ΤΕΧΝΟΛΟΓΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΥΠΡΟΥ

ΣΧΟΛΗ ΓΕΩΤΕΧΝΙΚΩΝ ΕΠΙΣΤΗΜΩΝ ΚΑΙ ΔΙΑΧΕΙΡΙΣΗΣ ΠΕΡΙΒΑΛΛΟΝΤΟΣ

Διδακτορική διατριβή

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Dietary habits of young adults, health benefits of mastic consumption and its functional stability in a probiotic yoghurt

της

Έλενας Χατζημπέη

Λεμεσός 2019

ΕΝΤΥΠΟ ΕΓΚΡΙΣΗΣ

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Επιβλέπων καθηγητής: ……

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Μέλος επιτροπής: ……

Μέλος επιτροπής: ……

Μέλος επιτροπής: ……

Τεχνολογικό Πανεπιστήμιο Κύπρου

Δεκέμβριος, 2019

Είναι απίστευτο τι μπορεί να κάνει μια αχτίδα του ήλιου στην ψυχή σου.

Φιοντόρ Ντοστογιέφσκι

Удивительно, что может сделать один луч солнца с душой человека.

Федор Достоевский

Στη γιαγιά μου

Πνευματικά δικαιώματα

Η έγκριση της διδακτορικής διατριβής από το Τμήμα Γεωπονικών Επιστημών, Βιοτεχνολογίας και Επιστήμης Τροφίμων του Τεχνολογικού Πανεπιστημίου Κύπρου δεν υποδηλώνει απαραιτήτως και αποδοχή των απόψεων του συγγραφέα εκ μέρους του Τμήματος.

Η διεξαγωγή και η υλοποίηση μιας διδακτορικής διατριβής απαιτεί σκληρή δουλειά, επίμονη προσπάθεια, χαλύβδινη θέληση, καθώς και ανθρώπους που θα είναι δίπλα σου, να σε στηρίζουν και να σε καθοδηγούν. Σε αυτούς τους ανθρώπους οφείλω και θα ήθελα να πω ένα μεγάλο ευχαριστώ γιατί χωρίς τη δική τους παρουσία τίποτα δε θα ήταν εφικτό.

Πρωτίστως, ευχαριστώ με όλη μου την καρδιά τον Επίτιμο Καθηγητή Βασίλη Γκέκα, ο οποίος πίστεψε σε μένα από την πρώτη στιγμή, παρέχοντας μου την ευκαιρία, την καθοδήγηση και τη στήριξη για την πραγματοποίηση ενός μεγάλου ονείρου.

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Θα ήθελα επιπλέον να ευχαριστήσω τον Καθηγητή Ιωάννη Γεροθανάση του τμήματος Χημείας στο Πανεπιστήμιο Ιωαννίνων, ο οποίος με δέχτηκε με χαρά στο εργαστήριο μαγνητικής φασματοσκοπίας όπου με τις γνώσεις και την εμπειρία του συνέβαλε στην επίλυση ουσιαστικών ερωτημάτων αναφορικά με τη λειτουργικότητα των γιαουρτιών. Επίσης ειλικρινείς ευχαριστίες στη διδακτορική φοιτήτρια Ελένη Αλεξανδρή για τη ζεστή υποδοχή και ανεκτίμητη βοήθεια.

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ΠΕΡΙΛΗΨΗ

Η αρχή της ενηλικίωσης αποτελεί σημαντική στιγμή για την εφαρμογή προγραμμάτων παρέμβασης και εγκαθίδρυσης μακροπρόθεσμων συμπεριφορών υγείας. Η δημιουργία ενός νέου τροφίμου που καταναλώνεται ευρέως από τους νεαρούς ενήλικες θα μπορούσε ενδεχομένως να χρησιμοποιηθεί ως τροφοφάρμακο, προσφέροντας έναν εναλλακτικό τρόπο πρόληψης των ασθενειών και προαγωγής της καλής υγείας. Η παρούσα εργασία αξιολογεί τις διατροφικές συνήθειες 193 Κυπρίων φοιτητών και περιγράφει τη δημιουργία ενός καινοτόμου λειτουργικού γαλακτοκομικού προϊόντος, χρησιμοποιώντας εκχυλίσματα ρητινών *Pistacia atlantica* και *Saccharomyces boulardii,* σε μια προσπάθεια συνδυασμού των ευεργετικών επιδράσεων του γάλακτος, του εκχυλίσματος και των προβιοτικών μικροοργανισμών. Πέντε διαφορετικά γιαούρτια παρασκευάστηκαν και αξιολογήθηκαν. Έγιναν μετρήσεις των βακτηρίων του γαλακτικού οξέος κάθε τρεις ημέρες, για 25 ημέρες. Η σταθερότητα των εκχυλισμάτων ρητινών *Pistacia* και των λιπαρών οξέων εξετάστηκε με ανάλυση NMR. Τέλος, τα δείγματα αξιολογήθηκαν οργανοληπτικά. Τα αποτελέσματα υποδεικνύουν τη μετάβαση από την παραδοσιακή υγιεινή διατροφή σε πιο ανθυγιεινά σχήματα διατροφής. Συγκεκριμένα, οι συμμετέχοντες καταναλώνουν μικρές ποσότητες φρούτων και λαχανικών, επισκέπτονται συχνά ταχυφαγείο και καταναλώνουν γλυκά πολλές φορές την ημέρα. Επίσης, περίπου οι μισοί νέοι ενήλικες δεν ασκούνται και το ένα τέταρτο από αυτούς καπνίζουν. Αναφορικά με το γιαούρτι, τα εκχυλίσματα ρητινών *Pistacia* προώθησαν την ανάπτυξη των οξυγαλακτικών βακτηρίων και τα καλύτερα αποτελέσματα λήφθησαν σε συνδυασμό με τη ζύμη *Saccharomyces boulardii*. Η φασματοσκοπία NMR αποκάλυψε ότι τα λειτουργικά λιπαρά και οι βιοδραστικές ενώσεις διατηρήθηκαν μέχρι το τέλος ζωής του γιαουρτιού. Με βάση την οργανοληπτική εξέταση δεν υπήρξαν σημαντικές διαφορές στη γεύση, την υφή και την εμφάνιση μεταξύ των διαφορετικών γιαουρτιών. Το νέο αυτό προϊόν θα μπορούσε να προωθηθεί στην αγορά ως πιθανό καινοτόμο λειτουργικό τρόφιμο.

Λέξεις κλειδιά: νεαροί ενήλικες, διατροφικές συνήθειες, γιαούρτι, ρητίνες *Pistacia atlantica, Saccharomyces boulardii*

ABSTRACT

Young adulthood may be an important time for intervening and establishing long-term health behaviors. The development of a novel food product widely consumed by young adults could potentially used as nutraceutical, offering an alternative way of disease prevention and wellbeing. The present study assess the dietary habits of 193 Cypriot students and describes the development of a novel functional goats' milk yoghurt, using *Pistacia atlantica* resin extracts and *Saccharomyces boulardii*, in an attempt to combine and expose the beneficial effects of the milk, extract and probiotic microorganisms. Five different yoghurt formulations were prepared and assessed. Enumerations of total lactic acid bacteria (LAB) were performed every three days for 25 days. Stability of *Pistacia* resin extracts and fatty acids was examined by NMR spectroscopy. Finally, the samples were also assessed organoleptically. Results support a shift from traditional healthy diets to more unhealthy eating patterns. Specifically study participants consume low quantities of fruits and vegetables, visit fast-food restaurant often, and consume sweets several times a day. Also about half of the young adults don't exercise and one quarter of them smoke. Regarding yoghurt, *Pistacia atlantica* resin extracts promoted the survival of lactic acid bacteria (LAB). However best results in viability of LAB were obtained in combination with *Saccharomyces boulardii*. NMR spectroscopy revealed that the functional fatty acids can be retained during the shelf-life of the yoghurts, alongside with the other bioactive compounds. The organoleptic assessment revealed that there were no significant differences in flavour, body, texture and appearance between the different yoghurts. This new product could be marked as potential innovative functional yoghurt.

Keywords: young adults, dietary habits, yoghurt, *Pistacia atlantica* resins, *Saccharomyces boulardii*

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ABBREVIATIONS

TPB:	Theory of Planned Behavior
GC-MS:	Gas Chromatography-Mass Spectrometry
TMEWP:	Total mastic extract without polymer
SGOT:	Serum glutamic oxaloacetic transaminase
SGPT:	Serum glutamic pyruvic transaminase
γ-GT:	γ-glutamyltransferase
CD:	Crohn's disease
IL-6:	Interleukin-6
TNF-a:	Tumor necrosis factor-alpha
CRP:	C-reactive protein
TAP:	Total antioxidant potential
NRI:	Nutritional risk index
MIF:	Migration inhibitory factor
LDL:	Low density lipoprotein
VCAM-1:	Vascular cell adhesion molecule
ICAM-1:	Intercellular adhesion molecule
SCID:	Severe combined immunodeficiency mice
VEGF:	Vascular endothelial growth factor
Erk1/2:	Extracellular signal-regulated kinases ½
AR:	Androgen receptor
LLC:	Lewis lung carcinoma
NF-κB	Nuclear factor kappa B
MMP-2:	Matrix metalloproteinase 2

BC:	Before Christ
USDA:	United States Department of Agriculture
RNI:	Reference nutrient intakes
COMA:	Committee on Medical Aspects of Food Policy
DNA:	Deoxyribonucleic acid
RNA:	Ribonucleic acid
ATP:	Adenosine triphosphate
FAD:	Flavin adenine dinucleotide
FMN:	Flavin adenine mononucleotide
CLA:	Conjugated linoleic acid
SFA:	Saturated fatty acids
BMD:	Bone mineral density
AAD:	Antibiotic-associated diarrhea
IBD:	Inflammatory bowel disease
HELENA:	Healthy Lifestyle in Europe by Nutrition in Adolescence
CVD:	Cardiovascular diseases
BMI:	Body mass index
HDL:	High density lipoprotein
T2DM:	Type 2 diabetes mellitus
IFN-γ:	γ-interferon
IgE:	Immunoglobulin E
MUFA:	Monounsaturated fatty acids
PUFA:	Polyunsaturated fatty acids
MCT:	Medium chain triglycerides
LAB:	Lactic acid bacteria

KIDMED index:	Mediterranean Diet Quality Index for children and adolescents
MRD:	Maximum Recovery Diluent
MRS agar:	de Man, Rogosa and Sharpe agar
DRBC agar:	Dichloran Rose Bengal Chloramphenicol agar
NMR:	Nuclear magnetic resonance
SD:	Standard deviation
ANOVA:	Analysis of variance
Vs:	Versus
CFU:	Colony forming units

INTRODUCTION

Poor dietary habits are an important public health issue that has large health and economic implications. Most of our food habits are formed in infancy and early childhood and these are difficult to change, however people make more and more independent eating decisions as they move through adolescence and the transition to independent living during college years is a good moment for changes in food habits as well.

Unfortunately literature shows that young adults tend to follow a poor diet, which is characterized by low intake of fruits and vegetables and high consumption of fast food, soft drinks and other sugar-sweetened beverages.

Young adulthood is an important transitional period from adolescence to adulthood, during which long-term health behavior patterns are formed and established. Young adults should have good dietary habits with adequate nutrient intakes for overall good health. This can influence not only their concurrent health but also their future risk for a number of chronic diseases at a later age.

Therefore this life stage is critical for promoting good health and also preventing the risk of chronic diseases. Tailored-made public health strategies targeting the young adult population would be warranted. An important alternative intervention method is food fortification.

Pistacia resins are a complex mixture comprising of different bioactive compounds, with the presence of triterpenes and essential oils being characteristic for these resins. Since ancient times, *Pistacia* resins have been used in traditional medicine of Mediterranean and Middle Eastern countries as herbal remedies. There is accumulating evidence that suggests that Pistacia resins may contribute to the reduction of many chronic diseases, such as gastrointestinal disorders, cardiovascular diseases, and some forms of cancer, while in

parallel promoting oral health and other physiological functions such as antimicrobial and antioxidant activities.

The characteristic taste in combination with health-promoting properties of *Pistacia* resins has attracted the interest of food industries. *Pistacia* resins are the main ingredient or used as additives in several foods. In particular, the resin and its by-products are exploited in a great variety of products such as bakery, traditional and gourmet sweets, snacks, chewing gums, liquor, flavored wines and filter coffees. In addition, the resins and their essential oils are used in cosmetics and pharmaceutical or parapharmaceutical products as toothpastes, soaps, shower gels, shampoos, sunscreen creams and nutritional supplements with diverse pharmacological properties.

Moreover, *Pistacia* resins can be used to produce innovative functional foods, by fortifying certain food products widely consumed such as breakfast cereals, nutrition bars, yoghurts and spreads, designed to promote beneficial physiological effects (e.g. reduce cholesterol, oral health, control *H. pylori*).

Clearly the studies reveal the beneficial effects of *Pistacia* resins in several pathological conditions and therefore there is accumulating evidence that needs to act as leverage to the food industry towards the development of novel food product formulations that could potentially be used as nutraceuticals, offering alternative ways of disease prevention.

Yoghurt could be a good vehicle for providing functional ingredients as it is widely consume by young adults and already contributes to good health because of probiotics, calcium and other minerals and vitamins. The stability of resin and its interaction with the product must be explored and thoroughly investigated in order to ensure functionality of the product throughout its commercial life period.

1 Dietary habits /food choice

Food choice is one of the most frequent behaviors and although seemingly simple, in fact it is an example of complex and complicated human behavior that is determined by many factors and interactions. Although over the last years the number of publications has grown very rapidly, food choice remains a topic that is not well understood. Specific food choices lay the groundwork for long term food habits. The food choice process incorporates not only decisions based on conscious reflection, but also those that are automatic, habitual and unconscious. Therefore a blend of many factors such as availability, convenience, taste, health, pleasure, cost, cultural values, parents' practices interact in complex and changing ways to the development of eating behaviors and dietary habits.

1.1 Determinants of food choice

A general model of the determinants of food choice split the factors into three major categories: 1) food characteristics, where factors are related to the food, such as sensory characteristics of foods including taste, texture, and appearance. 2) Individual characteristics, where factors are related to the individual such as the sensory, physiological and psychological processes. 3) Economic and social factors, where factors are related to the environment, e.g. culture, religious and social pressures. It seems that food and environmental characteristics influence the individual characteristics and all together produce food acceptance or rejection behavior, which results in food choices and food intake (Figure 1) (Conner, 1993).

Influences on food choices and even influences on changes in food choices are likely to be mediated by the beliefs and attitudes held by individual. This approach is derived from the Theory of Planned Behavior (TPB). This theory has been widely applied to many issues in

social psychology and also successfully applied to a range of food choice issues and eating behaviors such as intentions to consume breakfast, to eat genetically modified food, to follow a low fat diet, to consume fruits, beer, pizza etc. More specifically the TPB postulates that behavior is best predicted from an individual's intention to perform the behavior, with intention determined by three sets of factors: attitude towards the behavior, subjective norm and perceived behavioral control (Figure 2). Attitude towards the behavior is held to be the affective reaction to the situation and is measured overall by a positive-negative semantic differential. The overall attitude is held to be based on the individual's beliefs about the salient outcomes of the behavior (e.g. this food will taste pleasant; this food may increase my chances of coronary heart disease). The subjective norm or perceived social pressure to perform the behaviour in question, reflects the socio-psychological assumption that our intentions to perform behaviour are shaped by expectations which others hold for us as well as by personal attitudes. The subjective norm therefore is assumed to be a sum of the normative beliefs about what salient groups or individuals believe about the individual performing the behavior, each weighted by the motivation to comply with the expectations of relevant people. Finally the third determinant is the amount of control which the individual perceives themselves to have over the behavior in question. Perceived control is estimated from evaluations of the power of the factors to facilitate or inhibit the performance of the behavior, each weighted by their frequency of occurrence (e.g. personal skills, dependence on others, barriers) (Conner 1993, Shepherd 1999, Ajzen 1991).

In conclusion according to this theory, individuals are likely to choose and consume a particular food/or establish an eating behavior, if they believe that consumption of that food/or the establishment of that behavior will lead to particular outcomes which they value positively, if they believe that people whose views they value think that they should carry out the behavior, and if they feel that the action is easily brought under their own control.

Many studies have used the TPB to predict the behavior and to investigate the determinants that influence the behavioral intention. Verbeke and Vackier, (2005) showed that determinants as hypothesized by the TPB and personal characteristics influence fish consumption intention and frequency. Favourable attitude, high subjective norm and high perceived control had a positive impact on fish consumption decisions. Appreciation of the attribute taste emerged as the most important driver for eating fish, followed closely by health. Bones and price constituted the negative attitude factor, which however does not directly reduce behavioral intention. Individual determinants pertain to gender, age, children, income, education level and region. Similarly, Tuu et al. (2008) applied the conceptual framework of the TPB to explain the consumption of fish and the results indicated that attitude, social norms, descriptive norms and behavioral control all had significantly positive effect on behavioral intention. Finally, both intention and perceived behavioral control were highly associated with the frequency of fish consumption. Applying the TPB to fruit and vegetable consumption of young adults, the data fit the model well and appeared to be strong relationships between subjective norms or barriers and intentions as well as gender differences in the strengths of the relationships in the model (Lien et al. 2002). Understanding soft drink consumption among 707 female adolescents in Los Angeles, using the TPB, attitude, subjective norm and perceived behavioral control had statistically significant positive associations with intention, and were each significant predictors of intention to drink soft drinks. Since almost all the participants (96.3%) reported that currently drink soda and 50.1% reported drinking two glasses of soda or more per day during the past year, authors suggest that efforts to reduce soda consumption among adolescents should include parents and friends (subjective norm). It is also important that soda should not be excessively available at home or school (perceived control) and campaigns needed to reinforce the perception that there are other healthier drinks that quench thirst and that taste good as well

(attitude) (Kassem et al. 2003).

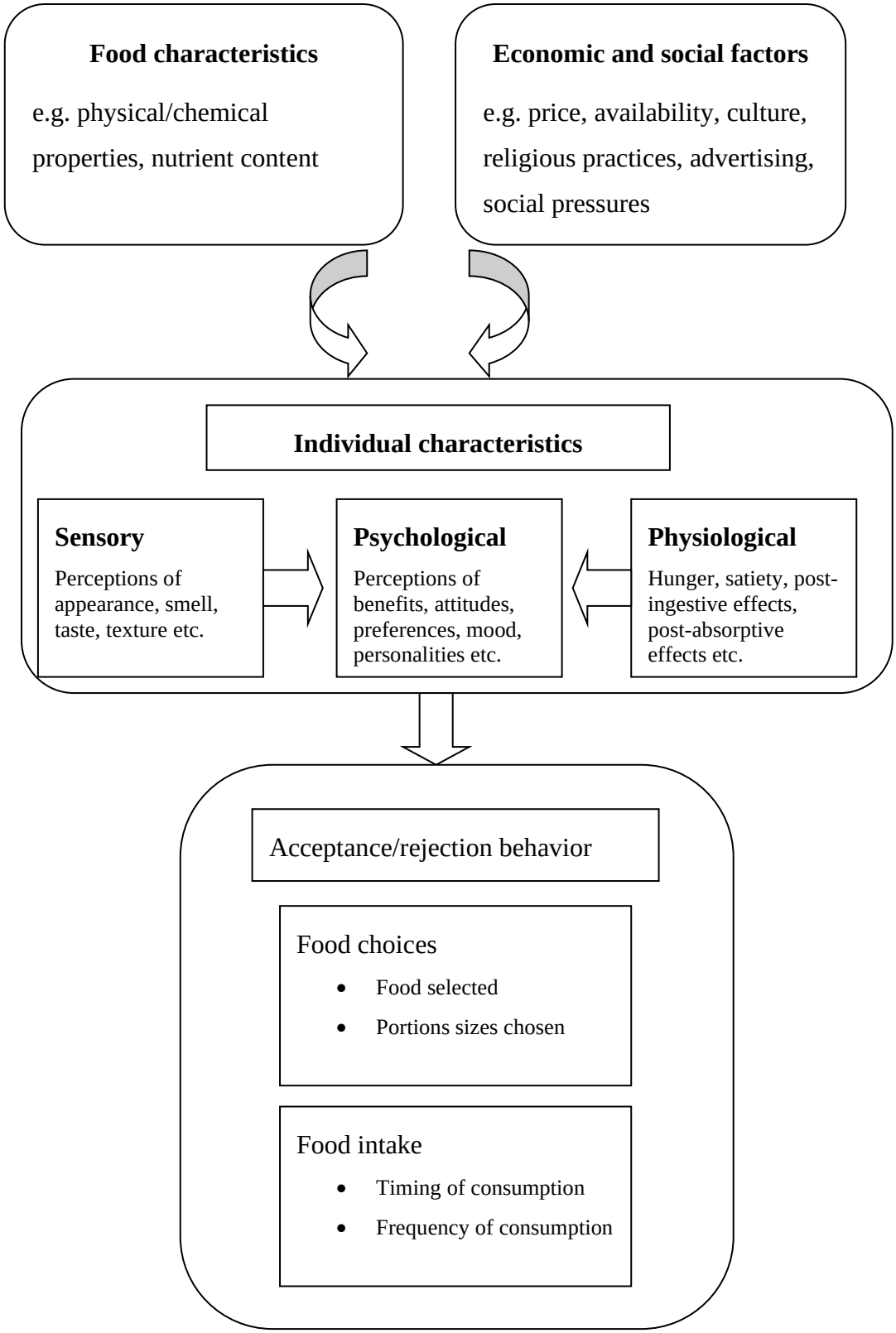

Figure 1. A General Model of the Determinants of Food Choice

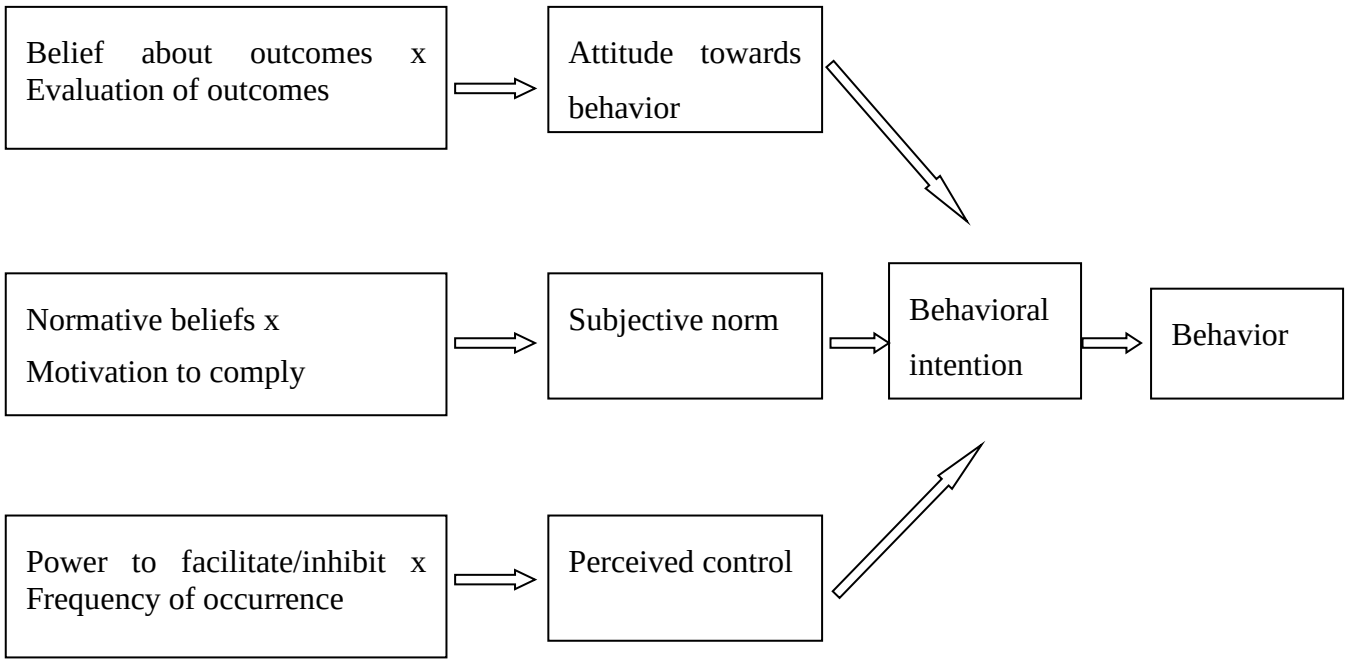

Figure 2. The Theory of Planned Behavior

1.1.1 Food characteristics

1.1.1.1 Physical properties

Unquestionably, color plays a key role in food choice by influencing taste thresholds, sweetness perception, food preference, pleasantness, choice and acceptability (Clydesdale 1993). A significant main effect for color indicated that children preferred foods that were red, green, orange, and yellow, in that order (Walsh et al. 1990). Wadhwani and McMahon, 2012 examined the effect of color on low-fat cheese flavor perception and consumer acceptability. Clearly, consumer liking and perception of flavor of low-fat cheese was influenced by cheese color and appearance and more specifically, consumer liking was negatively influenced when the cheese appearance was too translucent or too white.

