bosque-Sendra *et al.,* 2011). The advantages of the long-term effect of freezing on olive oil, compared with storage at room temperature, have been studied Mulinacci *et al.* (2013).

4.5.3 Preparation of Olive Oil Extracts

Ten samples of different olive oils were obtained from different areas of Cyprus listed in Table 4.1 together with two samples of commercial olive oil purchased from supermarkets in the UK. All oils were stored in 0.5-1L dark glass bottles at room temperature for 1-2 years, then 4°C for one year in refrigerator.

4.5.4 Fatty Acid Analysis

The fatty acid content of selected olive oils was measured using gas-liquid chromatography in LMU laboratories by Dr. Jhon. The oil was extracted three times with petroleum ether, and ether removed by rotary evaporation. Fatty acids in 50-100 mg of oil samples were converted into fatty acid methyl esters (FAME) by heating them in 10% BF3-methanol. FAME (5µl) were analysed using a Varian 2100 chromatograph on a fused silica capillary column MN FFAP (10 % diethylene glycol succinate, 50 mm x 0.32 mm i.d.; film thickness 0.25 µm), under the following temperature programme: 90 °C (7 min), 5 °C /min to 240 °C (15 min). The flow rate of nitrogen, used as carrier gas, was 6 ml/min. Temperature of both injector and flame ionization detector was 225 °C. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times (Tanilgana *et al.* 2007).

4.5.5 NMR Analysis

NMR analysis of crude olive oils was carried out in a Bruker 500MHz Nuclear magnetic resonance spectrometer by Mr John Crowder at London Metropolitan University. The oils were diluted in $CDCl_3$ with tetramethylsilane used as internal standard. Theoretical spectra were generated using prediction portal ACD/I-Lab housed at the UK National Chemical Database Service (cds.rsc.org).

4.5.6 Preparation of Polyphenol Extracts

The method described in Baiano *et al.* (2009) was used, with modifications. 20 mL of olive oil was mixed with 10 mL hexane and 10 mL methanol:water (7:3) by votexing. The mixture was centrifuged at 3000 rpm for 10 min and the upper phase of aqueous methanol, containing the polyphenols, transferred to a fresh tube. 10 mL hexane was added, the mixture vortexed and spun at 3000 rpm for 10 min. The upper phase of 70% methanol containing polyphenols was transferred to a fresh tube and stored at -20 °C. For some extracts the methanol and water were removed by rotary evapouration at 50 °C leaving a brown residue which was dissolved in 2.0 mL of methanol and transferred to a pre-weighed weighing boat. The methanol was allowed to evapourate to dryness and the residue was weighed. DMSO was added to a concentration of 10 mg/mL.

4.5.7 Total Polyphenol Determination

The method described in Baiano *et al.* (2009) was used. Briefly, 100µL of methanol: water (70:30 v/v) polyphenol extracts or phenolic standard (gallic acid – see below) were mixed with 100µL of Folin–Ciocalteau reagent (Sigma-Aldrich, Poole, UK). After 4 min, 800µL of 5% (*w/v*) Na_2CO_3 was added and the mixture was incubated at 40 °C for 20 min. The total phenol content was determined the colourimetrically at 750nm. The standard curve was prepared using solutions of gallic acid in a methanol: water solution (70: 30 v/v) in the range 0 to 250 µg/ml. The total phenolic content is expressed as µg of gallic acid equivalents per mL of oil (Baiano *et al.*, 2009). Depending on the olive oil, 10 to 20 mg of extract was obtained from 20 mL of olive oil.

4.5.8 Anti-oxidant activity

A spectrophotometric (quantitative) assay to determine the antioxidant capacity of selected olive oil polyphenol extracts (Braca *et al.*, 2002). In a 96-well plate, a series of duplicate quercetin standards were created by two-fold dilution in MeOH, starting with 100μg/ml. Similarly, a duplicate series of two-fold dilutions of each polyphenol sample were prepared using methanol. 100 μl of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (60 μg/mL in MeOH) was added to all wells, the plate shaken and incubated for 30 minutes at room temperature before absorbance at 517nm was measured in an Omega FluoStar plate reader. Antioxidant activity relative to quercetin was calculated from the linear slopes of the quenching reaction of DPPH by olive oil extracts compared to that of quercetin. An example of data is shown in Fig. 4.4.

4.5.9 Cytotoxicity analysis

Two human breast cancer cell lines were used in this study, MCF-7 and MDA-MB-231.

The MCF-7 breast cancer cell line was identified in 1973 at the Michigan Cancer Foundation (Holliday and Speirs, 2011). The MCF-7 cell line is a popular line used in cancer research due to its exquisite hormone sensitivity through expression of oestrogen, androgen, progesterone, and glucocorticoid receptors (Holliday and Speirs, 2011).

The MDA-MB-231 breast cancer cell line was originally isolated in the early 1970s and is widely used as a model for oestrogen receptor negative breast cancer with applications in the study of tumorigenic, metastasis, and cell invasion (Ghanbari *et al.*,

2016). It represents the triple negative, rare forms of breast cancer because it lacks oestrogen, androgen and progesterone receptors.

MCF7 and MDA-MB-231 cells were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 in DMEM medium containing 10% foetal calf serum. The cells are adherent and when ready for use were detached after removing medium using trypsin-EDTA. After removal of trypsin, cells were re-suspended in full medium to approximately 5 x 10^4 cells / mL. 0.5 mL of cells in full medium was applied to each well of a 24-well plate pre-treated with polyphenol or 70% MeOH as vehicle control, described below.

Methanol-water (70:30) polyphenol extracts were prepared from four olive oils as described in section 4.5.4. Extracts from two types of BOO (sample 1 Evtad BOO and sample 2 Karpaz BOO) and two types of EVOO (sample 3 Evtad EVOO cold press and Evtad Golden Drop EVOO) were tested for effect on growth of two breast cancer cell lines; MCF7 and MDA-MB-231.

Methanol-water (70:30) extracts (Section 2.5.6) were added to sterile 24-well cell culture plates and allowed to dry overnight at room temperature. Control wells received solvent only and the volume of solvent in all wells was adjusted to 150 µL. For each extract duplicate samples were added to give either 0.5 or 5.0 µg of polyphenol per well.

Cells were seeded the next day at an estimated concentration of 10^5 per ml. After 24 hours cell growth was assessed by microscopy. The MDA-MB-231 cells had grown sufficiently for assessment of growth by an MTT assay. The MCF7 cells grew more slowly and were allowed to grow for another four days.

An Olympus IX 51 inverted microscope was used to observe the changes in cells.

An MTT assay was used to measure cell growth (Menendez *et al.*, 2007; 2010). After cells had been allowed to grow for 1 or 4 days, 100μL MTT solution (5mg/mL in phosphate-buffered saline) was added to the medium in each well, and cells were further incubated at 37 °C for 3 hours. The medium with MTT was removed and 500 μL DSMO added to each well to dissolve the purple formazan crystals generated by the metabolically active cells. The lysed cells were incubated for 10 minutes at 37 °C and the absorbance at 590nm was read, corrected for light scattering by also measuring the absorbance at 620nm.

4.5.10 Statistical analyses

Data were analysed using GraphPad Prism 8.0. ANOVA was used to analyse differences between means of the control and the various treatment groups. Turkey's test for post-hoc comparison of means was carried out to determine significant differences between any pairs of samples.

4.6 Results

4.6.1 Chemical Analyses

4.6.1.1 Fatty Acid Analysis

The broad types of fatty acids three samples were obtained in the laboratory at London Metropolitan University (LMU) by gas chromatography and are shown with corresponding composition of commercial samples 4 and 11 of EVOO from the manufacturers' data in Table 4.2. Comparing the three oils analysed at LMU, BOO had more saturated fat, 19.6%, than the two Evtad samples, about 16% each. Conversely there was a slightly lower proportion, by about 3%, of unsaturated fatty acids in the BOO compared with the Evtad samples. The data from the commercial samples indicate lower proportions of saturated and mono-unsaturated fatty acids compared with the Cypriot samples.

Table 4. 2 Fatty acid types in selected olive oil samples

Sample	% SFA	% MUFA	% PUFA	% Total Fat
3. Karpaz BOO	19.6	71.7	6.8	98.1
3. Karpaz BOO*	19.4	72.6	6.8	98.8
4. Napolina EVO	13.1	66.7	7.5	91.4
5. FilippoBerio EVOO	15.5	66.1	9.7	91.3
7. Evtad Golden Drop	16.4	75.2	8.4	100
11. Evtad Cold press VOO	16.2	75.7	8.7	100.6

SFA – saturated fatty acid; MUFA – mono-unsaturated fatty acid; PUFA – polyunsaturated fatty acid. *Data for the second sample of Karpaz BOO are taken from Table 4.3.

A more detailed analysis of fatty acid composition was determined for sample 3, Karpaz BOO, and is shown in Table 4.3. Fatty acids commonly present in olive oils, including palmitic, palmitoleic, stearic, oleic, vaccenic, linoleic, linolenic, arachidic, behenic and gadoleic acids were all found in the BOO sample. As expected for olive oil, the major fatty acid in Karpaz BOO is oleic acid which makes up 68.85% of the total and the next most abundant fatty acid is the sixteen carbon atom palmitic acid at 15.9%, which is fully saturated.

Table 4.3 The fatty acid composition of sample 3 Karpaz BOO.

Fatty Acid	Carbon Chain	%
Palmitic acid	C16:0	15.94
Palmitoleic acid	C16:1 n-7	1.18
Stearic acid	C18:0	2.93
Oleic acid	C18:1 n-9	68.85
Vaccenic acid	C18:1 n-7	2.60
Linoleic acid	C18:2 n-6	6.17
Linolenic acid	C18:3 n-3	0.66
Arachidic acid	C20:0	0.39
Gadoleic acid	C20:1 n-9	0.29
Behenic acid	C22:0	0.13

4.6.1.2 Polyphenol content

The major constituents of olive oils are the fatty acids, discussed in 4.7.1.1, but olive oils contain polyphenols which provide another major health-promoting benefit to the oils. For example 98% (w/w) of EVOO is made of fatty acids and the remaining 2%

is made from over 230 chemical compounds which include aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and polyphenol antioxidants. Many phenolic compounds in virgin olive oil have been identified such as 3,4-dihydroxyphenylethanol (3,4-DHPEA, or hydroxytyrosol) and p-hydroxyphenylethanol (p-HPEA, or tyrosol), gallic, caffeic, vanillic, p-coumaric, syringic, ferulic, homovanillic, p-hydroxybenzoic, protocatecuic acids. The phenolic content of olive oil can be affected by the maturity of the olives, agriculture methods, pressing techniques, time since the harvest and storage of olives, and even cooking methods (Cicerale *et al.*, 2010; Baiano *et al.*, 2009; Perez *et al.*, 2014). Olive oil has been classified according to total polyphenol content as mild (below 180 mg/kg), medium (180-300 mg/kg) or robust (above 300 mg/kg) (Polyphenols and antioxidants in olive oil, 2014).

It was of interest therefore to assess the polyphenol content of BOOs in comparison with the common types of OO, especially the virgin OOs. The unique processing needed to create BOO could have an influence on the polyphenol content. The polyphenol content of 11 OOs is shown in Fig. 4.1. The four BOOs had much lower content, five times less, compared with the virgin OOs. The mean polyphenol content of the four BOOs was 56.1±20.0 μg/mL, whereas the seven VOOs had a mean content of 283.7±68.2 μg/mL. A Student's t-test to

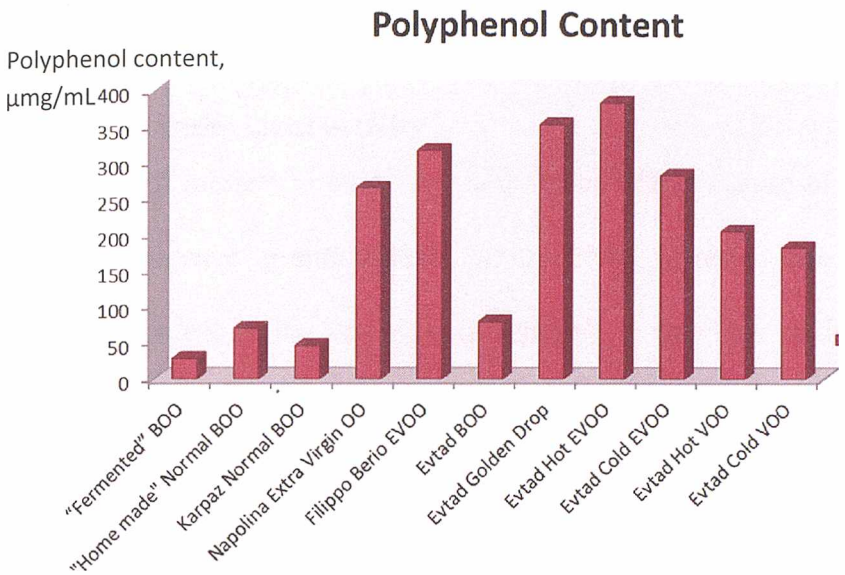


Figure 4.1 Comparison of polyphenol content of BOOs compared with VOOs.

compare the means gave $p < 0.0001$, confirming the significance of the difference. In particular the set of Evtad OOs, which are made from the same type of olives at the same manufacturing site, but by different processes, demonstrate that the processing needed to make BOO is likely to cause a large loss of polyphenol content.

Cypriot traditional black olive oil is obtained by boiling, but the other olive oils are obtained from pressing the olive fruit by the cold pressing method. Temperature will adversely affect olive oil's phenolic content (Baiano *et al.*, 2009; Attya *et al.*, 2010). Olive oil anti-inflammatory, antioxidant compounds are more stable at lower temperatures, and they cannot survive when the temperature is high. This could suggest that the reason why the total phenolic content between oils varies so much might be due to the differences in temperature, or in the fermentation process unique to BOO, during the manufacture of the oil.

4.6.2 Biochemical Analyses

4.6.2.1 Antioxidant activity

It was of interest to study AO activity of BOO because of the low polyphenol content. A difference in antioxidant (AOx) activity of tested oils may depend on the total phenol content in the varieties, as reported in previous studies (Barbarisi *et al.*, 2014; Sarolic *et al.*, 2014). Polyphenol extracts from four oils were assayed (Figs. 4.2 and 4.3). Relative antioxidant values were calculated using quercetin as a reference (Fig. 4.2) and are expressed per µg mass of polyphenol. The data indicated that BOO polyphenols are less protective than VOO polyphenols, although surprisingly the Evtad Golden Drop VOO also had lower antioxidant activity similar to the BOOs, despite having a much higher, by five times, polyphenol content (Fig. 4.1). Thus the processing involved with the production of BOO reduces both the quantity and anti=oxidant quality of the polyphenols.

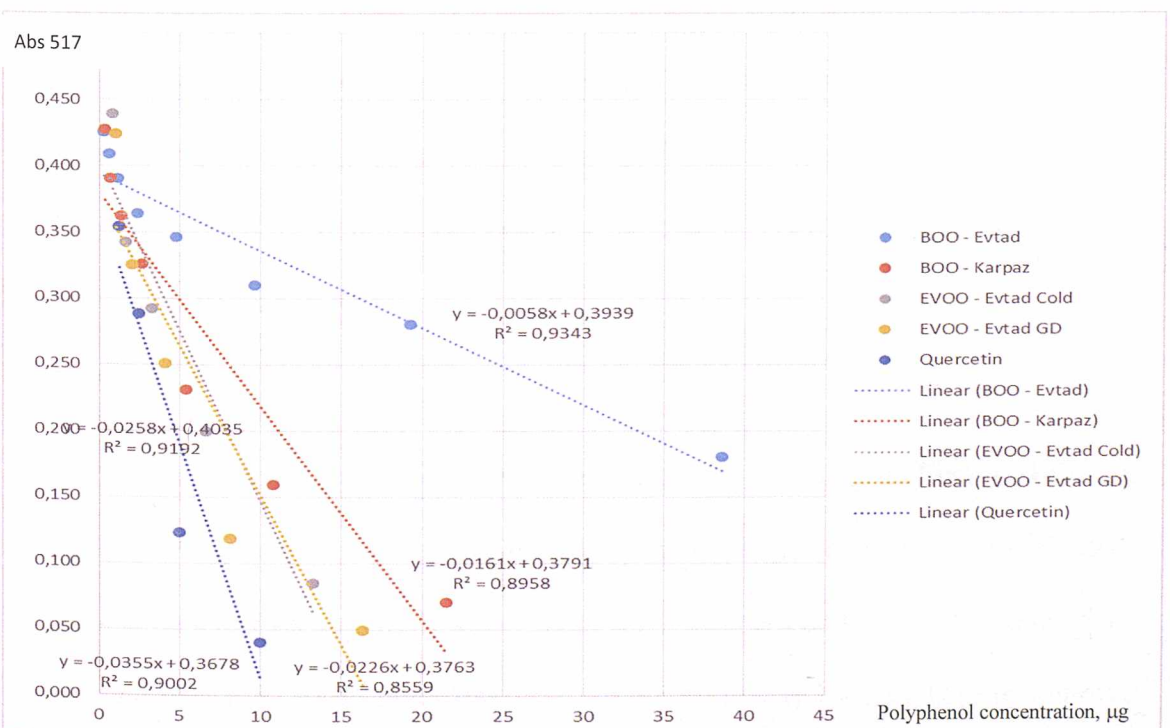


Figure 4.2 An example of an anti-oxidant assay of olive oil polyphenol extracts.

The assay measures the inhibition of oxidation of DPPH using serially diluted samples, and quercetin as the standard against which other samples are compared. Oxidation of DPPH induces an increase in absorbance. The linear correlation between concentration and amount of oxidation is determined for each sample and the slope calculated, as shown on the graph. The slopes are compared. In this set of data the slope for quercetin is -0.0355 and for Evtad BOO is -0.0058, which is 16% of the slope optained with quercetin.

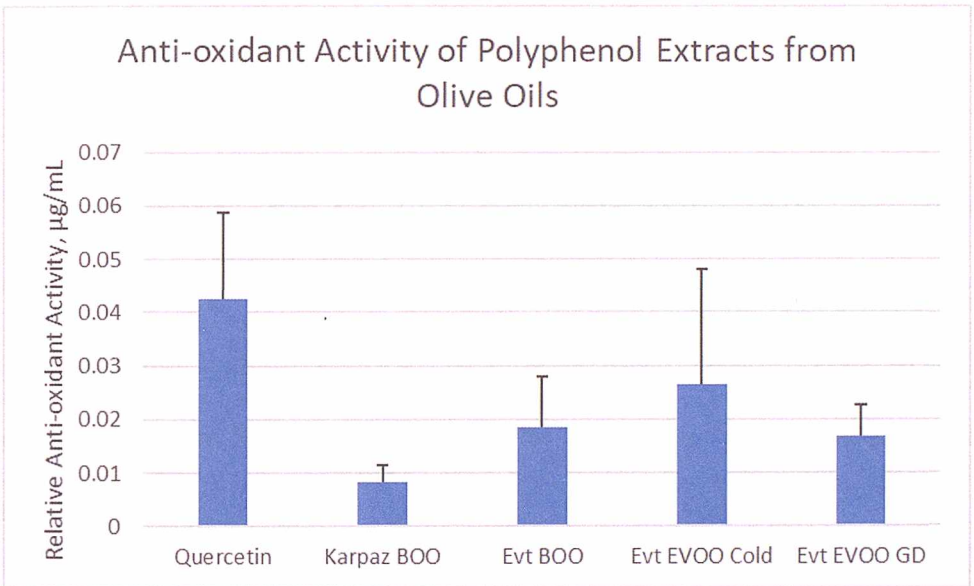


Figure 4.3 Antioxidant capacities of four samples of olive oil. Data are the mean + SD of three extracts from each olive oil. One-way ANOVA with *post-hoc* testing using the Tukey correction for multiple comparisons indicates a $p < 0.05$ comparing means of quercetin with Karpaz BOO.

4.6.2.2 Cell Toxicity

There are no reports in the literature on the characterisation of effects of BOO on cell growth. Polyphenol extracts of BOO were compared with extracts from traditional EVOO and VOO in a cytotoxicity assay using breast cancer cell lines. Since one of the aims of the research was to investigate the possibility that BOO may help to improve the outcome for breast cancer patients, two breast cancer cell lines MDA-MB-231 and

MCF-7, were chosen as targets initially and these experiments were followed up with tests on another cancer cell line, a human hepatoma line HepG2 as well as a cell line used as a non-cancer control PNT2.

MDA-MB-231 cell line

Figure 4.4 summarises data from four experiments. Polyphenol extracts from BOO inhibited MDA-MB-231 cell growth more than EVOO extracts. Polyphenol extracts from BOO inhibit cell growth about 50% at 1.0 µg/ml and 85-90% at 10 µg/mL, whereas extracts from EVOO have no effect at 1.0 µg/mL and about 50% inhibition at 10µg/ml. The data indicate presence of an inhibitory activity in BOO at higher concentration than in EVOO. Images of MDA-MB-231 cells treated with extracts are shown in Fig. 4.5 and show the inhibition of growth at the higher concentration of BOOs.

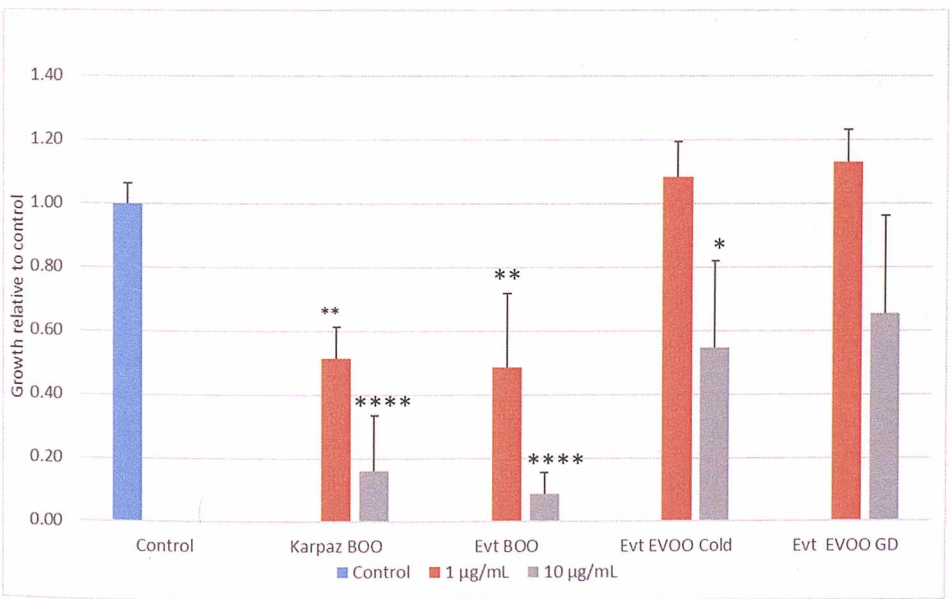


Figure 4.4 Effect of olive oil polyphenols on growth of MDA-MB-231 cells. Data, taken from MTT assays, are the mean+SD of four assays, expressed normalised with respect to vehicle-treated control. Nested 1 way ANOVA was used to compare the mean of the control with the mean of each treatment. *P* values less than 0.05 are indicated as follows: * < 0.05, ** < 0.01, **** < 0.0001.