Taste and flavour are important factors in consumer food choice and acceptance (Clark, 1998). In a study with a large number of sample (about 3000 adults), taste was the most important influence on their food choices. The importance of taste showed no significant relationship to age or income, but was more important to women (Glanz et al. 1998).

Similarly in another study, using focus-group discussions, taste and appearance were among the most important and crucial factors in making decisions about food choices. Moreover here, study participants extended the terms taste and appearance and discussed about the overall appeal of food and its influence on food choices. Therefore they discussed taste, familiarity with food, appearance, smell, how the food is prepared or served, temperature of food, whether or not foods ‘go together’, and variety (Neumark-Sztainer et al. 1999).

Stroebele and De Castro, (2004) adding even more information focusing in overall ambience correlated with food. Changes in intake can be detected with different levels of the number of people present, food accessibility, eating locations, food color, ambient temperatures and lighting, and temperature of foods, smell of food, time of consumption, and ambient sounds.

1.1.1.2 Palatable foods – rich in sugar and fat

Palatable foods, especially those rich in sugar and fat may involve with food reward system mediating from endogenous opioid peptides. As we eat not only for survival but also for pleasure, palatable aspects of food may determine dietary habits. Therefore hedonic response mediating from opioids influence the preference for high fat or sweet foods (Zhang, Gosnell and Kelley, 1998, Kampov-Polevoy et al. 2006).

Abnormalities in the endogenous opioid peptide system may be linked to elevated sensory preferences for sugar/fat mixtures and compulsive overeating of sweet high fat foods (Krahn et al. 1992).

Furthermore opioids seem to reduce activity in oxytokin systems that contribute to satiety and aversion, promoting the overconsumption of palatable foods. Also palatable food may produce a condition that resembles drug addiction (Gosnell and Levine, 2009).

Woolley, Lee and Fields, (2006) investigated whether opioid regulation of food consumption is based primarily on its macronutrient content or its flavor per se. The results showed that when given a choice between foods with identical macronutrient content and texture but different flavors, opioid signaling increases consumption of the food with the generally preferred choice.

Since the rates of obesity are growing worldwide an understanding of the role of opioid peptides in mediating food reward and promoting overconsumption of palatable foods may provide insights into new approaches preventing weight gain.

1.1.2 Individual characteristics

1.1.2.1 Physiological

1.1.2.1.1 Gender/age

Dietary habits may differ by gender. Caine-Bish and Scheule, (2009) performed a study in 1818 school-age children and adolescents and according to the results boys preferred meat, fish and poultry foods over girls and girls preferred fruits and vegetables over boys.

A study conducted in 23 countries in a large sample of young adults indicated that women were more likely than men to report avoiding high-fat foods, eating fruit and fiber, and limiting salt (to a lesser extent) in almost all of the 23 countries. They were also more likely to be dieting and attached greater importance to healthy eating. Gender differences in food choices therefore appear to be partly attributable to women's greater weight control involvement and partly to their stronger beliefs in healthy eating (Wardle et al. 2004).

Likewise in another study of 480 men and 515 women, aged 15-90 years, women over men and older women over younger have the best values of healthy-diet index. Women consumed cooked vegetables, green salad/shredded vegetables, fruit and fish more frequently than men. On the other hand men consumed meat and used fat spreads more frequently than women. Also increasing age was associated with a more frequent intake of cooked vegetables and fish (Dynesen et al. 2003).

Moreover comfort food preferences vary across gender and age. Males preferred warm, hearty, meal-related comfort foods (such as steak, casseroles, and soup), while females instead preferred comfort foods that were more snack related (such as chocolate and ice cream). In addition, younger people preferred more snack-related comfort foods compared to those over 55 years of age (Wansink, Cheney and Chan, 2003).

1.1.2.1.2 Hunger

Hunger or cravings are very important factors on the food choice. Often the first response individuals give, when they are asked why they eat a particular food, is hunger or craving. Usually when they are hungry they want something fast and something that will fill them up at least for a while and if they are craving a particular food, they will go in search of that food, even they aren't necessarily hungry (Neumark-Sztainer et al. 1999). Moreover hungry individuals (late in the afternoon) chose more unhealthy snacks than did satisfied ones (immediately after lunch), (i.e. fruit and junk food) (Read and Van Leeuwen, 1998).

1.1.2.1.3 Genetic predispositions

The genetic predispositions that initially constrain food preferences include: a) the preference for sweet and salty tastes and the rejection of sour and bitter tastes, b) the tendency to reject novel foods and c) the ability to learn food preferences based on the frequency of exposure and the postingestive consequences of eating (Birch, 1999).

This innate preference for sweet and salty tastes and the avoidance of bitter and sour tastes may reflect an evolutionary background: obtaining sufficient energy from plant sources, regulating sodium balance and predicting toxicity.

Rosenstein and Oster, (1988) examined the differential facial responses of newborns to four basic tastes. Facial responses to sucrose were characterized primarily by facial relaxation and sucking. On the other hand, lip pursing in response to sour and mouth gaping in response to bitter taste. There was no distinctive facial expression for sodium chloride solution.

Beauchamp and Moran, (1984) examined the preferences for sweet solutions versus water in 2 year old children. Children who had been regularly fed sugar water by their mothers consumed more sucrose solutions but not more water than did children whose mothers did

not feed them sugar water. However, when these children were tested with sucrose in a fruit-flavored drink base, prior exposure to sugar water was unrelated to consumption of sweetened or unsweetened fruit-flavored drink. Thus, the apparent effects of dietary exposure on sucrose acceptability were specific to the medium in which sucrose was dissolved.

Another series of tests evaluated response to salt with soup and carrots. Individual children who ingested more salty than plain soup also tended to ingest more salty compared with plain carrots.

There is a developmental change in response to saline solutions during human infancy. Infants less than 4 months of age were indifferent to sodium chloride solutions relative to water, consuming both in equal amounts. In marked contrast, infants 4-24 months exhibited heightened acceptance of saline solution relative to water. These data demonstrate not only a much earlier manifestation of salt acceptance in humans, but also that this acceptance does not initially depend on the interaction of the taste of salt with that of food. Moreover these data are consistent with the hypothesis developed from animal model studies that during early human postnatal development, transductive elements sensitive to saltiness mature (Beauchamp, Cowart and Moran, 1986).

Humans typically show some degree of avoidance to new foods, a trait that has been termed food neophobia, literally ‘fear of the new’. Neophobia may have adaptive value in reducing the possibility of poisoning from unfamiliar and toxic foods, although it will also have costs in terms of limiting dietary variety. Neophobia is minimal in infancy, increases through early childhood, and declines from early childhood to adulthood. By early childhood when children are increasingly mobile and independent and able to forage themselves, neophobia could serve a protective function. Neophobia is reduced as we learn that the food is safe to eat, therefore early experiences and learning can reduce the rejection of new foods.

Food preference is an increasing function of exposure frequency (Birch and Marlin 1982). Cooke, Wardle and Gibson, (2003) asked 564 mothers of 2-6 year-olds to report about their child's eating behavior. According to the results higher levels of neophobia were associated with lower consumption of vegetables, fruit and meat but were unrelated to consumption of sweet, fatty snack foods, starchy staples or eggs. As learning plays the major role in the development of a child's eating behavior, guiding parents in the technique of regular and repeated taste exposure has the potential to improve diets of young children. It is also important to teach parents about innate tendencies for some tastes and developmental stages in the hope that an understanding of these genetic predispositions will increase their awareness regarding feeding practices.

1.1.2.2 Psychological

Shaikh et al. (2008) reviewed the relationship between psychological predictors and fruit and vegetable intake in adults. Strong evidence was found for self-efficacy, social support and knowledge as predictors of adult fruit and vegetable intake. Weaker evidence was found for variables including barriers, intentions, attitudes/beliefs, stages of change, and autonomous motivation.

1.1.2.2.1 Body image

It seems that appearance and body image plays an important role in many aspects of our lives including the food choices. A study of Appleton, (2015) demonstrated the clear value of an appearance-based ("Eat fruit and help your waist") compared with a health-based ("Eat fruit and help your heart") health promotion poster for increasing fruit selection. Whitehead et al. (2012) in a commentary have concluded that appearance may act as a motivator for diet change. They highlighted that interventions advertising the consequences of behavior for appearance have been successful in modifying sun-exposure habits and tobacco use, therefore

such an approach might also facilitate dietary improvement. Authors have illustrated the impact of food pigments on normal skin color as a motivator to increase fruit and vegetable consumption.

1.1.2.2.2 Stress

Stress and emotions may compromise the health of susceptible individuals through negative stress-related changes in food choice.

The best predictors of stress-related eating and drinking at age of 31, were being single or divorced, a long history of unemployment, an academic degree, a low level of occupational education and the lack of emotional support. Stress-driven eaters tended to eat sausages, hamburgers, pizza, and chocolate more frequently and consumed more alcohol than other people (Laitinen et al. 2002).

During periods of high workload total energy intake and total fat intake were significantly higher (McCann et al. 1990).

Moreover the transition to college is a stressful period for both males and females. College norms that define certain types of behavior as "appropriate" under certain conditions, e.g., staying up all night to study for an exam or an assignment, may be stress inducing and may lead to less healthy practices. Therefore during the high-stress period health behaviors deteriorated and students follow less healthy diets and exercise less (Hystad et al. 2009, Bennett, Greene and Schwartz-Barcott, 2013, Weidner et al. 1996).

In a laboratory study Oliver et al. (2000) investigated whether acute stress alters food choice during a meal. The stressor that was used was the anticipation of a speech performance, after a midday meal. The control group instead, was given a nonstressful task of comparable duration, which was to listen to a passage of emotionally neutral text. Increases on blood

pressure and changes in mood confirmed the effectiveness of the stressor. Stress increased intake of sweet fatty foods in emotional eaters. In a similar study, healthy pre-menopausal women exposed in a stress session and in a control session on different days. Salivary cortisol samples were collected. High cortisol reactors consumed more calories in the stress day compared to low reactors, but ate similar amounts on the control day. In terms of taste preferences, high reactors ate significantly more sweet food (Epel, Lapidus and Mcewen, 2001).

1.1.2.2.3 Nutrition knowledge

Nutrition knowledge was significantly associated with healthy eating. Wardle et al. (2000) using 1040 adult participants, showed that respondents in the highest quintile for knowledge were almost 25 times more likely to meet current recommendations for fruit, vegetable and fat intake than those in the lowest quintile. Berg et al. (2002) investigated knowledge and beliefs of 181 schoolchildren, in relationship to breakfast choices. The results showed that knowledge concerning sources and health attributes of dietary fiber was associated with usual consumption of bread and breakfast cereals rich in fiber. Similarly, a positive attitude toward limited fat intake predicted consumption of reduced-fat milk products.

In a similar way, in another study of 445 students aged 4-16 years, nutrition knowledge was positively associated with pasta/rice, fish, vegetable and fruits intakes, and negatively with sweets, snacks, fried foods and sugary drink consumption. Also students with higher nutrition knowledge scores were less likely to have two or more snacks daily and to spend more than 3 hours in sedentary activities daily (Grosso et al. 2012). A recent study reported the effect of a group-based nutrition and physical activity intervention program on nutrition knowledge and eating habits in a cohort of people with obesity. Knowledge and reported consumption of healthier nutrition improved during the active intervention (6-month period) and was

maintained during the self-management period (another 6 months) for individuals who completed the program (Miedema et al. 2016).

On the other hand, many studies provided evidence of weak, positive association between nutrition knowledge and dietary intake (Spronk et al. 2014). Perceived benefits of the food (eg, for health, energy, body shape) is a factor of secondary importance on food choices in adolescents (Neumark-Sztaine et al. 1999).

1.1.2.3 Sensory

Sensory attributes of a product and individual sensory expectations are very important on consumer perception and food product acceptance. Visual sensory properties are of critical importance, as the 'first taste is almost always with the eye'. Therefore visual sensations will affect subsequent willingness to accept a product and will modify the subsequent flavour perception.

Moreover sensory and hedonic experiences interact with post-consumptional experiences to produce responses that feed-back into the consumer physiological state, the learning process and memory building. This process would in turn affect consumer expectation. Expectation can be generated from cues such as packaging, labeling, product information and stereotypes (Imram, 1999).

Consumers want to enjoy their food and in fact many foods are consumed almost entirely for pleasure. Therefore if food producers can optimize the perceived sensory attributes of a product, this will help to increase its perceived value among consumers (Clark, 1998).

Furthermore the eater has learnt consciously or unconsciously their best foods, especially those affecting mood. An obvious example is the lifting of mood, or calming of stressed

‘nerves’, by eating foods having hedonic sensory qualities that elicit pleasure and activation of its palliative neural substrates (Gibson, 2006).

Lampure et al. (2015) investigated the association between a liking for salty and sweet tastes and sociodemographic, psychological and lifestyle characteristics in 37,181 French adults. A liking for salt increased with age, whereas sweet liking decreased. Smokers and alcohol consumers showed a high liking for salt, whereas smokers had a low sweet liking. Furthermore, cognitive restraint was inversely associated with liking for sweet and salty tastes, whereas a liking for the two tastes was higher among uncontrolled eaters, and sweet liking was higher in emotional women.

It is of great interest the fact that sensory perceptions can change across the lifespan and thus can influence eating behaviors at life key time points. In early life, the senses play a crucial role in informing acceptance and rejection of foods. Although in adulthood the role of senses is often simplified into ‘likes’ and ‘dislikes’, there is a more functional role in guiding eating behaviors. A food’s perceptual properties are important for the detection of its nutrient content and through this, guide not only food choice but also habitual energy selection and consumption behavior. As we age, sensory acuity often declines for taste, smell and texture perception, and this can have an impact on food perception, preference and food intake. This creates an opportunity to apply an understanding of sensory influences on choice and intake to stimulate appetite during periods where nutrient intakes may become compromised (Boesveldt et al. 2018).

1.1.3 Environmental characteristics

1.1.3.1 Family

The strongest environmental factor of young child's dietary behavior is the family. Parents can influence children's eating practices by controlling availability and accessibility of foods, meal structure, food modeling, food socialization practices, and food related parenting style (Nicklas et al. 2001).

Children's dietary behavior is influenced by availability and accessibility. Availability concerns whether foods are present in an environment, whereas accessibility concerns whether foods are available in a form, location and time that facilitates their consumption. E.g. fruits and vegetables in the refrigerator's bin versus ready to eat fruits and vegetables pieces or sticks in a plastic bag on the front of a refrigerator shelf.

It seems that children tend to choose to eat foods that they are served most often and they prefer foods that are readily available in home. Therefore preference is an increasing function of exposure frequency (Birch and Marlin, 1982). Children's consumption of fruits and vegetables was related to home availability and accessibility (Cullen et al. 2003, Hearn et al. 1998).

A growing body of research demonstrates that children and adolescents who eat meals with family have better dietary habits. Gillman et al. (2000) examined the association between frequency of eating family dinner and diet quality among 16202 boys and girls aged 9 to 14 years who were children of participants in the ongoing Nurse's Health Study. Results showed that eating dinner with family was associated with healthful dietary intake patterns, including more fruits and vegetables, less fried food and soda, less saturated and trans fat, lower glycemic load, more fiber and micronutrients such as folate, calcium, iron, and vitamins B6, B12, C and E.

Similarly Hammons and Fiese, (2011) in a total sample of 182836 children and adolescents aged 2.8 – 17.3 years showed that children and adolescents who share family meals 3 or more times per week are more likely to be in a normal weight range and have healthier dietary and eating patterns than those who share fewer than 3 family meals together. Therefore study results have suggested that family mealtimes may act as a protective factor for many nutritional health-related problems during childhood and adolescence, including issues of overweight, unhealthy eating, and disordered eating.

In another study authors examined not only the associations between family meal patterns and dietary intake in but also with sociodemographic characteristics. Frequency of family meals was positively associated with intake of fruits, vegetables, grains and calcium-rich foods and negatively associated with soft drink consumption. Positive associations were also seen between frequency of family meals and energy, protein, calcium, iron, folate, fiber and vitamins A, C, E and B6. Sociodemographic characteristics associated with more frequent family meals included gender, school level, mother's employment status and socioeconomic status. Boys reported more family meals than girls; frequency of family meals was higher among middle school than high school students; the mean frequency of family meals was highest among students whose mothers were not employed and lowest among students whose mother worked full time; youths with higher socioeconomic status reported more family meals. (Neumark-Sztainer et al. 2003).

Study findings provide clear evidence of a strong positive association between frequency of family meals and quality of dietary intake. Family meals could improve diet quality in two ways. Family meals contain foods that are more healthful than children and adolescents would otherwise eat and eating meals together engenders conversations about nutritional topics and healthful eating practices.

Parental food modeling is very important tool in learning children optimal behaviors towards food. Parents can best encourage health behaviors such as breakfast consumption, fruit and vegetable intake etc by acting as role models. Fisher et al. (2002) indicated that parents who consume fruits and vegetables may encourage fruit and vegetable consumption in their daughters, leading to higher micronutrient intakes and lower dietary fat intakes. On the other hand parental pressure to eat may discourage fruit and vegetable consumption among young girls. Gibson, Wardle and Watts, (1998) showed that children's fruit intake was related to mothers' frequency of fruit consumption as well to mothers' nutritional knowledge, and mothers' attitudinal conviction that increasing fruit and vegetable consumption by their children could reduce their risk of developing cancer. Young children are more likely to accept unfamiliar foods when adults are also eating the food they offer to them. Moreover more children put food in their mouths when the adult eating the food is their mother instead of anybody else (Harper and Sanders, 1975). A systematic review from 24 published papers on family correlates of children and adolescent's breakfast consumption showed that parental eating breakfast and living in two-parent families were the correlates supported by the greatest amount of evidence. (Pearson et al. 2009).

Therefore the bibliography supports a positive association between parental eating behaviors and eating behaviors of children. Parents should be encouraged to be positive role models to their children by targeting their own dietary behaviors.

Parents often use methods in order to control or modify children's behavior and thus resulting three parenting styles: permissive, authoritarian and authoritative. Permissive feeding might be described as letting the child eat what he or she wants in whatever quantities they want. Authoritarian feeding is characterized by attempts to control the child's eating, through commands, instructions, directives or coercion. Authoritative feeding represents a balance between permissive and authoritarian feeding such that the child is encouraged to eat healthy

foods but also is given some choices about eating options. (Patrick and Nicklas, 2005, Nicklas et al. 2001).

The evidence seems to suggest that an authoritative feeding style is associated with better fruit and vegetables consumption in the childhood and adulthood years (Blissett 2011, Kremers et al. 2003). Parental use of positive reinforcement and monitoring was associated with children's healthy eating and exercise. Also parent's use of appropriate discipline styles was associated with healthier eating, while parental use of control styles was associated with unhealthy eating. The negative impact of controlling parenting style is higher in daughters compared with sons (Arredondo et al. 2006).

1.1.3.2 Partner

Partners are a significant influence on individuals' health behaviors, including dietary habits. Romantic partners (especially women) exert control over their partners' eating behaviors. Men's and women's attempts to regulate their significant others' eating behaviors were associated with their partners' weight status and the extent to which they were satisfied with their partners' bodies (Markey, Gomel and Markey, 2008). Jackson, Steptoe and Wardle, (2015) examined the influence of partner's behavior on making positive health behavior change. Results indicated that when one partner changed to a healthier behavior (newly healthy), the other one partner was more likely to make a positive health behavior change than if their partner remained unhealthy.

Gorin et al. (2008) examined whether a weight loss program deliver to one spouse has beneficial effects on the untreated spouse. As expected, spouses of intervention participants reported a greater decrease in the number of high-fat foods available at their homes and an increase in the number of low-fat foods compared to spouses of control participants. Reduced availability of high-fat foods was associated with greater reductions in energy intake and

better weight losses in spouses, underscoring the potential role home food cues play in weight control.

Adoption of exercise or diet modifications in one individual is likely to spread to others, creating a social environment characterized by mutual reinforcement on healthy behavior (Perry et al. 2016).