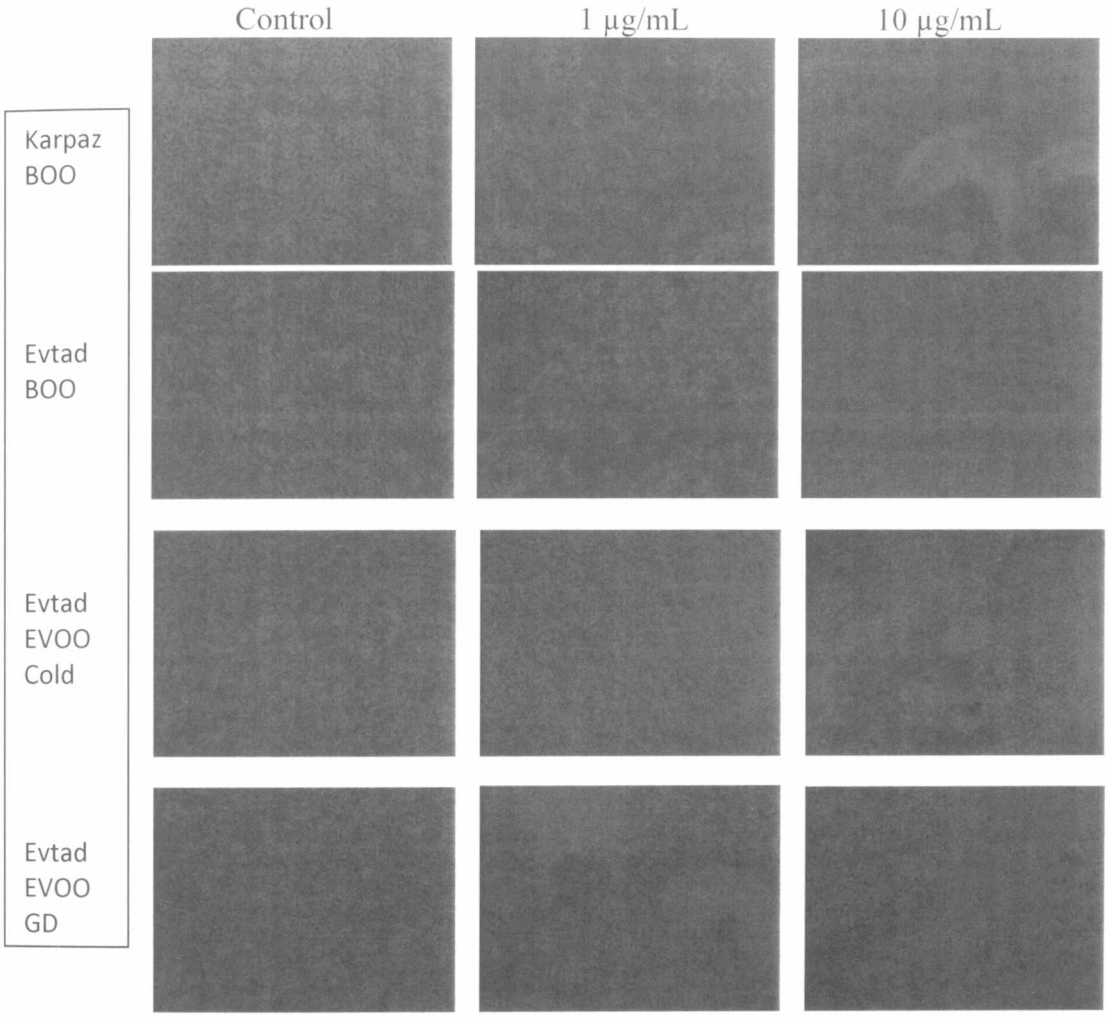


Figure 4.5 Effect of olive oil polyphenols on growth of MDA MB 231 breast cancer cells. Phase contrast images were taken at a magnification of 100x. Cells treated with BOOs at 10 µg/mL are much less dense compared with controls or cells treated with EVOOs at the same concentration.

MCF-7 cell line

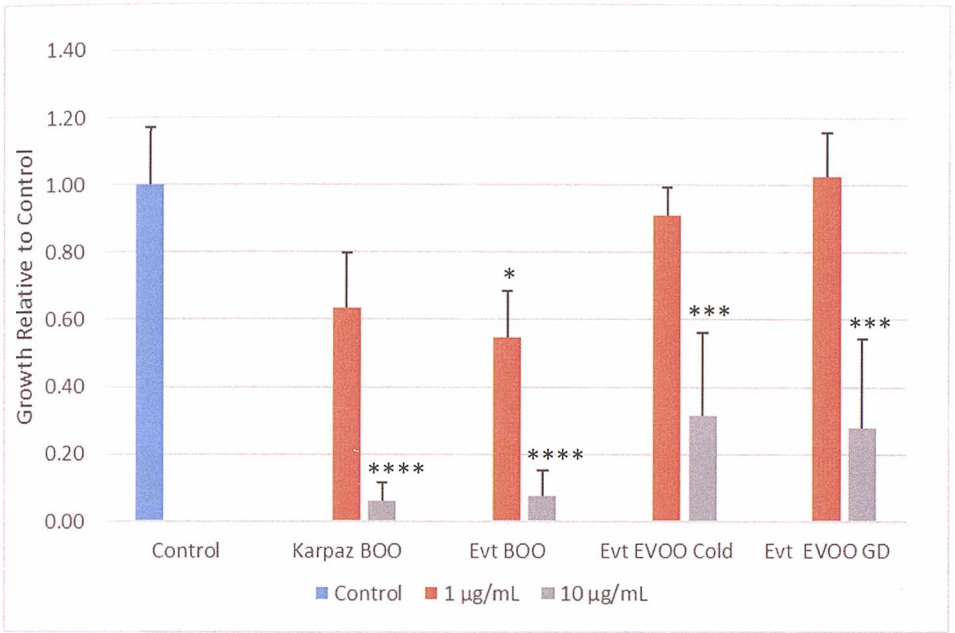


Figure 4.6 Effect of olive oil polyphenols on growth of MCF-7 cells. Data, taken from MTT assays, are the mean+SD of three assays, expressed normalised with respect to vehicle-treated control. Nested 1 way ANOVA was used to compare the mean of the control with the mean of each treatment. *P* values less than 0.05 are indicated as follows: * < 0.05, *** < 0.001, **** < 0.0001.

MCF-7 cells are taken from a breast cancer where oestrogen receptors are present and there is no ERBB2 amplification. In contrast MDA-MB-231 cells are a model for the triple negative type of breast cancer, in which no estrogen receptor is expressed. It may be expected that the two types of cell could respond differently to the olive oil extracts. However the response of the two cell types to the four extracts is broadly similar, with strong inhibition in both cases by BOO extract at 10 µg/mL.

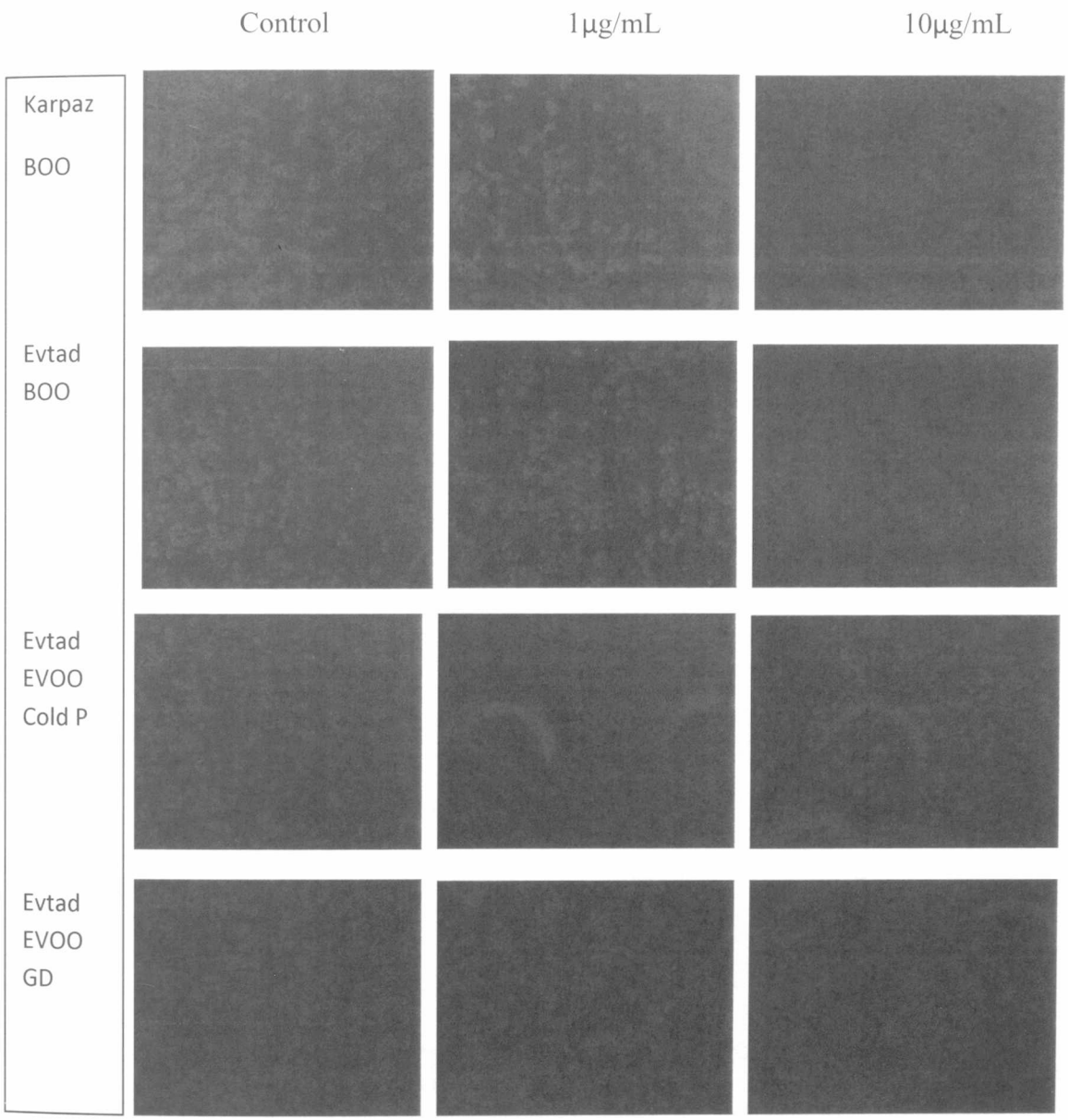


Figure 4.7 Effect of olive oil polyphenol on growth of MCF-7 breast cancer cell line Phase contrast images were taken at a magnification of 100x. Cells treated with BOOs at 10 µg/mL are much less dense compared with controls or cells treated with EVOOs at the same concentration.

HepG2 and PNT cell lines

The finding that the BOO extracts were cytotoxic towards breast cancer cells suggetsed further experiments should be tried with other cell lines. One of these was HepG2 a human hepatoma, and, to test whether the effect was selective for cancer cells only, the human prostate cell line PNT 2 was also used. PNT2 cells are immortalised by transformation with a virus but do not grow into tumours when injected into mice. Similar results were found with the extracts on HepG2 cells as with the breast cancer cell lines – the BOO extracts were both strongly cytostatic at the higher concentration used (Fig. 4.8).

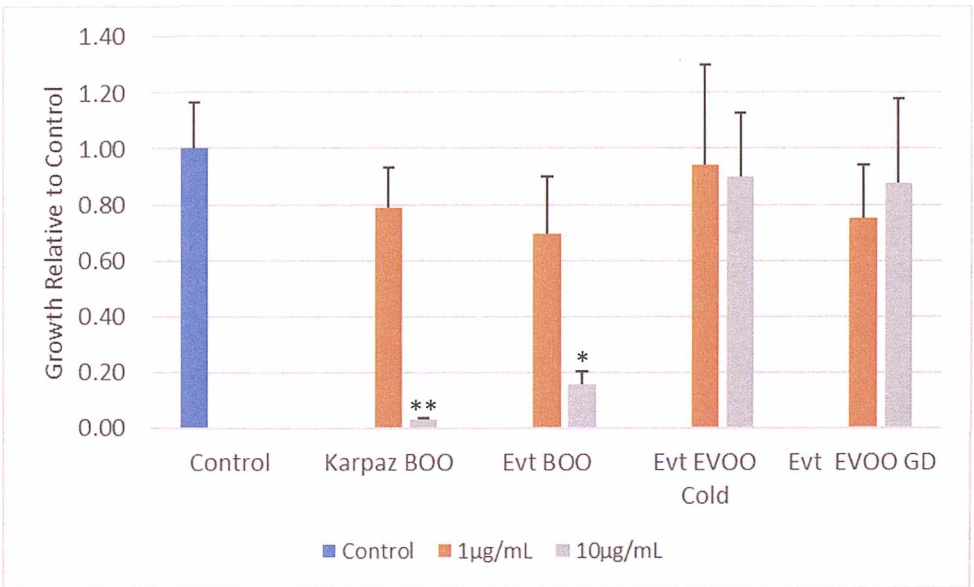


Figure 4.8 Effect of olive oil polyphenols on growth of HepG2 cells. Data, taken from MTT assays, are the mean+SD of two assays, expressed normalised with respect to vehicle-treated control. Nested 1 way ANOVA was used to compare the mean of the control with the mean of each treatment. *P* values less than 0.05 are indicated as follows: * < 0.05, ** < 0.01.

Figure 4.8 Effect of olive oil phenols sample C on growth of Hep G2

Interestingly there was a clear difference in the effect of the extracts on PNT2 cells in that three of the extracts were not effective at either concentration used, but the Evtad BOO extract alone showed strong, 90%, inhibition of growth at 10µg/mL (Fig. 4.9).

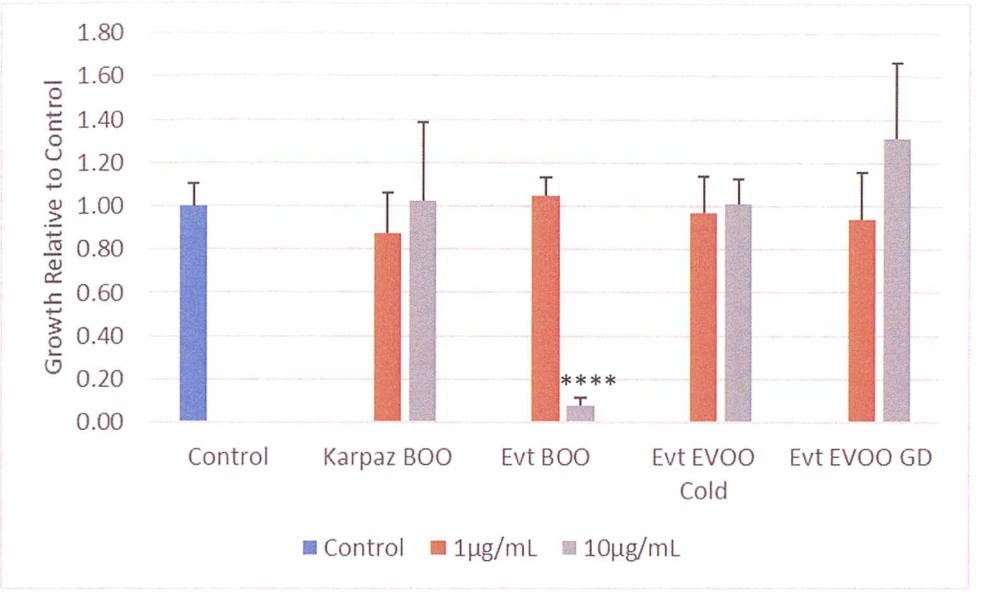
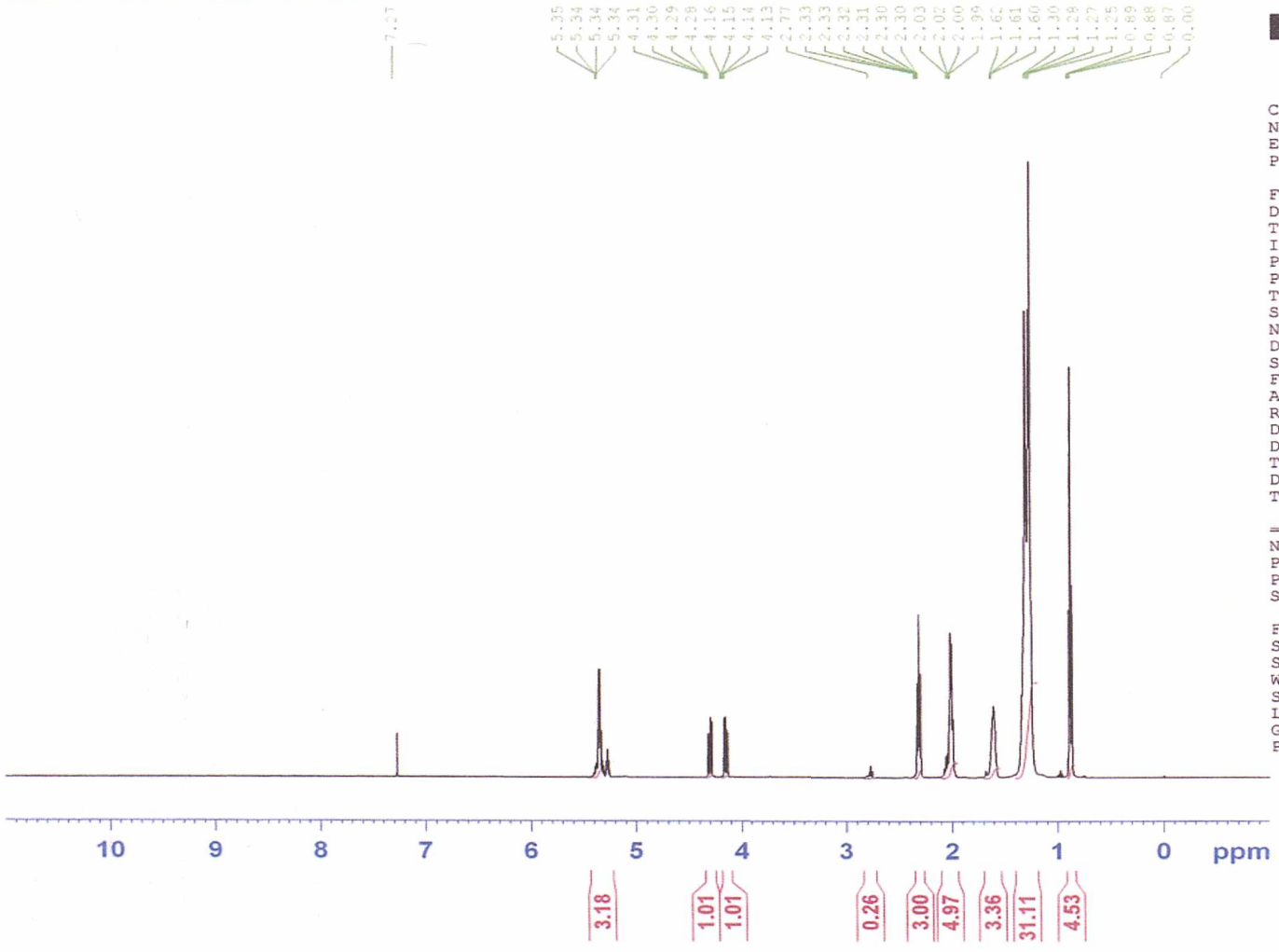


Figure 4.9 Effect of olive oil polyphenols on growth of PNT2 cells. Data, taken from MTT assays, are the mean+SD of five replicates in a single assay, expressed normalised with respect to vehicle-treated control. 1 way ANOVA was used to compare the mean of the control with the mean of each treatment. *P* value less than 0.0001 is indicated by ****.

4.6.2.3 NMR Analysis

^{1}H-NMR spectra of five olive oils were compared to identify differences between BOOs and EVOOs. Representative spectra of one type of BOO and one type of EVOO across the range of 0 to 10 ppm, shown in Fig. 4.12, appear identical. When the spectra are amplified

Ayse Okan sample O1A in cdcl3
Black Olive oil (Evtad)



BRUKER

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SOLVENT CDCl3
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DS 4
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FIDRES 0.157632 Hz
AQ 3.1719923 sec
RG 45.2
DW 48.400 usec
DE 6.00 usec
TE 293.0 K
D1 10.00000000 sec
TD0 1

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PL1 -1.00 dB
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PC 1.00

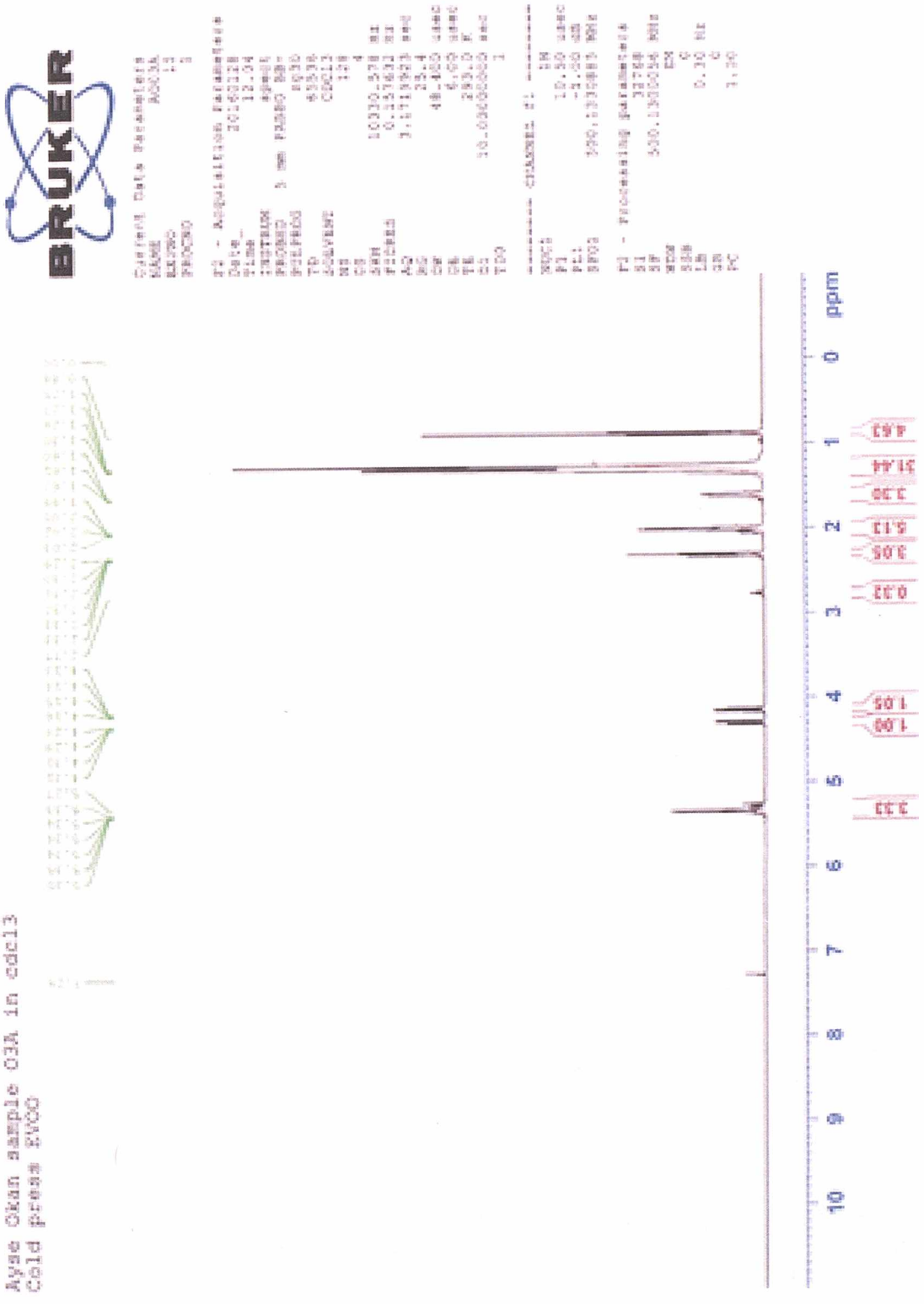
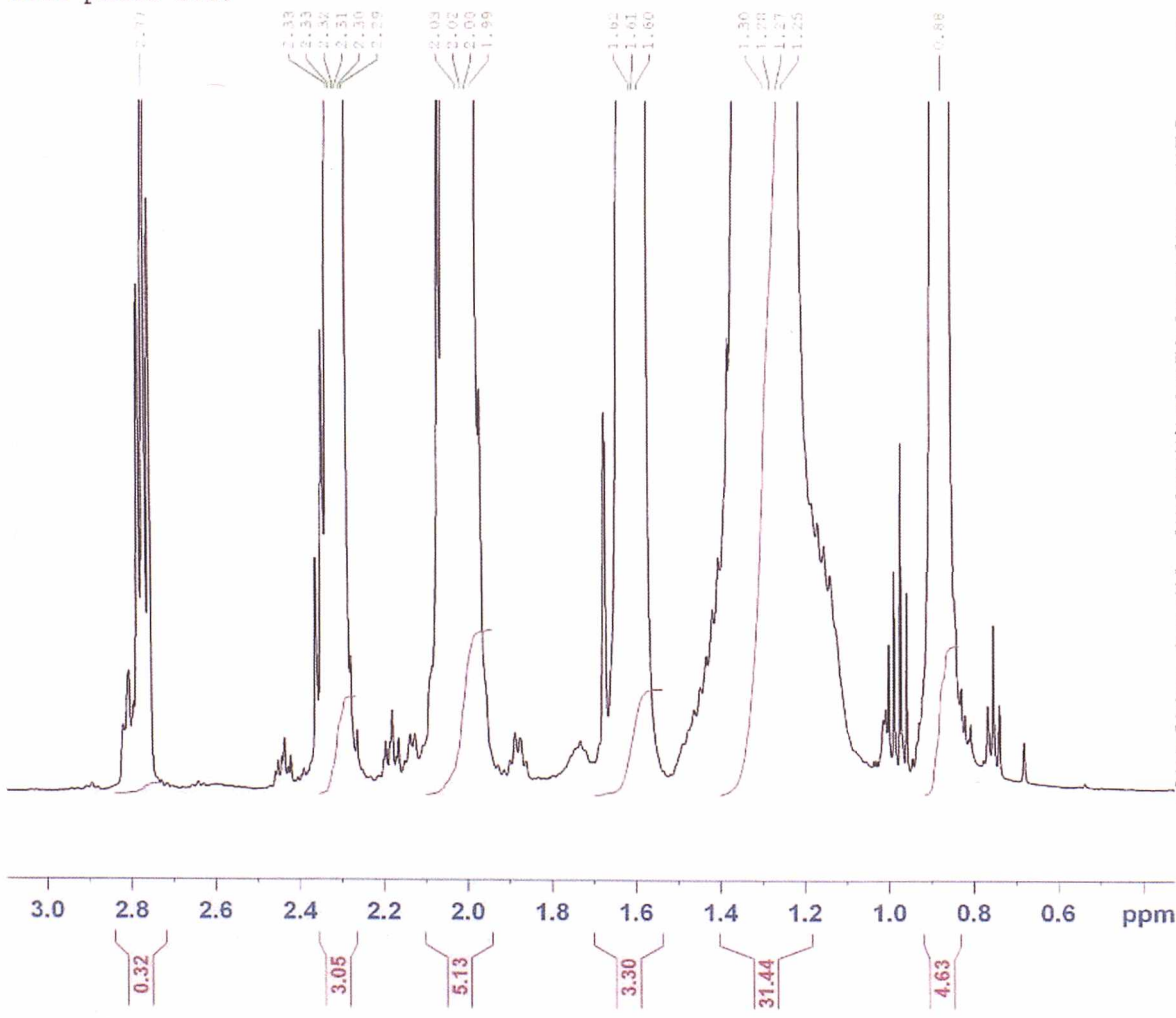


Figure 4.10 Representative ^{1}H-NMR Spectra of Olive Oils. The spectrum from the Evtad BOO, upper panel, is identical to the spectrum from EVOO, lower panel, produced by cold-pressing Evtad olives. Spectra of all five OOs analysed are given in Annex 8.