1.1.3.3 Price

In a survey of about 3000 adults, price of food, followed taste among the most important influences on their food choices. The importance of cost was highest for younger respondents, women and people with lower incomes (Glanz et al. 1998). Economic constraints may contribute to the unhealthy food choices observed among low socioeconomic groups. Thus a simple cost constraint influences food selection in ways that decrease nutrient densities (Darmon ,Ferguson and Briend 2002). Diets high in fat, sugar, and grains were associated with lower diet costs. For most levels of energy intake, each additional 100 g of fats and sweets was associated with a €0.05–0.40 per day reduction in diet costs. In contrast, each additional 100 g of fruit and vegetables was associated with a €0.18–0.29 per day increase in diet costs. Therefore diets high in fats and sweets represent a low-cost option to the consumer, whereas the recommended “prudent” diets cost more (Drewnowski, Darmon and Briend, 2004). Added sugars and added fats are far more affordable than are the recommended “healthful” diets based on lean meats, whole grains, and fresh vegetables and fruit. There is an inverse relationship between energy density of foods (kJ/g) and energy cost ($/MJ), such that energy-dense grains, fats, and sweets represent the lowest-cost dietary options to the consumer (Drewnowski and Darmon, 2005).

1.1.3.4 Culture/Religion

The geographical, historical and economic contexts of a culture all shape its food preferences. Religion too, can play a role in food preferences. Nowadays as consumers become more affluent they move from satisfying basic physiological needs to fulfilling social and psychological needs that are shaped by the nations and sub-cultures to which they belong. Also the continuing rise of vegetarianism in affluent countries, especially amongst young women, could also be related not only to a heightened sense of animal welfare but to a feminine desire to control body weight. Whilst some cultures, such as Hindu, have always been vegetarian, in most societies the eating of meat has traditionally had masculine, assertive connotations.

There is a divergence in culture and taste among countries. For instance French chefs turn their attention to the creation of delicate and complex meat dishes, whereas in England they concentrate on the production of plain roast meats. In Britain food became a taste of necessity for many, rarely of good quality and often in short supply. On the other hand the French retained stronger links with the peasantry, so they supply of fresh food from the countryside was maintained and the preparation and eating of food remained a very important and hedonistic ritual. Furthermore the strong family and community ties are significant in some countries, such as Greece and Cyprus. The great majority of Greeks stay with their parents until the time they leave to create their own families and in many cases, parents live with their children's family. Families gather on Sundays for meals and celebrate together national events and birthdays, especially in the smaller towns and villages (Wright, Nancarrow and Kwok, 2001). In countries like Morocco, Tunisia and Algeria of North Africa female fatness is viewed as a sign of social status, and is a cultural symbol of beauty, fertility and prosperity. Health institutions in these countries have an enormous challenge to change cultural norms that do not recognize obesity (Mokhtar et al. 2001). Traditional African-American fare,

sometimes referred to as ‘soul food’, is based in part on food practices and specific customs. For example corn is a mainstay food item and meats are often breaded and fried (Kulkarni, 2004).

Religion has a dynamic influence in a range of dietary restrictions across cultural groups. For example fasting is integrated into the lifestyle of many persons and is perceived as a way of retaining the traditions of a culture and setting examples for children to follow. In a study examined the socio-cultural and environmental influences in eating habits in Australian migrant communities an Australian/Greek mother mentioned: “Our children sometimes fast for one week. My husband fasts for one week. Some men fast. The elderly fast. We try to keep our tradition and set examples for kids”. (Green et al. 2003).

1.1.3.5 Advertising

The power of food advertising on eating behavior and especially in children is of critical interest. Unfortunately, media food advertising does not support the recommendations for healthy eating and therefore the viewers exposed to an environment that predominantly promotes foods high in fat, sugar and salt.

Halford et al. (2004) showed that exposure to food adverts promoted food consumption in all three groups of children (lean, overweight and obese) aged 9-11 years old. Moreover obese and overweight children had the greater consumption and also these children recognized a greater number of food adverts compared to lean ones. Similarly Halford et al. (2007) also demonstrated that exposure to food adverts increases energy intake in young children (5-7 years old) regardless of their weight status. While the greatest increase in calories came from the intake of sweet foods, advert exposure significantly increased the intake of all study foods, with the exception of fruit. Critically, as none of the study foods were the brands shown in the adverts and were presented without any packaging, in this situation, advert

exposure clearly produced effects on children's feeding behavior 'beyond-brand', producing a net increase in consumption.

Harris et al. (2009) have extended food advertising research beyond children, to adults. The results demonstrated that children consumed 45% more when exposed to food advertising. Adults consumed more of both healthy and unhealthy snack foods following exposure to snack food advertising compared to the other conditions (food advertising that promoted nutrition benefits, or no food advertising). In both experiments, food advertising increased consumption of products not in the presented advertisements, and these effects were not related to reported hunger or other conscious influences.

1.2 Dietary and behavioral habits of young adults

Young adulthood, defined as 18-25 years of age, is an important transitional period from adolescence to adulthood, during which long-term health behavior patterns are formed and established. This life stage is critical as many changes occur during it, such as the development of self-identity, leaving home and increased autonomy in decision-making (Nelson et al. 2008). Especially the transition to university is a critical period for young adults, who are facing first opportunity to make their own food decisions and therefore young adults are an important population, which is at a key crossroads in nutritional health.

Young adults should have good dietary habits with adequate nutrient intakes, not only for overall good health, but also because the skeletal development continues during that period. Although approximately 90% of peak bone mass is attained by the age of 18 years, bone mass keeps growing until around age 30 (Manifold 2014). However young adults tend to follow a poor diet, marked by low consumption of fruits and vegetables and high consumption of fast food and sugar-sweetened beverages (Pelletier et al. 2014). This can influence not only their concurrent health but also their future risk for a number of chronic diseases at a later age.

Young adults eat much less than the recommended amounts of fruits and vegetables.

A survey conducted by McLean-Meyinsse et al. (2013) on 305 college students reported that only 13% of students consumed fruits and vegetables at least two times per day, with 50% of the students consuming no fruits and 52% consuming no vegetables daily. Another recent study conducted on university students reported that two thirds of students are not eating fruits and vegetables at all daily (Avram and Oravitan, 2013).

Many young adults have the habit of skipping breakfast; with values ranging between 57% in American young adults (Merten et al. 2009) and 72% in Medical students in Ghana

(Ackuaku-Dogbe and Abaidoo, 2014), indicating a possible relationship between a heavy study load and skipping breakfast. In another study of adolescents and young adults in Sao Paulo, those who ate breakfast were shown to have a significantly higher mean calcium, vitamin D and dairy products intake than those who did not (Peters and al. 2012).

Larson et al. (2009) studied the changes in calcium and dairy intake during the transition from middle adolescence to young adulthood in a five year follow-up study. According to their results, daily mean total intakes of dairy products were reduced by approximately 0.5 servings in both genders between baseline and follow up. Also mean daily calcium intakes of females and males decreased by an average 153 mg and 194 mg respectively. Reports in the literature further suggest that consumption of dairy products by children and adolescents in many countries has waned in recent decades and declines further with age (Dror and Allen 2013, Green et al. 2015, Gopinath et al. 2014).

Students tend to visit often fast food restaurants. Avram and Oravitan, (2013) reported that 26% of students visit a fast food restaurant 2-3 times per week. Niemeier et al. (2006) in a prospective study of 9919 adolescents and young adults concluded that fast food consumption and breakfast skipping increased during transition to adulthood, and both dietary behaviors were associated with increased weight gain from adolescence to adulthood.

Coffee consumption is frequent among young people with males to consume more coffee than females (Lim and Kim 2012, Demura et al. 2013). Coffee consumption may affect diet quality in female college student. The average intakes of dietary fiber, vitamin A, beta-carotene and folate in the non-coffee group have reported to be significantly higher than those in the light coffee (<250ml) and moderate coffee (≥250ml) groups. Also the non-coffee group consumed a significantly higher amount of vegetables compared to the light coffee group (Bae and Kim 2009).

With regards to alcohol consumption, young adults are occasional drinkers (Gotia et al. 2013, Loxton et al 2015). Reasons for drinking in youth include: helping with their shyness, escaping their inhibitions or as a way of being accepted by their peers. The main occasions of alcohol consumption are reported to be participation in social events and going out with friends (Gotia et al. 2013). Moreover a recent article indicated that students of permissive parents drank more beer and this was associated with more alcohol related problems. This study also suggested that young women drank significantly less beer than young men (Whitney and Froiland 2015).

Smoking prevalence among young adults is high. A study conducted in a sample of 1205 Greek university students indicated that 47% of them were smokers and 30% of the smokers had already started smoking at the age of 16 years. The most important factor associated with smoking prevalence was friendship with smokers and maternal smoking (Alexopoulos et al. 2010). Studies suggest that more young males than females smoke cigarettes (Mudhovozi et al. 2012, Muge et al 2014) with even further implications as Atalay et al. (2014) have shown that nicotine dependence may be a factor that affects physical activity among young people.

Young adults do not exercise regularly. A recent study conducted in Spain in a large sample size reported that whereas the majority of children (72.2%) and adolescents (56.4%) reached their recommended physical activity levels, only a 40% of young adults managed to do so. Most active were students in primary schools, with university students being the least active (Cocca et al. 2014). Young men were more active that young women (Sabau 2014, Olchowska-Kotala and Chromik 2013).

Lieberman et al. (2015) indicated that 42% of college students used multivitamins/multiminerals and 17% protein/amino acids. College students appear more likely to use dietary supplements than the general population in order to promote general

health, provide them with more energy, increase muscle strength and enhance performance. While the intake of vitamins supplements in recommended doses may not pose a risk, protein supplements may pose a real risk. Excess dietary protein can adversely affect bone through urinary calcium loss (Barzel and Massey 1998) and high protein diets can accelerate renal disease progression (Lentine and Wrone 2004, Brandle et al. 1996).

It seems that body dissatisfaction is pervasive among young adults and as perhaps expected, is a particular issue for young women, with more women than men desiring a body shape slimmer than their current one (Cheung et al. 2011, Olchowska-Kotala and Chromik 2013).

A birth cohort study in 4100 subjects aged between 22 and 23 years in Brazil showed that the prevalence of body dissatisfaction reached 64%, with 42% of the subjects reporting feeling larger and 22% reported feeling smaller than the desired body size (Mintem et al 2015). While body dissatisfaction may start even before adolescence, longitudinal studies have shown that body dissatisfaction increases between middle and high school and increases even further during the transition to young adulthood (Bucchianeri et al. 2013), making this an important period for future perceptions and self confidence.

2 Pistacia Resins

Pistacia is a genus of flowering plants belonging to Anacardiaceae family and is widely distributed in Mediterranean basin (Browicz 1987). *Pistacia* plants yield resins making longitudinal incisions at close intervals from the base of the trunk up to the thicker branches and allowing the sap to drip onto the specially prepared ground below (Freedman 2011). *Pistacia lentiscus* L. variety chia produces the well-known resin called mastic gum which is globular, with pyriform or elongated tears, 4-8 mm in diameter, pale yellow, clear and glassy when fresh and becoming dull and brittle during keeping. Mastic gum is characterized by an aromatic odour and distinct taste (Ansari et al. 2012). Related species such as *P. atlantica*, *P. palaestina*, *P. terebinthus* native to Asia and the Mediterranean can also produce a resin similar to mastic (Bozorgi et al. 2013).

Resins from *Pistacia* have been extensively used in Mediterranean and Middle Eastern countries, both as dietary supplement and as herbal remedy, since ancient times. Mastic gum is the most well-known *Pistacia* resin in traditional medicine. Ancient Greek physicians including Hippocrates, Theophrastus, Dioscorides, and Galenus recommended its use for the treatment of various gastrointestinal malfunctions (Giaginis and Theocharis, 2011). According to Dioscorides, *P. terebinthus* resin has also been used as antidotal, aphrodiasiac, stimulant, diouretic and suitable to treat the leprosy (Andrikopoulos et al. 2003). Iatrosophikon, a collection of prescriptions from a monastery in Cyprus written down during the island's Ottoman period (1571–1878), also described the use of *Pistacia* resins as analgesic against heart-, belly-, head- aches, to treat problems with ears, eyes, tooth, bad breath, and to heal diseases related with skin, gastrointestinal tract and rheumatics. The health benefits of *Pistacia* resins were exploited under different mode of applications such as oral, external topical application and fume (Lardos, 2006).

The health promoting effects of *Pistacia* resins have attracted the attention of researchers and their phytochemical composition has been explored thoroughly in order to correlate individual phytochemicals with possible health claims (Papageorgiou et al. 1991, Katsoudaki et al. 2005).

2.1 Bioactive compounds

Pistacia resins are a complex mixture of different bioactive groups with the presence of triterpenes and essential oils being characteristic for these resins. The bioactive compounds are classified in Figure 3 based on their chemical structure.

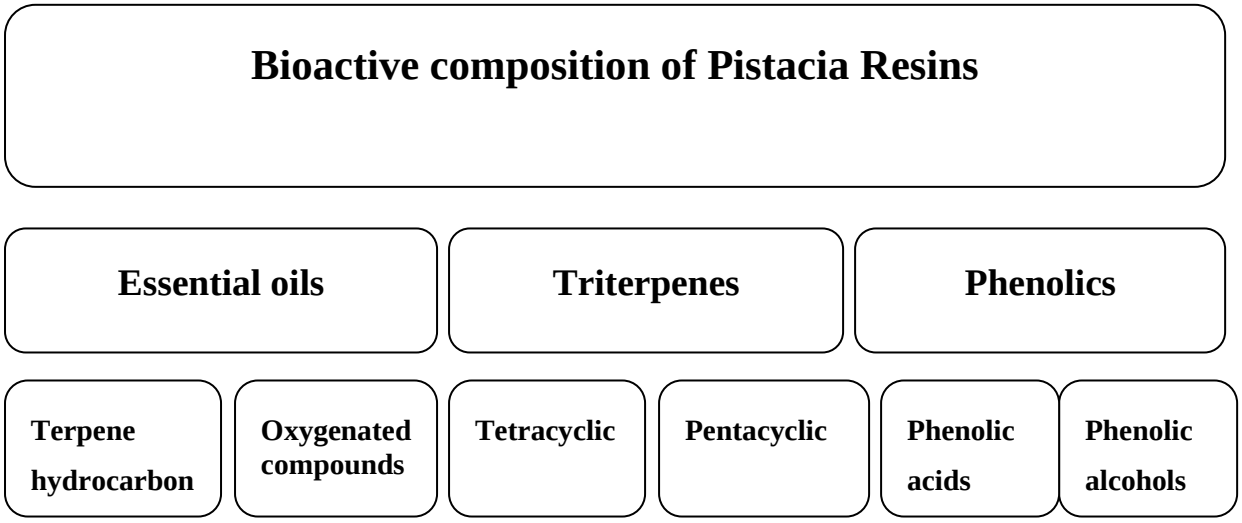

Figure 3. Main groups of bioactive compounds found in Pistacia resins

Essential oils constitute the most studied group of *Pistacia* species components and significant qualitative and quantitative differences were found in terms of chemical composition. Essential oils can be divided into two groups of chemical constituents, the terpene hydrocarbons (monoterpenes and sequiterpenes) and the oxygenated compounds which are mainly phenols, alcohols (monoterpene and sequiterpene alcohols), aldehydes,

ketones, esters, lactones, coumarins, ethers and oxides. Monoterpene content of mastic gum is high, while α-pinene (~80%) is the predominant essential oil of the gum of *Pistacia lentiscus* var.chia. Beta-pinene, camphene, myrcene and limonene have been also detected in their essential oils (Papageorgiou et al. 1991, Koutsoudaki et al. 2005; Kokolakis et al. 2010). The major sequiterpene present in mastic gum is β-caryophyllene and the major monoterpene alcohol is linalool.

Several health claims have been associated with the presence of penta- or tetra-cyclic triterpenes from the oleana(e)ne, dammarane, lupa(e)ne and tirucalla(e)ne skeletons (Assimopoulou and Papageorgiou, 2005a). A comprehensive gas chromatography – mass spectrometry (GC-MS) analysis of resin reported the identification of 36 triterpenes (Assimopoulou and Papageorgiou, 2005b). The main triterpenes in resins were in the following order: isomasticadienonic acid, masticadienonic acid and 28-norolean-17-en-3-one. In particular, the acidic fraction included the major triterpenic acids: masticadienonic acid, masticadienolic acid, morolic acid, oleanonic acid, ursonic acid and their derivatives have been detected in acidic fractions of *P. lentiscus*, *P.terebinthus* and *P.atlantica resins*. Triterpenoid compounds have been also isolated from neutral fraction of *P. lentiscus* and *P. terebinthus* resins such as tirucallol, dammaradienone, β-Amyrin, lupeol, oleanolic aldehyde and 28-norolean-12-en-3-one (Giaginis and Theocharis, 2011).

On contrary to fruit and leaves, *Pistacia* resins are poor in phenolic compounds. Phenolic acids are the main phenolic compounds in the resin. In particular, *p*-hydroxy-phenylacetic, vanillic, gallic and *trans*-cinnamic acids detected in mastic gum. Regarding phenolic alcohols, tyrosol has been reported as the major alcohol in mastic gum (Kaliora et al. 2004).

2.2 Pistacia Resins and Human Health

Numerous studies have revealed several physiological responses to *Pistacia* resins that may be relevant to the promotion of health and the prevention or treatment of some chronic diseases. Figure 4 summarizes the health claims that are correlated with *Pistacia* resins.

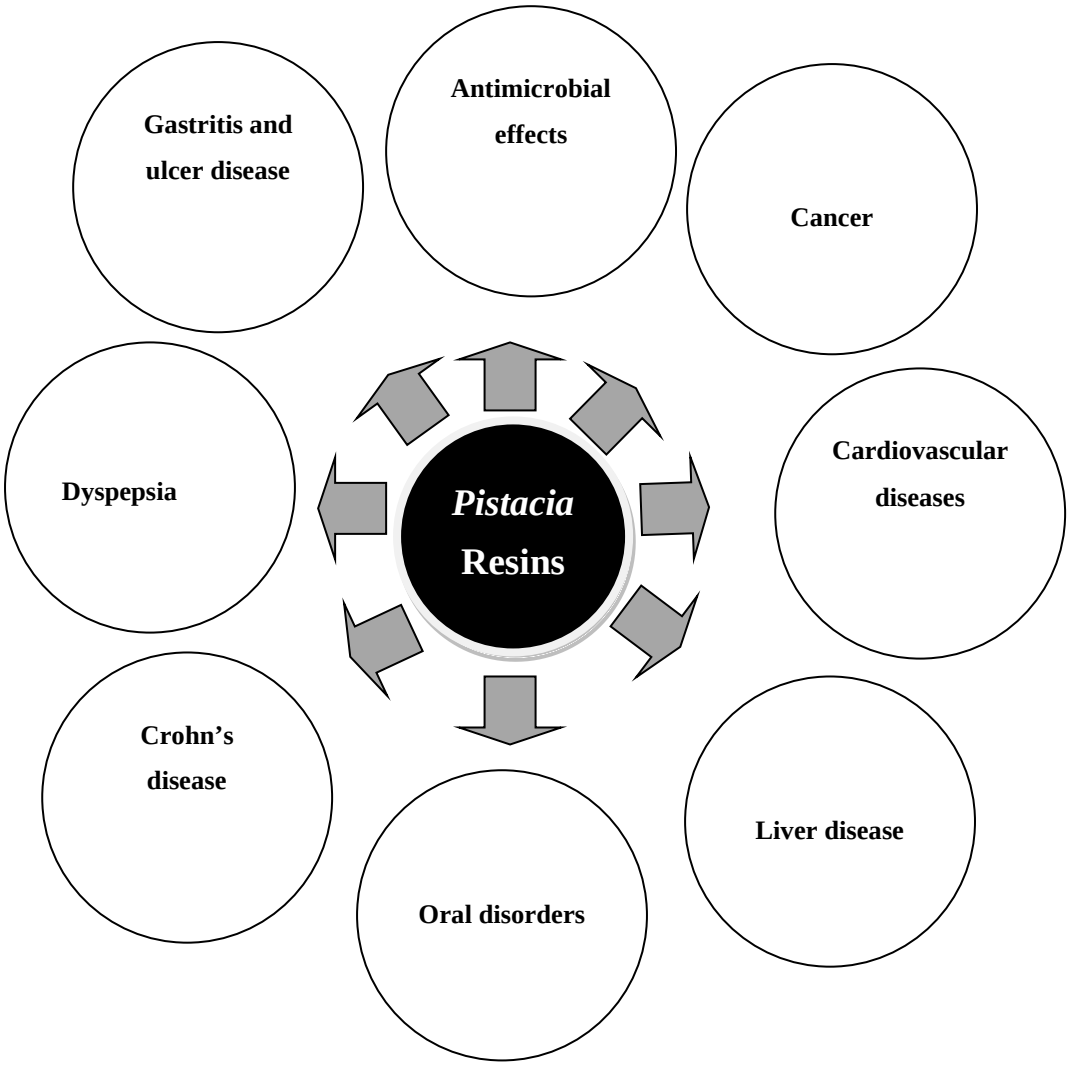

Figure 4. Diseases and disorders potentially prevented or treated by *Pistacia* resins.

2.2.1 Antimicrobial effects

Many studies reported that the mastic gum exhibits antimicrobial activity. A strong antimicrobial effect of mastic gum oil has been found against *Staphylococcus aureus, Lactobacillus plantarum, Pseudomonas fragi, Salmonella enteritidis, Escherichia coli,*

Bacillus subtilis and *Rhizoctania solani* (Tassou and Nychas, 1995, Katsoudaki et al. 2005, Duru et al. 2003).

In general, the inhibition was greater on Gram positive bacteria compared to Gram negative bacteria (Tassou and Nychas, 1995). Terpenes such as verbenone, α-terpineol and linalool contribute significantly to the antimicrobial activity of mastic gum oil. In addition, the antimicrobial potential of isolated terpenes showed that the antibacterial efficacy of mastic gum oil is attributed to its mixture of components working synergistically (Katsoudaki et al. 2005).

Thus mastic gum oil exhibits a significant antimicrobial activity and promising, since no toxic effects have been reported.