Ayse Okan sample O3A in cdcl3
Cold press EVOO



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FIDRES             0.157632 Hz
AQ                 3.1719923 sec
RG                     25.4
DW                   48.400 usec
DE                    6.00 usec
TE                   293.0 K
D1              10.00000000 sec
TD0                       1

======== CHANNEL f1 ========
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PL1                   -1.00 dB
SFO1           500.1330885 MHz

F2 - Processing parameters
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LB                     0.30 Hz
GB                        0
PC                     1.00
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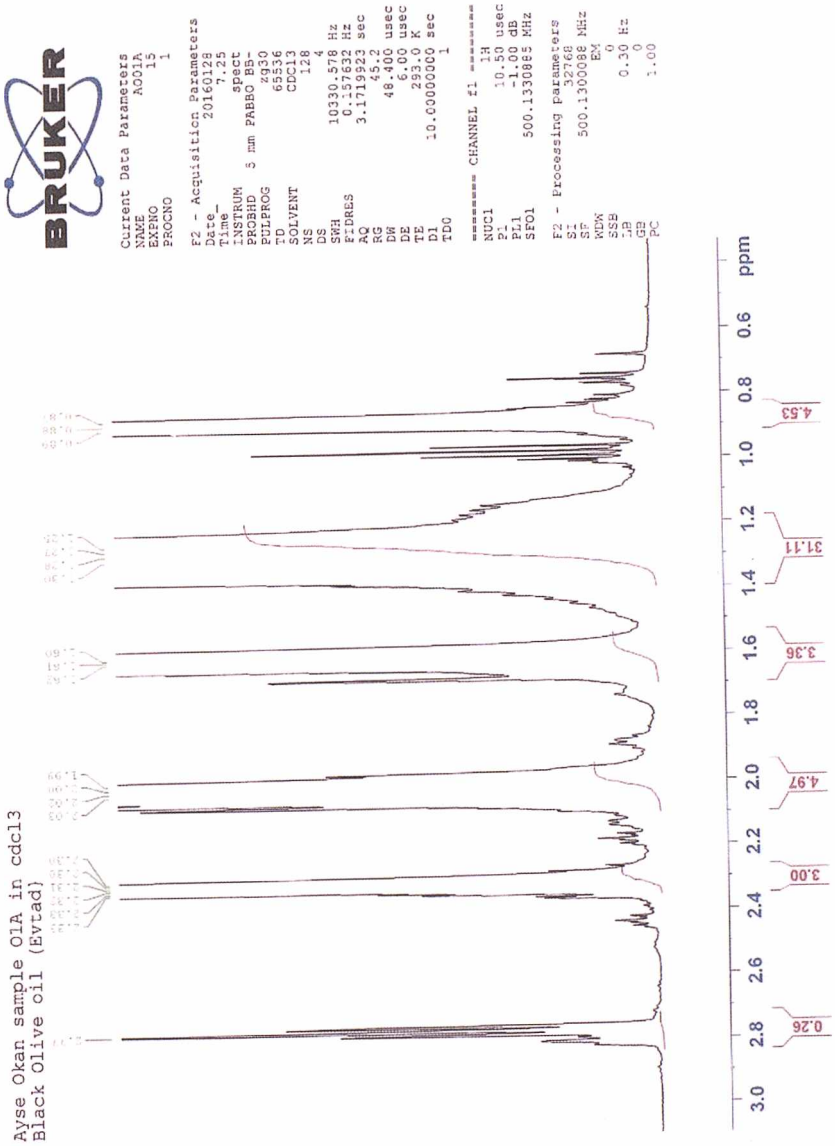
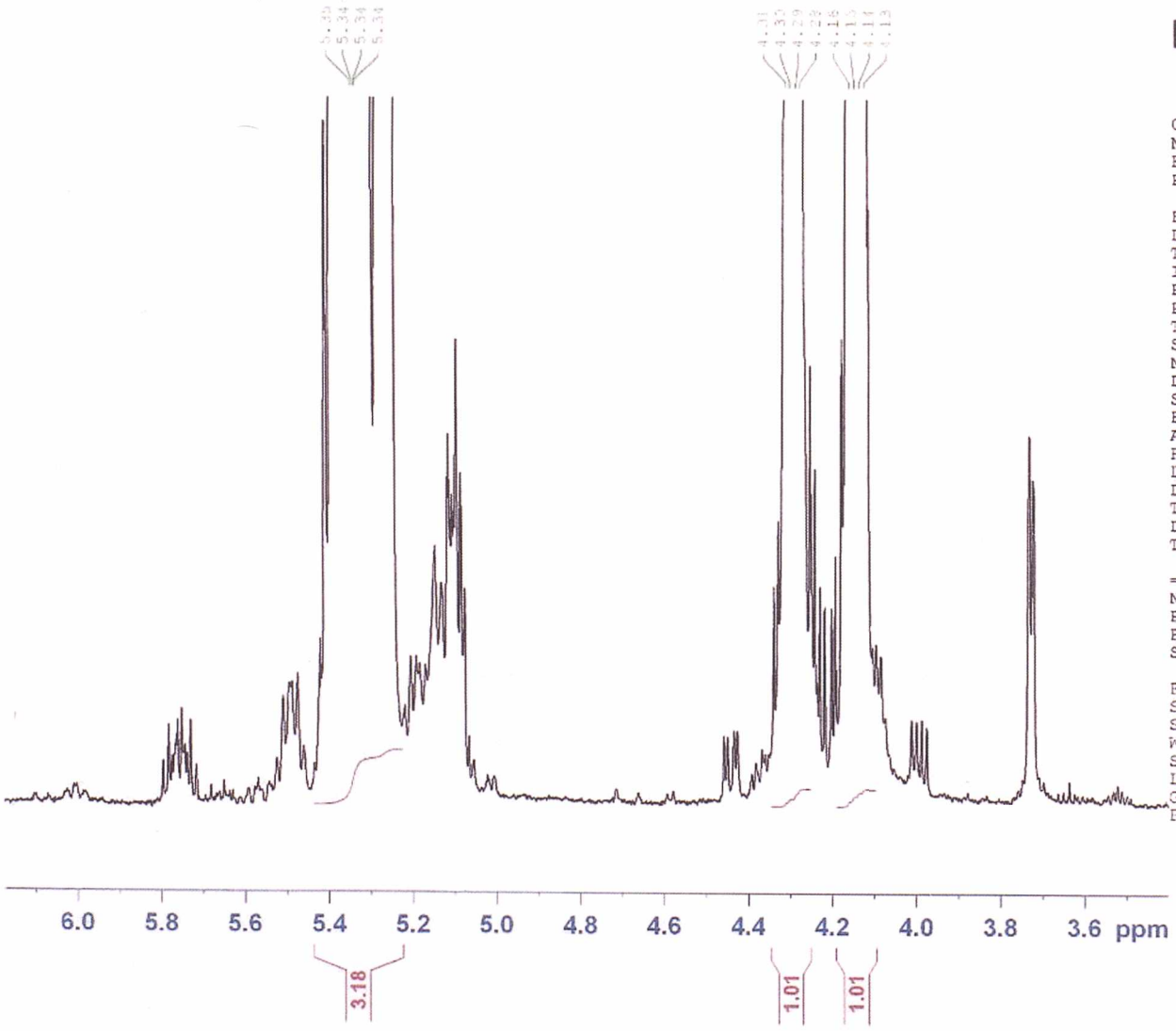


Figure 4.11 Expanded ^{1}H-NMR Spectra of Representative Olive Oils. The expanded spectrum in the range 0 to 3.0 ppm from the Evtad BOO, upper panel is identical to the spectrum from Evtad EVOO. 0 to 3.0 ppm spectra of all five OOs analysed are given in Annex 8.

There is no clear difference between BOO and EVOO across the range 0 to 3.0 ppm (Fig. 4.11), but there are differences in the region of 5.6 to 5.8 ppm (Figs. 4.12 and 4.13).

Ayse Okan sample O1A in cdcl3
Black Olive oil (Evtad)



BRUKER

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PROCNO                    1

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DS                        4
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FIDRES            0.157632 Hz
AQ                3.1719923 sec
RG                     45.2
DW                   48.400 usec
DE                    6.00 usec
TE                   293.0 K
D1             10.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                    10.50 usec
PL1                   -1.00 dB
SFO1           500.1330885 MHz

F2 - Processing parameters
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WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```

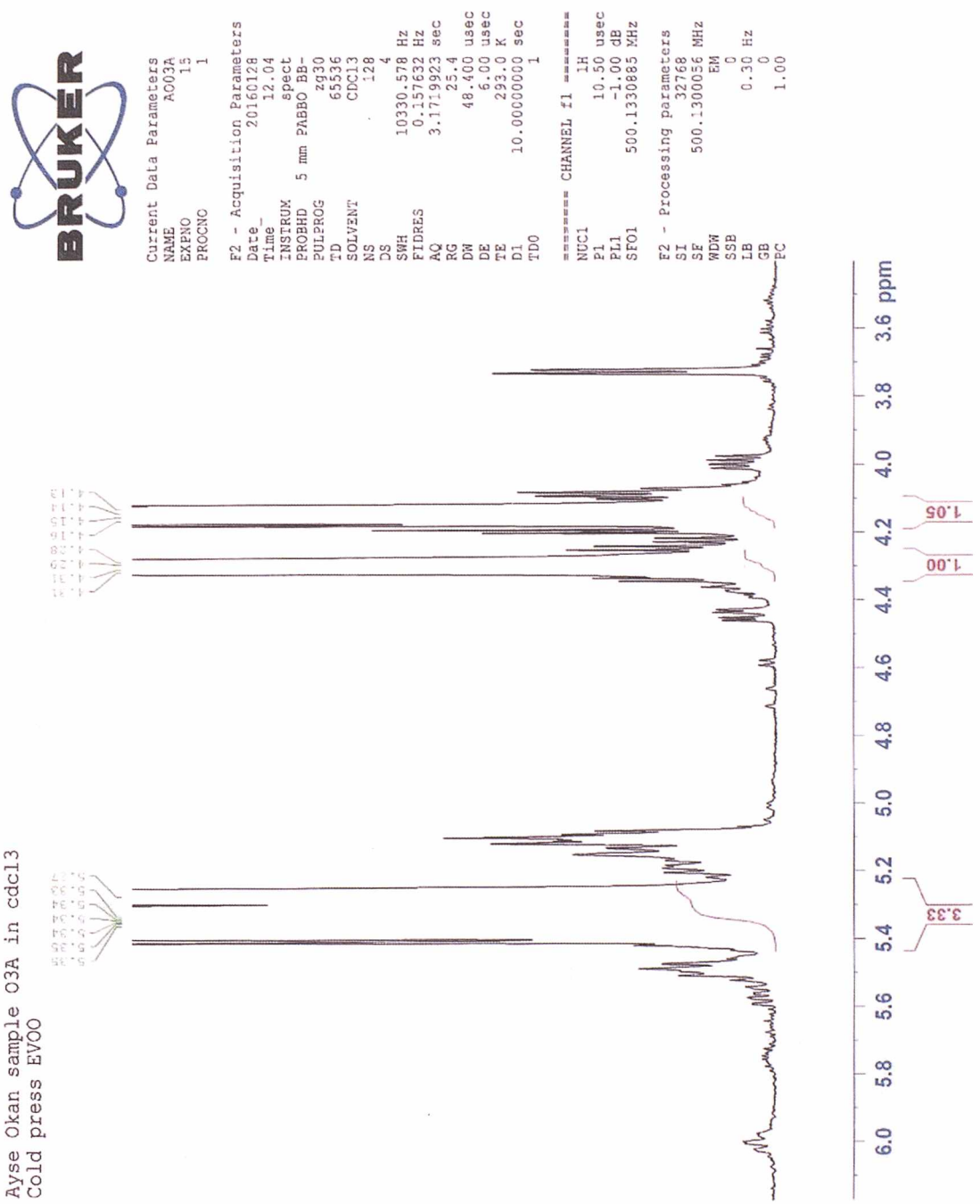

Figure 4.12 Expanded ^{1}H-NMR Spectra of Representative Olive Oils. The expanded spectrum in the range 3.0 to 7.0 ppm from the Evtad BOO, differs from Evtad EVOO in the region 5.7 to 5.8 ppm. 3.0 to 7.0 ppm spectra of all five OOs analysed are given in Annex 8.

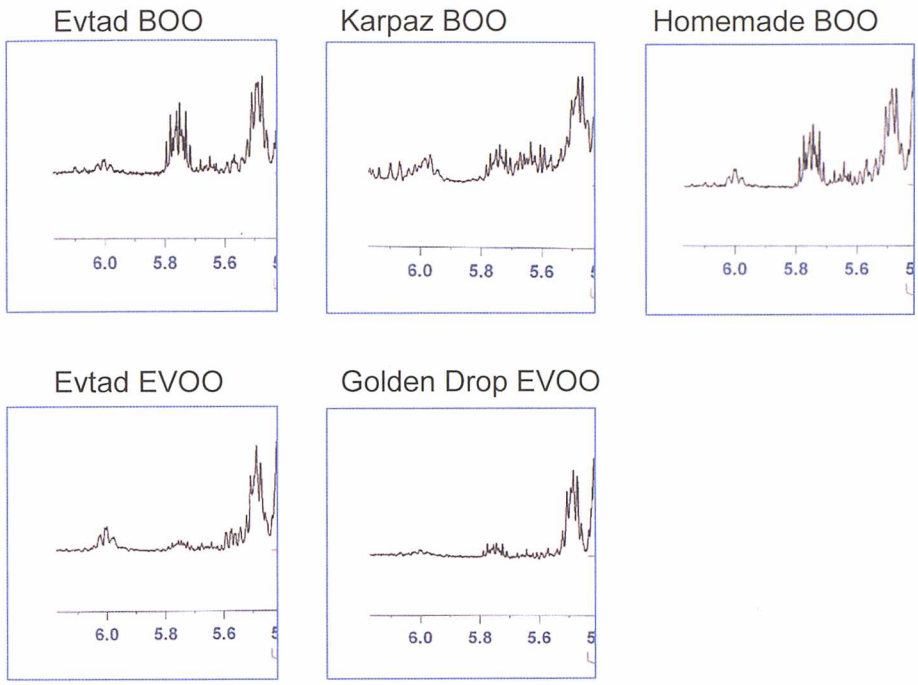

Figure 4.13 Olive oil ^{1}H-NMR spectra expanded in the region 5.4 to 6.2 ppm. The top panels are spectra of BOOs and the bottom panels of EVOOs. BOOs have signals in the region 5.7 to 5.8 ppm that are weak or absent in EVOOs. The set of full spectra expanded over 3.0 to 6.0ppm are given in Annex 8.

To analyse the spectra further it is helpful to identify peaks due to H atoms on oleic acid, the major constituent of both BOOs and EVOOs. A theoretical 1H-NMR spectrum of oleic acid taken at 500 MHz is shown in Fig. 4.13, aligned with spectra from a BOO and EVOO each.

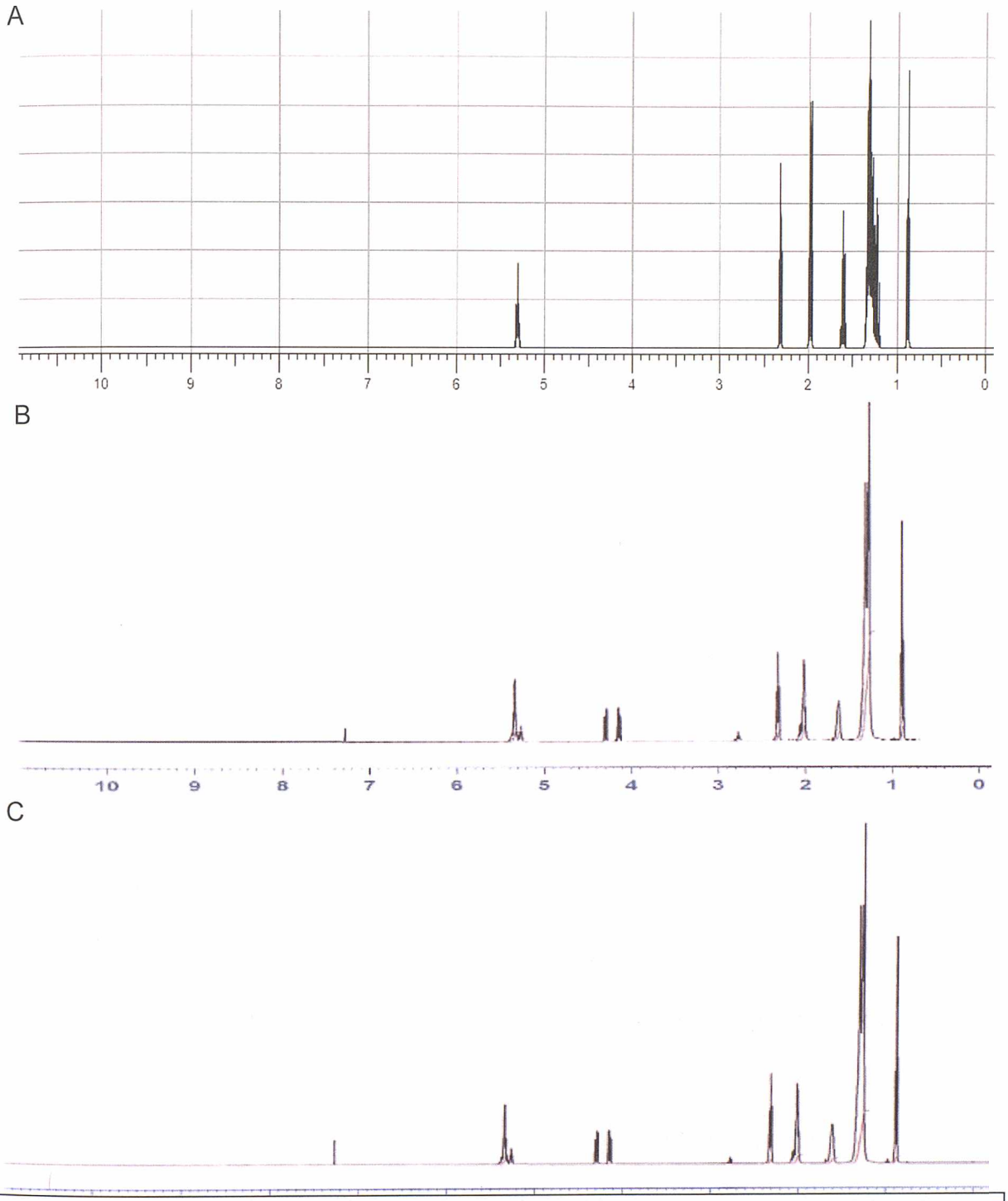

Figure 4.14 Identifcation of oleic acid components in ^{1}H-NMR spectra of olive oils. A theoretical 1H-NMR spectrum of oleic acid generated ACD/I-Lab (methods)(A) and is aligned with spectra from Evtad BOO (B) and Evtad EVOO (C). The six peaks of oleic acid are the major peaks of both OOs. In addition both OOs have five minor peaks.

The six major peaks of the olive oils are all at the same position as peaks for oleic acid. In addition the olive oils contain five smaller peaks, which are assumed to include polyphenols. Characterisation of the smaller signals in the ^{1}H-NMR spectra of olive oil, has been described (Alonso-Salces *et al.* 2010, Dais and Hatzakis 2013), including resonance peaks in the region 5.6 – 5.8 ppm. Peaks in the range 0.85 – 5.30 are due to fatty acid or glycerol protons (Dais and Hatzakis 2013) and from 5.97 – 6.95 ppm are attributed to phenolic protons (Alonso-Salces *et al.* 2010), but no structural attribution was made to peaks in the range 5.6 – 5.8 ppm.

Overall the analysis indicates broad similarities between BOO and EVOO but with some significant differences in the region 5.6 to 5.8 ppm. Further work to characterise these differences could include assessment of NMR profiles of polyphenol preparations, analysis and purification by HPLC followed by structural characterisation by NMR and mass spectrometry.

4.7 Discussion

The aim of the work described in this chapter was to identify any constituents of black olive that are distinct from normal olive oils. Various types of olive oil made from olives gathered from differing areas of Cyprus and processed at different times were analysed. They are broadly either variant of black olive oil or of virgin olive oil. As described in section 4.3, the BOO produced in Cyprus is processed differently to VOOs and entails boiling the olives and then allowing them to undergo natural maturation in the sun. This alters the phytochemical constituents and hence creates a unique taste. The question arises which constituents are involved and what proportion are changed.

Initial analysis involved an assessment of fatty acid components, described in section 4.6.1.1. The level of total saturated, mono-unsaturated and polyunsaturated fatty acids as % of total fat content of BOO were 19.55%, 1.74%, and 6.83% respectively. Perhaps the most interesting part of this analysis is the higher content of unhealthy saturated fatty acids in BOO, almost 20%, compared with other OOs, which 13-16%. It would be of interest to compare the fatty acid composition of BOO and VOO prepared from the same olives, to see if the difference in unsaturated fat can be attributed to variation in content of different batches of olives or as a result of the different modes of preparation.

The most abundant fatty acid was oleic acid (68.85 %) followed by palmitic (15.94%), linoleic (6.17%), stearic (2.93 %), palmitoleic (1.18%) and linoleic (0.66 %). The olive 'Kalamata', which grows in Khyber Pakhtunkhwa/Pakistan, has similar oleic acid content, 65.2%, to BOO (68.85%) (Muhammed *et al.,* 2013). Tanilgana *et al.* (2007) analysed the fatty acid composition of olive oils collected from five different areas of Turkey, Gemlik, Kilis, Ulus, Tirilye and Ayvalik. The results were similar to BOO, with oleic acid present at the highest concentration (65.7-83.6 %), followed by palmitic (8.1-15.2 %) [lower than BOO], linoleic (3.5-15.5 %), stearic (2.0-5.6%) and linolenic (0.1-3.0 %) acids. In another study compositions of fatty acids were analysed in four samples of olive oil from different locations in Turkey, Edremit, Gemlik, Domat and Sarıulak (Matthaus and Ozcan, 2011). According to fatty acids composition, the major fatty acids found in Turkish olive oil was oleic, linoleic, palmitic, and stearic (Matthaus and Ozcan, 2011). The detailed analysis of fatty acids from a single sample of BOO (Table 4.3) confirmed that the profile of fatty acids was similar to that found for normal OOs. From these studies we can conclude that levels of oleic acid in olive

oil are similar across samples from many areas and are present as the highest proportion of all fatty acids in olive oil, including BOO.

Another important component of olive oil is the polyphenols, comprising up to about 0.1% of the mass of olive oil. Cypriot black olive oil samples were found to have five times less phenolic compounds compared to the extra virgin olive oils, with a mean content of 56.1 ± 20.0 μg / mL, compared with 283.7 ± 68.2 μg / mL for EVOOs (Section 4.6.1.2). In a study that was conducted in Iran, total polyphenol and fatty acid composition was measured in five different olive oils (Zard, Coratia, Frangivento, Beledy and Arbequina). As has been found in all other studies, the fatty acid present in the highest concentration was oleic acid and there was some variation in total phenol content of the five olive oils, ranging between 148.42μg/kg (Frangivento Olive oil) and 181.63mg/kg (Zard Olive oil) (Hashempour *et al.,* 2010). In contrast in the present study the total phenol content of the Cypriot olive oils analysed showed a much broader range, between 28.6mg/kg and 382.8mg/kg. The maximal content of polyphenols in the Cypriot olives is twice that of the Iranian, perhaps attributable to differences in climate, variety, agricultural practices, ripeness at harvest, maturity of the olives, agricultural methods, pressing techniques, time from the harvest and storage of olives, and even cooking methods (Baiano *et al.,* 2009; Chehap *et al.* 2013; Cicerale *et al.,* 2010; Erel *et al.* 2013; Esmaeili *et al.* 2012; Perez *et al.,* 2014).