2.2.2 Oral disorders

Oral diseases usually including dental caries, periodontal disease and tooth loss are a common health problem. Nutrition and microbiological infection seem to play an important role on oral health (Palmer et al. 2010). Mastic gum appears promising as a potential antibacterial against oral bacteria. Chewing mastic gum decreases the salivary concentrations of cariogenic bacteria. Chewing mastic gum reduced significantly the bacteria colonies during the 4 hours, while the mastic group showed a significant reduced plaque and gingival index compared to the placebo group (Takahashi et al. 2003) Furthermore, studies reported that chewing mastic gum had significant antibacterial activity against *Streptococcus mutans* and *mutans streptococci*, *Porphyromonas gingivalis* and *Prevotella melaninogenica* (Aksoy et al. 2006; Sakagami et al. 2009). Aksoy et al. (2007) confirmed these results showing that chewing mastic gum decreased the total number of viable bacteria, *Streptococcus mutans* and *lactobacilli* in saliva in orthodondically treated patients with fixed appliances. Moreover their findings demonstrated that mastic stimulates the survival of oral polymorphonuclear

leukocytes by preventing their apoptosis and thus may contribute in the potentiation of natural immunity such as elimination of bacteria from oral cavity. Finally mastic may have protective role of oral malodor (Sterer et al. 2008).

2.2.3 Dyspepsia

Dyspepsia or indigestion is a general term that is frequently used to describe discomfort in the upper digestive tract. Symptoms may include abdominal pain, bloating, nausea, vomiting and belching. Symptoms of prolonged dyspepsia may be related to underlying problems such as gastrointestinal reflux, gastritis, peptic ulcer disease, delayed gastric emptying, gallbladder disease or cancer. Dyspepsia in the absence of any specific pathology is referred to as functional dyspepsia. Chios mastic gum is traditionally used in dyspepsia. Recently a double-blind placebo controlled trial assessed the effects of this natural remedy in patients with functional dyspepsia. It is worth noting that 77% of the patients receiving 350mg Chios mastic gum three times daily for three weeks, reported significant improvement of symptoms. Therefore mastic gum appears to be effective in the treatment of functional dyspepsia. (Dabos et al. 2010a).

2.2.4 Gastritis, ulcers and liver disease

Gastritis and peptic ulceration may result when microbial, neural or chemical abnormalities disrupt the factors that normally maintain mucosal integrity (Mahan and Escott-Stump 2000). The most common cause is *Helicobacter pylori* infection. *H. pylori* is a Gram–negative, microaerophilic, spiral bacterium that colonizes the stomach. Infection with *H. pylori* is associated with gastritis, peptic ulceration, gastric lymphoma and adenocarcinoma (Versalovic 2003).

The *in vitro* antibacterial activity of mastic gum against *H. pylori* has been described (Huwez et al. 1998, Marone et al. 2001). However, other *in vivo* experiments showed that mastic gum

has no effect on *H. pylori* (Bebb et al. 2003, Louglin et al. 2003). The explanation came later when researchers prepared a total mastic extract without polymer (TMEWP). The crude resin contains an insoluble polymer that reduces the biovailability of the bioactive mastic constituents. Administration of TMEWP over a period of 3 months in infected mice was effective in reducing *H. pylori* colonization levels by 30-fold. The anti-*H. pylori* activity of mastic gum is mainly attributed to the presence of triterpenic acids such as oleanonic acid, moronic acid, 24Z-masticadienonic acid, 24Z-isomasticadienonic acid, 24Z-masticadienolic acid and 24Z-isomasticadienolic acid, but a synergistic effect was found (Paraschos et al. 2007). Recently, Dabos and co-workers (2010b) confirmed that mastic gum possesses *in vivo* antibacterial activity against *H. pylori* and is able to eradicate it from patients.

Two early studies have shown some effect of mastic gum on gastric and duodenal ulcer. The first study was conducted on experimentally induced gastric and duodenal ulcers rats. Mastic administration produced a significant reduction in the intensity of gastric mucosal damage and a significant decrease of free acidity (Al Said et al. 1986). A double-blind clinical trial carried out on patients with symptomatic and endoscopically proven duodenal ulcer. The results showed increased symptomatic relief in patients receiving 1 g mastic daily over a period of 2 weeks, compared to placebo group, while endoscopically proven healing occurred in 70% of the patients on mastic (Al Habbal et al. 1984).

Mastic gum has been reported to have hepatoprotective effect. Chios mastic powder resulted in a decrease in serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and γ-glutamyltransferase (γ-GT) levels in human population. Thus Chios mastic powder could have a hepatoprotective role in vivo in humans (Triantafyllou et al 2007).

2.2.5 Crohn's disease

Crohn's disease (CD) is a chronic granulomatous inflammatory disease of unknown etiology involving any part of gastrointestinal tract from mouth to anus (Garrow et al. 2003). The gastrointestinal tract is a major immune organ in that it is lined with large numbers of immune cells, such as macrophages and T lympocytes, that are capable of triggering nonspecific and specific immune responses that result in the release of potent proinflammatory cytokines {such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-a)}, eicosanoids and destructive oxygen radicals (Mahan and Escott-Stump 2000). Kaliora et al. (2007a) examined the effectiveness of mastic supplementation on the clinical course and plasma inflammatory mediators of patients with active CD. Despite the relatively small sample size, they achieved statistically significant results.

Four-week mastic administration improved the clinical features of the disease, regulated the inflammation and antioxidant status in CD patients. It significantly decreased the activity index (an index that evaluates the severity of disease) and the plasma lecels of IL-6 and C-reactive protein (CRP), while it increased plasma total antioxidant potential (TAP). Also, nutritional risk index (NRI), one of the clinically useful measures of nutritional status in CD, which is calculated based on serum albumin levels and body weight, was improved.

Moreover in the same subjects conducted another study. This time is assessed the effectiveness of mastic administration on cytokine production of circulating mononuclear cells of patients with active Crohn's disease.

The results showed that mastic act as an immunomodulator on peripheral blood mononuclear cells, acting as a TNF-a inhibitor and as a macrophage migration inhibitory factor (MIF) stimulator. Therefore, mastic gum might serve well in the regulation of immunity in CD patients (Kaliora et al. 2007b).

2.2.6 Cardiovascular diseases

It is well-known that cardiovascular diseases due to atherosclerosis are one of the main causes of death worldwide. They include heart attack, stroke and gangrene of the extremities. Oxidative modification of low density lipoprotein (LDL) by free radicals is an important determinant in the development of atherosclerosis. Oxidative modification accelerates the uptake of LDL by macrophages, which is the beginning of formation of foam cells and eventually of fatty streaks. Mastic gum is believed to offer cardiovascular protection.

Chios mastic gum consumption led to a significant reduction of total cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein(a), apolipoprotein B in human subjects (Triantafyllou et al. 2007). Moreover mastic gum may affect the cardiovascular function through mechanisms of action related to LDL-cholesterol oxidation. Andrikopoulos et al. (2003) demonstrated that Chios mastic gum inhibits LDL oxidation *in vitro*. It has also been shown that the saliva secreted during chewing of mastic gum exhibits protective effect against LDL oxidation (Andrikopoulos et al. 2002). The anti-inflammatory effect of Chios mastic gum on endothelium has been also investigated (Loizou et al., 2009). Mastic extract inhibited the expression of endothelial adhesion molecules (vascular cell adhesion molecule [VCAM]-1 and intercellular adhesion molecule [ICAM]-1). Adhesion of monocytes to endothelium is a crucial step in the pathogenesis of atherosclerosis where this procedure requires the expression of adhesion molecules. Thereafter monocytes converted into macrophages. In another study mastic gum downregulated the expression of CD36, which it is a scavenger receptor of oxidized LDL. Since macrophages attract oxidized LDL to scavenger receptors and through endocytosis they convert into foam cells, this study shows that mastic exert antiatherogenic effect (Dedoussis et al. 2004).

In summary, Chios mastic gum has been associated with cardiovascular protection because of its hypocholesterolemic, antioxidant and anti-inflammatory effects.

2.2.7 Cancer

The role of mastic gum in protection against cancer has been supported by ample evidence from studies in cell culture and animal models. Mastic gum constituents that appear to be mainly responsible for its anticancer potential are triterpenoids (Giaginis and Theocharis, 2011). Chios mastic gum may exert anticancer activity in several types of cancer, including leukemia, prostate, lung and colon cancers. Balan et al. (2005) indicated that the hexane extract of Chios mastic gum can “kill” human colon cancer HCT116 cells in vitro by a mechanism that includes several features of apoptosis. Hydroalcoholic extract (1:1, water-ethanol) of Chios mastic gum also inhibited *in vitro* proliferation and induced death of HCT116 human colon cancer cells. Treatment with the ethanolic extract induced G1-arrest of the cells, detachment of the cells from the substrate, activation of pro-caspases -8, -9 and -3 and further caused morphological changes typical of apoptosis in cell organelles (Balan et al. 2007). The previous studies were extended to investigate the *in vivo* anticancer activity of the hexane extract of mastic gum against human colon tumor, by the use of a colon cancer/ immunodeficient mouse model.

The hexane extract of mastic gum administered at a dose of 200mg/Kg daily for 4 consecutive days (followed by 3 days without treatment) suppressed tumor growth about 35% without any side effects after 35 days. Thus the hexane extract of mastic gum demonstrated a capability to delay the growth of colorectal tumors developed from HCT116 cells xenografted into severe combined immunodeficiency (SCID) mice (Dimas et al. 2009). Mastic oil was also demonstrated to inhibit cell proliferation and survival of human leukemia K562 cells and decrease the release of vascular endothelial growth factor (VEGF) from K562

and B16 mouse melanoma cells. Moreover, mastic oil caused inhibition of endothelial cell proliferation without affecting cell survival and a significant decrease of microvessel formation both *in vitro* and *in vivo*.Investigation of mechanisms of chemopreventive effects reported that mastic oil reduced in K562 cells the activation of extracellular signal-regulated kinases ½ (Erk1/2) known to control leukemia cell proliferation, survival and VEGF secretion. Mastic oil also reduced the activation of RhoA, an essential regulator of neovessel organization in endothelial cells.

These data suggest that mastic oil is a tumor and angiogenesis inhibitor targeting components of critical signaling cascades in both leukemia and endothelial cells (Loutrari et al. 2006). Furthermore there is an increased evidence showing that mastic gum possesses antiprostate cancer properties. He et al. (2006) reported that mastic gum inhibits the expression and function of the androgen receptor (AR) in prostate cancer cells. It seems that androgens and therefore androgen receptor play an important role in the development of prostate cancer. These authors indicated that mastic gum constituents inhibited the expression of androgen receptor at the transcriptional level, resulting in the downregulation of both androgen receptor mRNA and protein levels. The suppression of the androgen receptor function was verified by the reduced expression of all 3 androgen-regulated genes, namely NKX3.1, hK2 and prostate-specific antigen.

Another study by the same research group examined the effect of mastic gum on androgen-independent prostate cancer. They used prostate cancer PC-3 cells where AR expression is absent. Mastic gum inhibited PC-3 cells growth and blocked cell cycle in the G1 phase by suppressing NF-kB activity and NF-kB signal pathway. Therefore mastic gum inhibits prostate cancer development in both androgen-dependent and androgen-independent prostate

cancer cells (He et al, 2007a). Finally, He et al. (2007b) also indicated that mastic gum increases maspin expression in prostate cancer cells.

Maspin is a tumor-suppressing gene for prostate cancer. Mastic gum at a dose of 8μg/ml increased maspin expression about 1,5-fold. It was further demonstrated that maspin mRNA and protein expression was significantly increased by mastic gum. Thus the authors concluded that the increased effects of mastic gum on maspin expression mainly occur at the transcriptional level. Mastic oil is also effective chemopreventive agent to lung cancer. Magkouta et al. (2009) examined the effects of mastic oil on the experimental growth of an aggressive lung cancer type, Lewis lung carcinoma (LLC).

Treatment of immunocompetent mice bearing LLC tumors with mastic oil (45mg/Kg body weight, intraperitoneally, 3 times/week for 3 weeks) significantly inhibited tumor growth without toxicity. This effect was associated with increased apoptosis, reduced neovascularization and inhibition of chemokine expression. Recently a novel study also addressed the anti-metastatic actions of mastic oil. Authors indicated that treatment of LLC cells with mastic oil significantly limited tumor cell invasiveness and migration capabilities in transwell assays, reduced the levels of secreted matrix metalloproteinase 2 (MMP-2), restricted phorbol ester-induced actin remodeling and limited the length of neo-vessel networks in tumor microenvironment. (Loutrari et al. 2011).

Combination of gemcitabine, an antitumor agent used for solid tumors such as ovarian, non-small cell lung and pancreatic cancers, and mastic gum was also supported to be an effective strategy for pancreatic cancer. Huang et al. (2010) found that gemcitabine combined with mastic gum causes potent growth inhibition and apoptosis of pancreatic cancer cells. BxPC-3 and COLO 357 cells were treated with grated concentrations of gemcitabine (0,01-100μg/ml) or mastic gum (10-50 μg/ml) for 72 hours. Overall, 10 μg/ml of gemcitabine or 40 μg/ml

mastic gum produced maximal growth inhibition of 55,55% and 49,75% respectively in BxPC-3 cells.

After co-treatment with these two agents, cell proliferation was inhibited to a much greater extent that with either agent alone. Treatment of COLO 357 cells was also effective. Regarding apoptosis the results were similar, showing that compared with single-agent treatment, the combination resulted in apoptosis in a much higher percentage. Remarkably when cells were treated with gemcitabine in combination with mastic gum, the IkBa level was increased, whereas NF-kB activation was blocked. Moreover the expression of Bax protein was substantially increased, and Bcl-2 protein was down-regulated.

3 Yoghurt

Yoghurt is among the most popular fermented foods in the world, which has great consumer acceptability due its taste and health benefits. It can be used as accompanier of the main meal or as a snack during the day.

Mediterranean diet pyramid suggest the daily consumption of moderate amounts of dairy products, principally yoghurt and cheese (Willett et al. 1995).

The word yoghurt probably comes from the Turkish word “yogurmak”, which means to thicken, coagulate or curdle.

It is believed that milk products were incorporated into the human diet around 10000-5000 BC, with the domestication of milk-producing animals. However, milk spoiled easily, making it difficult to use. At that time, herdsmen in the Middle East carried milk in bags made of intestinal gut. It was discovered accidentally that contact with intestinal juices caused the milk to curdle and sour, preserving it and allowing for conservation of a dairy product for extended periods of time.

In the Bible, Abraham owed his longevity and fecundity to yoghurt consumption. Known scientists in early ages, such as Hippocrates, considered fermented milk a medicine and they prescribe it for curing disorders of the stomach and intestines.

In 1905, a Bulgarian medical student, Stamen Grigorov, was the first to discover *Bacillus bulgaricus* (now *L. bulgaricus*), a lactic acid bacteria that is still used in yoghurt cultures today. Based on Grigorov’s findings, in 1909, the Russian Nobel laureate, Yllia Metchnikoff, from the Pasteur Institute in Paris, suggested that lactobacilli in yoghurt were associated with longevity in the Bulgarian peasant population. The principle of his theory was that the lactic

acid bacteria resulted in the displacement of toxin producing bacteria normally present in the intestine, resulting in prolonged life.

The first industrialized production of yoghurt was taken place in 1919, in Barcelona, Spain at a company named Danone (Fisberg and Machado, 2015;Lourens-hattingh and Viljoen, 2001;Weerathilake et al. 2014).

3.1 Nutritional value

Yoghurt is milk that has been fermented and acidified by viable bacteria, usually *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* creating a thickened product with an extended shelf life. Yoghurt is a rich source of calcium that provides significant amounts of calcium in bio-available form. It is also a good source of phosphorus, potassium, vitamin A, vitamin B2 and vitamin B12. It is also provides high biological value proteins and essential fatty acids. Therefore yoghurt is a nutrition-dense food and a probiotic carrier. A basic nutrient report is given in Table 1.

Table 1. Basic Report, Yoghurt, plain, whole milk

Nutrient	**100gr**	**1 cup (245gr)**
Water (g)	87.90	215.35
Energy (Kcal)	61	149
Protein (g)	3.47	8.50
Total lipid (g)	3.25	7.96
Carbohydrate (g)	4.66	11.42
Minerals		
Calcium (mg)	121	296

Magnesium (mg)	12	29
Phosphorus (mg)	95	233
Potassium (mg)	155	380
Zinc (mg)	0.59	1.45
Vitamins		
Riboflavin (mg)	0.142	0.348
Vitamin B12 (μg)	0.37	0.91
Vitamin A (IU)	99	243
Vitamin D (IU)	2	5

Source: USDA, National Nutrient Database for Standard Reference, 2016

One cup of yoghurt (245gr) covers 40% of Reference Nutrient Intakes (RNI) in calcium, 40% in phosphorus, 10% in potassium, 10% in vitamin A, 30% in vitamin B2 and 60% in vitamin B12 for males and females aged 19 – 50, as conducted by Committee on Medical Aspects of Food Policy (COMA).

Calcium is needed to permit optimal gains in bone mass and density in the prepubertal and adolescent years and continues to be important for maintenance of bone health throughout life cycle. In addition to its role in building and maintaining bones and teeth, calcium also has a number of metabolic roles in cells in all other tissues. Calcium influences the transport functions of cell membranes, the transmission of ions across membranes of cell organelles, the release of neurotransmitters at synaptic junctions, the function of protein hormones and the release or activation of intracellular and extracellular enzymes. Calcium is also required for nerve transmission and regulation of heart muscle function. Moreover calcium ions serve as required cofactors for several enzymatic reactions, including the conversion of prothrombin to thrombin.

Phosphorus, as phosphates, participates in numerous essential functions in the body. DNA and RNA are based on phosphate. The major cellular form of energy, (adenosine triphosphate) (ATP), contains high-energy phosphate bonds, as done creatinine phosphate. As part of phospholipids, phosphorus is present in every cell membrane in the body. Finally, phosphates combine with calcium ions to form hydroxyapatite, the major inorganic molecule present in bones and teeth.

Potassium is the mainly extracellular cation. Along with sodium, it is involved in maintaining normal water balance, osmotic equilibrium and acid-base balance. Along with calcium, it is important in the regulation of neuromuscular activity. Potassium also promotes cellular growth.

Vitamin A has essential role in vision, growth and development and maintenance of epithelial tissue, immune functions, and reproduction.

Vitamin B2 is essential for the metabolism of carbohydrates, amino acids and lipids and also supports antioxidant protection. It discharges these functions as the coenzymes flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN). FMN is also required for the conversion of pyridoxine (vitamin B6) to its functional form pyridoxal phosphate. FAD is also requires for the biosynthesis of the vitamin niacin from the amino acid tryptophan.

Vitamin B12 plays important roles in the metabolism of propionate, amino acids and single carbons. These steps are essential for normal function in the metabolism of all cells, especially for those in the gastrointestinal tract, bone marrow and nervous tissue.

Yoghurt is a good source of fatty acids such as Conjugated linoleic acid (CLA), linolenic acid (ω-3) and linoleic acid (ω-6).

At present there is a great interest in CLA because it appears to exhibit many health benefits related to anti-obesitic, anti-carcinogenic, anti-atherogenic, anti-diabetagenic, immunomodulatory, apoptotic and osteosynthetic effects (Benjamin and Spener, 2009).

Linolenic acid (ω-3) and linoleic acid (ω-6) belong to polyunsaturated fatty acids and are essential fatty acids. In diet is important to use a ratio of ω-6 to ω-3 polyunsaturated fatty acids of about 1-2:1 instead of a radio 20-30:1 that appears in Western diets. ω-3 fatty acids have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties. These beneficial effects may contribute to the prevention of many chronic diseases, such as, coronary heart disease, hypertension, type 2 diabetes, renal disease, rheumatoid arthritis and inflammatory bowel diseases, as reviewed by Simopoulos (1997).

Serafeimidou et al. (2012) determined the fatty acid composition of various yoghurts, come from cow, sheep or goat milk and that are found in Greek market. The fat content of yoghurts was in the order of goat < cow < sheep. The c-9, t-11 CLA isomer was the predominant one present in yoghurts. CLA content ranged between 0.128 - 1.501, 0.405 - 1.250 and 0.433 - 0.976 (g/100g fat) in cow, sheep and goat milk yoghurts respectively. Low fat yoghurts showed lower values of c-9, t-11 CLA content on lipid basis compared to full – fat yoghurts. Samples from mountain areas showed average c-9, t-11 CLA content higher than those from prairie districts. Interestingly the highest amounts of saturated fatty acids (SFA) were found in low-fat yoghurts.

Yoghurt is a probiotic carrier and can be categorized into two different groups namely, standard culture yoghurt and bio- or probiotic yoghurt. Standard yoghurt refers to those made with *L. bulgaricus* and *S. thermophilus*. Bio yoghurts are manufactured by culturing additional beneficial microorganisms, typically the probiotic strains of Bifidobacteria and *L. acidophilus*.

Probiotics have multitude beneficial effects on human health including immune system enhancement (Perdigon et al. 1995, Galdeano and Perdigon 2006), treating diarrhea (Guandalini 2011, Szajewska et al. 2001), treating inflammatory bowel diseases such as Crohn's disease and ulcerative colitis (Sheil et al. 2007, Campieri and Gionchetti 1999), relieving from the symptoms of irritable bowel disease (Moayyedi et al. 2008), preventing cancer (Kumar et al. 2010, Rafter 2003) and lowering cholesterol (Ooi and Liong 2010).

It is recommended to consume 3 servings of milk or milk products daily in order to meet nutritional requirements especially for calcium and all these important nutrients. Although it is possible to meet calcium intake recommendations without consuming dairy foods, nondairy alternatives can result in other nutrient shortfalls. The use of nondairy calcium replacement foods (fortified soy beverage, fortified orange juice, bony fish, leafy greens) is not a realistic option because most replacement foods are rarely consumed. Thus replacing actual dairy product intake with a calcium-equivalent amount of nondairy foods does not result in a nutritionally equivalent diet (Fulgoni et al. 2011).

3.2 Types of yoghurt

Yoghurt products can be classified into about five categories:

1. Set type yoghurt is incubated and cooled in the final package and is characterized by a firm jelly-like texture.

2. Stirred type yoghurt is incubated in a tank and the final coagulum is broken by stirring prior to cooling and packing. The texture of stirred yoghurt will be less firm than a set yogurt, somewhat like a very thick cream.

3. Drinking type yoghurt is very similar to stirred yogurt, having the coagulum broken prior to cooling. In a drinking yoghurt the agitation used to break the coagulum is severe. Little if any reformation of the coagulum will occur after packing.

4. Frozen type yoghurt is inoculated and incubated in the same manner as a stirred yogurt. However, cooling is achieved by pumping through a whipper/chiller/freezer in a fashion similar to ice cream. The texture of the finished product is mainly influenced by the whipper/freezer and the size and distribution of the ice crystals produced.

5. Concentrated yoghurt is inoculated and fermented in the same manner as a stirred yoghurt. Following the breaking of the coagulum, the yogurt is concentrated by boiling off some of the water; this is often done under vacuum to reduce the temperature required. Heating of low pH yoghurt can often lead to protein being totally denatured and producing rough and gritty textures. This is often called “strained” yoghurt because the liquid that is released from the coagulum upon heating used to be strained off in a manner similar to making soft cheese (Kim and Oh, 2013).