Fermented BOO had the lowest, and Evtad Hot press Extra virgin olive oil had the highest, total phenol contents in all samples. The phenolic content of all BOO samples was four to five times lower than other (E)VOOs. Polyphenols are considered to confer health benefits, and this may be attributable to anti-oxidant activity. Lower

polyphenol content would be expected to give rise to lower antioxidant activity. Thus it may be expected that, once a polyphenol fraction has been isolated from olive oil, it should have comparable anti-oxidant activity to fractions from other oils when comparing equivalent amounts. Comparison of polyphenol extracts from four OOs, two BOO and two EVOO, revealed BOO polyphenol fractions to have lower or similar anti-oxidant activity (Fig. 4.3). Interestingly three of the polyphenol fractions were prepared at the same location, Evtad (which is the company name) from the same type of olive. The BOO and the EVOO anti-oxidant activities were not significantly different, but the poly-phenol content was. In contrast a BOO prepared in a different location, Karpaz, had significantly less anti-oxidant activity than Evtad BOO. It remains to be seen whether this is due to variation in batches of olives or to method of preparation of the BOO – the Karpaz method did not involve fermentation but the Evtad method did (Table 4.1).

Baiano *et al.* (2009) assessed changes in phenolic content and antioxidant activity of EVOOs during storage. They measured total phenolic content of oils at production and at 6 and 12 months of storage. On average there was a 16% loss of content after 6 months storage in the dark at room temperature, which increased to 72% loss after 12 months of storage (data calculated from Baiano *et al.* 2009), averaged from 15 different olive oils sampled from around Italy. In our study however, we stored our samples in a dark cupboard at room temperatures for almost one year, and determined total phenolic compound of samples at 6 month uninterrupted periods. Similar to the findings of Baiano *et al.* the content of phenolic compounds decreased about 43% over 9 months, averaged for the 11 olive oils. Moreover it has been shown that phenolic compounds and antioxidants in VOO can decrease by 40-50% during the cooking

process of frying (10min. 180 °C) (Gomez-Alonso *et al.*, 2003). These data suggest that polyphenols are slightly unstable and will naturally decompose even at room temperature. This suggests that heating to around 100 °C could easily account for the lower recovery of polyphenols in BOO. Further work is needed to test this idea, especially by comparing different processes on the same batch of olives.

The final type of chemical analysis carried out was a comparison of the NMR spectra of the whole olive oils, comparing BOO and EVOO. No obvious differences were found (Figs. 4.12, 4.13) until a careful expansion of the scale revealed some peaks present in BOO but not in EVOO (Figs. 4.14 and 4.15). These peaks are just outside one of the six peaks due to oleic acid (Fig. 4.16). Further work is required to identify the compound(s) that cause these peaks, starting with a comparison of spectra from the polyphenol fractions.

Generally the analytical work can be continued by purifying the non-fatty acid components of olive oil using solid phase extraction, followed by HPLC to separate mixtures and finally NMR and mass spectrometry to identify structures (Alarcon-Flores *et al*. 2013; Dias and Hatzakis 2013).

Whilst it is important to know the chemical constituents of the olive oils, the biological effects of them need to be found as well. The theme of this research is the potential for improvement of breast cancer through diet, with a focus on the contribution that BOO might make. The data described so far indicate differences in the polyphenol profile between the BOOs and EVOOs which suggested further comparison using a biological assay.

The possibility that olive oils may contain chemicals that are cytotoxic to cancer cells was tested by treating two breast cancer cell lines, representing two types of breast cancer, with the polyphenol extracts from two BOOs and two EVOOs. This set of olive oils has been analysed for polyphenol content (Section 4.6.1.2) and anti-oxidant activity (Section 4.6.2.1). One BOO was made at Evtad where the two EVOOs were also prepared. The other BOO, from Karpaz, by a different procedure to the Evtad BOO that did not involve fermentation. At the higher concentration used the BOO extracts strongly inhibited growth of both breast cancer cell lines (Figs. 4.4 to 4.7) as well as a liver hepatoma cell line HepG2 (Fig. 4.8). Interestingly the Evtad BOO, but not the Karpaz BOO, could also inhibit PNT2 cells (Fig. 4.9), a prostate cell line that is not tumourigenic, but has been immortalised by transformation with a virus. In other words the Karpaz BOO extract is selectively toxic at 10μg/ml towards the three cancer cell lines but not towards the non-tumourigenic cell line. The extracts of EVOO showed some inhibition of growth of the breast cancer cells, but not the hepatoma or the prostate cells. Overall the BOO extracts show some promising effects on cancer cell growth and these can be explored further on other cell lines, especially non-cancer cells. The mechanism of inhibition of cell growth could be explored – it could cytostasis, where cell growth is stopped, or apoptosis, where cells are triggered to undergo cell death. It would also be useful to identify the active component or components of BOO that are cytotoxic.

4.8 Limitations

The results described in this chapter show some new and interesting properties of BOO, but need to be treated with caution. The olive oils were stored for periods of several months, or over a year in some cases, before being extracted for analysis. The

last batches of oils were processed immediately on receipt, and in future work care will be taken to ensure this always happens. It was not possible to do this in the first part of the project because of the limited time that was spent at LMU.

Constraints of time also meant that the measurements of cytotoxicity could not be completed for all cell lines, especially PNT2. The use of PNT2 cells as a non-cancer control cell line may not be the best choice, because of its immortal phenotype. Alternative non-cancer control cells could be used, including MCF-10A, MCF-12A immortalised normal human breast epithelial cells, HDFs, HUVECs, MRC5, HEK293, and HaCaT cells.

Time was the biggest challenge in this study. For cell culturing, more time was needed to get more replicates of results.

4.9 Conclusion and Future Work

The data described in this chapter indicate that EVOOs contain about 5 times more polyphenols than BOOs, and the polyphenol constituents differ between BOO and EVOO. The data are very clear, but more samples are needed to be analysed. There are a variety of ways in which OOs can be prepared at small enterprises in Cyprus, for both BOO and EVOO. To compare the products from these different processes carefully it would be necessary to have a single batch of olives split into sub-batches and each processed by a different method. To simplify this approach it could be possible to focus on two manufacturers, at Evtad and Karpaz, and compare batches of BOO and EVOO from each.

Although, the total polyphenol content in BOO is markedly lower than in EVOO its effects on inhibiting growth of breast cancer cells was higher, approximately up to 10 times, than extracts from EVOOs. This indicates the presence of different chemicals, or key cytostatic chemicals in different proportions, in the BOO and further detailed analysis of the extracts is required to identify and quantify the compounds, and to establish the relationship between the processing of the olives and the constituents of the oil extracted from them. The very preliminary, but interesting, results described in this chapter provide the basis for a deeper exploration of BOO that could indicate some unique health benefits of this unusual olive oil.

5. Chapter V-Overall Discussion

This study was conducted on the Mediterranean island of North Cyprus taking into consideration olive oil which forms the basis of the Mediterranean diet. As has been stated throughout the research, this study is the first one to analyse and look into the traditional olive oil which is characteristic of Cyprus, black olive oil. Two opposite perspectives comprise the community's view on black olive oil. Its distinct taste and smell is what results in these two opposing views. The interviews in the third section verify BOO's unique taste and smell. Taking olive oil as a base, the study was conducted in three different phases.

Firstly, breast cancer patients' recovery and olive oil consumption, plus adherence to other features of the Mediterranean diet were examined. Later, in the second section interviews with 14 breast cancer patients were conducted in order to provide further insight into perceptions and practices regarding diet and breast cancer, and contextualise these studies' results. In the third section, the black olive oil particular to the TRNC, and its effects on breast cancer and other cells, were analysed. The results obtained from all three sections are interconnected, as mentioned above, and support each other in addressing the research questions.

From the analyses presented in Chapter 4, the results section, it is clear that the nutritional and polyphenol values of black olive oil are low because of the cooking procedure applied before obtaining oil, and support our hypothesis that black olive oil should be treated differently to standard extra virgin olive oil. This study examined this insight and looked at breast cancer patients' views and consumption of black olive oil, and found that a section of the population reduced their consumption and preference for

black olive oil due to hearing gossip regarding adverse effects on health. There is a lack of previously published evidence to support or contest this claim.

5.1 Key findings from the three studies in relation to the overall study aims

The TRNC population, in contrast to the Mediterranean diet, has a low consumption of fish, and a high consumption of meat and grain (cereal). In many "Mediterranean" countries the populations' diet differs considerably from the "Mediterranean Diet" which is based on a 1920s notion of the MD (Arvanitakis, 2014). This was the first study to directly compare the current NC diet to the MD. This study examined the effects of diet on breast cancer biomarkers of BC patients. From the results obtained in Chapter Two, the results section, it was identified that those with a significantly higher intake of vitamin B1, fibre, oleic acid and MUFA had improved CA153 biomarkers. A higher consumption of VitB1, fibre, oleic acid and MUFA led to a reduction in the biomarker and hence suggested a positive effect on breast cancer recovery. A small sample sized double blind study conducted on newly detected BC patients investigated the effect of raw yellow onion on cancer biomarkers. In CEA and CA-125 they saw statistically decreasing values (Jafarpour-Sadegh *et al.,* 2015).

From Study Two, which examined the breast cancer patients' views and consumption of black olive oil, findings suggest that a section of the population reduced their consumption and preference for black olive oil due to hearing gossip regarding adverse effects on health. There is a lack of previous published evidence to support or contest this claim. In the qualitative study presented in Chapter Three a key theme indicated that individuals realized that there was a link between nutritional consumption (diet) and cancer. All individuals gave positive responses to olive oil consumption and

expressed that their consumption patterns before and after cancer detection had changed. Many participants had increased consumption of olive oil and some stopped the use of olive oil as cooking oil, instead adding it directly to food on the plate.

From the analyses presented in Chapter Four, sections 4.3, 4.5.2, 4.5.6 and 4.6.1.2, it is clear that breast cancer patients would be right to view black olive oil as distinct. The nutritional and polyphenol values of black olive oil are low because of the cooking procedure applied before obtaining oil, and support our hypothesis that black olive oil should be treated differently to standard extra virgin olive oil.

5.2 Comparisons of the Mediterranean Diet and the NC Diet

Although North Cyprus is a Mediterranean Island, some of the eating habits contrast with the Mediterranean Diet. When considering the results obtained in the second and third sections, a large percentage of patients diagnosed with breast cancer were aware of the significance of diet in relation to cancer, and had sought information on the topic from a variety of sources. However, it was observed that individuals were not always successful in applying what they had learnt. Most of the participants stated that they changed their eating habits after being diagnosed.

In contrast to other Mediterranean regions, the North Cyprus population has a preference for meat and poultry rather than fish. Their preference for oils is, however, the same - olive oil.

Some find it surprising that North Cyprus has a high percentage of cancer patients, because the image of quality of life in a Mediterranean lifestyle is perceived as healthy and protective against cancer.

When one takes Cyprus's history into consideration one can realize that many wars have taken place here and many mineral fields are located in the soil due to war on both sides of the Island. It is argued that a major section of the soil on the island is either heavily contaminated with minerals and other detritus of war. A survey of soil for the presence of carcinogenic heavy metals was conducted between 2003 and 2005 all over the Island in a collaboration between the Cancer Research Foundation in North Cyprus and the Frederick Institute of Technology in South Cyprus (Akun *et al.*. 2010, Djamgoz *et al.,* 2017). Three possible carcinogenic heavy metals were surveyed - lead, cadmium, and arsenic and one trace mineral, selenium - which shows potential against cancer were examined. Atomic absorption spectroscopy was used to analyze soil taken from different areas around the Island. The results showed lead and selenium levels to be 'normal'. Arsenic and cadmium levels proved to be close to internationally accepted levels in some areas, while in other areas they were higher than accepted levels. Possible carcinogenic cadmium is known to be present in more than 50% of phosphate fertilizers and fossil fuels that mankind is exposed to. Interestingly, cadmium is an estrogens impersonator and in the TRNC, this could contribute to the number of younger age breast cancer incidents (Djamgoz *et al.,* 2017). Consequently, this leads us to believe that the soil quality on the island is low even though the heavy metal levels are lower in Cyprus than in other European countries (Hadjiparaskevas 2001). This could be one of the reasons why there is a high percentage of cancer incidences in Cyprus.

Also, Farazi (2014) discusses risk factors of cancer in Cyprus, and highlights similar issues to those in this study. As a Mediterranean Island, Cyprus has different dietary preferences compared with the Mediterranean diet, as shown by the KIDMED

study which Farazi mentions in his study. It is established that Cypriot children have a low MD score. More needs to done to look at the influence of diet on cancer incidence in Cyprus. Farazi also mentions the possibility of genetic input, because the Cypriot population is fairly homogeneous and isolated.

5.3 Physical Activity

Another factor found in this study was that the level of physical activity is lower than previously thought. Recent research indicates a strong relationship between physical activity and cancer incidence (Chapter 1, section 1.8). Exercise, especially in women who are approaching menopause, can assist/result in reducing hormone levels such as insulin and insulin-like growth factors I, enhancing the response of the immune system, as well as helping to reduce body fat and body mass through a balanced weight (National Cancer Institute-Physical Activity and Cancer). Studies show that physically active women face a lower risk of developing breast cancer than women who undertake little physical activity (Zang *et al.*, 2015).

5.4 Cooking Methods

Interviews indicate that some people use unsuitable cooking methods in Cyprus. Even if individuals choose healthy food products, it is observed that there are mistakes in either the preparation or the cooking method. The most obvious in North Cyprus is cooking food on a coal BBQ when socializing. As mentioned in section three, burnt foods or foods cooked close to charcoal leads to increased PAH formation, which is linked to cancer incidence. Similarly, olive oil burns fast, thus when exposed to prolonged fire it burns faster than other vegetable oils and leads to the production of

carcinogenic substances. Although most participants indicated a preference for olive oil, they also indicated that they used it as a base in cooking.

Another aspect of the current research study focuses on EVOO's polyphenols (antioxidant properties) and oleic acid's relationship with breast cancer cells. *In vitro* studies showed a positive relationship between inhibiting breast cancer cell growth and treatment with olive oil polyphenols and oleic acid (Menendez *et al.,* 2010). The use of the hot press in the production of black olive oil changes the nutrient content. The current study suggests that BOO compared to traditional EVOO contains nearly one third the amount of polyphenol and antioxidant quantities.

However, it is interesting to note that when investigating the effect of this on breast cancer cells, although the polyphenol in BOO is lower, it results in a significantly faster rate of cancer cell death. This surprising result led to us to investigate the effects of black olive oil on other cells. The results were exactly the same. Black olive oil not only leads to the death of breast cancer cells but also of hepatoma and virally transformed cells. NMR analyses of pure olive oils show that there are meaningful differences between black olive oil and EVOOs. However, to obtain clearer answers on what these differences are requires further investigation. This was the first study of its kind to analyse and compare NC BOO, so there are no published findings to compare with.

On this matter, regarding the lab results, black olive oil prepared in Karpaz, but not Evtad, seems to have a therapeutic potential regarding breast cancer cells. Polyphenol extracts of BOOs from both locations were more toxic to cancer cells compared with extracts from EVOOs, but the extract from Karpaz showed better

selectivity for cancer cells. There is scope for further work to examine more carefully the production process of BOOs and to identify key phytochemicals that could be altered during production. Moreover it would be of great interest to understand the molecular basis of the selectivity.

In the participant study people expressed confusion about BOO; some heard it might not be good but are not really sure. Results of this study do not clearly inform us on whether BOO can protect against or be good for BC. BOO extracts help kill the cancer cells to a much higher degree than extracts fromother olive oils, but on the other hand it contains the lowest levels of polyphenols and antioxidants which evidence suggests are protective anti-carcinogens. Perhaps guidelines can be generated for manufacturing methods of BOO to adhere more to the standard EVOO procedure. Black olive oil was the main point of starting this journey, and so far the study has revealed some unique qualities of BOO from analysis of fatty acid, polyphenol, antioxidant activity, NMR spectra and cancer cell work.

Although the general subject of this research investigates what may be a common subject, it is actually unique from beginning to end. As mentioned in chapter I, part one (chapter II) looks at the relationship between the Mediterranean diet and olive oil consumption in breast cancer patients in only in the North of Cyprus. Moreover, it leaves its mark as being the first research to investigate cancer and nutrition in Cyprus. In the third chapter, qualitative techniques are used to consolidate and strengthen the information obtained in previous sections. This is the first piece of research to use this technique on Nutrition/Health Research in North Cyprus. The last section (chapter IV) looks at the analysis of black olive oil which is the main role player in the emergence and continuation of extensive research in black olive oil, and its

effects were tested on breast cancer cells for the first time both in the research itself and the field of research. It not only established awareness of black olive oil in the world of science, but also confirmed the eating habits of this Mediterranean island, and created a statistical data base of breast cancer patients that can be utilised in further research at the social level.

Results from the current study suggest health professionals need to give much more detailed advice on how cancer patients can use foods in order to protect us from cancer, based on the latest evidence base. NC health professionals need to be provided with educational resources to increase awareness that the healthy MD has well-documented and evidenced health benefits and is actually very different from the current average NC diet. Dietetic tips are needed in order to make the NC diet healthier, in order to help achieve dietary changes and aid recovery.

5.5 Overall Limitations

In the process of the research many difficulties posed a challenge for continuing. The biggest problem was obtaining reliable data from a less-than-flourishing health system, as Cyprus is an underdeveloped country. The lack of computerized patient records limited the amount of data that could be obtained on the patients. Moreover, to date, hospital patient data is stored in paper files in archives, and flooding a short time before the research began caused irreversible damage to these. The researcher intended to reach a larger target population during the research process to ensure stronger and more significant research results. Similarly, the intention to investigate cell line research using the olive oil samples emerged as the study's findings were revealed. If this aspect could have been planned and thoroughly organized from the very beginning,

there would have been more time to repeat and exted the obtained analyses and thus reach more conclusive results concerning the components of the OO and their mechanism of action. It would also have been helpful to compare a wider selection of cancer cell lines. However, in this respect it is interesting that cell lines representing two different types of breast cancer, MCF-7 from the most common type of breast cancer and MDA-MB-231 representing the rare triple negative type of breast cancer, were both inhibited by BOO extracts.

If there were a chance to change the study, the following could be considered:

-Increasing the sample size;

-Including more questions on the consumption of black olive oil;

-Including three day 24h recall instead of one day for increased generalisability;

-Adding a third control group comprising of healthy individuals;

-Assigning more time in the laboratory for further characterisation of phytochemicals in black olive oils and their effect on cancer cells;

- Comparing the differences in the preparation of individual black olive oils, and how these could affect the polyphenol or phytochemical content.

6. Chapter VI-Overall Conclusion

In conclusion, the results of this study suggest that BOO has lower levels of protective polyphenols and antioxidants than EVOO, yet black olive oil has a toxic effect on selected cancer cell lines. However, the cause of this toxic effect is unknown and needs further research. There is however, a strong suggestion of a relationship between nutritional diet and breast cancer recovery, and the type of olive oil consumed.

This study also observed that as food and nutritional awareness increases the attitudes towards diseases may also be changing. Therefore, the approaches of health professionals need to recognise this change and tailor health promotion more effectively. This research is the first to examine the link between breast cancer biomarkers and nutritional elements. Even though this study cannot state that there is a definite direct link between the two aspects above, the study can be confident that dietary habits have statistically established effects on the biomarkers, and hence potentially on the recovery from BC. This study, similar to previous research, indicates that a 'traditional' Mediterranean diet, as opposed to the current NC diet, including olive oil, has positive effects on breast cancer. Further work is needed to establish the role, if any of BOO in the recovery from BC.

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ANNEX 1-Survay

Diet, olive oil intake and breast cancer recovery in North Cyprus

ATTENTION! This information is just for use in scientific research and it is not going to be shared with third parties.

Hospital no: Survey no:
Address: Tel.:

I.Personal Information

Age (year): ..... Education;.....

1. Do you smoke? 1. No, Never smoked 2. Smoked and left 3. Yes, still smoking
2. When did you first detect BC/when were you first diagnosed with BC;
3. Has your treatment stopped? 1. Yes 2. No if yes when? .....
4. Did you get chemotherapy during your treatment? 1. Yes 2. No
5. Did you get radiotherapy during your treatment? 1. Yes 2. No
6. Do you use any medicine; .....
7. Did you have any symptoms? 1. Sore mouth 2. Sore stomach 3.Amnesia 4. Pain
8. Your last blood tests; CEA: ..... CA15-3: .....
9. Are you in menopause? 1. Yes 2. No
10. If yes, when did it happen? .....

Anthropometric measurements	**Lower (last 5 year)**	**Now**	**Higher (last 5 year)**
Weight (Kg)			
Height (cm)			
BKI (kg/m^2)			

II. Physical Activity

11. How often do you do the sports below?

• Running • Brisk walking • Cykling • Swimming • Football • Jump reap	• Volleyball • Tennis • Badminton • Squash • Weight lifting • Hockey	• Step • Aerobics • Gymnastics • Dance • Fight sports • Pilates • Yoga	• Heavy garden works • Carpentry • Forest work • Digging

1) Every day or 5 days in a week 2)2-3 days in a week 3)Once a week 4)1-3 days in a month 5) non

III. MDS

12. 1. Do you use olive oil as the principal source of fat for cooking? 1. Yes
13. 2. How much olive oil do you consume per day (including that used in frying, salads, meals eaten away from home, etc.)? 1. >4
14. 3. How many servings of vegetables do you consume per day? Count garnish and side servings as 1/2 point; a full serving is 200 g. 1. >2
15. 4. How many pieces of fruit (including fresh-squeezed juice) do you consume per day? 1. >3
16. 5. How many servings of red meat, hamburger, or sausages do you consume per day? A full serving is 100–150 g. 1. <1
17. 6. How many servings (12 g) of butter, margarine, or cream do you consume per day? 1. <1
18. 7. How many carbonated and/or sugar-sweetened beverages do you consume per day? 1. <1
19. 8. Do you drink wine? How much do you consume per week? 1. > 3
20. 9. How many servings (150 g) of pulses do you consume per week? 1. >3
21. 10. How many servings of fish/seafood do you consume per week? (100–150 g of fish, 4–5 pieces or 200 g of seafood) 1. >3
22. 11. How many times do you consume commercial (not homemade) pastry such as cookies or cake per week? 1. <3
23. 12. How many times do you consume nuts per week? (1 serving = 30 g) 1. >1
24. 13. Do you prefer to eat chicken, turkey or rabbit instead of beef, pork, hamburgers, or sausages? 1. Yes
25. 14. How many times per week do you consume boiled vegetables, pasta, rice, or other dishes with a sauce of tomato, garlic or onion? 1. >2

IV. Qualitative questions (Eating Habits)

26. How ordinary is you'r dailying meals; Main..... Snack.....
27. are you eating breakfast regularly? 1. Yes 2. No
28. When you found out about your illness did you change your diet? 1. Yes 2. No
29. If yes.....

1. Reduce fat 2. Reduce salt 3.reduce CHO 4. High protein diet 5. I went to dietician and got suitable diet therapy 6. Chose organic products 7. High vegetable and fruit 8.

Give up eating light products 9. Give up eating tinned products 10. Other (specify.....)

30. Who suggested to you to do these changes in your diet?

    1.My self 2. My friend 3. Doctor 4. Nurse 5. Dietician 6. Social media 7. Other

31. Do you use BOO? 1. Yes 2. No

32. If yes what brand ;.....

33. How often do you use;.....

V. 24h recall

ÖĞÜNLER MEALS	YEMEK veya BESİN ADI VE İÇİNDEKİLER FOOD NAME and İNGREDIENTS	NET MİKTAR AMOUNT
Sabah Breakfast		
Kuşluk Late Morning		
Öğle Lunch		
İkindi Afternoon		

Akşam Dinner		
Gece Night		

VI. Food Frequency

Food Groups	Non	Monthly	Weekly			Daily			
		1-2	1	2-4	5-6	1	2-3	4-6	6+
Milk Products									
Whole milk Semi skim milk Skimmed milk Condensed milk Cream or milk Cream Cottage cheese Cheese into portions or creamy White or fresh cheese Other cheese Yoghurt Light yoghurt Sundaes Custard									
Meat, egg and fish products									
Chicken and turkey with skin Chicken and turkey without skin Veal or beef									

Pork Lamb Rabbit Liver Ham Processed meat Hamburgers Blue fish White fish Salted fish Oyster ..... Calamari.... Prawn									
Vegetables									
Spinach Cauliflower, Broccoli Lettuce Tomato Carrot Green bean Eggplant, Cucumber Pepper Asparagus Onion Garlic Parsley, thyme Potato Mushroom									
Food Groups	**Non**	**Monthly**	**Weekly**			**Daily**			
		1-2	**1**	**2-4**	**5-6**	**1**	**2-3**	**4-6**	**6+**
Fruits									
Orange, mandarin, grapefruit Banana Apple, pear Strawberry Cherry Peach, apricot, nectarine Watermelon Melon Kiwi Grape Olive Fruits in syrup									

Dried fruits Almond, chestnuts...etc. How many days a week do you take fruit for dessert?									
Legumes and cereals									
Lentil Bean Chickpea Pea White bread Whole grain bread Breakfast cereals Rice Macaroni Pizza									
Food Groups	**Non**	**Monthly**	**Weekly**			**Daily**			
		1-2	**1**	**2-4**	**5-6**	**1**	**2-3**	**4-6**	**6+**
Pastry									
Cookies Doughnut Fibre biscuits Biscuits with chocolate Croissant Cake Chocolates, bonbons Nutella									
Mix									
Croquette Cream Mayonnaise Tomato paste, ketchup Tabasco Salt Marmalade Honey Sugar Chips									
Drinks									

Tea Cola Whisky, vodka, gin, liquor Vine Natural fruit juice Grape juice Beer Coffee without caffeine Coffee									

34. Can I contact you again? 1. Yes 2. No

ANNEX 2-Interview schedule

Time Available: 30-45 minutes

1. This question is for introduction and warm up.

Q1 When were you first diagnosed with Breast Cancer (BC)

2. Today scientific research shows that there is a significant relation between cancer and our food choices. We are investigating if patients have awareness of any relation between their disease and nutrition. Also, patients usually get their health information from doctors. In this questionnaire, we are attempting to find out if participants have any knowledge of cancer nutrition.