3.3 Manufacturing procedure of yoghurt

The generalized process of yoghurt making is comprised of modifying the original composition of milk, pasteurizing the yoghurt mix, fermentation at thermophilic temperatures (40-45 ºC) and cooling. The production steps in manufacture of stirred- and set yoghurt are illustrated in the Figure 5 (Weerathilake et al. 2014).

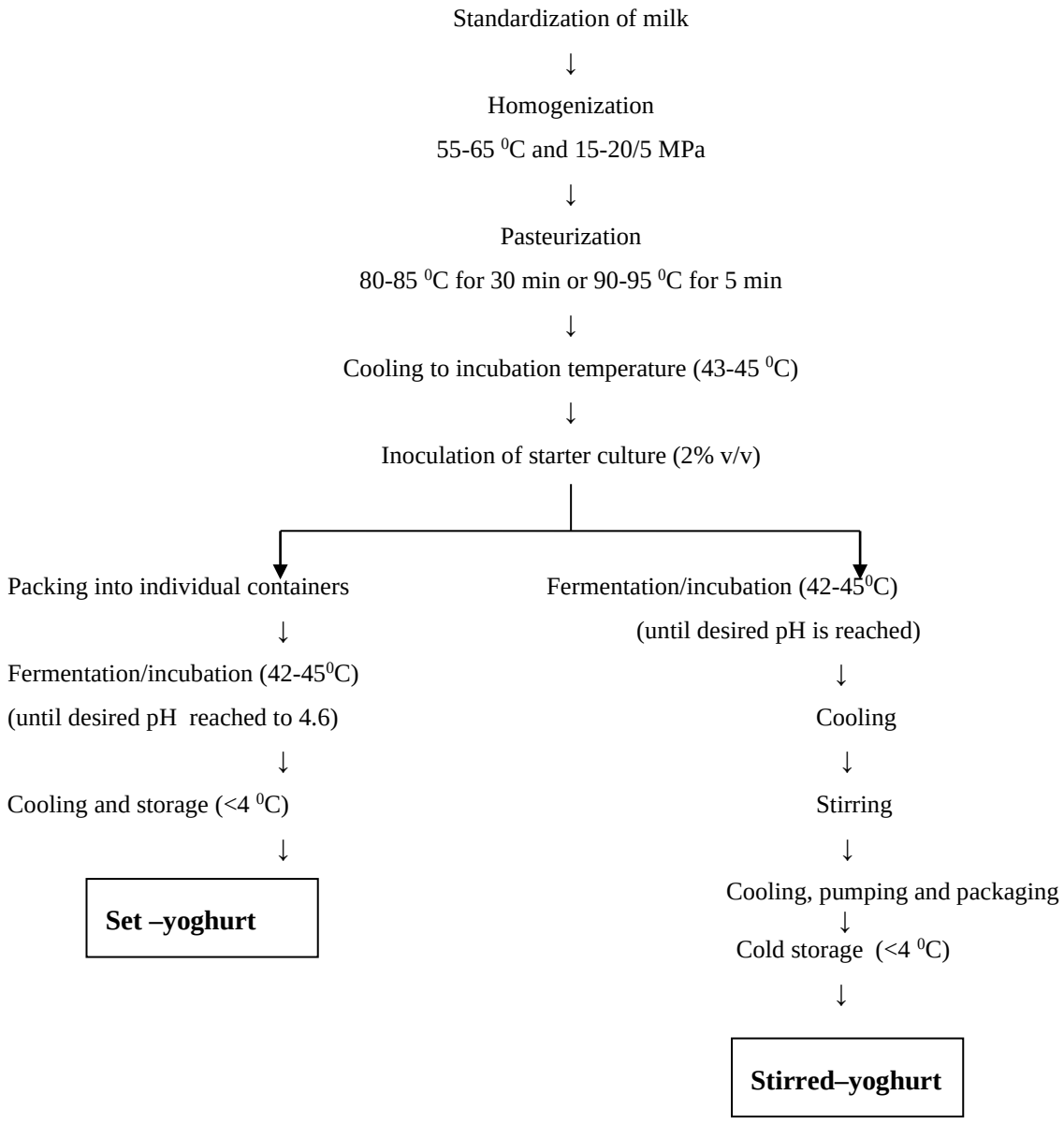

Figure 5. Manufacturing process of set- and stirred- yoghurt

3.4 Yoghurt and health

Yoghurt consumers tend to follow a better, quality diet, to have higher potassium, vitamins B12 and B2, calcium, magnesium and zinc intakes. In addition yoghurt consumers are associated with lower levels of circulating triglycerides, glucose, and lower systolic blood pressure and insulin resistance (Wang et al. 2013).

3.4.1 Bone health

Osteoporosis is an increasingly common, chronic condition which is characterized by decreased bone mineral density (BMD) with a significantly increased risk of fracture and, subsequently, morbidity and mortality.

In 2506 men and women from the Framingham Offspring Study, participants with high yoghurt intake (>4 servings per week) had higher BMD at the trochanter compared to those with no intake, while no significant associations were observed for other bone sites (Sahni et al. 2013).

In a recent cohort study of 4310 adults higher yoghurt intake was associated with increased BMD and physical function scores. More specifically a higher percentage of females were yoghurt consumers with mean daily yoghurt servings significantly higher than males (0.42 vs 0.32/day, respectively). In females, yoghurt intake was a significant positive predictor of BMD at all regions. Total hip and femoral neck BMD in females were 3.1–3.9% higher among those with the highest yoghurt intakes (>once per day serving) compared to the lowest (<once per week serving/never). In males, vertebral BMD was 4.1% higher in low yoghurt consumers compared with non-consumers. Each unit increase in yoghurt intake in females was associated with a 31% lower risk of osteopenia and a 39% lower risk of osteoporosis and in males, a 52% lower risk of osteoporosis (Laird et al. 2017).

3.4.2 Gut health

Yoghurt consumption showed promising health benefits for certain gastrointestinal conditions, including lactose intolerance, constipation, diarrheal diseases, colon cancer, inflammatory bowel disease, Helicobacter pylori infection, and allergies (Adolfsson, 2004).

Antibiotic-associated diarrhea (AAD) is a common complication in individuals treated with antibiotics. Although preventive measures include the use fermented products such as yoghurt, its efficacy remains unclear since the studies' results are controversial. A systematic review and meta-analysis indicated no consistent effect of yoghurt consumption for preventing AAD (Patro-Golab et al. 2015).

Moreover, even the yoghurt contains extra probiotics, not all placebo-randomised controlled trials demonstrated the efficacy of probiotic yoghurt in AAD. Pereg et al. (2005) recruited randomly 541 young males to receive either a yoghurt containing *Lactobacillus casei* or a nonprobiotic yoghurt. Authors demonstrated a nonsignificant trend for reduction of the incidence of diarrhea among healthy young adults consuming yoghurt containing Lactobacillus casei. Similarly, in a three-arm study (bio yoghurt, commercial yoghurt, no yoghurt), yoghurt has not any significant effect on AAD (Conway et al. 2007). On the other hand, Fox et al. (2015) study the efficacy of a probiotic yoghurt containing *Lactobacillus rhamnosus GG, Bifidobacterium lactis* and *Lactobacillus acidophilus* compared to a pasteurised yoghurt (200 g/day) for the prevention of antibiotic-associated diarrhea in children aged 1-12 years. The probiotic yoghurt was proven an effective method for reducing the incidence of ADD in children.

Promising are the effects of yoghurt on acute diarrhea in 6-24-month old hospitalized infants. Infants in the case group received at least 15ml/kg/day of pasteurized cow milk yoghurt orally plus routine hospital treatment. According to the results significant differences were

observed in mean hospitalization days, reduction in diarrhea frequency and weight gain (Pashapour and Iou, 2006).

Lorea Baroja and Kirjavainen, (2007) assessed the anti-inflammatory effects of probiotic yoghurt in subjects with inflammatory bowel disease. All the subjects consumed *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 supplemented yoghurt for 30 days. The proportion of $CD4^+$ $CD25^{high}$ T cells increased significantly in IBD patients after treatment, but non-significantly in controls. The basal proportion of tumour necrosis factor (TNF)-a^+/ interleukin (IL)-12^+ monocytes and myeloid DC decreased in both subject groups, but of stimulated cells only in IBD patients. Also serum IL-12 concentrations and proportions of IL-2^+ and $CD69^+$ T cells from stimulated cells decreased in IBD patients.

A multi-centre, randomized, double blind, controlled trial, set in 13 general practices within central England, evaluated the effect of a probiotic vs nonprobiotic dairy product on symptoms in irritable bowel syndrome which included constipation as a feature.

Significant improvements were reported for most outcomes in all trial participants but improvement did not differ by group. Therefore this study recommended the inclusion of a fermented dairy product and the requirement of a probiotic one was not supported (Roberts et al. 2013).

Furthermore the efficacy of pasteurised yoghurt in improving chronic constipation was proven in a double-blind, randomised, placebo-controlled study of 118 constipation subjects, where fed a placebo or pasteurised yoghurt, with non viable microorganisms, for 7 weeks. The subjects who ingested pasteurised yoghurt showed a significant increase in their defecation frequency following one week of the intervention. Constipation symptoms such as straining, lumpy or hard stool, and sensations of incomplete evacuation and anorectal blockage were all ameliorated. The numbers of faecal bifidobacteria and lactobacilli, and the

short-chain fatty acid concentrations increased significantly in the treatment groups (Liu et al. 2015).

In another randomized controlled clinical trial, this time the researchers studied the the effect of probiotic yoghurt on constipation in pregnant women: A treatment group received 300 g of yoghurt enriched with *Bifidobacterium* and *Lactobacillus* while the control group received conventional yoghurt , which also contained *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* for 4 weeks. Bowel performance was improved after consumption of probiotic yoghurt and conventional yoghurt and no significant difference happened between the treatment and control groups. Given that constipation is caused by a combination of mechanical and hormonal factors during pregnancy, it is therefore recommended to include dairy products especially probiotics containing food products in their daily routine as a dietary supplement (Mirghafourvand et al. 2016).

3.4.3 Cardiovascular diseases

There were early indications that yoghurt supplementation of diet causes a significant reduction of serum cholesterol and therefore reduces the risk of cardiovascular diseases (Hepner et al. 1979).

In the HELENA study of adolescents in Europe, an inverse association was observed between consumption of yoghurt and of milk- and yoghurt-based beverages and some CVD risk factors, especially total and abdominal excess body fat (Moreno et al. 2015).

Yoghurt consumption was associated with lower body weight, waist-to-hip ratio, and circumference and tended to be associated with a lower BMI. Also consumers had lower levels of fasting total cholesterol and insulin. Interestingly, overweight/obese individuals who were consumers of yoghurts exhibited a more favorable cardio-metabolic profile

characterized by lower plasma triglyceride and insulin levels than non-consumers within the same range of BMI (Cormier et al. 2016). Farvid et al. (2017) in the Golestan Cohort Sudy, where 42403 men and women participated, noted 11% lower all-cause mortality and 16% lower cardiovascular disease mortality risk with high yoghurt intake.

Babio et al. (2015) indicated that participants in the highest tertile of whole-fat yoghurt consumption had a lower risk of several components of metabolic syndrome, including abdominal obesity, hypertriglyceridemia, low HDL cholesterol, high blood pressure, and high fasting plasma glucose. The associations with low-fat yoghurt were in the same direction as those with total and whole-fat yoghurt, but inverse associations were limited to hypertriglyceridemia, low HDL cholesterol and high fasting plasma glucose.

3.4.4 Diabetes

In recent years, the role of dairy products in the etiology of type 2 diabetes mellitus (T2DM) has created considerable attention in research fields. A meta-analysis of cohort studies indicated an inverse association of daily intake of dairy products with T2DM, suggesting a beneficial effect of dairy consumption in the prevention of T2DM (Tong et al. 2011).

Yoghurt also seems to be promising agent in the management of diabetes. In a randomized, double-blind, controlled clinical trial, 64 patients with type 2 diabetes mellitus, 30 - 60 years old, were assigned to two groups. The patients in the intervention group consumed 300 g/d of probiotic yoghurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 and those in the control group consumed 300 g/d of conventional yoghurt for 6 wk. The consumption of probiotic yoghurt decreased fasting blood glucose and hemoglobin A1c and improved antioxidant status in type 2 diabetic patients (Ejtahed et al. 2012). Moreover the consumption of probiotic yoghurt improved total cholesterol and LDL cholesterol

concentrations in type 2 diabetic people and may contribute to the improvement of cardiovascular disease risk factors (Ejtahed, Niafar and Mofid, 2011).

In another randomized controlled clinical trial, (Asemi et al. 2012) found that compared with conventional yoghurt, consumption of 200 gr per day probiotic yoghurt (*Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12), for 9 weeks in the third trimester of pregnancy prevented the increase in serum insulin levels and the development of insulin resistance.

3.4.5 Immunity

The role for fermented milk products in the modulation of the immune system has been supported by many studies. (Van de Water, Keen and Gershwin, 1999) followed the health of a college-aged (20-40 y) and senior-aged (55-70 y) population during chronic yoghurt consumption. Subjects were asked to eat 200 g of plain yoghurt every day for 1 year; a group that ate heat-inactivated yogurt and a group that ate no yogurt served as controls. Yoghurt consumption, especially for the live-culture groups, was associated with a decrease in allergic symptoms in both age groups. Seniors in the control group experienced an increase in both total and LDL cholesterol, whereas those in the yogurt groups remained stable during the course of the study. There was little effect on γ-interferon (IFN-γ) and Immunoglobulin E (IgE) production, although seniors in the yoghurt group had lower levels of total IgE throughout the year.

Moreover chronic high levels yoghurt consumption (450 gr per day for 4 months) increased the production of IFN-γ by isolated T cells (Halpern et al. 1991).

Daily yoghurt intake in young women had a stimulating effect on cellular immune functions, but the probiotic product did not perform better than the traditional one. In both groups, the expression of CD69 on T lymphocytes increased after yogurt consumption, especially on CD8+ and to a lesser extent on CD4+. The cytotoxic activity also augmented following the intake and this effect persisting after cessation of consumption (Meyer et al., 2006).

Marcos et al. (2004) demonstrated that consumption of milk fermented with yoghurt cultures plus *Lactobacillus casei* DN-114001, by university students, resulted in modulation of the altered immune response caused by psychological stress during the examination period. More specifically the fermented milk was able to modulate the number of lymphocytes and CD56 cells.

3.5 Goat milk yoghurt

Goat milk and its products of yoghurt, cheese and powder have three-fold significance in human nutrition: 1) feeding more starving and malnourished people in developing world than from cow milk; 2) treating people afflicted with cow milk allergies and gastro-intestinal disorders, which is a significant segment in many populations of developed countries; and 3) filling the gastronomic needs of connoisseur consumers, which is a growing market share in many developed countries (Haenlein, 2004).

The basic composition of goat milk is similar to that of cow milk and on average contains 12,2% total solids, consisting of 3,8% fat, 3,5% protein, 4,1% lactose and 0,8% ash. Goat milk has slightly less total casein but more non-protein nitrogen than cow milk. Goat milk also has higher amounts in vitamins and minerals than cow milk (Park 2007). The nutrient composition of goat milk can be greatly influenced by several factors such as season, stages of lactation, breed, diet, individual animal and environmental conditions (Haenlein, 2004).

Goat milk differs from cow or human milk in higher digestibility, distinct alkalinity, higher buffering capacity, and certain therapeutic values in human medicine and nutrition (Kim and Oh, 2013).

The fat of goat milk is more digestible than that of cow milk because the fat globules of goat milk are smaller and have a greater surface area, and lipases in the gut are supposedly able to attack lipids more rapidly (Jandal, 1996). Goat milk exceeds cow milk in monounsaturated (MUFA), polyunsaturated fatty acids (PUFA) and medium chain triglycerides (MCT), which are all known to be beneficial for human health, especially for cardiovascular conditions (Haenlein, 2004). The presence of relatively high levels of MCT in goat milk could be responsible for its stronger flavor. The proteins in goat milk are also digested more rapidly and their constituent amino acids absorbed more efficiently than those of cow milk (Jandal, 1996).

Eissa et al. (2010) evaluated the nutritional, microbial and sensory quality before and after storage of goat milk yoghurt with reference to cow milk yoghurt. Compared to the composition of fresh cow milk, goat milk had higher fat, protein and total solids. Preparation of yoghurt slightly changed the level of protein, fat, ash, total solids and moisture for both goat and cow milk products, suggesting the effect of the indigenous microflora on such constituents. *Lactobacillus* count in goat yoghurt was observed to be higher than that of cow yoghurt. After 5 days of cold storage a significant increase in acidity and a decrease in pH for both yoghurts types were observed. The magnitude of increase in acidity was high in goat yoghurt. After 15 days of storage goat milk yoghurt had a higher acidity and lower pH compared to cow milk yoghurt. A higher significant value of viscosity was obtained for cow milk yoghurt compared to that of goat yoghurt. Viscosity of both yoghurt types significantly decreased with increasing storage time. Therefore continuous activity of microflora in

yoghurt suggests changes in the micro-structure of the media and hence affecting viscosity. Finally the scores of all sensory attributes of goat milk yoghurt were significantly lower than those of cow milk yoghurt. Goat milk yoghurt was evaluated as less consistent and more acid, with non-typical yoghurt taste and flavor.

3.6 Yoghurt as functional food

During the last years the knowledge of the dietary influence on health and well-being has been highly increased which has led to design new and healthier foods reducing the risk of several chronic diseases. The foods thus designed are called functional foods modified in such a way that they have health benefits compared to the non-modified. The market and interest for functional foods is increasing globally, with an estimation of 90.5 billion US$ in 2013 (Kaur and Daus 2011).

Functional foods have been developed virtually in all food categories and mainly in the dairy products, cereals, juices, spreads and eggs and also in baby-food market.

Most early developments of functional foods were those of fortified with vitamins and/or minerals such as vitamin C, vitamin E, folic acid, zinc, iron and calcium. Subsequently, the focus shifted to foods fortified with various micronutrients such as omega-3 fatty acids, phytosterol and soluble fiber and more recently, food companies have taken further steps to develop food products that offer multiple health benefits in a single food (Siro et al. 2008).

As milk is a natural, multi-component, nutrient rich beverage, market trends indicate that milk-based beverages are ideal vehicles for newly discovered bioactive food ingredients targeting lifestyle diseases (Sharma 2005). Bibliography suggests that the market for yoghurt continues to expand and new varieties of yoghurt with novel ingredients emerge.

Firstly *Bifidobacterium* and *Lactobacillus*-enriched probiotic yoghurts are among the most common type of functional food products marketed worldwide. Yoghurt is also used commercially as a carrier for prebiotics (Allgeyer et al. 2010) and cholesterol lowering plant sterols and stanols (Noakes et al. 2005). Recently several attempts have been undertaken to produce yoghurts fortified with various plant extracts, such as extracts from artichoke (*Cynara scolymus* L.), strawberry-tree fruit (*Arbutus unedo* L.), cherry (*Prunus avium* L.) (Cossu et al. 2009), *Aloe barbadensis* and *Aloe arborescens* (Chiodelli et al. 2017), green tea (*Camellia sinensis*) (Amirdivani and Baba, 2015), grape seed (*Vitis vinifera*) (Chouchouli et al. 2013), seaweed (*Ascophyllum nodosum, Fucus vesiculosus*) (O'Sullivan et al. 2016).

Furthermore functional foods not only need to be safe for consumption at the end of their self life, but also need to remain functional. Therefore in the yoghurt probiotic market, survival of the health-promoting microorganisms throughout the commercial life of the product is an important consideration. The food industry in general has adopted the minimum recommended level of 10^6 CFU ml−1 viable cells at the time of consumption (Rubka and Kailasapathy, 1995). Several factors during processing and storage affect the viability of probiotic organisms and thus the development of foods with adequate doses at the consumption time is a challenge. The identified factors include food parameters (pH, water activity, presence of salt, sugar, artificial flavoring, coloring agents), processing parameters (heat treatment, incubation temperature, cooling rate of the product, packaging materials and storage methods) and microbiological parameters (strains of probiotics, rate and proportion of inovulation) (Tripathi and Giri, 2014).

Many studies have been carried out aiming to enhance the survival of probiotics by enriching milk with various food substances required for the growth of lactic acid bacteria.

Growth of some probiotic bacteria was significantly improved in milk supplemented with whey protein hydrolysate (WPH). However, by day 28 of refrigerated storage, the populations of the probiotic cultures that had been grown in samples supplemented with WPH were similar or below those in the control samples. (McComas and Gilliland, 2003). Results from another study showed that some pulse ingredients may have beneficial effects on probiotic and yoghurt starter cultures. Minor benefits of milk supplementation with pea products, particularly pea fibre, were noted for yoghurt starter cultures. However, with probiotic bacteria, lentil flour demonstrated the greatest ability to increase the acidification rates as did soy flour. Pea protein, chickpea flour and pea fibre also showed some promise (Zare et al. 2012). Agil et al. (2013) demonstrated that green lentils selectively enhanced the number of probiotic bacteria in yoghurt in the initial stages of storage and maintained overall microbial counts (starter cultures and probiotics) over a 28-day storage period. Kailasapathy, Harmstorf, and Phillips, (2008) evaluated the effect of commercial fruit preparations (mango, mixed berry, passion fruit and strawberry) on the viability of some probiotic bacteria. Apart from yoghurts containing 10 g/100 g added passion fruit and mixed berry fruit, the addition of 5 g/100 g and 10 g/100 g of the four different fruit mixes to the yoghurt base did not affect the viability of probiotics compared to plain yoghurts. Citrus fiber presence in fermented milks also enhanced bacterial growth and survival of the tested probiotic bacteria. This study indicates that citrus fiber enriched fermented milk have good acceptability and are good vehicles for a variety of commercial probiotics (Sendra et al. 2008).

Another possible supplement for the poor survival of probiotic bacteria is the use of probiotic yeasts such as *Saccharomyces boulardii.* It seems that the use of probiotic yeast *Saccharomyces boulardii* promoted the growth of LAB and its concentration remained steady over 28-day storage period tested. Also this new product had demonstrated relatively good organoleptic scores (Karaolis et al. 2013).

Obviously the addition of these different ingredients in milk affects the physic-chemical characteristics and the sensory properties of yoghurt. Especially sensory aspects are not to be neglected since they are critical to consumer acceptance.

4 Mathematical modeling

The aim of Mathematical Modeling in Applied Sciences is the possibility of prediction of the result of a process without the need each time to perform additional experiments. In our case the product is a yoghurt functional food and the process consists in various treatments trying to obtain a final product of high and stable LAB bacteria. Therefore our Response variable is the LAB population. Microbial populations are expressed in log(cfu) units.