Q2 To what extent (how much) do you think nutrition affects your BC?

- **Do you think there is a link between nutrition and BC?**
- **Where did you hear of this?**
- **Were you aware of this previously (before being diagnosed)?**

3. Adequate and healthy nutrition has been shown to prevent chronic disease (Shahril *et al.*, 2013). Even small changes in diet have a positive impact on recovery from chronic disease (Budzynska *et al.*, 2013). Nutrition therapy is becoming the most important preventive treatment for chronic disease. Today, as technology is developing it is easy to access any type of information. However, every source that we find cannot be reliable.

Q3 Did you change your diet after being diagnosed?

- **Which changes did you make?**

- **Who gave you this advice?**

- **What sources do you trust the most (what is your primary source of information)?**

4. The terms "complementary" and "alternative" are sometimes used to refer to non-traditional methods of diagnosing, preventing, or treating cancer or its symptoms (American Cancer Society-www.cancer.org). Cancer patients investigate alternative medicine to manage their cancer-related symptoms or side effects such as acupuncture, detox, Chinese medicine, herbal treatments (Philippou *et al.,* 2013, Vinjamury *et al.,* 2013, Towler *et a.,* 2013).

Q4 Do you receive any alternative treatment to help with your illnesses outside of hospital?

- **Do they give you any nutritional advice?**

- **Tell me a bit about it.**

5. This is an exploratory question in order to identify key concerns experienced by Cypriot breast cancer patients.

Q5 When you were first diagnosed what were you most concerned about?

- **Did you change anything due to this?**

6. The symptoms of cancer can vary depending on the cancer type and the person. Chemotherapy can affect normal cells too. This damage to normal cells causes side

effects. During their treatment and recovery patients can get a sore mouth, sore stomach, amnesia, pain…. etc. Also some anticancer drugs may affect cells of vital organs, such as the heart, kidney, bladder, lungs, and nervous system (American Cancer Society-www.cancer.org). These can affect patients' eating habits, social life or totally decrease their quality of life. Due to this they have to change their habits imperatively.

Q6 Did you have any symptoms during your treatment and did you change anything due to this?

- **How did that make you feel?**
- **Do these symptoms affect your lifestyle? Your diet?**

7. Exposure to heat, light or oxygen will alter the nutrients found in food. Some cooking methods such as heating, steaming, microwaving, boiling, stir-frying, using acid, and blanching, affect foods' nutrients and health-promoting compounds, and also can cause loss or decrease of foods' nutritional ingredients (Yuan *et al.,* 2009). The way that food is cooked is absolutely essential for avoiding unnecessary nutrient loss. Cooking temperature and time can make an enormous difference in the nutritional quality of a meal.

Q7 Did you change any cooking methods?

- **Did this affect your eating out at friends/family/restaurants?**

8. The type of fat is important in prevention of BC (Solanas *et al.,* 2010). Extra virgin olive oil is believed to have chemo preventive characteristics for BC (Solanas *et al.,* 2010). Up until now there hasn't been any research on black olive oil. On this question

we want to get the opinion of the patients whether they use black oil and do they trust traditional black oil. Do they compare black oil with other olive oils?

Q8 Do you feel that Cypriot black oil (BO) is good for health?

- **Why?**
- **Do you prefer BO to Green olive oil**

9. In this question we are looking at the view of patients about nutrition.

Q9 Do you feel you have enough information about nutrition?

- **Did you experience any confusion about the right things to eat?**

Q10 Do you have any comments you would like to make / add?

Example Interview

[+Me -Patient] [Turkish]

+ When were you first diagnosed?

- In 2011

+ in 2011

- I was diagnosed in March. You can go ahead with your questions, ask anything..

+I'll ask my questions as we go along.

- Yes, please do. As I said, I was first diagnosed in 2011.

+Were you treated at Near East University Hospital?

- No, I wasn't treated at N.E.H. but we went there to get an opinion, as part of the pre-diagnosis.

- I was scanned there; actually all the examination took place there. About the treatment, whether there will be a need for operation or not… luckily, I didn't need chemotherapy

+That's great

- Why didn't I need chemotherapy? The tumour in my breast was 2cm and the lymph was clear.

+Early diagnosis

- -erken tesis aslinda ben cok erken fark ettiydim, cok cok. Iki sene once fark ettiydim, daha dogrusu bir bucuk sene once fark ettiydim. Cok minicik boyle toplu ignenin basiykenden fark ettim. Iste giddim ultrasound taramalari falan sinirlari duzgun bir kitle gibi gorundu ilk etapda. Ben da sey yapmadim tabii. Dr. sinirlari duzgundur kesinlikle bir tumor degildir. Degildir deyinca benda cok usdune dusmedim. Aslinda bana 6 ay sonra tekrar gel gorelim falan dedi ben usdune dusmedim gitmedim. Odan sonra ne zaman ki buyumeye basladi cunku yuzeyseldi cok benim.
- Yes, it was early diagnosis. I realised it very quickly, almost two years ago, or maybe one and a half years.. I realised it when it was as small as a pinhead. I had check-ups, ultrasound and so on, it seemed like a regular lump. So I didn't do anything about it. I didn't think it was important because the doctor said it was a regular lump with a clear-cut shape. Later on it started to get bigger, it was tangential.

+Was it visible?

- onun icin elimle cok daha rahat.... Gozle gorulmese bile deriye cok yakin bir yerde oldugu icin elimle cok rahat bulurdum kendini. Iste buyumeye baslayinca dedim artik gideyim bird aha chek up olayim nedir hani nereye varacak bu buyumenin sonu hani en azindan aldiririm seyse. O zaman sekli sey oldu
- I could easily feel it with my hand. I couldn't really see it but because it was really close to the skin I could easily spot it. When it started to grow, I went to have another check-up. I thought I could have it removed. Then its shape started to....

+You mean there was a change in its shape?

- -tabii cok korkunc derecedeydi yani sekli bilirsiniz....
- Yes, there was a horrible change, you know how it is..

+It doesn't have a regular structure

- -yani kanserli hucrelerin ilk goruntuleri sekilleri ilk duzensiz bir sekilde buyur ve iste tesis konduk dan sonra turkiyeye gittim ben .
- Cancerous cells grow in a very irregular shape. Then I went to Turkey after I was diagnosed.

Turkiyede ameliyatimi oldum yuzeysel oldugu icin benim oyle gogsumun buyuk bir kismi vey a yarisi cok az bir kismi alindi ve hemen estetik ameliyat yapildi

I had an operation in Turkey and because it was very close to the surface, they only removed a small part of my breast and I also had plastic surgery.

+You mean at the same time?

- Ayni anda estetik ameliyat da yapildi, belli belirsiz yani benim. Ondan sonra kemoterapi almadim aslinda sinirdaydim alip almama konusunda.
- Yes, the plastic surgery was also done during the operation, it's barely visible now. I didn't have chemotherapy afterwards, but actually it was a borderline case.

+And the blood tests? Were they also borderline?

- -ben amerikada genin, genomar diye gen testi yaparlar bu hastaliklarla ilgili hani kemoterapi almam vucudumun kemoterapiye ihdiyaci var mi yok mu diye bu test yapilir
- In America, they do genome testing for these illnesses. They do this test to find out whether your body needs chemotherapy or not.

+I see.

- -ordada sinirda cikdim. Bende kendi insifiyatifimi kullanarak kemoterapi almamayi uygun buldum. O kadar cok arastirma yaptim ki bu kemoterapiyle ilgili. O kadar cok yani eeee…. Cok sicak bakmadim, cok ornek var kemoterapi alan insanlarin. Oyle sinirda oldugum icin doktorumla da konustum dr. bana birakti. Bende kemoterapi almamayi uygun gordum kendi capimda. Tabi bud r.un onerisi degildi. Ben dedim almaycagim bir ornek de ben olayim kemoterapi almadan o seyi
- The test results were borderline. So it was up to me to to decide if I wanted chemotherapy and I decided not to. I did lots of research on chemotherapy. Lots! I didn't like the idea. There are many examples, so many people who go through chemotherapy. . Because my case was borderline, I consulted my doctor too and he said it should be my choice. And I decided against it. Of course, this was not the doctor's recommendation. I said I won't do it, I wanted to be one of the cases that refuse chemotherapy.

+But you had medical treatment, right?

- -sadece ilac tedavisi aldim. Isde 5 yillik bir tamoksifen diye bir hap vardr agizdan alinan bu mem kanserlilernin tumune verilen ir ilacdir. Hapdir yani gunde bir defa alnir. Smdi 2014un martinda benim 3 yilim dollar 2 yilim daha kaldi 5 yil doldukdan sonra zaten butun tedaviler kesilecek . tabii ameliyatdan sonra ki tip tedavi sekli vardir bir kemoterapi ikincisida radyoterapi isin tedavisi. Ben isin tedavisi aldim
- Yes. There's a medicine called tamoxifen, it's an oral tablet. It's the medicine that they give to all breast cancer patients. It's a pill that you take once a day. In March 2014, it'll be three years, and then I'll have two more years left. At the end of five years, the treatment ends. Of course there are two types of treatment after the operation, radiotherapy and chemotherapy. I had radiotherapy.

+You had radiotherapy?

- -Radyoterapiyi redetmedim, cunku on da birazda goruntume cok onem verdigim icin herzamanda boyleydim hastaykende boyleydim.
- I didn't refuse radiotherapy because I care about my physical appearance, I always did.

+Are there any differences in your weight before and after the illness?

- Aaam simdi kontrol altina girdim kendim cunku hastaligmi ogrendikden sonra kesinlikle seker ve sekerli gidalarin kanseri tetikledigni ogrendim, tumour besledigni ogrendim, hatta ve hatta pet taramasinda yapilan damaran enjekde edilen bir sivi vardir pt e girmeden onada radyoaktive bir maddeyle glikoz

verildigni ogrendim seker. Cunku seker tumor olan vucutta sekeri gorurkenden seker ona yapisirmis onu emermis. Ha onuda ogrendikden sonra ben bir de kullanacagim bu tamoksifen haplarinin hormonlari bloke ettigini ogrendim. Ostrojeni baskiladigini ogrendim

- I take care of myself now. After my illness, I learned that sugar and sweet things trigger cancer; it feeds the tumour. I even found out that in PET scan, they inject some liquid which is radioactive glucose. Apparently, the tumour finds the sugar in the body and feeds on it. I also found out that these pills that I'm taking, tamoxifen, block the hormones. They boost oestrogen.

+You mean it's anti-oestrogene?

- Antiostrojen. Tabii ki bunlarinda kilo artisina yol acacagni da bildigim icin ben kendi kendime bir diyet programi uyguladim. Bir diyetisyene gitmedim. Aslinda benimda size cok sorularim olacak cok merak ettigim seyler vardir.
- Yes. Of course I knew that these would cause me to put on weight so I went on a diet. I didn't see a dietician, though. Actually, I've got a lot of questions to ask you, too.

+Sure, you can ask anything.

- Kilomda cok artis olmadi ogrendikden oncesi ve sonrasi cunku zaten kiloluydum 82 kiloydum su an ben 73 kiloyum tabiii bu 3 yil zarfirnda degil son bir senedir
- I didn't put on any weight because I was already overweight, 82 kilos. Now I'm 73 kilos, of course I wasn't always 73, only since last year.

+So you're 73 kilos since last year, what was your weight before then?

- Son bir senedir 73 oldum. Ondan once hep 82-83 78 boyle hep oynardim. Yaklasik bir yildir gecen yazdan beri siki bir seye girdim ben daha dogrusu cok da siki degil aslinda kendi capimda bilincli beslenme.
- Yes. Before then, I was 82-83. I've been on a strict diet for a year, since last summer. It's not actually very strict, I'm just trying to eat healthy.

+What kind of changes are there in your diet? What's the difference between your diet before and after your illness?

- Ha yani o kadar cok bilinclendim ki bu hastalikla ilgili etkenleri ogrendik sonra ben nerde yanlis yaptigmi da ogrendim. Yani ben niye meme kanseri oldum ne sebep oldu da meme kanseri oldum
- I've become very conscious, I learned a lot about this illness, its causes and so on.. I've become aware if my own mistakes. Why did I get breast cancer? What caused it?

+You started to question?

- Kendi kendimi sorgulamaya basladim. Sorgulayinca sigaranin meme kanserni tetikledigini ogrendim ben 20 sene sigara ictim ama yaklasik 7-8 sene once kesdim. Demekki bana yapacagi zarari yapmis. Sekerin tumour belsedigini ogrendim.
- Yes, and I found out that smoking triggers breast cancer. I had smoked for 20 years but I quit 7-8 years ago. But apparently, it had already caused some damage. I also found out that sugar is bad for tumour.

+Did you cut down on sugar totally?

- Tamamen kesdim. Evet meme kanserinden once asiri derecede tatli bagimliligim vardi hic kendimi durduramazdim. Yemekten cok tatli yerdim
- Yes. I used to eat a lot of sugar before I got breast cancer, I couldn't cut down on it. My diet was based on sugar.

+Did you use to have dessert instead of main course, for instance?

- Ogun niyetine hep oyle. Yani evde bir tatli olsun ogun yemegim oydu uc ogun. Tatli buzlukda bitecek.
- Yes, I used to do that all the time. if there was dessert at home, I'd have dessert for three meals a day.

+Were these mostly desserts with syrup, or sorbet?

- Her cesit yani kendim yapayim disardan gelen nen olursa. Dondurma yazlari sinirsizdi yani sinir koymayi bilemezdim o kadar cok bagimliligim vardi tatliya.
- All kinds of desserts.. I used to make desserts at home and I also used to buy a lot of dessert. Especially in the summer I had a lot of ice-cream. I was addicted to sugar, I had no limits.

+What other changes have you made in your diet than cutting down on sugar?

- Karbonhidrati kesdim.
- I cut down on carbohydrates.

+When you say you cut down on carbohydrates, what did you stop eating?

- Pirinc, beyaz pirinci aslinda son bunu nasil degerlendireyim ben size, bu meme kanseri oldukdan sonraki surec degil. Meme kanseri oldukdan sonra iki sene gene ben cok bakmadim kendime. Boyle bir bocalama pisikoljik olarak ck kendimi yikmadim. Zaten ben bunu hastalaik olarak gormedim. Bu hastaliga yakalanan herkese tavsiyem en basta bu seyi kesinlikle bunu hastalik olarak gormesinler. Ben hastalik olarak gormedim ben ule oynaya giddim seye turkiyeye. Tabii ilk ogrendigim an dnyalar basima yikildiydi ama o bir gundu ondan sonra kendimi topladim. Ameliyatdan cikdim kendimi topladim butun ne kadar alisveris merkezi varsa o halde sargili yedim icdim gezdim, bitirdim dedim hayatimda artik bitmistir geride kaldi ve ben onu hayatimdan cikardim. Hicbir zamanda kanser hastasiyim diey veya tekaradan nuks edecek diye oyle bir dusunce hic olmadi kafamda. Hic . ne olum korkusu ne dedigim gibi yani beynimde bitirdim sigarayi da oyle kesdim ben. Bir gunde . evdekilerde halen da icer ve irademin guclu oldugunu dusunurum hastaligmida oyle yendigmi dusunurum. Diyet konusundada karbonhidratlari kesdim. Benim 8-9 kilo vermemin seyi yaklasik bir yilki surece dagilir ama ara ara bozarim haftada bir bozarim.

- Rice, especially white rice. Actually, this is not the process that I followed just after the breast cancer. After I got breast cancer, I didn't take good care of myself for two years. I didn't know what to do but I didn't collapse psychologically. I didn't really take it as an illness. My advice to everyone who has this illness is that they should not consider this as an illness. I was in a very

good mood when I went to Turkey. But of course when I first found out about it I felt horrible. But that lasted only for a day, and then I pulled myself together. As soon as I got out of the operation, I pulled myself together and went out shopping and had fun. I thought it was over, I went through this thing and I left it in the past. I never thought I'm a cancer breast or that it will recur. Never. I wasn't scared of dying either. That's what I did when I quit smoking, too. I just left it behind, I did it in one day. Everyone in my family still smokes but I have a strong will and I believe that's how I overcome this illness too. as for my diet, I cut down on carbohydrates. It took almost a year for me to lose 8-9 kilos, but of course I do eat a lot sometimes.

+When you say carbohydrates, what do these include?

- Pirinc tuketmem beyaz ekmek tuketmem
- I don't eat rice, I don't eat white bread.

+Do you eat brown bread and bulgur rice?

- Evet onlari tuketirim. Makarna arada cok nadir. Belki 15 de bir. Ha oncelik sekeri kesdim porsiyonlarimin miktarini azalttim. Karbonhidradi cok azalttim kesme olmaz cunku bilirim onu. Isde kepekli ekmek
- Yes, I do. I also have pasta once in a while, maybe once in two weeks. Most importantly, I cut down on sugar and reduced my portions. I cut down on carbohydrates but of course I didn't stop eating them at all, I know that's not good. I replaced it with brown bread.

+So you actually you didn't cut down on carbohydrates but started to have healthy carbohydrates?

- Aynen oyle kesdim denemez saglikliya gecdim.
- Yes, exactly.

+How much fruit and vegetables do you eat?

- Meyveyi kesdim. Meyveyi cok azalttim. mesela benim bir gunde yediklerim belki bir meyve yerim. Dun mesela hic meyve yemedim.
- I cut down on fruit. I don't eat fruit much. Maybe one portion of fruit a day only. For instance, I didn't have fruit at all yesterday.

+Why did you cut down on fruit?

- Sebep olarak fruktozunda sekere donustugunu glisemik indeksi yukselttigini, onu ogrendim. Ben bunlari internetten dergilerden gazetelerden hep okuyarak ogrendigim seylerdir.
- I found out that fructose also turns into sugar in the body and raises glycemic index. These are things I learnt through the internet, newspapers and magazines.

+Did you receive any alternative treatment or support?

- Hayir hayir hayir zaten ben tedavimi oldum kemoterapi almadim randyoterapiden sonrada hap tedavisi uygulandi bana zaten psikolojim gayet yerindeydi oyle bir seyim olmadi.
- No, I had the medical treatment. I didn't take chemotherapy and after radiotherapy, I received treatment with pills. I didn't have psychological problems.

+Are there any fruit that you don't eat at all?

- Hayir tum meyveleri yerim ama gunde bir tane meyve yerim. Mesela yazda karpuzun cok tatli cok sekerli meyvelerinde zararli oldugunu ogrendim . yani oyle duydum belki yanlis bilirim bu konulari sizinle daha sonra detayli konusalim. Meyveyi azalttim belki bir muz bir mandalina kis aylarinda. Yazlarida karpuz hellim uc ogun oyle porsiyonlar dolu dolu onar bitti azalttim.

- No, I like all kinds of fruit but only one portion a day. For instance, I found out that watermelon has a lot of sugar in it and that sweet fruit are not good for you. That's what I heard, maybe it's not true, we should talk about this in detail later. I cut down on fruit, I sometimes have one banana or a mandarin a day in the winter. I cut down onw watermelon and halloumi in the summer.

+Have you made any changes in the way you cook your meals?

- Pisirme yontemlerimde bir degisiklik yapmadim . simdi diyet yapmadigim surecde kizartmada yerdim hersey yerdim ama normal sulu yemeklerimi hep zeytin yaginda yaparim zeytin yagini cok kullanirim salata cok yerik biz.
- No, I haven't changed anything. When I wasn't on a diet, I used to eat a lot of fried food. But I always used olive oily with other meals. We use olive oil a lot, we have a lot of salad.

+Do you have salad with every meal?

- Her ogunde yapmam sulu yemegin yaninda salata yapmam. Ama izgara yemeklerin yaninda mutlaka salata olur. Sulu yemekleri de zeytin yagiynan

yaparim ve kizartmadan . mesela etli taze fasulye ornek gosderim eti veya domatesi hicbirsekilde kizartmam cunku zeytinyaginin kizarmadigi bilirim. Onu hicbirsekilde ya isitmam ya yakmam. Sadece onu suyuna katarim kapattiktansonra. Etleri sogani kizarmadan direk hepsini bir tencerenin icine koyup suyunu koyup usdune zeytinyagini eklerim ve o sekilde pirisirim yemeklermi yani o sekilde yaparim hep yemeklemi.

- Not with every meal. Btu if we have grilled food, then we definitely have some salad. I use olive oil with other meals. For instance, when I make green beans with meat I never fry the meat or the tomatoes because I know that you're not supposed to fry olive oil. I never heat or fry it. I only add it to the water after the dish is cooked. I put the meat and the onions in the pan but I don't fry them, I just put olive oil on them and cook it.

+Do you use black oil?

- Kara yag kullanmam. Cunku kokusundan dolayi. Arada bir kullanirim severim aslindada kokusunu cocuklar cok sevmedigi icin. Salatalara koyarim ben zeytin yagi kara yag farki daha yogundur. Cokluk onu tercih etmem.
- No, I don't. I don't like it's smell. I use it once in a while, I actually like it's smell but the kids don't. I put it in salad. But I mostly prefer olive oil, black oil is a bit heavy. I don't prefer it.

+Where do you buy the black oil?

- Bize hep organic gelirdi yaglarmiz bildigmiz bir yerden cikarillardi kendileri getirillerdi bize.yaklasik bir yildir o durdu simdi marketten karpaz kara yagini

alirim. En iyisini almaya calisirim, iyi oldugunu soyledikleri icin. Belki sizing tavsiye edecegniz bir marka varsa onu da kullanabilirim.

- We always had organic oil. They used to bring us organic oil that they produce themselves. Now they stopped, so we buy the Karpaz black oil at the supermarket. I try to pick the best brand. They say Karpaz is good. If you have other recommendations, I'd like to try it.

+Did you use to cook with olive oil before?

- Daha oncedenda hep zeytinyagi. Aslinda yemeklerim hafiftir benim.
- Yes, always. I actually cook light dishes.

+How much fast food do you have?

- Bir yil oncesine kadar cok kotuytu cok tuketirdim. Surekli yani haftada iki uc , belki rum tarafina gecdigim zaman mc doladsi cok severdim. Burdan pizzadir haftada 2-3 kez cagirirdik
- Until one year ago I used to have a lot of fast food. Two or three times a week, when I went to the Greek side I used to have McDonalds. Here, we used to have pizza 2-3 times a week.

+Did you pay attention to buying organic food other than olive oil?

- Bal I organic almaya calisirim. Simdi ben sekeri kesdim dedim ya yaklasik uc Aydan beidir, sadece bal kullanirim tatlandirmak amaci ile cayimi veya sutumu. Sabah kahvaltisinda yarim yagli sut yarm bardak yarim tatli kasik bal bir haslanmis yumurta her gun bir de kepekli peksemet veya bir dilim kare kepekli ekmek lerden tost yaparim.

- I try to buy organic honey. As I said, I cut down on sugar, so I only have honey with milk and tea. For breakfast, I have semi-skimmed milk with half a spoon of honey, one boiled egg and a slice of toasted brown bread.

+Do you toast the bread or other things a lot?

- Hayir kesinlikle ben yaniklarin yanik yemeklerin de kansere yol acdigini bildigim icin bnu uzun suredir hicbir sekilde ne yerim ne da yedirdirim. Ekmeklerim olsun yemek olsun mesela mangal yapdigmizda kesinlikle etler yanik olsun kesinlikle onlarida dikkat ederim yememeye calisirim. Sebzeyi cok artirdim.
- No, never, I'm aware that burnt food causes cancer too. So stopped having burnt food a long time ago. For instance, when we have barbecue, I never eat burnt meat. I started to eat a lot of vegetables.

+Do you eat canned food? Salami? Pepperoni?

- Hayir. Kesinlikle belki bir yildir eve eskiden jambon turu bolibif buzdolabimda hep bulundururdum simdi ne yerim ne yedirdirim. Yani onlarinda zararlarini ogrendigim icin. Conserve yok, fast food yok denecek kadar az oldu, kizartma zaten bizim evde kofte cips olayi vardi sadece o . daha oncedenda hastaligimdan oncedenda bilincliydim ama ben uymazdim bu duruma. Simdi bu uc ay zarfinda 8 kilo verdim, kendim degisdirdim .
- No. I used to have canned beef and ham in the fridge all the time but now I never buy them. I learnt about how unhealthy they are. No canned food, no fast food or very little, as for fried food, we only used to fry meatballs and potatoes

anyway. I knew about all these before my illness too of course but I never did anything about it. In these last three month,s I lost 8 kilos, I changed myself.

+Where do you do your research?

- Internetten arastiririm, karatay diyetini uyguladim halen daha belki uyguladigim odur kitaplarni aldim cok benimsedim benim mantigma cok uydu. Ama tabii bilemem ne dere dogru.
- On the internet. I followed the Karatay diet, I still do at times. I bought his books, I really liked his diet, I think it makes sense. Of course I don't know how accurate it is.

+How long have you been following the Karatay diet?

- Okudugumdan belli yaklasik bir yildir, ama siki 5 aydir siki girdim. Haftada bir yazda belki bir top dondurma tatli da mecbur olurum bir yerde bir dilim. Eve hic tatli almam gelenleri paketi acilmadan geri gonderirim sirf gormeyim diye.
- Since I first read about it, for about a year now. But for 5 months I followed it very strictly. In the summer time I have ice cream maybe once a week. I never buy desserts anymore.

+Did you have any side effects after the operation or during your treatment such as nausea?

- Hic bir yan etkisini ben gormedim. Biraz bas agrisi oldu. Bir de radyoterapi alirken yuzeysel distan 2. Derece yanik olduydu ileri derecede onu iki uc ay cekdim te gecsin . tamoksifein bas agrisi yaptigini bilirim. Bugunlerde yine

kontrolum var bundan sonra artik yilda bir kontrlum olacak momografi kan tahlilleri falan.

- No, I never had any side effects. Just a slight headache. During radiotherapy, the surface of my skin was burnt; it was a severe burn, second-degree. It took three months to recover. I know that tamoxifen causes headache. One of these days I'll have another check-up and then I'll have check-ups only once a year; mammogram, blood tests and so on.

+Is there something you'd like to add?

- Beni birakirsan sabah kadar anladirim ama ozet olarak budur yani basimdan gecenler beslenme oykum yaptigim degisiklikler temel olrak bunlardir.
- Well, I can say a lot more but this is the summary of what I've been through. These are the basic changes I made in my diet.

ANNEX 3-Patient Information Sheet

London Metropolitan University
PATIENT INFORMATION SHEET

We would like to invite you to take part in a research study. Before making a decision it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

To date there are no studies about the nutrition habits of breast cancer in North Cyprus before. We don't know the eating habits of breast cancer patients and the possible effects of them. The study aims to recruit 1000 women to discover breast cancer patients' nutritional states, particularly in age 19-65 year women. We hope it will help us to understand the cause of the disease in breast cancer patients.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to change your mind or withdraw at any time and without giving a reason. This will not affect the standard of treatment or care you receive.

What will happen to me if I take part?

If you do agree to participate, there are several parts to the study: asking you some questions using a questionnaire about your daily lifestyle, physical activity, Mediterranean diet and food intake; a request to view your medical records; we are going to take your anthropometric measurements; we will record your 24 hour food consumption with the help of a photographic food catalogue. All data will remain anonymous and you will not be identified in any way.

What are the possible disadvantages of taking part?

There are no specific risks associated with taking part in this study.

What are the possible benefits of taking part?

If we can discover more about the nutritional causes of breast cancer we may be able to prevent disease or detect it at an earlier stage. It will provide the first nutritional data for breast cancer in North Cyprus.

What will happen to the results of the study?

The overall results of the study will be made available publicly through presentation of results at research meetings and publication in scientific journals. No participant in the study will be identified in these presentations or publications.

Will I be contacted again?

We may wish to re-contact you to discuss your form in more detail, for further information or if a point on the form requires clarification. We would like to offer to re-contact you if a research finding was relevant to you.

Will my taking part in the study be kept confidential?

All data will be strictly confidential and kept in a locked filing cabinet on the hospital site.

Who is organising and funding the research?

This research forms part of a PhD study based at London Metropolitan University. No funding has yet been acquired but may be sought from funding streams.

Who has reviewed the Study?

The study has been reviewed by London Metropolitan University Ethics Committee, the Research and Graduate School Committee at London Metropolitan University and by the doctors who are responsible for your care. Dr. Özlem Gürkut and Dyt. Ayşe Okan

ANNEX 6- Ethical Approval

Ethics Approval

A process of ethics evaluation must be undertaken for all projects, including those within taught modules (not just project modules). Usually, ethical approval will only be needed if, in the course of the research, data from human subjects will be collected and stored, or they may be given samples of food to eat, they may be subjected physical or psychological testing, or body fluids or tissue samples may be collected (only under medical supervision). For research projects, students should fill in this form and discuss it with their supervisor. This process MUST be followed for ALL student projects – undergraduate (BSc), postgraduate (MSc, MRes, PhD). They also need to read the University Code of Practice. See: http://www.londonmet.ac.uk/research/the-graduate-school/research-ethics/documents.cfm

When completed, the form should be sent to the Chair of the Research Ethics Review Panel (RERP) for recording.

Basic project information

Name: Ayse Okan **Date:** 04/2012

Course: PhD (International Public Health Nutrition) **Year:** 1
Supervisor: Dr. Anne Majundar

Title of project: Diet, olive oil intake and breast cancer recovery in Northern Cyprus

Main aim of study and hypothesis to be tested:

-To explore the nutritional intake of cancer patients in Northern Cyprus (NC) and compare the nutritional intake of Cypriot cancer patients to the traditional Mediterranean diet
- To explore dietary changes that can be observed in cancer patients under treatment
-To examine the effects of diet and olive oil intake on breast cancer tumour markers in breast cancer (BC) patients
-To determine the chemical characterization of black olive oil (BOO) and compare it with other olive oils to identify any special characteristics of BOO

I. Project details

1. Are you using people in your research? Delete as necessary:

YES

2. If YES, number of research subjects: 1000

If NO, please go to question 5.

3. Who are the research subjects?

Women had and have breast cancer. Group 1; Active treatment continues n:550 (including chemotherapy, radiotherapy), Group 2; Active treatment completed within 2 years (follow-up) n:150 (including chemotherapy, radiotherapy and surgery), Group 3; If patient is clear after >2 years since treatment stops n:300

5. Research Procedures

Please complete the following:

a. When and where will the research be conducted?

Patients will be recruited from the two biggest hospitals of Northern Cyprus; Dr. Burhan Nalbantoglu Government Hospital and Near East University Hospital. The study will start in July 2012

b. How will you collect the data? Eg Questionnaires, Interviews (face-to-face phone, by email etc), Physiological measurements (please specify), Other (please specify)

Patients will initially be interviewed for 25-35 minutes. 24-hour food records, food frequency, weight and waist circumference will be collected with face to face interviews with the nutritionally trained researcher. Also from patient's medical records: Tumour biomarkers – CEA and CA 15-3

Personal information – address, telephone, age

Weight (kg)

Height (cm) will be collected.

Every eligible patient will be given an information sheet (Patient Inform Sheet [PIS]) regarding the study. If a patient is willing to participate in the study, they will be asked to sign content a form to show they agree to participate.

Their general information, nutritional habits, physical activities and anthropometric measurements will be obtained directly from the participants, who will personally answer face to face interviews conducted by the main researcher and qualitative answers will be collected from the interviews. To evaluate their nutritional status 24-hour recall and food frequency questionnaire (FFQ) will be analysed. Also a Mediterranean diet is going to be measured by using validated Mediterranean Diet Adherence Screener (MDAS) which includes 14 questions

You may need to describe your research procedures in more detail. Please discuss this with your supervisor.

d. Are any of these procedures likely to cause discomfort, anxiety, stress or embarrassment?	YES	NO
e. Is this unavoidable?	YES	NO

If you answered 'YES' to either or both questions (d) and (e), please give details and explain how you will seek to minimise the impact of this. Append a separate page to the form.

Continued over……

Please provide the following information and discuss the details with your supervisor. You may be asked to add more detail or change it.

i. Will you provide a written/oral explanation of the project to the subjects *Attach the explanation to this form*	YES	NO
ii. Will you obtain freely given, explicit and informed consent, preferably in writing, before the research begins? *Attach the draft consent form*	YES	NO
iii. Will you minimise any risks to your research subjects?	YES	NO
iv. Will you explain to the subjects that you are a student and undertaking degree studies.		
v. Will you explain to the research subjects that they may not benefit personally from your study.	YES	NO
vi. If you are using tape or digital recording and other data collection methods, will you explain this to your subjects or explain why you are not going to tell them.	YES	NO
vii. Will your research subjects should be given the opportunity to decline to take part?	YES	NO
viii. Will you offer your research subjects the opportunity to withdraw at any stage?	YES	NO
ix. Will you be working through a third party or gatekeeper, or using minors (under 18) or the elderly as subjects? *If so, explain how you have considered issues relating to this.*	YES	NO

x. Will you ensure that all data will be treated with absolute confidentiality?	YES	NO
xi. Will you ensure complete anonymity?	YES	NO
xii. Have you read the provisions of the Data Protection Act. Indicate how you will comply with it. See Appendix below for what the Act requires.	YES	NO
xiii. Will you dispose of personal data when the project has been completed.	YES	NO
xiv. If you are working for a commercial client, have you agreed ethical issues with them and also how intellectual property rights have been assigned?	YES	NO
xv. Will you provide participants with details of the results? Please indicate how you will do this.	YES	NO

If you have answered YES to questions (*d*) and (*e*), please provide a written statement of why you have done so. You should also provide details under sections *i* to *xv* as necessary

The ***completed form and any additional supporting information*** MUST be sent to the Research Ethics Review Panel for your subject area for recording, review and comment.

This can be done by email to Richard Marshall r.marshall@londonmet.ac.uk copied to

ALL RESEARCHERS
Please read the following statement and sign that you have read it.

I , Ayse Okan 09021735 (insert your name, and student ID if appropriate) have assessed the ethics of my research project as indicated above and have also read the University guidance on research ethics.

If there are NO issues, please tick the following paragraphs and sign below

I also confirm that:

1. I have discussed my project with my supervisor and ***we agree*** that there are no issues that need to be considered by the Research Ethics Review Panel (RERP).

2. I have read and understood the University's Research Good Practice and Code of Practice.]

3. I have read and understood other Codes of Practice that are relevant to this project (ESRC, BBSRC, NHS, MRS, PFSG/IFST etc).]

Signed:.....*(Student)*
Date: 04/1012

Supervisor: I confirm that there are no issues that need to be considered by the RERP.

Signed:..... *(Supervisor)*
Date:.....

Where issues have been identified, please tick the box and sign below:

I/we are seeking/have obtained advice from the RERP. The project will not commence until the advice has been received and any issues of ethics have been dealt with appropriately.]

Signed:.....*(Student)*
Date:.....

Supervisor:

Signed:..... *(Supervisor)*
Date:.....

ALL completed forms (whether ethics issues have been identified or not) MUST be sent (by email) to the RERP for recording.

Appendix

Data Protection Act

There are eight key principles as follows

Data must be

- fairly and lawfully processed;
- processed for limited purposes;
- adequate, relevant and not excessive;
- accurate;

- not kept longer than necessary;
- processed in accordance with people's rights;
- kept secure;
- not transferred abroad without adequate protection.

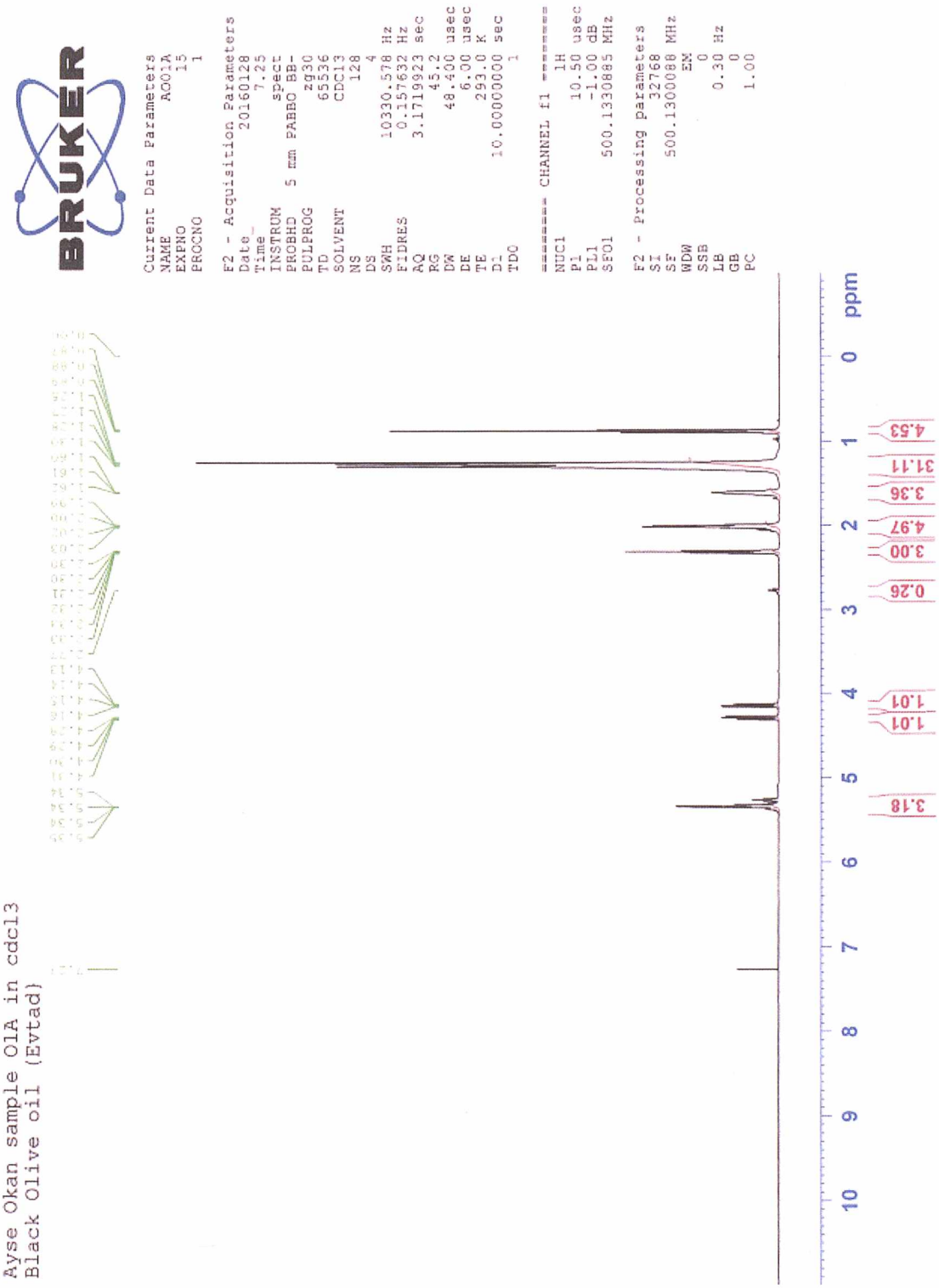
In addition, people whose data is recorded have the right to view that data ('right of subject access'), make corrections or have it deleted.

For details see: http://www.direct.gov.uk/en/RightsAndResponsibilities/DG_10028507

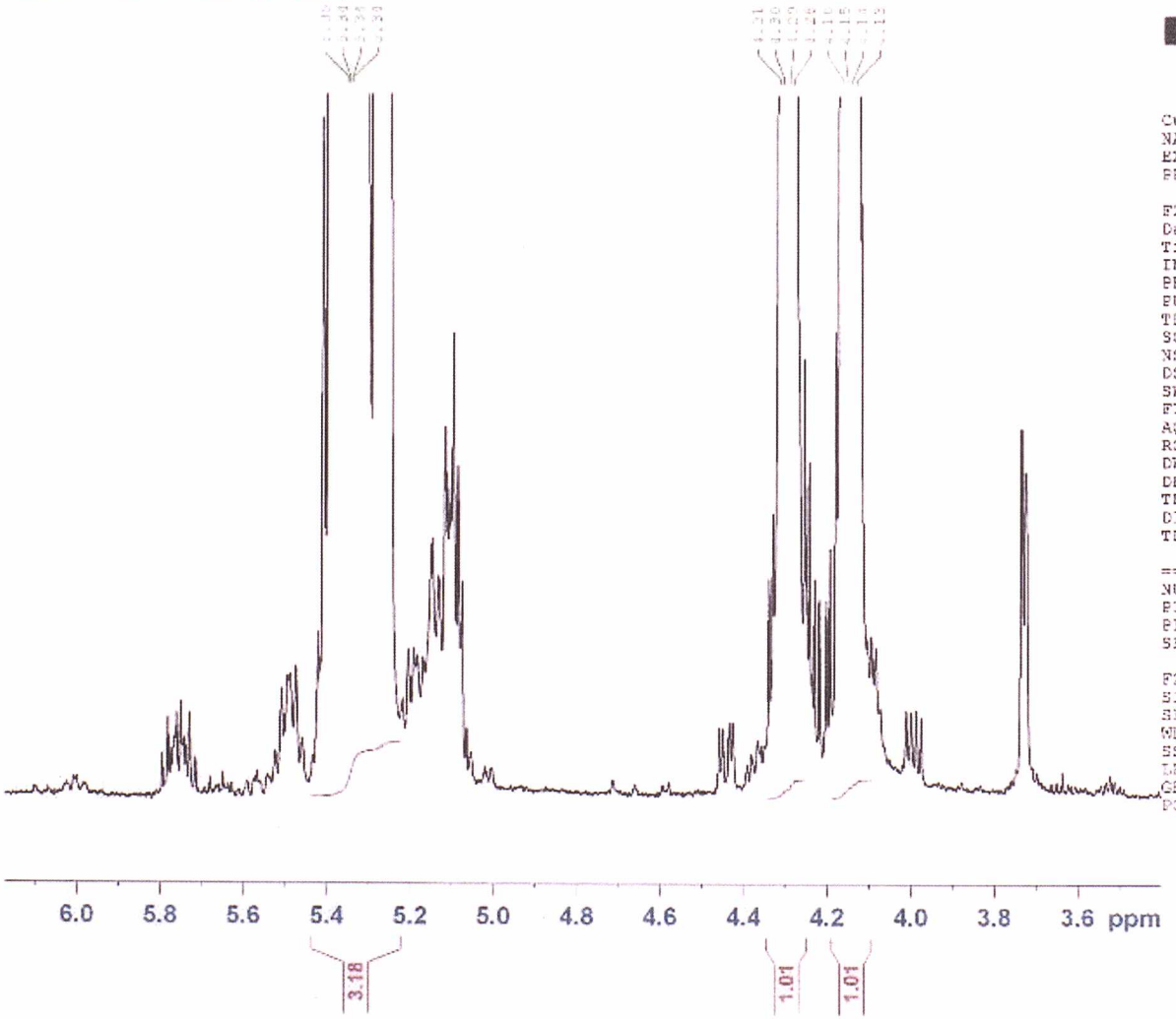
An example of a Participant Information Sheet and a Participant Consent Form are also available for download from the School Web Pages

Annex 8-1H-NMR Spectra of three BOOs and two EVOOs

Evtad BOO



Ayse Okan sample O1A in cdcl3
Black Olive oil (Evtad)



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RG                      45.2
DW                   48.400 usec
DE                    6.00 usec
TE                   293.0 K
D1            10.00000000 sec
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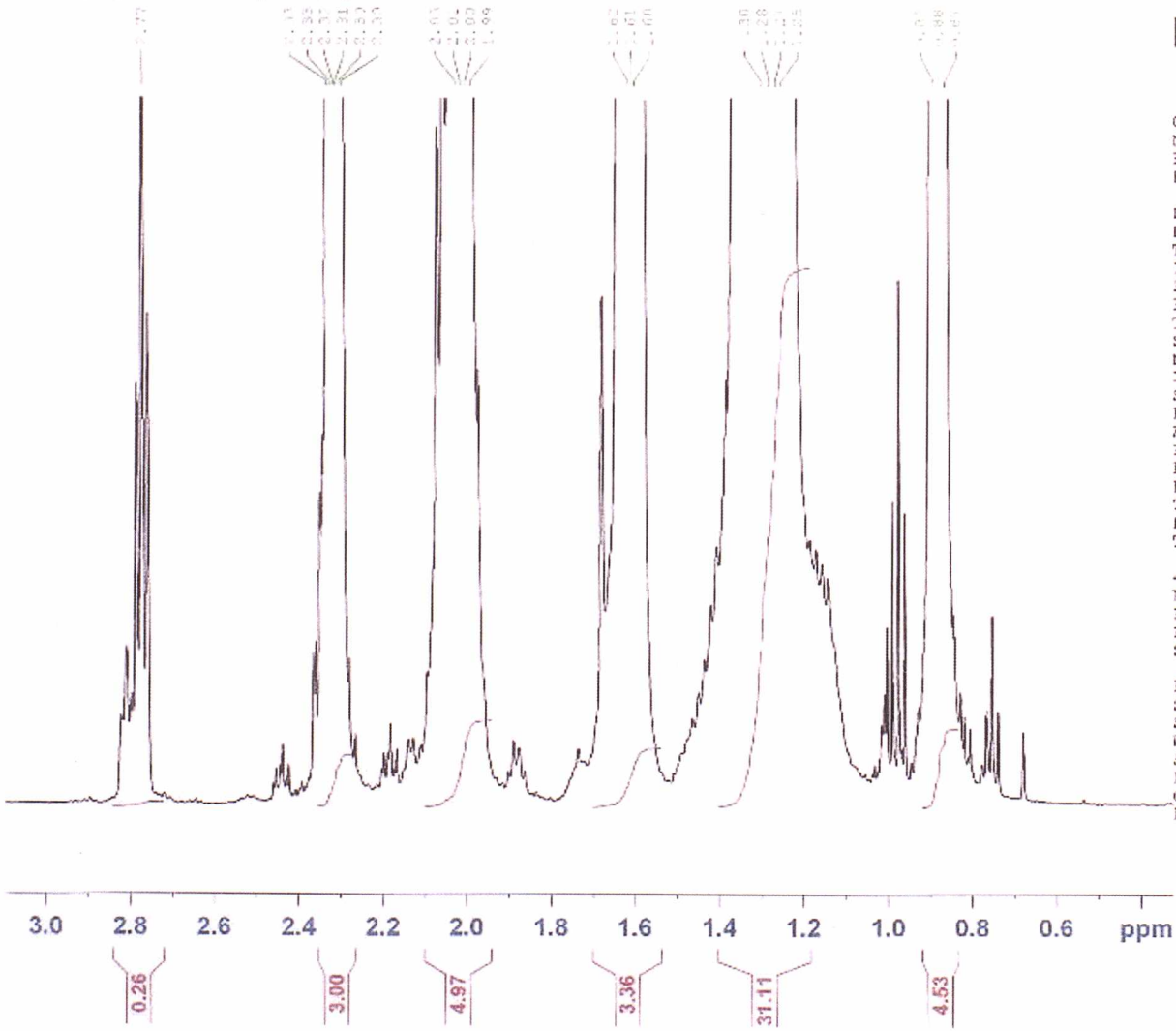

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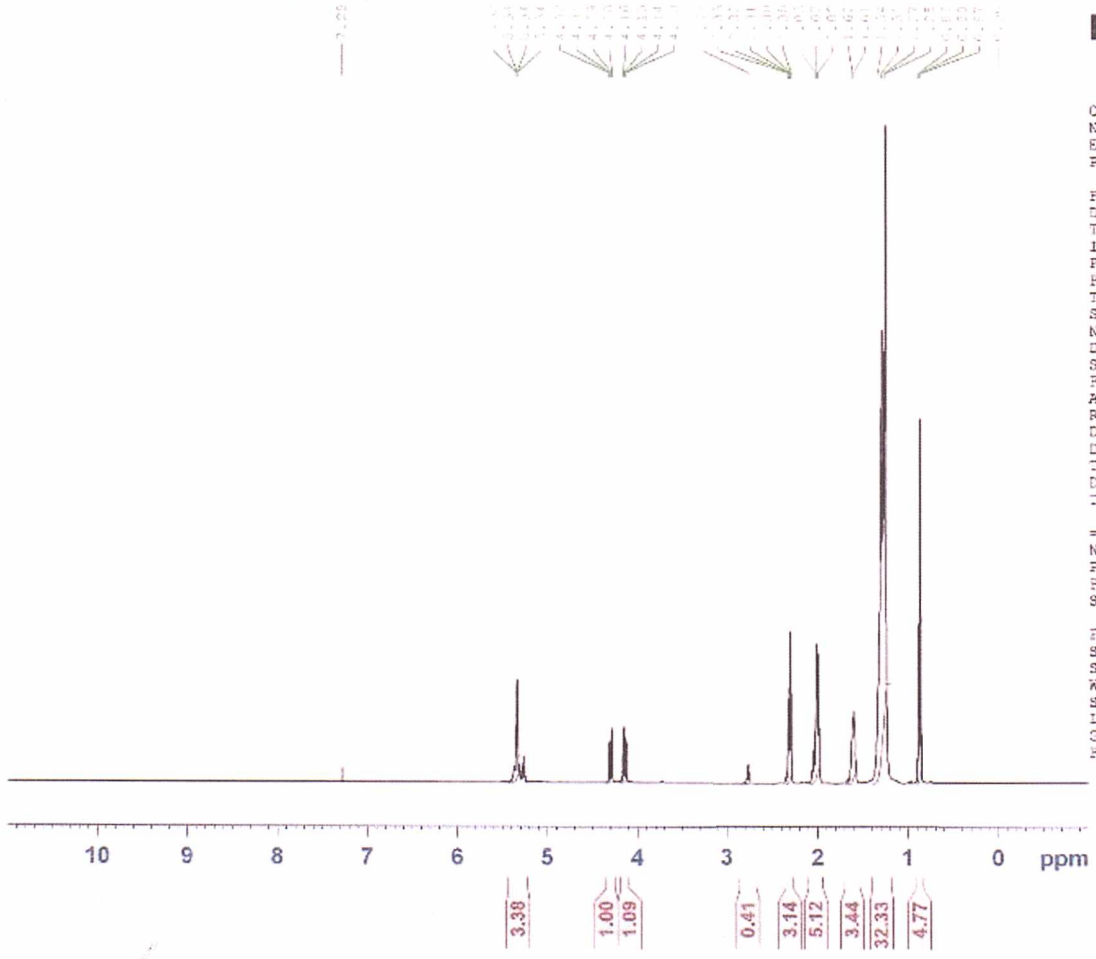