The dependent variable being the LAB population the main factors, independent variables, are Time and Temperature at which the treatment has applied. In the general case of modeling microbial population in Thermal Death Kinetics, where our aim is at obtaining the maximum or a considerable reduction of the population an elaborate system of mathematical modeling has been developed considering tenfold reductions and the use of parameters, such as Z, D, F. The Time was found to affect logarithmically, the Temperature linearly.

4.1 Thermal action

The essence of our approach is that we combined the two factors in one by defining the product of Temperature times the logarithm of Time as thermal Action

The name Action has its roots to the known term of Physics Action as a product of Energy times Time and bearing in mind that Temperature is Concentration of Energy.

The rationale for the definition of the quantity Thermal Action, is based on the fact that Time, t, and temperature, Θ are main factors influencing many phenomena in Food Technology and Preservation, upon thermal treatment. Temperature is dimensionally the concentration of heat that is of the thermal energy, under constant pressure, according to the dimensional analysis equation (1)

[Q] = [ρCρ].[Θ]…… equation (1)

Where Q: being the heat; ρ: the product of the density; Θ: Temperature; Cp: is the specific heat capacity

Since ρ. Cp remains a constant during many processes, temperature instead of heat could be used in the definition of Thermal Action. There is an overwhelming evidence that the effect of the temperature on the result of the thermal treatment is linear, whereas the effect of time is not linear but a logarithmic one (the logarithm with the base of ten is traditionally used). Therefore we coin the concept of Thermal Action SΘ as shown in equation (2).

$S\Theta = \Theta . log\ t$ ……..equation (2)

In a plot of t versus Θ the obtained curves are, thus, isodrastic curves (from Δράσις, pronounced drasis, and the Greek word for Action). Furthermore in a plot of log t versus Θ the obtained isodrastic curves become parallel straight lines. Examples are given below.

4.2 Application in death kinetics

Death kinetics is important to the Food scientist/technologist in a two-fold purpose. There are pathogenic or spoilage microorganisms, the population of which should be kept in a minimum, and on the other hand there are beneficial microorganisms the population of which should be protected. The study of their kinetic inactivation is useful in both cases. *Clostridium botulinum* is a pathogenic organism belonging to the first category mentioned above. In Figure 6 the isodrastic curves are shown based on raw data taken from literature (Heldman and Hartel, 1997). Each point in one of the curves is a pair of temperature and time values which were applied in the thermal treatment of the studied microorganism and yields the same degree of inactivation m. For example, m=12 shows the exponential degree of

reduction of the microorganism's population. If we start with an initial population of the microorganism in concern of 10^{12}, only one individual microorganism will be left active. If instead of time, the decadic logarithm of time is in the Y-axis, the family of the isodrastic curves become parallel straight lines. Also, this result is shown in Figure 7, where the isodrastic lines concern degrees of inactivation m equal to 12, 9 and 6 respectively. Analogous diagrams are shown for two more species of microorganisms, namely *Bacillus stearothermophilus* for low acidity foods of pH >4.5, Figure 8 and Figure 9 and *Bacillus coagulans* for acidic foods of pH <4.5, Figure 10 and Figure 11. The isodrastic curves are thus the loci of the points which are pairs of temperature and time values for which equivalent results are obtained in the reduction of the pathogenic microorganism.

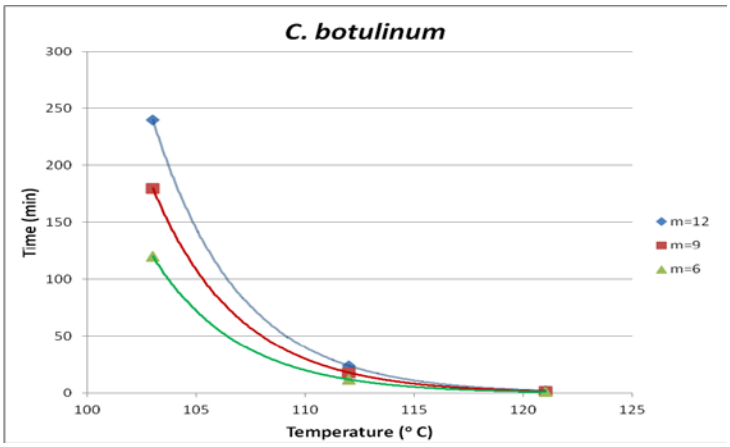

Figure 6: Isodrastic curves of *Clostridium botulinum* in a diagram Time-Temperature for m=6, 9 and 12 respectively.

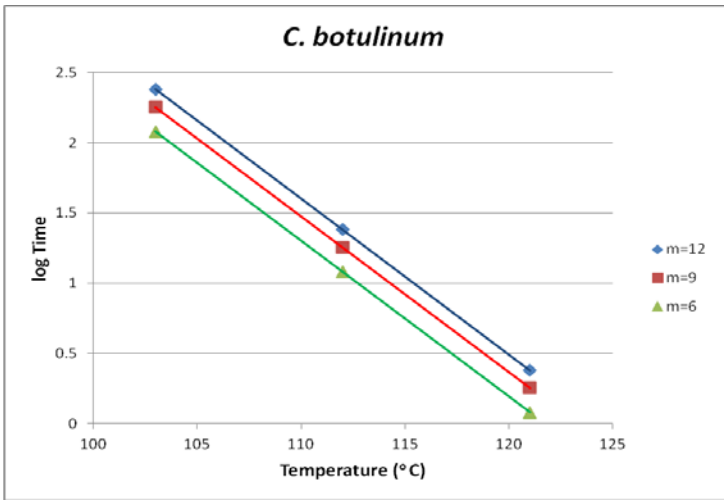

Figure 7: Isodrastic lines of *Clostridium botulinum* in a diagram log(Time)-Temperature for m=6, 9 and 12 respectively.

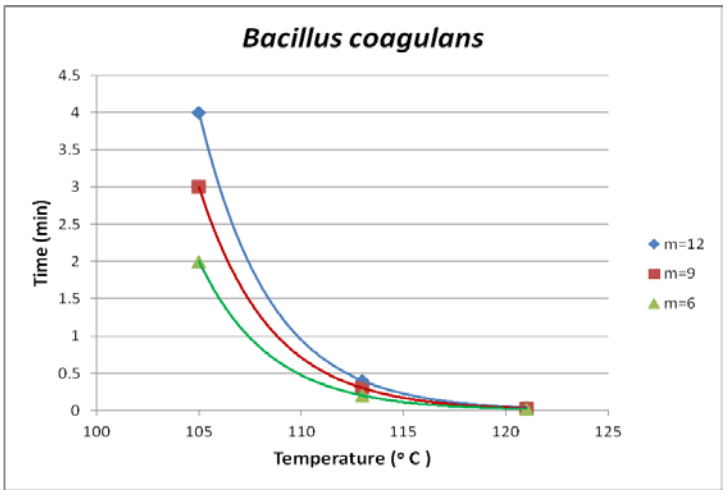

Figure 8: Isodrastic curves of *Bacillus coagulans* in a diagram Time-Temperature for m=6, 9 and 12 respectively.

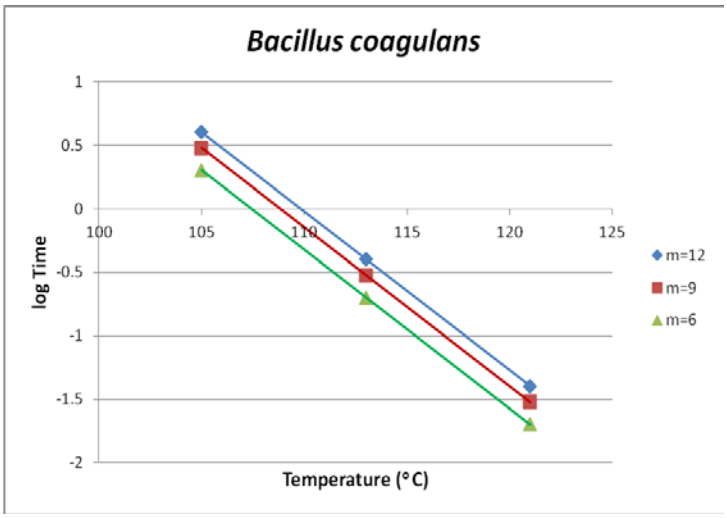

Figure 9: Isodrastic lines of *Bacillus coagulans* in a diagram log(Time)-Temperature for m=6, 9 and 12 respectively.

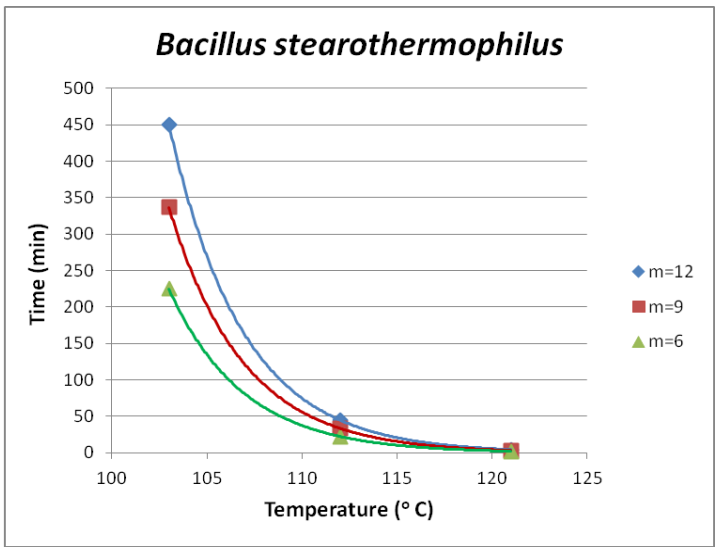

Figure 10: Isodrastic curves of *Bacillus stearothermophilus* in a diagram Time-Temperature for m=6, 9 and 12 respectively.

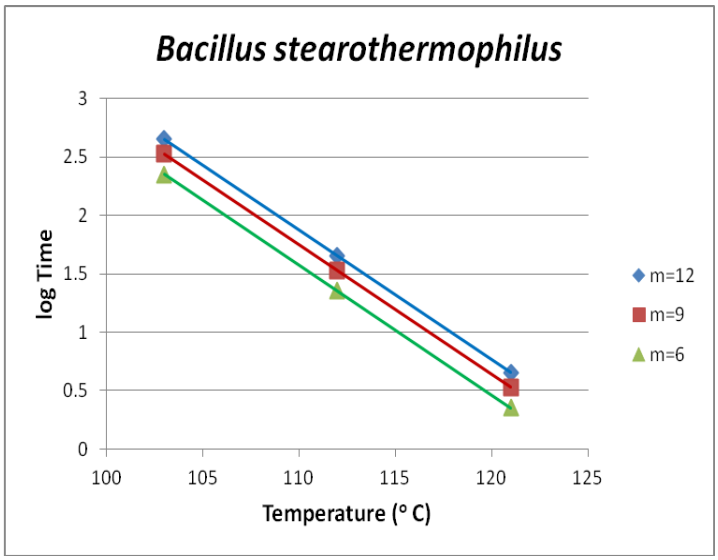

Figure 11: Isodrastic lines of *Bacillus stearothermophilus* in a diagram log(Time)-Temperature for m=6, 9 and 12 respectively.

Apart from the application in death kinetics of pathogenic and spoilage microorganisms thermal action can also be applied when investigating the survival of probiotic bacteria in functional food products.

4.3 Bayes approach

The Bayesian approach is one of the significant tools of prediction also in the Science and Technology. There are four basic elements in Bayesian approach:

1. The prior probability of a hypothesis p(H)
2. The posterior probability given an evidence E, p(H/E)
3. The likelihood i.e. given the hypothesis is fulfilled what is the probability this to be owed to the presence of the evidence E
4. The normalization constant p(E)

Then, the well known Bayes formula connects these four elements:

p(H/E) = p(E/H).p(H)/p(E)

It is possible that we have more pieces of evidence E1, E2… and the equation can be easily generalized to take into account all those evidences.

Another useful relation derived from the one above gives the ratio of the two probabilities the posterior and the prior as the so called Bayes factor which equals the ratio of the likelihood to the normalization constant.

PURPOSE OF THE STUDY

The primary objective of this study was to investigate the dietary and behavioral habits and also the level of adherence to the traditional Mediterranean diet pattern, among Cypriot young adults identified through universities, thus providing much needed evidence for intervention programs targeting this often overlooked age group.

It was considered necessary thought the evaluation of the dietary habits of young adults to also identify the appropriate substrate, a food that is widely consumed by young adults, for the subsequent study and creation of a new innovative functional food, promoting good health.

Therefore, the final aim of this work was to investigate the potential application of *Pistacia* resins alone or in combination with *S. boulardii* in goats' yoghurt investigating how it will affect the LAB normally present in the yoghurt and also the survival of the yeast into yoghurt for a period of 25 days, representing the life cycle of live yoghurt. Moreover, ^{1}H NMR spectroscopy was used to screen the stability of *Pistacia* resin extracts and fatty acid profile. Yoghurts were also assessed organoleptically to reveal the acceptability by young adults. This will lead to the development of a new functional dairy product that does not exist in the market in order to promote good health in young adults.

Mathematical modeling was based on Bayesian approach, and also on an innovative approach. The basis of the innovative approach will be the physical term of Action in applications where the product Temperature Time or Temperature logarithm of Time (which we define as the Thermal Action), is expected to give isodrastic results.

Using action we potentially aimed at modeling the survival and death of the probiotic bacteria in an attempt to be in position to predict not only the conventional shelf life of the product,

but most importantly its functional shelf life. The functional shelf life would be the period of time during which the numbers of the probiotic in concern will be sufficient enough to have an impact on the health of the consumer.

Materials and methods

1 Study participants

A total of 193 young adults aged 18-25 years participated in the present study from October to December 2014 through a convenience sampling. During this period a study researcher visited two university campuses, one public (Cyprus University of Technology, Limassol) and one private (University of Central Lancashire in Cyprus, Larnaca) asking students to self-complete an anonymous questionnaire (appendix 1) on baseline characteristics, including the following questions: “are you currently on a diet” (yes/no), “do you currently exercise” (yes/no), “do you currently take protein supplements” (yes/no), “do you currently smoke” (yes/no), “how often do you drink coffee, tea, wine, beer, whiskey-vodka” (4-6 times/day; 2-3 times/ day; 1 time/day; 1-4 times/week; 1-3 times/ month; few times/ year or never). All data were collected anonymously and completion of the questionnaire was considered informed consent.

Adherence to the Mediterranean diet was evaluated by the KIDMED index (Mediterranean Diet Quality Index for children and adolescents) (Serra-Majem et al. 2004) which includes 16 questions based on the principles of the Mediterranean diet, where those denoting a positive aspect with regards to the Mediterranean diet are assigned a value of +1, and those with a negative aspect -1. A total score ≤ to 3 implies a very low diet quality, a score between 4 and 7 implies a diet that needs improvement to adjust intake to Mediterranean patterns and a score ≥ 8 indicates optimal adherence to the Mediterranean diet. Since young adulthood is a transitional period from adolescence to adulthood, the use of KIDMED index was preferred instead of the Med diet score, which is used in adults, because young adulthood is closer to adolescence. KIDMED score gives more emphasis on fast food and sweets consumption that

Med diet score does, behaviors that are closer to students than older adults. On the other hand KIDMED questionnaire is easier in completion.

Moreover a ‘health habit’ score was created based on information from five important behavioral characteristics (breakfast consumption, eating fried food, eating away from home, exercise and smoking) ranging from 0 to 5, with questions denoting a positive behavior being assigned a value of +1 and those with a negative behavior being assigned a value of 0. Specific questions used were: "Do you skip breakfast?" (yes, no) ; "Do you currently smoke?" (yes, no); "Do you currently exercise?" (yes, no)"; "How often do you consume meals away from home?" (daily, 4-6 times/week, 1-3 times/week, 1-3 times/month, a few times/year or never); "How often do you consume fried food?" (daily, 4-6 times/week, 1-3 times/week, 1-3 times/month, a few times/year or never).

Weight and height were also measured with the use of a portable digital scale and stadiometer at the standing position without shoes by the same study researcher. Body mass index (BMI) was calculated as weight/height^2 (Kg/m^2) and used in the assessment of overweight and obesity among young adults according to the International Obesity Task Force (IOTF) age and sex-specific BMI cut offs (Cole et al. 2000).

2 Extraction process

The extractions of total mastic, neutral and acid fractions were performed according to Paraschos et al. 2007 with slight modifications. A quantity of 50 g mastic gum was diluted in 50 ml ethyl acetate and then 150 ml methanol was added. The mixture was stirrered and applied ultrasound irradiation for 2 hours. The clear solution was obtained by filtration using Bucher funnel and was centrifuged for 10 min and then was evaporated in a rotary evaporator ($T<65^oC$).

The total mastic extract was re-dissolved using 100 ml Na_2CO_3 5% w/v and 350 ml ether was added. Fractionation was performed through liquid-liquid extraction. The organic phase was re-extracted three times with 100ml Na_2CO_3 5 % w/v and afforded the neutral fraction of mastic. The aqueous phase was acidified with 300 ml HCl 1N and then the acidic solution was re-extracted with 600ml diethyl ether and the organic phase afforded the acid fraction of mastic. Finally the two fractions were evaporated to obtain dry extract.

3 Yoghurt manufacture process

Five different yoghurts using goats' milk were made: Yoghurt 1- Control was the control one with only the starter culture added; Yoghurt 2 – Acidic, with acid fraction of mastic extract; Yoghurt 3 – Neutral, with neutral fraction of mastic extract; Yoghurt 4 – Total, with total mastic extract; and Yoghurt 5 – Combined, with total mastic extract and yeast added simultaneously (Table 2). For the manufacture of the five yoghurts, a volume of 500 ml of fresh goat milk was poured in each of five sterilized 1 L Scott Duran® bottles and preheated at 55 ^{o} C. Calcium caseinate (2% w/w) was then added to the milk and thoroughly homogenized (IKA ®T25 digital Ultra Turrax) with a rotating speed of 12000 rpm for 3 min. A heat treatment at 90 ^{o} C for 5 min was carried out, and all five bottles containing the milk were cooled to 46 ^{o} C in a water bath. 2 gr of the starter culture was then added into all five bottles containing the milk. Three of the bottles (Yoghurt 2, Yoghurt 3 and Yoghurt 4) were inoculated in parallel with 300 mg of total mastic extract, neutral fraction of mastic extract and acid fraction of mastic extract respectively, where one of the bottles (Yoghurt 5) was inoculated in parallel with 300 mg of total mastic extract and 200 mg of probiotic yeast *S. boulardii* (Bioflor®; Biocodex, France) and all five were incubated at 46 ^{o} C for 15 min. The yoghurts were then poured into sterile plastic pots and sealed. A volume of 50 ml was poured

in each pot resulting in ten different pots for each Yoghurt, 1, 2, 3, 4 and 5 respectively. All pots were then incubated at 46 ^{o} C for 3 h and were then transferred and stored at 6 ^{o} C. A total of five replicates were made, and starting from Day 0 (Yoghurt manufacture day), one pot from each of the five different yoghurts was analyzed microbiologically and organoleptically up until day 25, which was the maximum storage period tested.

Table 2 The kinds of yoghurts manufactured in the present study

Yoghurts	Name	It contains
1	Control	Starter culture
2	Acidic	Starter culture + acid fraction of the mastic extract
3	Neutral	Starter culture + neutral fraction of the mastic extract
4	Total	Starter culture + total mastic extract
5	Combined	Starter culture + total mastic extract + *Saccharomyces boulardii*

Moreover fresh batches of yoghurt were made using 500 ml of fresh goat's milk and 300 mg total mastic extract following the same procedure as described above. This time the yoghurt pots stored at three different temperatures. Four pots stored at 6 ^{0} C, four pots stored at 10 ^{0} C and four pots stored at 14 ^{0} C. A total of three replicates were made, and starting from Day 0 (Yoghurt manufacture day), one pot from each of the three different temperatures was analyzed microbiologically every one week, until day 28.

4 Enumeration of total lactic acid bacteria and *Saccharomyces boulardii*

Plating and enumeration were performed using a modification of the methods described by Lourens-Hattingh and Viljoen (2001). From each pot of the five different yoghurts 10 g was

aseptically weighted and transferred into sterile stomacher bags, where mixed with 90 ml sterile Maximun Recovery Diluent (MRD; Oxoid, UK) and homogenized in stomacher for 1 min. Serial dilutions were prepared (1:10) using MRD down to 10^{-6}. A volume of 100μL from each dilution was inoculated and dispersed onto MRS agar (Oxoid, UK) for the enumeration of LAB and DRBC agar (Oxoid, UK) for the yeast in duplicates. To create microaerophilic conditions, the MRS agar was overlaid with molten MRS agar at 48 ^{o}C. MRS plates were incubated at 37 ^{o}C and DRBC plates at 25 ^{o}C for 3 days. This procedure was repeated every 3 days, for 25 days, and the total LAB and probiotics yeast *S. boulardii* were enumerated.

5 Organoleptic assessment

Two separate organoleptic assessments were performed; the initial, which was performed by two industry experts, and the young consumer's which was performed by a panel of twenty young potential consumer's aged 18-25. The purpose of the initial organoleptic evaluation was to examine the effects of different mastic extracts and also the effect of the yeast in the product throughout its tested shelf life and select the better of the three batches congaing mastic extracts (Yoghurt 2, 3, 4) for testing in a young potential panel. The yoghurts were evaluated with an organoleptic score (total 100) proposed by Nelson and Trout (1981). In detail, the 100 points were allocated for flavor (50), body and texture (40) and appearance (10). The initial organoleptic assessment followed the tested storage period and was performed in parallel with the microbiological analysis for LAB and *S. boulardii*. Following the results of the initial assessment Yoghurt 4 was selected. Fresh batches of Yoghurt 1, Yoghurt 4 and Yoghurt 5 were made, and the panel evaluation was performed on the third day following manufacture. The twenty young panel assessors were selected on the basis of

regular purchasing of yoghurt, with all of them consuming yoghurt at least twice a week. The tasters were asked to assess the first (Yoghurt 1- Control), the second (Yoghurt 4- Total mastic extract) and the fifth batch (Yoghurt 5- Total mastic extract and yeast added simultaneously). The young tasters were asked to complete a questionnaire examining the parameters proposed by Nelson and Trout (1981), and a spider diagram was plotted to examine differences and assess the acceptability of the mastic extract alone and with combination with yeast containing yoghurts against the plain traditional yoghurt that currently exists in the market.