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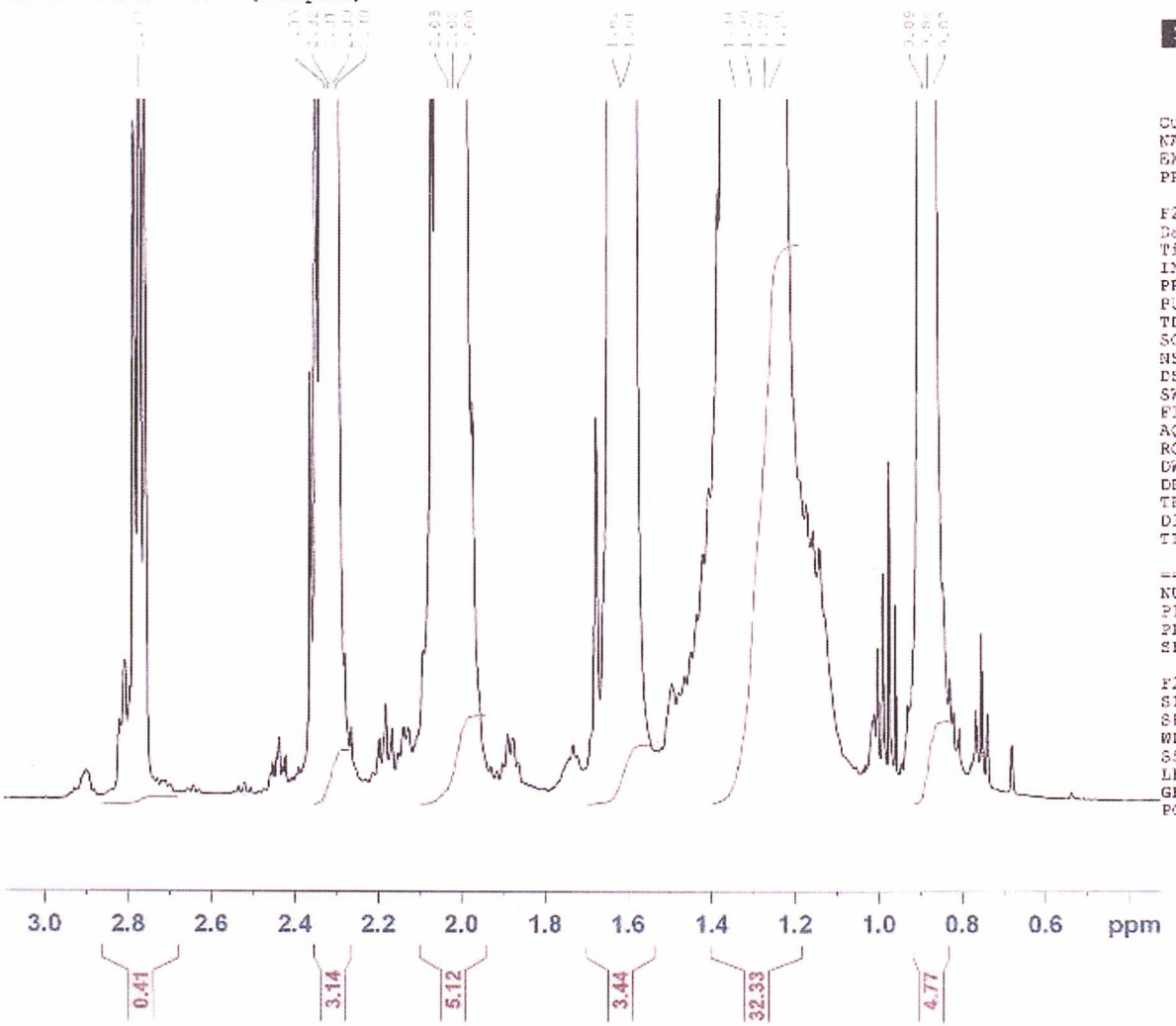
Ayse Okan sample O2A in cdcl3
Black Olive oil (Karpaz)



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RG                      25.4
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DE                    6.00 usec
TE                   293.0 K
D1              10.00000000 sec
TD0                       1

======== CHANNEL f1 ========
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PL1                  -1.00 dB
SFO1          500.1330885 MHz

F2 - Processing parameters
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SF              500.1300041 MHz
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LB                     0.30 Hz
GB                        0
PC                     1.00
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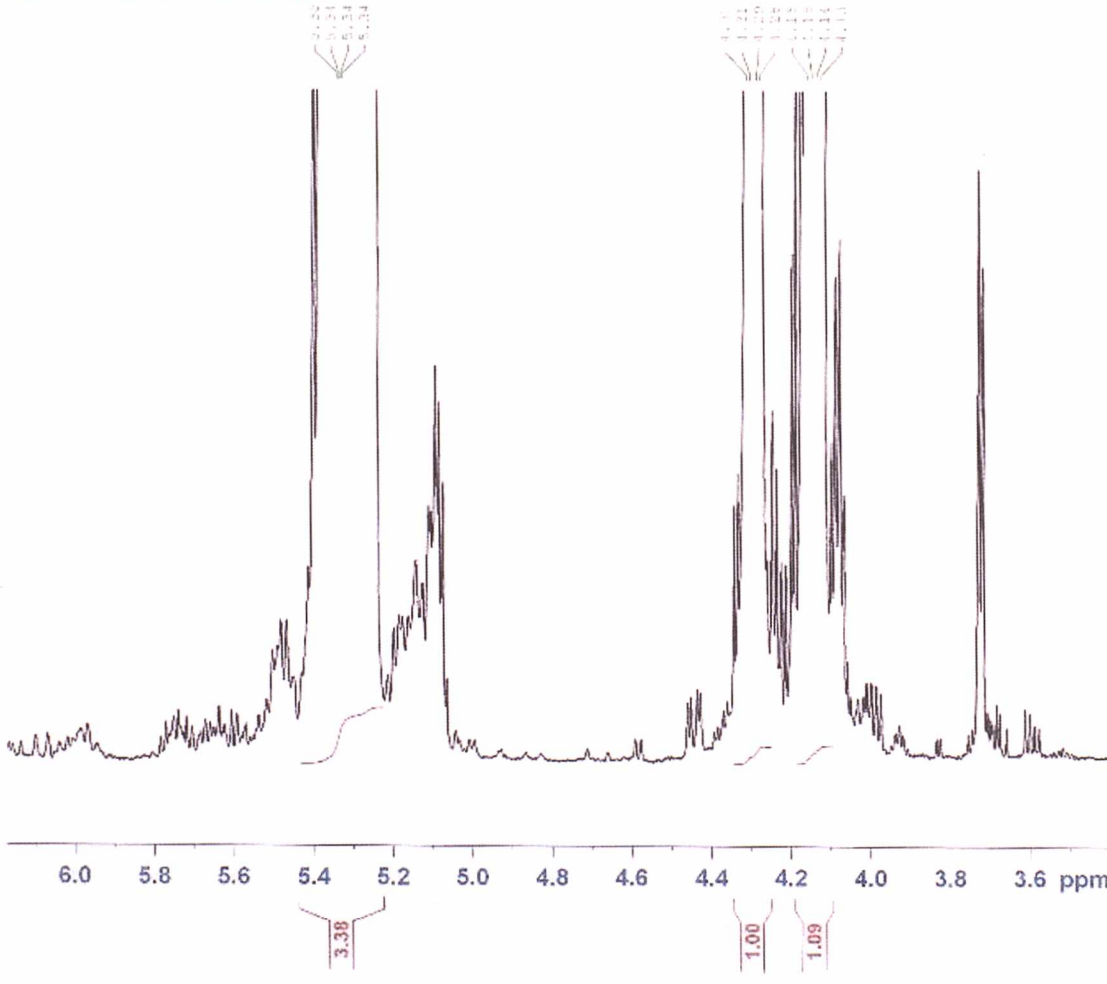
Ayse Okan sample O2A in cdcl3
Black Olive oil (Karpaz)



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AQ                3.1719923 sec
RG                     25.4
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TE                   293.0 K
D1                10.00000000 sec
TD0                       1

======== CHANNEL f1 ========
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P1                    10.50 usec
PL1                   -1.00 dB
SFO1          500.1330885 MHz

F2 - Processing parameters
SI                    32768
SF            500.1300041 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```
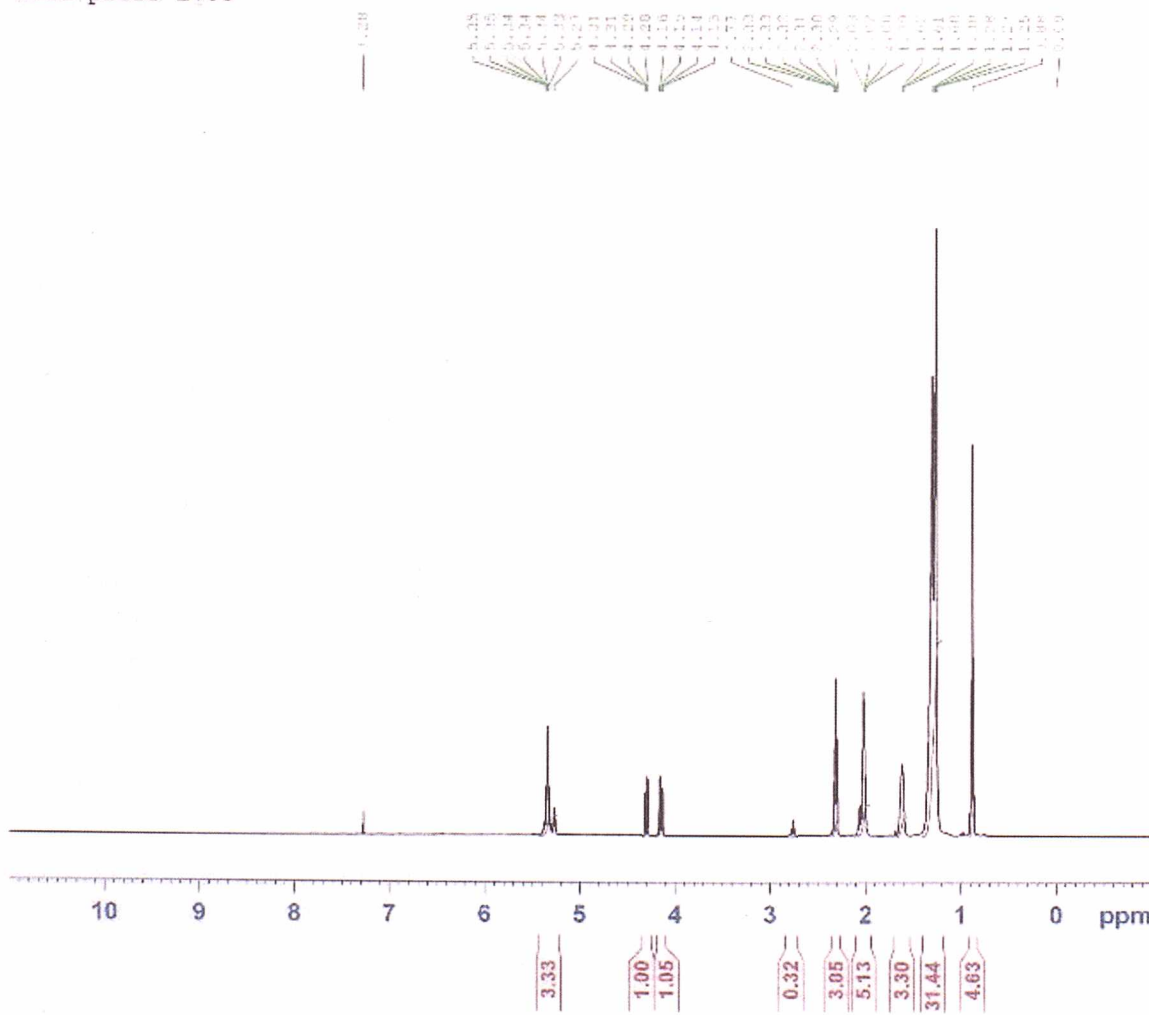

EvtadEVOO

Ayse Okan sample O3A in cdcl3
Cold press EVOO



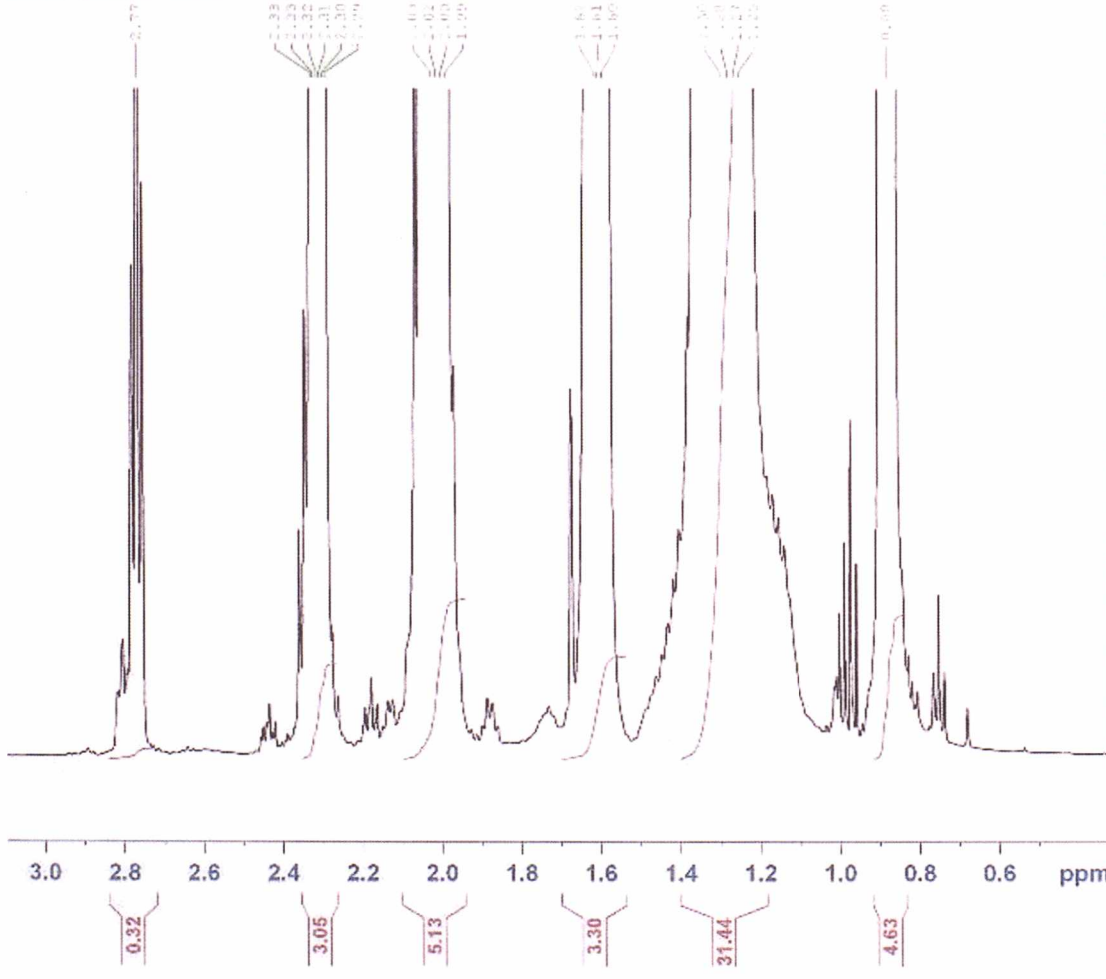
Current Data Parameters
NAME AO03A
EXPNO 15
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160128
Time 12.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719923 sec
RG 28.4
DW 48.400 usec
DE 6.00 usec
TE 293.0 K
D1 10.00000000 sec
TD0 1

-------- CHANNEL f1 --------
NUC1 1H
P1 10.50 usec
PL1 -1.00 dB
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300056 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Ayse Okan sample O3A in cdcl3
Cold press EVOO



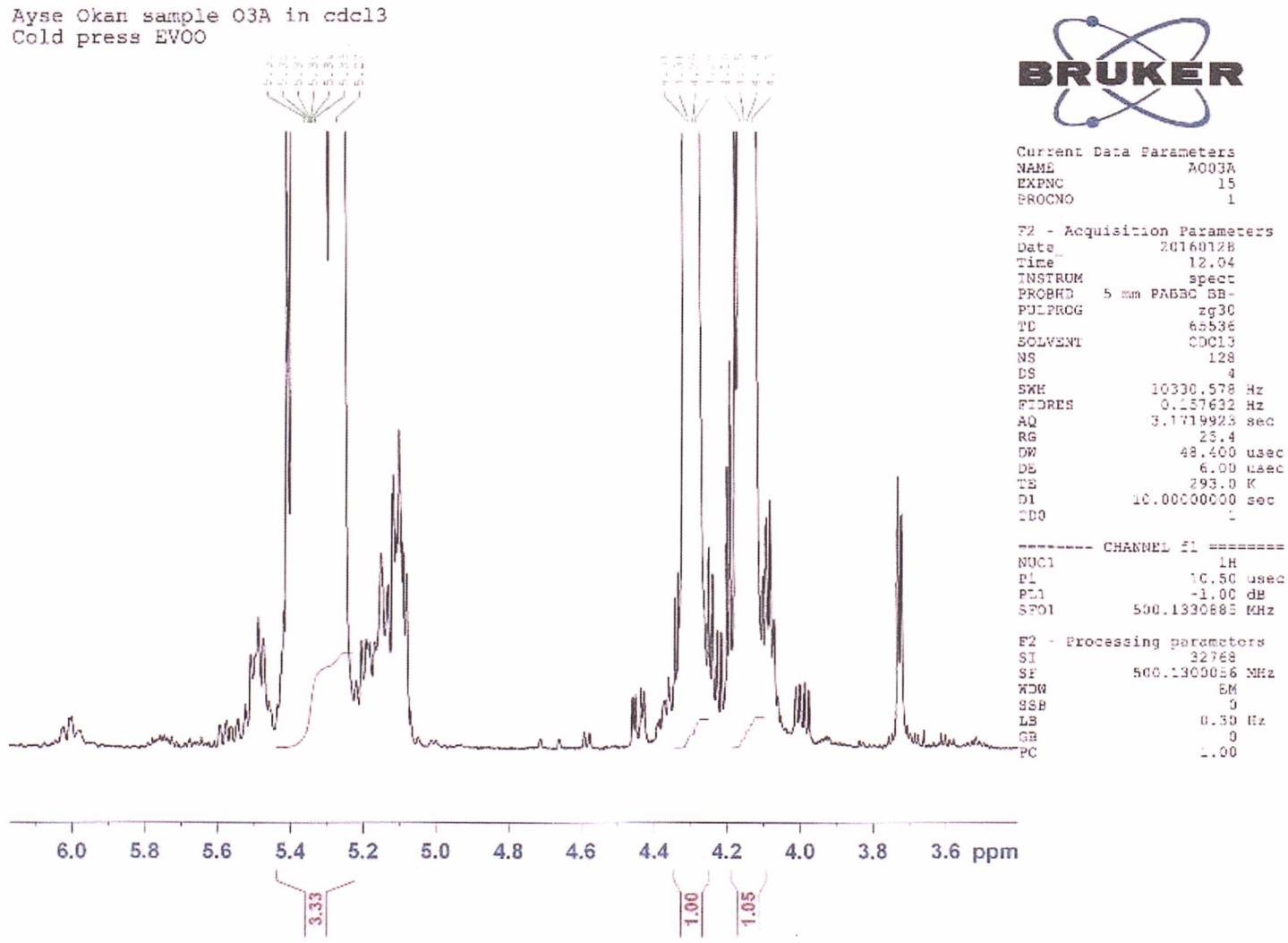
Current Data Parameters
NAME AO03A
EXPNO 15
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160128
Time 12.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719923 sec
RG 25.4
DW 48.400 usec
DE 6.00 usec
TE 293.0 K
D1 10.00000000 sec
TD0 1

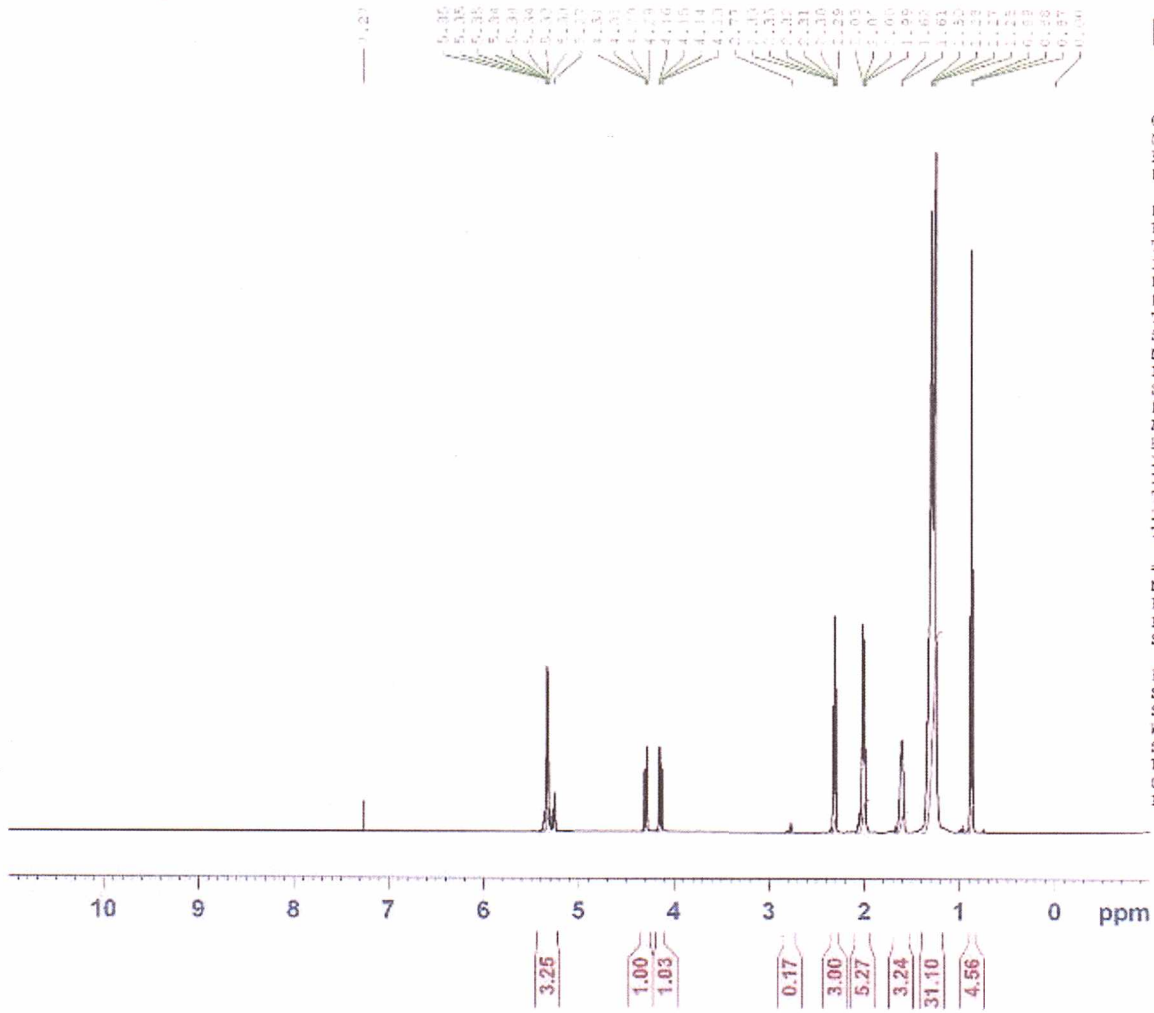
======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -1.00 dB
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300056 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Golden Drop EVOO



Ayse Okan sample C4A in cdcl3
Golden drop EVOO



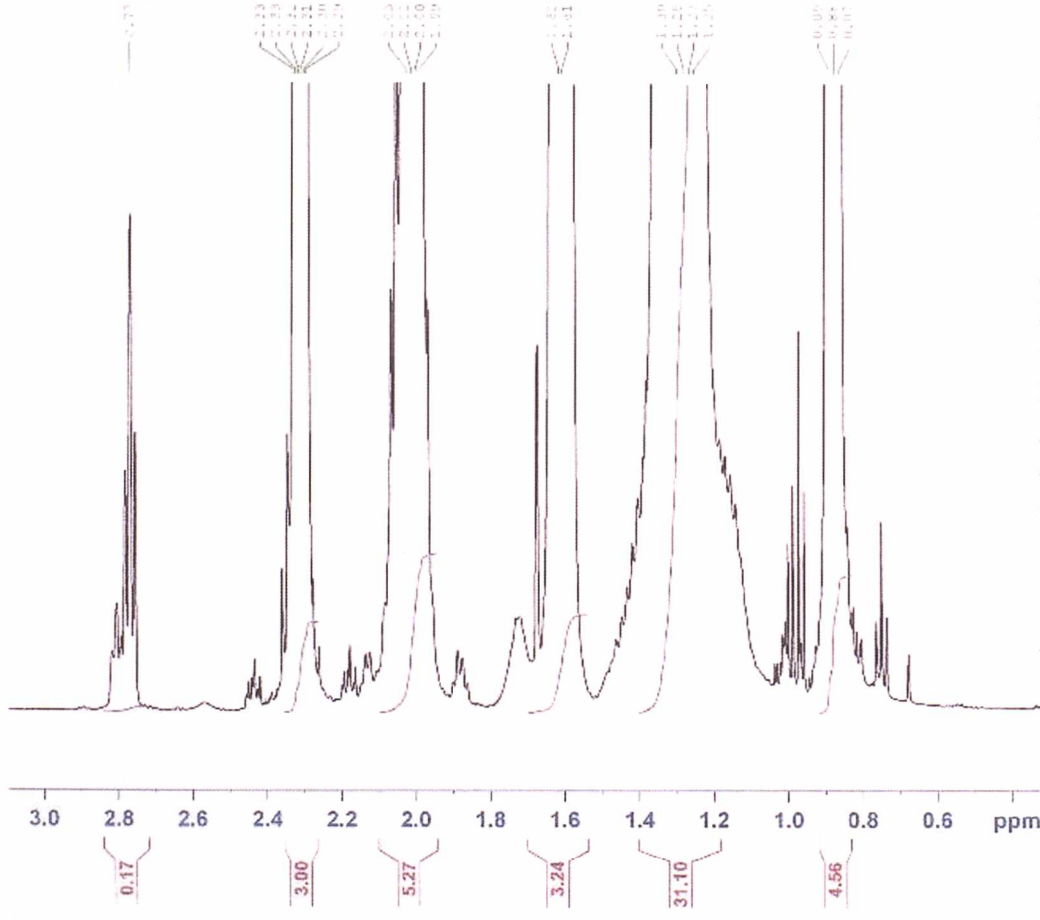
Current Data Parameters
NAME A004A
EXPNO 15
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160128
Time 12.39
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719923 sec
RG 28.5
DW 48.400 usec
DE 6.00 usec
TE 293.0 K
D1 10.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -1.00 dB
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300064 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

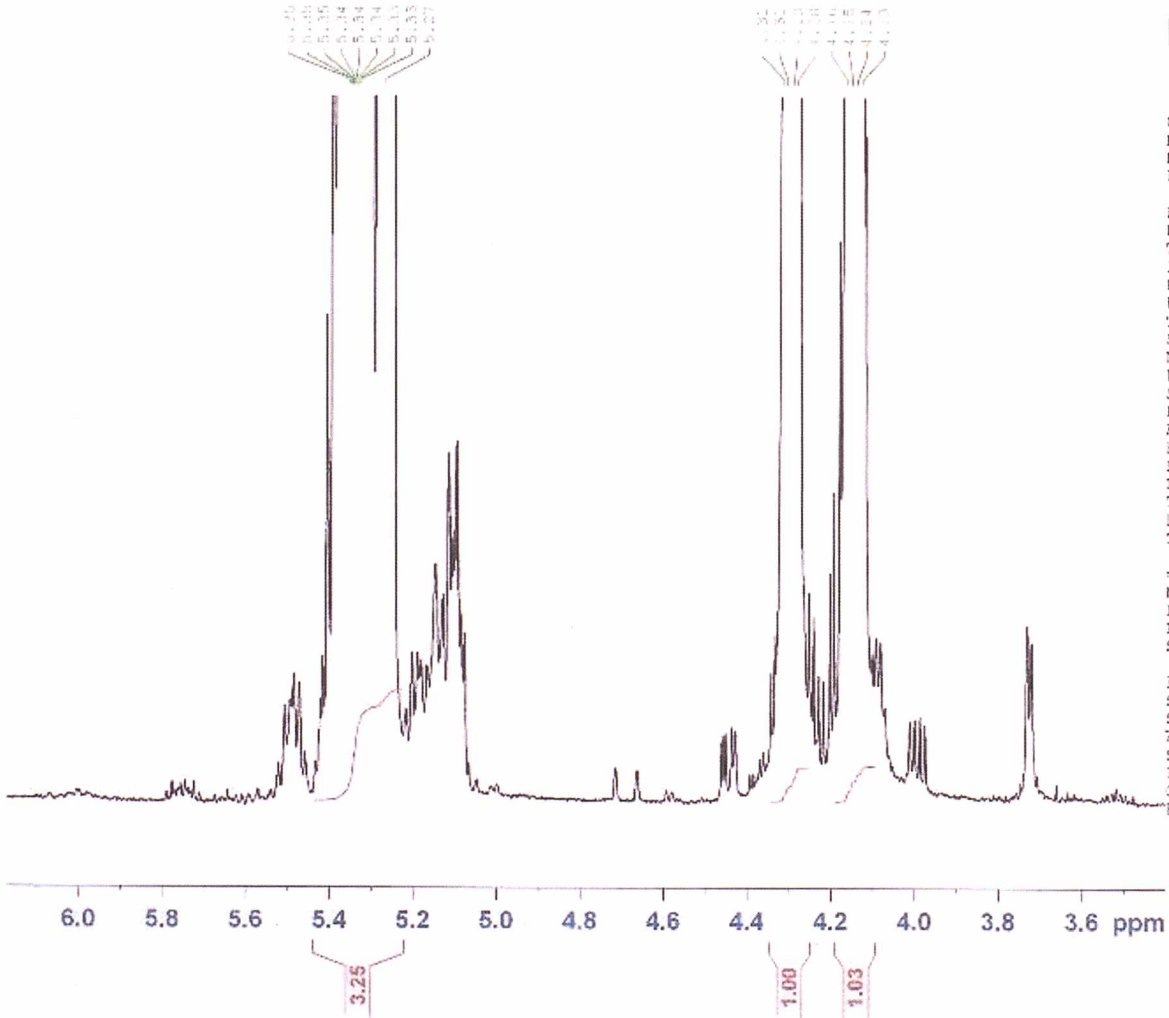
Ayse Okan sample O4A in cdcl3
Golden drop EVOO



BRUKER

```
Current Data Parameters
NAME                  AOO4A
EXPNO                    15
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160128
Time                  12.39
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                      128
DS                        4
SWH               10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719923 sec
RG                     28.5
DW                  48.400 usec
DE                   6.00 usec
TE                  293.0 K
D1               10.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                      1H
P1                    10.50 usec
PL1                   -1.00 dB
SFO1          500.1330885 MHz

F2 - Processing parameters
SI                    32768
SF            500.1300064 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```

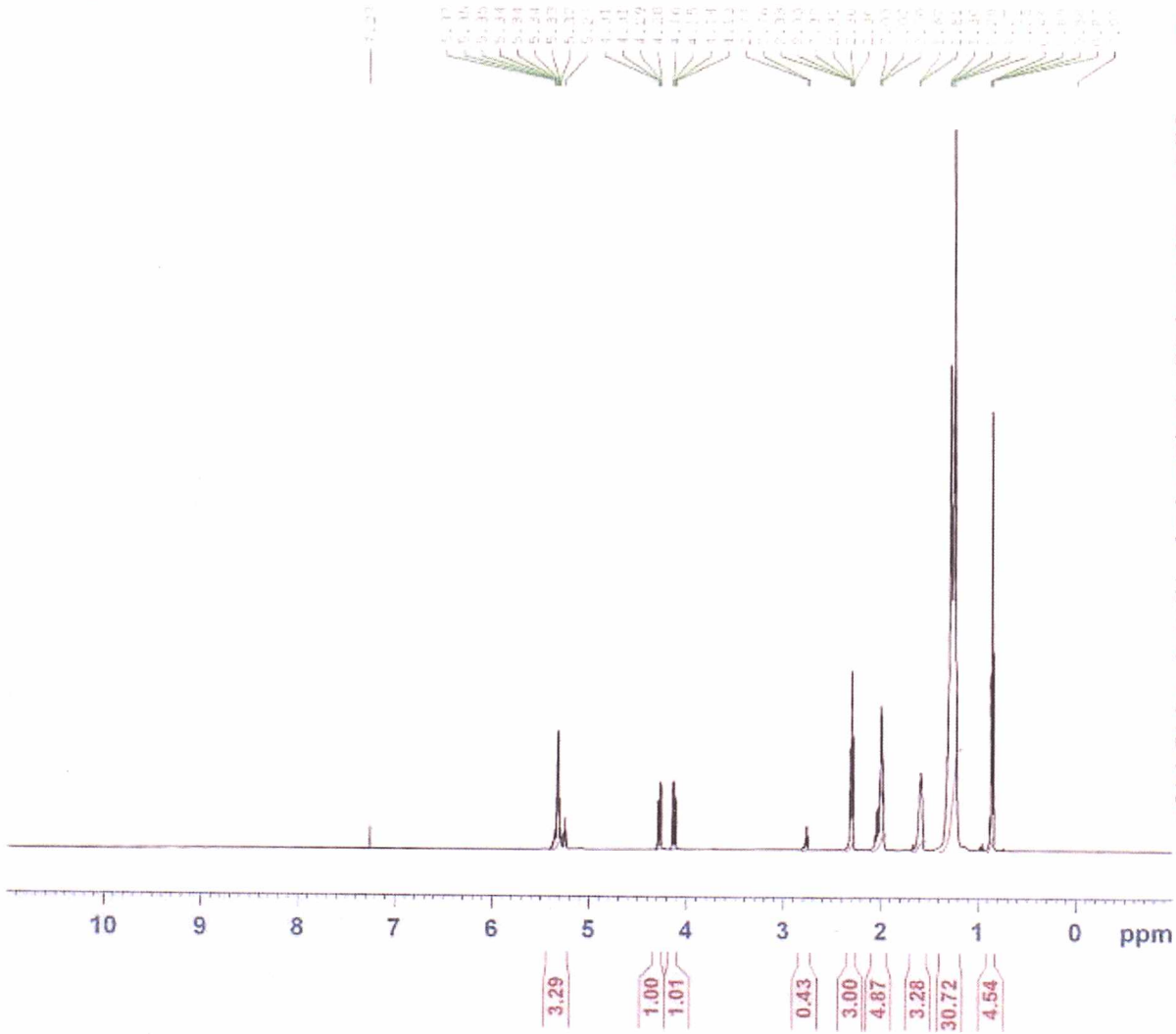
Ayse Okan sample 04A in cdcl3
Golden drop EVOO



BRUKER

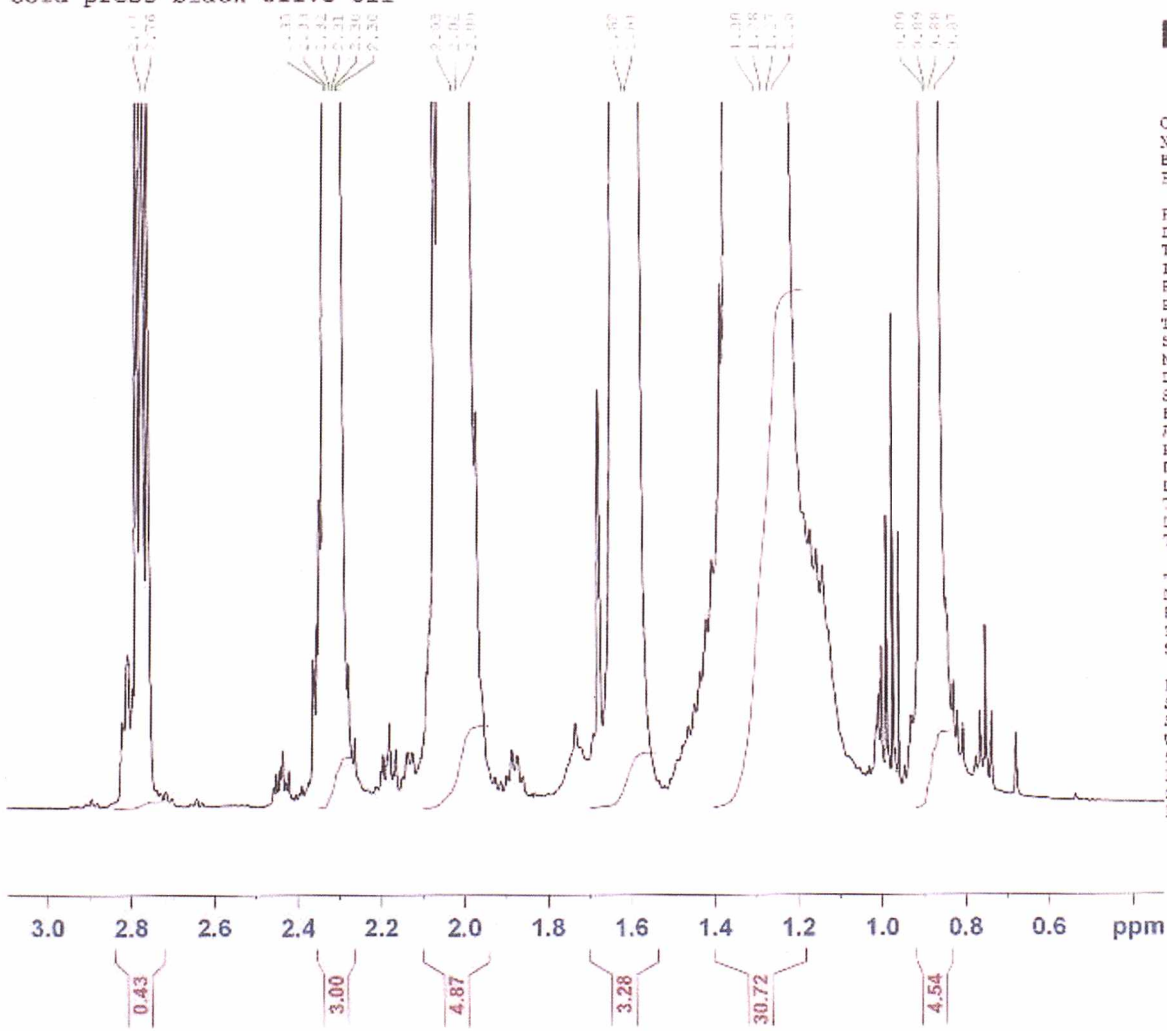
```
Current Data Parameters
NAME                  A004A
EXPNO                    15
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160128
Time                  12.39
INSTRUM                spect
PROBHD     5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                      128
DS                        4
SWH               10330.578 Hz
FIDRES                0.157632 Hz
AQ                    3.1719923 sec
RG                       28.5
DW                    48.400 usec
DE                     6.00 usec
TE                     293.0 K
D1                10.0000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                    16.50 usec
PL1                   -1.00 dB
SFO1             500.1330885 MHz

F2 - Processing parameters
SI                       32768
SF                500.1300064 MHz
WDW                        EM
SSB                         0
LB                     0.30 Hz
GB                          0
PC                       1.00
```

Ayse Okan sample 12A in cdcl3
Cold press black olive oil




```
Current Data Parameters
NAME                  AO12A
EXPNO                    15
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160128
Time                  13.19
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                      128
DS                        4
SWH               10330.578 Hz
FIDRES             0.157632 Hz
AQ                3.1719923 sec
RG                       32
DW                  48.400 usec
DE                   6.00 usec
TE                  293.0 K
D1              10.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                   10.50 usec
PL1                  -1.00 dB
SFO1           500.1330885 MHz

F2 - Processing parameters
SI                    32768
SF             500.1300067 MHz
WDW                      EM
SSB                       0
LB                   0.30 Hz
GB                        0
PC                     1.00
```

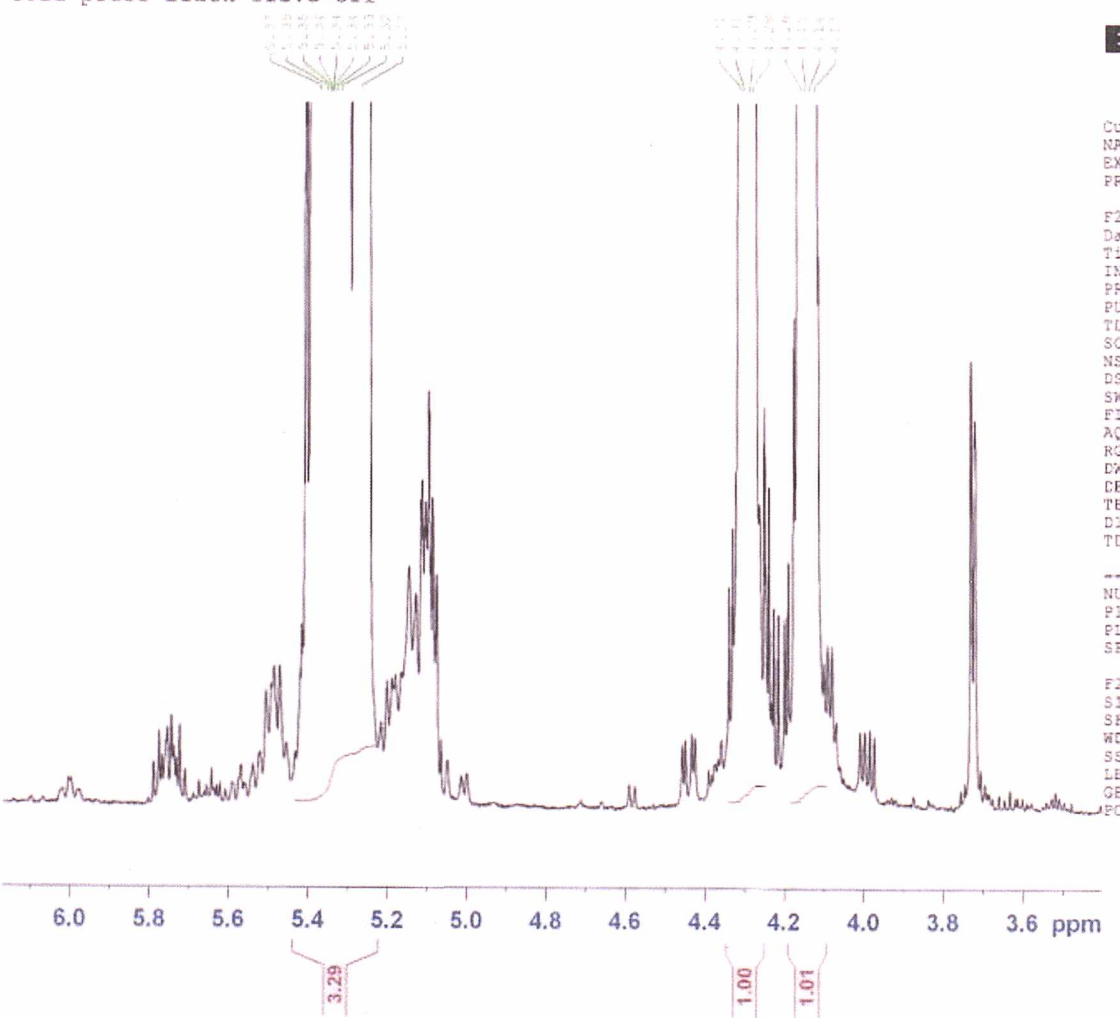
Ayse Okan sample 12A in cdcl3
Cold press black olive oil



BRUKER

```
Current Data Parameters
NAME                  AC12A
EXPNO                    15
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160128
Time                  13.19
INSTRUM               spect
PROBHD   5 mm BABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                      128
DS                        4
SWH               10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719923 sec
RG                       32
DW                  48.400 usec
DE                   6.00 usec
TE                  293.0 K
D1             10.00000000 sec
TDC                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                   10.50 usec
PL1                  -1.00 dB
SFO1          500.1330885 MHz

F2 - Processing parameters
SI                    32768
SF            500.1300067 MHz
WDW                      EM
SSB                       0
LB                   0.30 Hz
GB                        0
PC                    1.00
```

Ayse Okan sample 12A in cdcl3
Cold press black olive oil



BRUKER

```
Current Data Parameters
NAME                  A012A
EXPNO                    15
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160128
Time                  13.19
INSTRUM               spect
PROBHD   5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                      128
DS                        4
SWH               10330.578 Hz
FIDRES             0.157632 Hz
AQ                 3.1719923 sec
RG                       32
DW                   48.400 usec
DE                    6.00 usec
TE                   293.0 K
D1            10.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                    10.50 usec
PL1                   -1.00 dB
SFO1           500.1330885 MHz

F2 - Processing parameters
SI                    32768
SF             500.1300067 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```

Annex 9- Conference representations

Oral presentation at **8th European Congress for Integrative Medicine. Copenhagen, Denmark 25-27 September 2015**

Diet, black olive oil and breast cancer recovery in northern Cyprus, an exploratory study

Okan A[1], White K[1], Majumdar A[1]

The incidence of breast cancer (BC) in Northern Cyprus (NC) is higher than seen internationally. There is little extant literature on the influence of diet on breast cancer recovery among Cypriot women. Black Olive Oil (BOO) a unique product of Cyprus that is consumed heavily and differs in manufacturing processes is widely consumed in NC. The unique properties of BOO are undocumented. The aim of the current study was to investigate the influence of dietary factors including the locally made unique BOO on BC recovery in NC.

Method: patients who had been treated for BC at two hospitals in NC were invited to take part in the study. N= 140 participants were recruited. At each scheduled follow up visit following the completion of active treatment, Participants' BC biomarkers (CA15-3 and CEA), food frequency information, med diet score and 24 hour recall were taken. Appropriate descriptive and influential statistics were used to identify patterns on patients at matched stages of treatment.

Results: correlation was seen between monounsaturated fatty acid (MUFA) and oleic acid consumption only with CEA ($p<0.05$). There was statistically significant between MUFA and oleic acid with CA15-3 in active treatment group. Also there was statistically significant with consumption of BOO and CA15-3 in active treatment group, where no correlation seen in treatment stopped group. Further investigation into diet and BC biomarkers is warranted in North Cypriot BC recovery.

CAMSTRAND 2016, 8th June 2016 in Warwick, UK

Factors affecting Mediterranean Diet Score of breast cancer patients in North Cyprus. By A. Okan[1], A. Majundar[2], K. White[1], [1]School of Social Sciences, Nutrition and Dieteteics Department, London Metropolitan University, London, United Kingdom and [2] St. Mary's University, London, United Kingdom

A traditional Mediterranean Diet (MD) is associated with a lower risk of Breast Cancer (BC)[(1)]. However BC in North Cyprus is the number one cause of morbidity; a cohort study showed that BC was the most common cancer in women with 30% of the population being diagnosed with it [(2)]. This study aimed to review BC patients' MD score (MDS) and identify factors that influence the score.

This was a cohort study on breast cancer patients seen in the two main hospitals in NC. Study population includes 140 BC suffers. To evaluate their nutritional status, a 24-hour recall and food frequency questionnaire (FFQ) have been used. The Mediterranean diet was measured using validated a Mediterranean Diet Adherence Screener (MDAS) which includes 14 questions[(3)] and validated FFQ was used [(4)].

Average MDS of the population is 7.7±2.4. MDS was correlated significantly correlated with age, education, physical activity, total energy intake, protein, fat, carbohydrate, SFA, MUFA, PUFA and oleic acid, cholesterol. There was no statistical significant link between menopause, physical activity, black olive oil, alcohol and vegetables with total MDS.

This was the first study to determinate the relation between BC patients and MDS in NC. This study will inform future studies and interventions on BC in NC.

Glossary

Black olive oil is a unique olive oil of Cyprus which is too dark green. Olive oil i made from boiling olives.

Cyprus is an island country in the Eastern Mediterranean. Cyprus has two separate republics on the island.

North Cyprus is north side of Cyprus, Turkish Republic of North Cyprus.

Greek Cypriot is given name of south Cyprus citizens.

Cypriot is given name of both side Cyprus citizens

Ayran is a yogurt drink which is made from mixing yogurt and water.