6 ^{1}H NMR spectroscopy

^{1}H NMR analysis was performed in all yoghurts samples containing *Pistacia* resins alone or in combination with *S. boulardii* (Yoghurts 2, 3, 4 and 5). From each Yoghurt were prepared three samples representing Day 0, Day 14 and Day 25. Firstly yoghurt extracts were prepared by diluting in 100 ml ethyl acetate, stirring for an hour in magnetic stirrer. This procedure was repeated 3 times. The solution was then evaporated in a rotary evaporator and sublimated in freeze dryer. All NMR data were recorded using the spectrometer "Digital NMR Bruker Avance 400MHz". The samples containing a solution of 20 mg of each yoghurt extract in 0,6 ml Chloroform-D_1. NMR analysis was also performed for the total mastic extract, the acid fraction and the neutral fraction of mastic extract, where 5 mg of each extract was diluted in 0,5 ml Chloroform-D_1. The NMR system was controlled by the software TopSpin 3.5

Statistical Methods

Continuous variables are presented as mean ± SD, whereas categorical variables are presented as frequencies. The normality of variables distribution was tested with the Kolmogorov-Smirnov test. The chi-square test was used to evaluate associations between the categorical variables and the Students independent t-test and ANOVA methods were applied to evaluate differences in mean values of continuous variables as appropriate. A two-sided p value of less than 0.05 was considered statistically significant. Data were analyzed using SPSS v.20.0 (SPSS Inc. Chicago Ill) and Microsoft Office Excel 2007.

RESULTS

1 Demographic characteristics of the participants

Out of 193 participants, 87 (45.1%) were men and 106 (54.9%) were women with a mean age of 20.56 (±1.85) years. The mean height was 169.06 (±9.53) cm and the mean weight was 67.09 (±14.9) Kg. The majority of participants came from the districts of Larnaca and Limassol, reflecting the location of the universities sampled. Overall characteristics of the subjects and for men and women separately are presented in Table 3.

Table 3: Baseline characteristics of study participants.

Variables	**All** 193	**Males** 87 (45.1%)	**Females** 106 (54.9%)	***P* value for difference between sexes**
Age (yr)	20.56 ± 1.85	21.20 ± 1.59	20.05 ± 1.89	<0.001
Height (cm)	169.06 ± 9.53	176.66 ± 6.25	162.82 ± 6.86	<0.001
Weight (Kg)	67.09 ± 14.90	78.18 ± 12.37	57.98 ± 9.75	<0.001
BMI (Kg/m^2)	23.31 ± 3.98	25.05 ± 3.68	21.89 ± 3.65	<0.001
BMI categories				
≤ 18.5 (Kg/m^2)	6.2%	2.3%	9.4%	
18.5 – 25 (Kg/m^2)	64.8%	51.7%	75.5%	
25 – 30 (Kg/m^2)	24.9%	41.4%	11.3%	<0.001*
>30 (Kg/m^2)	4.1%	4.6%	3.8%	
Type of education				
Public university	94 (48.7%)	44 (50.6%)	50 (47.2%)	
Private university	99 (51.3%)	43 (49.4%)	56 (52.8%)	0.638
Currently Employed				
Yes	47 (24.6)	27 (31.4%)	20 (19%)	
No	144 (75.4%)	59 (68.6%)	85 (81%)	0.049
Region of main residence				
Nicosia	34 (17.6%)	20 (23%)	14 (13.2%)	
Larnaca	75 (38.9%)	34 (39.1%)	41 (38.7%)	
Limassol	65 (33.7%)	22 (25.3%)	43 (40.6%)	
Paphos	4 (2.1%)	2 (2.3%)	2 (1.9%)	
Famagusta	15 (7.8%)	9 (10.3%)	6 (5.7%)	
				0.121*
Currently Exercising	107 (55.4%)	60 (69%)	47 (44.3%)	0.001
Currently Smoking	46 (24%)	27 (31%)	19 (18.1%)	0.037
Protein supplements	24 (12.4%)	19 (21.8%)	5 (4.7%)	<0.001

Data are presented as mean ± SD and categorical variables as percentages in parentheses.

* *p* value for trend.

2 Body Mass Index

The mean Body Mass Index (BMI) of study participants was 23.31 (± 3.98) Kg/m^2 with 6.2% of them being classified as underweight, 64.8% as normal weight, 24.9% as overweight and 4.1% as obese (Figure 12). Differences between sexes were significant for all categories with more women being in the "underweight" category (9.4%) compared to men (2.3%) (p<0.001) and more men in the "overweight" category (41.4%) compared to women (11.3%) (p<0.001). BMI groups for all participants and by sex are shown in Table 3.

Participants who were on a diet at the time of the study had significantly higher mean BMI values (25.54 ± 5.18Kg/m^2) compared to those who weren't (22.94 ± 3.62Kg/m^2);(p=0.001).

When looking at other dietary variables that could be associated with an individual's BMI, such as breakfast consumption, fruit and vegetable consumption, fast food consumption, smoking, exercise and taking protein supplements, none of them was found to be significantly associated with mean BMI and results were similar for men and women, although more men were current smokers or exercised. Data are shown in Table 4.

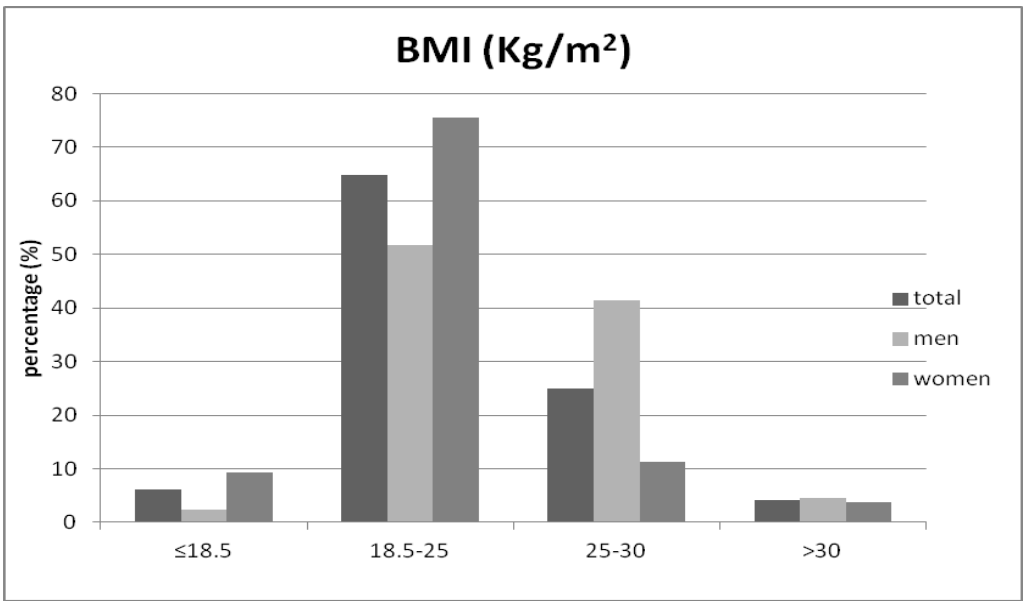

Figure 12: Distribution of BMI categories in all study participants and in men and women separately

Table 4: Association between dietary/personal habits and mean BMI in all study participants

Question asked	BMI	*P**
Do you have a fruit or fruit juice daily?		
Yes (73.1%)	23.65 ± 3.94	
No (26.9%)	22.40 ± 3.98	0.054
Do you have a second serving of fruit daily?		
Yes (31.6%)	23.76 ± 3.07	
No (68.4%)	23.10 ± 4.33	0.292
Do you eat fresh or cooked vegetables daily?		
Yes (56.5%)	23.33 ± 3.70	
No (43.5%)	23.30 ± 4.34	0.961
Do you eat fresh or cooked vegetables >1/day?		
Yes (29.5%)	24.14 ± 4.70	
No (70.5%)	22.97 ± 3.60	0.061
Do you skip breakfast?		
Yes (36.8%)	23.13 ± 4.40	
No (63.2%)	23.42 ± 3.73	0.626
Do you eat >1/week at a fast-food restaurant?		
Yes (25.9%)	23.85 ± 4.70	
No (74.1%)	23.13 ± 3.70	0.269
Do you currently smoke?		
Yes (24%)	22.69 ± 3.41	
No (76%)	23.53 ± 4.14	0.215
Do you currently exercise?		
Yes (55.4%)	23.30 ± 3.34	
No (44.6%)	23.32 ± 4.67	0.974
Are you currently on a diet?		
Yes (14.5%)	25.54 ± 5.18	
No (85.5%)	22.94 ± 3.62	0.001
Do you currently take protein supplements?		
Yes (12.4%)	23.74 ± 3.05	
No (87.6%)	23.25 ± 4.10	0.578

**P* for difference in mean BMI in those who answered “YES” Vs those who answered “NO “ in the above questions regarding dietary/personal habits

3 Adherence to the Mediterranean diet (KIDMED score)

The median KIDMED score in the study participants was 6.00 (IQR: 4 to 8), with men having a slightly, but statistically non-significant higher score compared to women (5.95 Vs 5.46 respectively, p=0.25). When looking at categories of adherence in all, about half (51.3%) were in the "intermediate adherence" to the Mediterranean diet category (total score between 4 and 7), 21.8% were in the "low adherence" category (total score of ≤3), and 26.9% were in the "high adherence" category (total score ≥8) (Figure 13). Again, there were differences between men and women, with more men in this age group being in the "high adherence" category compared to women (35.6% Vs 19.8% respectively; p=0.015). KIDMED score categories for all and men and women separately are shown graphically in figure 13.

Moreover students who skip breakfast and smoke had a lower KIDMED index value compared to students who consume breakfast and do not currently smoke (3.46 Vs 6.98; p=0.000 and 4.61 Vs 6.0; p=0.005).

Students who feel happy with their body weight had a higher KIDMED index compared to those who didn't (6.3 Vs 5.05, p=0.003).

In addition to overall adherence to the Mediterranean diet pattern, specific dietary categories included in the KIDMED index and their possible differences between men and women were also studied. With regards to the main nutritional categories of the Mediterranean diet, 73.1% of all study participants consumed a fruit/fruit juice daily, 56.5% consumed fresh or cooked vegetables daily and 47.7% consumed pulses >1 per week. Additionally, 76.2% ate two yogurts and/or 40g cheese daily, with 88.1% consuming a dairy product for breakfast, whereas 87.6% use olive oil at home. As perhaps expected in university students, ~70.0% ate pasta or rice almost daily (≥5 times per week), ~40% skipped breakfast and 26% ate at a fast

food restaurants >1 per week. Consumption of the above mentioned categories did not differ between men and women. Statistically significant differences in consumption between men and women were observed only in having a second serving of fruit daily (p=0.020) and regular nut consumption (at least 2-3 per week) (p<0.000) with higher values in men for both categories, whereas women tended to consume sweets more often (p=0.04). Results are shown in detail in Table 5.

When looking at other personal characteristics that could be associated with adherence to the Mediterranean diet, not smoking (p=0.005) and currently exercising (p<0.001) were significantly associated with a higher adherence score (KIDMED score for not smoking = 6.01 ± 2.93 Vs 4.61 ± 2.82 for smoking and 6.54 ± 2.87 Vs 4.62 ± 2.70 for not exercise), indicating an overall healthier lifestyle. "Currently being on a diet" was not associated with adherence (5.96 ± 2.08 Vs 5.64 ± 3.08; p=0.59).

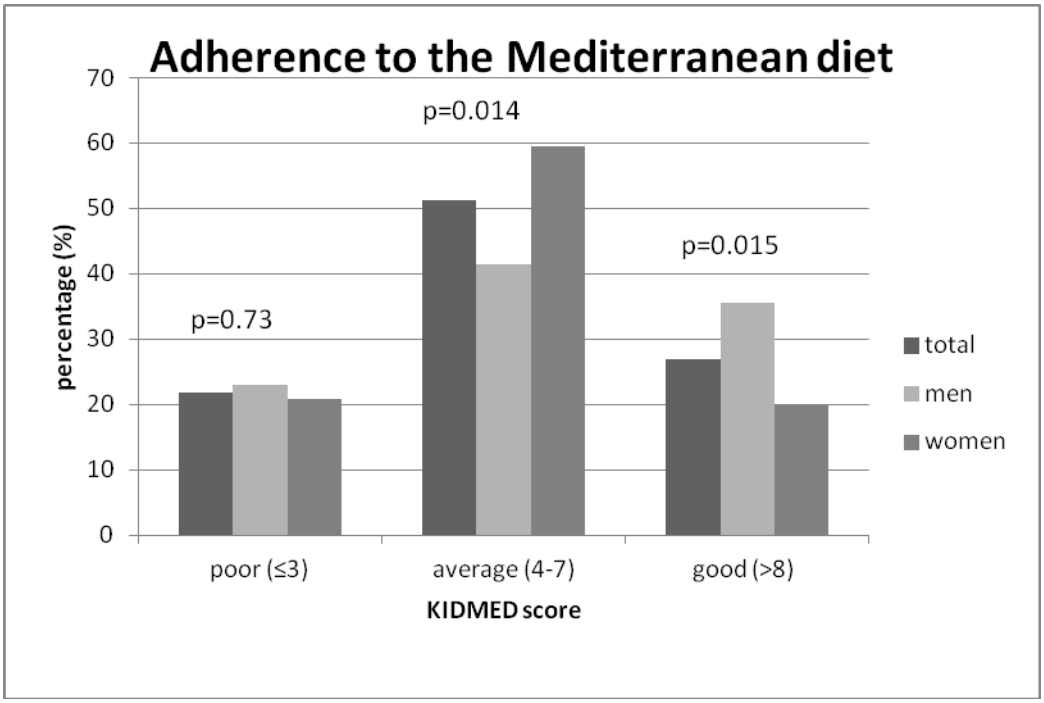

Figure 13: Distribution of KIDMED score categories in all and men and women separately

Table 5: KIDMED index questions in all, and men and women separately. Positive answers are given as percentages (%)

KIDMED index questions	All (%)	Males (%)	Females (%)	*p**
Fruit or fruit juice daily	73.1	77	69.8	0.26
Second serving of fruit daily	31.6	40.2	24.5	0.02
Fresh or cooked vegetables daily	56.5	55.2	57.5	0.74
Fresh or cooked vegetables >1/day	29.5	32.2	27.4	0.47
Regular fish consumption (at least 2-3/week)	28	33.3	23.6	0.13
>1/week fast-food (hamburger) restaurant	25.9	31	21.7	0.14
Pulses >1/week	47.7	50.6	45.3	0.46
Pasta or rice almost daily (≥5/week)	69.9	67.8	71.7	0.56
Cereal or cereal product for breakfast	73.1	70.1	75.5	0.40
Regular nut consumption (at least 2-3/week)	31.1	47.1	17.9	<0.001
Use of olive oil at home	87.6	87.4	87.7	0.94
No breakfast	36.8	36.8	36.8	0.99
Dairy product for breakfast	88.1	88.5	87.7	0.87
Commercially baked goods or pastries for breakfast	30.6	34.5	27.4	0.29
Two yoghurts and/or 40 gr cheese daily	76.2	72.4	79.2	0.27
Sweet and candy several times a day	30.6	24.1	35.8	0.04

**P* value for comparison between sexes from chi-square test

4 Beverage consumption

The majority of participants (32.5%) consumes coffee 2-3 times per day, with another 23% consuming coffee once per day. With regards to tea, about half of study participants (49.2%) drink tea 1-3 times per month or even more rarely. Regarding alcohol consumption, the majority of participants (38.7%) drink wine a few times per year with another 26.7% drinking wine 1-3 times per month and 27.2% drinking wine 1 to 4 times per week; the majority (37.2%) drinks rarely beer with another 27.7% drinking beer 1-3 times per month and 26.2% drinking beer 1 to 4 times per week; about half (50.3%) of all study participants drink whiskey or vodka a few times per year or never with another 25.1% drinking these beverages 1-3 times per month.

Statistically significant differences in beverage consumption between men and women were observed for beer (p=0.000) and whiskey-vodka consumption (p=0.012) with higher values in men for both categories (Table 6).

Table 6: Coffee, tea and alcohol consumption in all, and men and women separately.

Coffee consumption	All (%)	Men (%)	Women (%)	*p**
4-6 times/day	8.4	11.5	5.8	
2-3 times/day	32.5	32.2	32.7	
1 time/day	23	25.3	21.2	
1-4 times/week	15.2	16.1	14.4	
1-3 times/month	7.3	9.2	5.8	
Few times/year or never	13.6	5.7	20.2	0.067
Tea consumption				
4-6 times/day	2.6	3.4	1.9	
2-3 times/day	7.9	8	7.7	
1 time/day	17.3	14.9	19.2	
1-4 times/week	23	29.9	17.3	
1-3 times/month	20.9	20.7	21.2	
Few times/year or never	28.3	23	32.7	0.329
Wine consumption				
4-6 times/day	1	1.1	1	

2-3 times/day	0.5	0	1	
1 time/day	5.8	5.7	5.8	
1-4 times/week	27.2	25.3	28.8	
1-3 times/month	26.7	35.6	19.2	
Few times/year or never	38.7	32.2	44.2	0.178
Beer consumption				
4-6 times/day	2.1	4.6	0	
2-3 times/day	0.5	1.1	0	
1 time/day	6.3	11.5	1.9	
1-4 times/week	26.2	39.1	15.4	
1-3 times/month	27.7	24.1	30.8	
Few times/year or never	37.2	19.5	51.9	<0.001
Whiskey-vodka consumption				
4-6 times/day	1	2.3	0	
2-3 times/day	1	2.3	0	
1 time/day	2.6	4.6	1	
1-4 times/week	19.9	25.3	15.4	
1-3 times/month	25.1	27.6	23.1	
Few times/year or never	50.3	37.9	60.6	0.012

**P* value for comparison between sexes from chi-square test

5 Breakfast consumption

The majority of participants (63.2%) reported consuming breakfast on a regular basis. However about a third of those reported eating commercially baked goods or pastries (30.6%) for breakfast, a widely used practice in Cyprus and other Mediterranean countries such as Greece and Italy and which may not represent the healthiest choice for breakfast.

6 Meal patterns

About half (47.2%) of young adults consumed three or fewer meals per day while the rest consume more than four meals per day. The main person responsible for preparing meals at home were the parents (63.7%), with only 29% of young adults preparing food by

themselves. The majority of young adults (45.6%) consume meals away from home 1-3 times per week.

With regards to fried foods, half of study participants (50.3%) reported consuming fried food 1-3 times per week, with another 29% consuming such foods only 1-3 times per month. Most of them however use little extra salt at the table (44.6%).

As expected, given body size, men drank more water than women, with 40% of men consuming ≥ 8 glasses of water Vs 24% of women (p=0.017).

7 Exercise

A little over half of study participants (55.4%) reported currently exercising, with men exercising significantly more than women (69% Vs 44.3%, p=0.001). Noticeably, about a fifth of men surveyed (21.8%) reported taking protein supplements compared with only 4.7% of women (p<0.001), and about 20% of study participants took vitamins/minerals supplements (p=0.3 for men Vs women).

8 Smoking

Tobacco use was relatively high among this group of young Cypriots (24%) and especially among males, with 31% of them reporting current smoking, Vs 18.1% of young females (p=0.037).

9 Body image

Only half of young adults feel happy with their body weight, with males being overall happier than females (62.1% Vs 41.5%, p=0.004). However, out of those who feel unhappy with their body weight only 23.2% were currently on a diet and 43.2% exercised.

Students that consumed breakfast and exercised reported feeling happy with their body weight more often, compared to students that skipped breakfast (p=0.035) and did not

exercise (p=0.001), while there were no statistically significant differences with regards to smoking (p=0.239), eating fried food (p=0.531) and eating away from home (p=0.221), for both males and females.

Behavioral characteristics are presented in detail in Table 7.

Table 7:

Behavioral characteristics

	Total (%)	Males (%)	Females (%)	*p*
No breakfast	36.8	36.8	36.8	0.999
Currently Exercising	55.4	69	44.3	0.001
Taking Protein Supplements	12.4	21.8	4.7	0.000
Taking Vitamins/minerals supplements	21.8	18.4	24.5	0.304
Currently Smoking	24	31	18.1	0.037
Meal preparation by:				
Self	29	29.9	28.3	0.813
Parents	63.7	63.2	64.2	
Grandparents	5.7	4.6	6.6	
Other	1.6	2.3	0.9	
Meals per day				
≤ 3	47.2	41.4	51.9	0.146
≥ 4	52.8	58.6	48.1	
Meals away from home				
Daily	9.8	8	11.3	0.949

4-6 times/week	13	13.8	12.3	
1-3 times/week	45.6	47.1	44.3	
1-3 times/month	24.4	24.1	24.5	
Few times/year or never	7.3	6.9	7.5	
Fried food consumption				
Daily	0.5	1.1	0	0.311
4-6 times/week	5.2	6.9	3.8	
1-3 times/week	50.3	55.2	40.2	
1-3 times/month	29	24.1	33	
Few times/year or never	15	12.6	17	
Extra salt at the table				
Much	16.1	14.9	17	0.716
Little	44.6	42.5	46.2	
Not at all	39.4	42.5	36.8	
Water consumption				
≤ 3 glasses	17.1	5.8	26.4	0.017
4 -7	51.8	54.7	50	
> 8	30.6	39.5	23.6	

*P value for comparison between sexes from chi-square test

10 Health habit score

Having a higher health habit score, which included combined information on breakfast consumption, eating fried food, eating away from home, exercise and smoking, was significantly associated with higher adherence to the Mediterranean diet (p for trend<0.001), with a one unit increase in the health score being associated with a 0.614 increase in the KIDMED index (95% CI : 1.07 – 1.55).

11 Survival of total lactic acid bacteria

Total LAB were enumerated for all five yoghurts made (1,2,3,4 and 5) from day 0 to day 25. Results are presented in Tables 8-12. The initial numbers were 6.25 $\log_{10}$cfu/g, 6.20 $\log_{10}$cfu/g, 8.72 $\log_{10}$cfu/g, 8.73 $\log_{10}$cfu/g and 8.31 $\log_{10}$cfu/g for yoghurts 1,2,3,4 and 5 respectively.

Total LAB numbers remained significantly higher and steady at about 8 $\log_{10}$cfu/g in yoghurt 5 for 25 days.

Total LAB were followed similar trends in yoghurts 1 and 2 and in yoghurts 3 and 4 respectively. The numbers of total LAB remained in significantly higher values for yoghurts 3 and 4 until about day 18 compared with yoghurts 1 and 2. However at the last day of storage yoghurts 1 and 2 had significant higher values (about 5 $\log_{10}$cfu/g) compared with yoghurts 3 and 4 (about 4 $\log_{10}$cfu/g). Results are graphically presented in Figure 14.

Table 8 Results of the enumeration of LAB in Yoghurt 1- Control

Days	Yoghurt samples	Dilution	Number of colonies	CFU/g	Log_{10}CFU/g	Log_{10}CFU/g, average	stdev
0	1	10^{-3}	179	1790000	6.25	6.25	0.001711
	2	10^{-3}	180	1800000	6.25		
4	1	10^{-3}	146	1460000	6.16	6.16	
	2	10^{-3}	140	1400000	6.15		0.012887
7	1	10^{-3}	172	1720000	6.24	6.23	0.001791
	2	10^{-3}	171	1710000	6.23		
11	1	10^{-3}	104	1040000	6.02	6.04	0.033534
	2	10^{-3}	116	1160000	6.06		
14	1	10^{-3}	109	1090000	6.04	6.04	0
	2	10^{-3}	109	1090000	6.04		
18	1	10^{-3}	71	710000	5.85	5.89	0.044233
	2	10^{-3}	82	820000	5.91		
21	1	10^{-3}	71	710000	5.85	5.82	0.041625
	2	10^{-3}	62	620000	5.79		
25	1	10^{-3}	41	410000	5.61	5.62	0.014626
	2	10^{-3}	43	430000	5.63		

Table 9 Results of the enumeration of LAB in Yoghurt 2- Acidic

Days	Yoghurt samples	Dilution	Number of colonies	CFU/g	Log_{10}CFU/g	Log_{10}CFU/g, average	stdev
0	1	10^{-3}	167	1670000	6.22	6.20	0.037091
	2	10^{-3}	148	1480000	6.17		
4	1	10^{-3}	141	1410000	6.15	6.17	0.023069
	2	10^{-3}	152	1520000	6.18		
7	1	10^{-3}	195	1950000	6.29	6.3	0.022758
	2	10^{-3}	210	2100000	6.32		
11	1	10^{-3}	144	1440000	6.16	6.17	0.020618
	2	10^{-3}	154	1540000	6.19		
14	1	10^{-3}	140	1400000	6.15	6.13	0.015752
	2	10^{-3}	133	1330000	6.12		
18	1	10^{-3}	123	1230000	6.09	6.04	0.069777
	2	10^{-3}	98	980000	6.0		
21	1	10^{-3}	114	1140000	6.06	6.04	0.022344
	2	10^{-3}	106	1060000	6.03		
25	1	10^{-3}	46	460000	5.67	5.67	0.006604
	2	10^{-3}	47	470000	5.67		

Table 10 Results of the enumeration of LAB in Yoghurt 3- Neutral

Days	Yoghurt samples	Dilution	Number of colonies	CFU/g	Log_{10}CFU/g	Log_{10}CFU/g, average	stdev
0	1	10^{-5}	542	542000000	8.73	8.72	0.022932
	2	10^{-5}	503	503000000	8.70		
4	1	10^{-4}	1020	102000000	8.0	8.0	0.006081
	2	10^{-4}	1000	100000000	8		
7	1	10^{-4}	932	93200000	7.97	8.0	0.036609
	2	10^{-4}	1050	105000000	8.02		
11	1	10^{-4}	420	42000000	7.62	7.6	0.038925
	2	10^{-4}	370	37000000	7.57		
14	1	10^{-4}	100	10000000	7	6.99	0.022286
	2	10^{-4}	93	9300000	6.97		
18	1	10^{-4}	11	1100000	6.04	6.08	0.051301
	2	10^{-4}	13	1300000	6.11		
21	1	10^{-3}	30	300000	5.48	5.38	0.140267
	2	10^{-3}	19	190000	5.28		
25	1	10^{-2}	55	55000	4.74	4.73	0.011375
	2	10^{-2}	53	53000	4.72		

Table 11 Results of the enumeration of LAB in Yoghurt 4- Total

Days	Yoghurt samples	Dilution	Number of colonies	CFU/g	Log_{10}CFU/g	Log_{10}CFU/g, average	stdev
0	1	10^{-5}	554	554000000	8.74	8.73	0.014761
	2	10^{-5}	528	528000000	8.72		
4	1	10^{-4}	1160	116000000	8.06	8.06	0.010776
	2	10^{-4}	1120	112000000	8.05		
7	1	10^{-4}	1352	135200000	8.13	8.11	0.036625
	2	10^{-4}	1200	120000000	8.08		
11	1	10^{-4}	450	45000000	7.65	7.68	0.032355
	2	10^{-4}	500	50000000	7.70		
14	1	10^{-4}	220	22000000	7.34	7.27	0.107545
	2	10^{-4}	155	15500000	7.20		
18	1	10^{-4}	18	1800000	6.26	6.30	0.061624
	2	10^{-4}	22	2200000	6.34		
21	1	10^{-3}	13	130000	5.11	5.01	0.149096
	2	10^{-3}	8	80000	4.90		
25	1	10^{-2}	43	43000	4.63	4.59	0.063216
	2	10^{-2}	35	35000	4.54		

Table 12 Results of the enumeration of LAB in Yoghurt 5- Combined

Days	Yoghurt samples	Dilution	Number of colonies	CFU/g	Log_{10}CFU/g	Log_{10}CFU/g, average	stdev
0	1	10^{-5}	205	205000000	8.31	8.31	0.007583
	2	10^{-5}	200	200000000	8.30		
4	1	10^{-5}	210	210000000	8.32	8.31	0.010411
	2	10^{-5}	203	203000000	8.31		
7	1	10^{-5}	203	20300000	8.31	8.35	0.063954
	2	10^{-5}	250	250000000	8.40		
11	1	10^{-5}	109	109000000	8.04	8.04	0.002805
	2	10^{-5}	110	110000000	8.04		
14	1	10^{-5}	215	21500000	8.33	8.33	0.00287
	2	10^{-5}	213	213000000	8.33		
18	1	10^{-5}	95	95000000	7.98	7.94	0.052774
	2	10^{-5}	80	80000000	7.90		
21	1	10^{-5}	65	65000000	7.81	7.84	0.035645
	2	10^{-5}	73	73000000	7.86		
25	1	10^{-5}	50	50000000	7.70	7.71	0.017894
	2	10^{-5}	53	53000000	7.72		

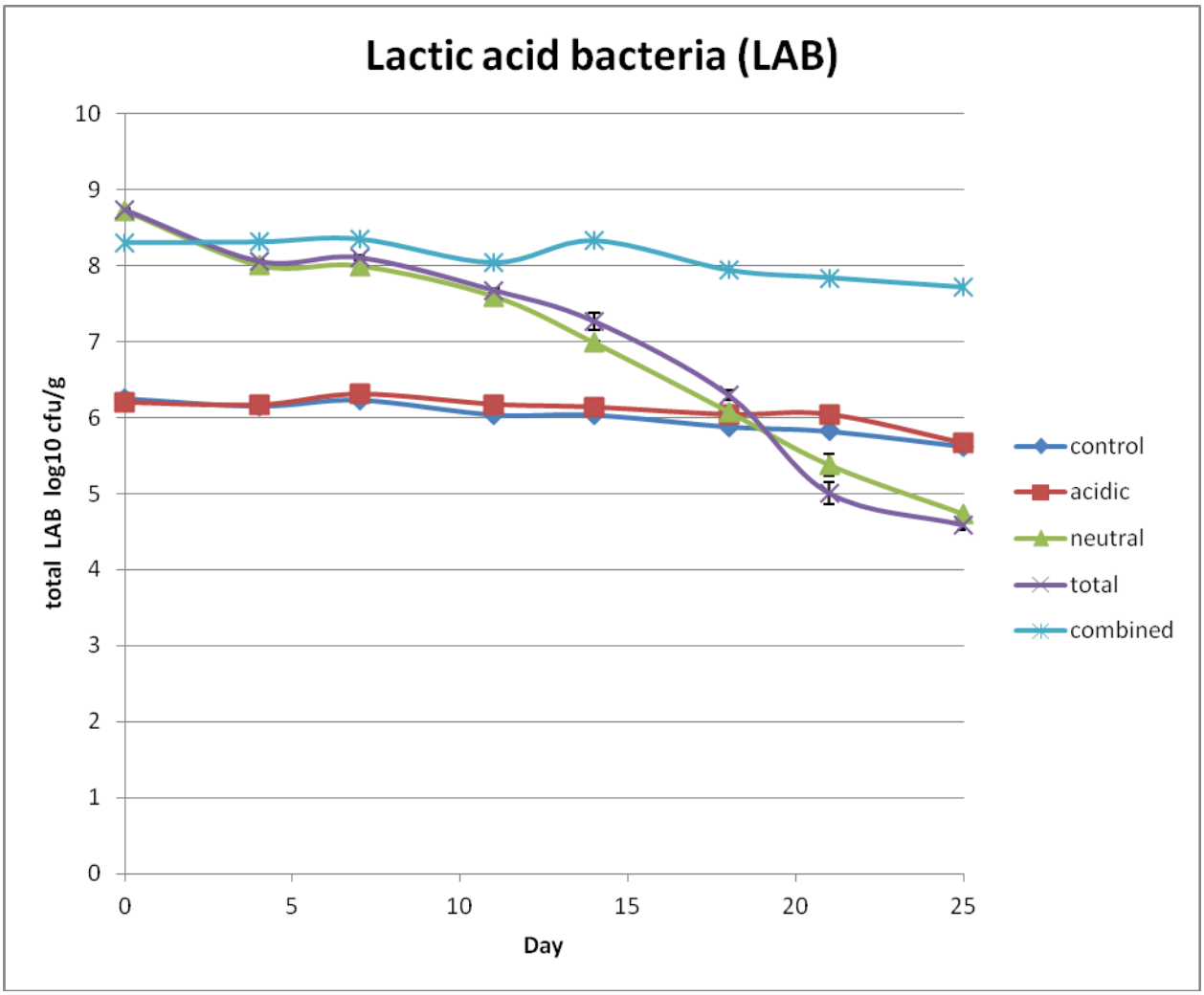

Figure 14 Survival of total lactic acid bacteria (LAB) in yoghurt formulations. Means of duplicates. Day 0 = day of inoculation

12 Survival of *Saccharomyces boulardii*

Yoghurt 5 was inoculated in parallel with 300 mg of total mastic extract and with 200 mg of probiotic yeast *S. boulardii*. Along with total LAB, *S. boulardii* was also enumerated. The numbers of *S. boulardii* remained steady at about 6.7 $\log_{10}$cfu/g. Results are presented in Table 13 and graphically in Figure 15.

Table 13 Results of the enumeration of *S. boulardii* in Yoghurt 5- Combined

Days	Yoghurt samples	Dilution	Number of colonies	CFU/g	Log_{10}CFU/g	Log_{10}CFU/g, average	stdev
0	1	10^{-4}	60	6000000	6.78	6.76	0.0323554
	2	10^{-4}	54	5400000	6.73		
4	1	10^{-4}	65	6500000	6.81	6.80	0.014511
	2	10^{-4}	62	6200000	6.80		
7	1	10^{-4}	54	5400000	6.73	6.72	0.0236342
	2	10^{-4}	50	5000000	6.70		
11	1	10^{-4}	55	5500000	6.74	6.71	0.0482705
	2	10^{-4}	47	4700000	6.67		
14	1	10^{-4}	59	5900000	6.77	6.77	0
	2	10^{-4}	59	5900000	6.77		
18	1	10^{-4}	37	3700000	6.57	6.63	0.0924671
	2	10^{-4}	50	5000000	6.70		
21	1	10^{-4}	40	4000000	6.60	6.64	0.0495243
	2	10^{-4}	47	4700000	6.67		
25	1	10^{-4}	42	4200000	6.62	6.63	0.007226
	2	10^{-4}	43	4300000	6.63		

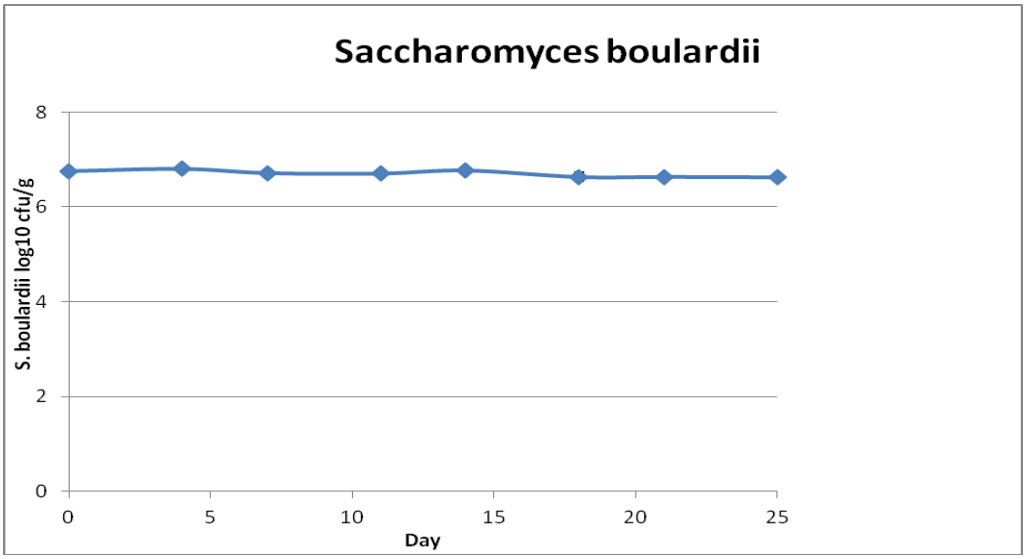

Figure 15 Survival of *Saccharomyces boulardii* in yoghurt 5. Means of duplicates. Day 0 = day of inoculation.

13 Initial organoleptic assessment

The progress of organoleptic assessment regarding the organoleptic properties of the five different yoghurt batches during their storage for 25 days and the total organoleptic score are presented in table 14 and in figure 16. Best results were observed in yoghurts contained mastic extract (total, acidic, neutral). Yoghurt 1, which was the control, had a goaty flavor and odour in contrast to Yoghurts 2, 3 and 4. On the other hand Yoghurt 5, which contained the probiotics yeast demonstrated a not pleasure taste. Yoghurt 4 with total mastic extract was selected to be tested against the control Yoghurt 1 and to be further analysed using NMR. Although Yoghurts 2, 3 and 4 were about the same organoleptically acceptable we choose Yoghurt 4 because contained all the components of mastic and the survival of total lactic acid bacteria at least until 18 days is in high levels.

Table 14 Initial organoleptic assessment

Yoghurts		Yoghurt 1-Control	Yoghurt 2-Acidic	Yoghurt 3-Neutral	Yoghurt 4-Total	Yoghurt 5-Combined
Days	Organoleptic properties					
0	Flavor and odour (50)	45	50	50	50	40
	Body and texture (40)	40	40	40	40	40
	Appearance (10)	10	10	10	10	10
	Total (100)	**95**	**100**	**100**	**100**	**90**
4	Flavor and odour (50)	45	50	50	50	35
	Body and texture (40)	40	40	40	40	40
	Appearance (10)	10	10	10	10	10
	Total (100)	**95**	**100**	**100**	**100**	**85**
7	Flavor and odour (50)	45	50	50	50	35
	Body and texture (40)	40	40	40	40	40
	Appearance (10)	10	10	10	10	10
	Total (100)	**95**	**100**	**100**	**100**	**85**
11	Flavor and odour (50)	45	50	50	50	35
	Body and texture (40)	40	40	40	40	40
	Appearance (10)	10	10	10	10	10
	Total (100)	**95**	**100**	**100**	**100**	85
14	Flavor and odour (50)	43	50	50	50	35
	Body and texture (40)	40	40	40	40	38
	Appearance (10)	10	10	10	10	9
	Total (100)	**93**	**100**	**100**	**100**	**82**
18	Flavor and odour (50)	43	48	49	50	35
	Body and texture (40)	39	40	40	40	36
	Appearance (10)	10	10	10	10	9
	Total (100)	**92**	**98**	**99**	**100**	**80**
21	Flavor and odour (50)	43	47	49	49	35
	Body and texture (40)	38	39	39	40	34

	Appearance (10)	10	10	10	10	8
	Total (100)	**91**	**96**	**98**	**99**	**77**
25	Flavor and odour (50)	42	45	47	48	34
	Body and texture (40)	37	38	38	39	33
	Appearance (10)	8	9	9	9	8
	Total (100)	**87**	**92**	**94**	**96**	**75**

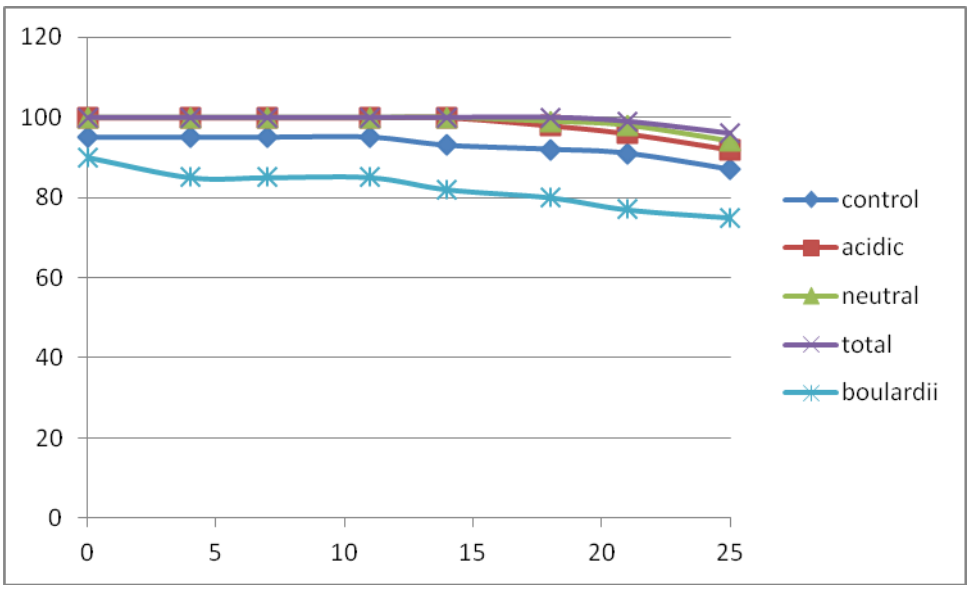

Figure 16 Organoleptic score

14 Group organoleptic assessment

The results of the group organoleptic assessment of Yoghurts 1, 4, and 5 are presented in Table 14 and graphically as spider diagrams for the organoleptic properties in figure 17, and for the organoleptic score in figure 18. Regarding the surface's appearance the majority of panel reported for all yoghurts flat and thin surface without bubbles and without synaeresis. However more persons reported a not flat surface for Yoghurt 5 compared with Yoghurts 1 and 4. Synaeresis was observed more in Yoghurts 4 and 5. The colour was defined as white in all samples. For the purposes of evaluation, the texture was divided in two categories: (i)

on the spoon, at which was assessed by the majority for all Yoghurts as thin, cohesive, not sticky without synaeresis after cutting, and (ii) mouth feel, which was evaluated as thin, cohesive, not sticky to the palate and not grainy. However more persons reported not thin texture on the spoon and not cohesive texture in the mouth for Yoghurt 5 comparing with the other two Yoghurts. With regards to the taste and odor the majority of testers defined it as pleasant and satisfying for all Yoghurts tested. Only few persons mentioned a goaty aftertaste (eight for the Yoghurt 1, two for Yoghurt 4 and one for Yoghurt 5). Moreover six tasters noted the mastic flavor in Yoghurt 4 and only one taster noted a taste of yeast in Yoghurt 5. Anova analysis was carried out to statistically examine the differences among the mean scores of the three yoghurt, regarding flavor, body and texture and appearance. Figure 18 in a spider diagram presenting the results of the organoleptic score. No significant differences were observed for all the parameters, flavor (p=0.708), body and texture (p=0.292), appearance (0.537) and the total organoleptic scores (p=0.422). Regarding the acceptability, Yoghurt 1 was preferred by three tasters (15%), Yoghurt 4 was preferred by ten tasters (50%) and Yoghurt 5 was preferred by seven tasters (35%). Yoghurt 1 was preferred by the tasters because as they justified it had a pleasant, sour taste, nice appearance and odor. Yoghurt 4 was preferred due its pleasant taste and odor and also due its characteristic mastic taste. Moreover tasters mentioned that this yoghurt was less sour. Yoghurt 5 was preferred due its pleasant taste and odor, increased acidity. Tasters also reported that this yoghurt was more pure and traditional.

Table 15 Group organoleptic assessment

			Yoghurts					
			1st : Control		2nd : Total		3rd : Combined	
Characteristics			Yes	No	Yes	No	Yes	No
Surface appearance	Flat		17	3	19	1	14	6
	Thin		20	0	18	2	19	1
	Bubbles		2	18	3	17	1	19
	Syneresis		3	17	1	19	4	16
	Other defect		1	19	0	20	17	3
Colour	White		19	1	19	1	19	1
Texture	In the spoon	Thin	17	3	18	2	16	4
		Cohesive	19	1	20	0	19	1
		Sticky	5	15	5	15	5	15
		One mass	18	2	19	1	18	2
		Continues phase during cutting	19	1	20	0	19	1
		Syneresis after cutting	2	18	1	19	1	19
	In the mouth	Thin	17	3	19	1	15	5
		Cohesive	17	3	17	3	17	3
		Sticky	2	18	3	17	4	16
		Grainy	3	17	4	16	5	15
Taste	Off taste		3		2		5	
	Moderate		5		3		2	
	Acceptable-pleasure		12		15		13	
Odour	Off taste		0		1		4	
	Moderate		6		3		2	

	Acceptable-pleasure	14		16		14	
Organoleptic score	Flavor and odour (50)	36.55 ± 9.87		38.9 ± 10.36		36.15 ± 12.67	
	Body and texture (40)	35.25 ± 5.59		37.15 ± 5.65		34.35 ± 5.71	
	Appearance (10)	8.8 ± 1.63		8.9 ± 1.68		8.35 ± 1.60	
	Total (100)	80.6 ± 13.30		84.95 ± 14.5		78.85 ± 16.72	

Acceptance		
Which yoghurt do you prefer and why;		
Why the 1st ?	3 (15%)	pleasant, sour taste, nice appearance and odor
Why the 2nd ?	10 (50%)	Pleasant taste and odor, characteristic mastic taste
Why the 3rd ?	7 (35%)	Pleasant taste and odor, increased acidity, more pure and traditional yoghurt
Do you feel any flavor?	Goaty after taste :8 persons for the 1st yoghurt, mastic flavor: 6 persons for the 2nd yoghurt, yeast: 1 person for the 3rd yoghurt	
Suggestions, comments		

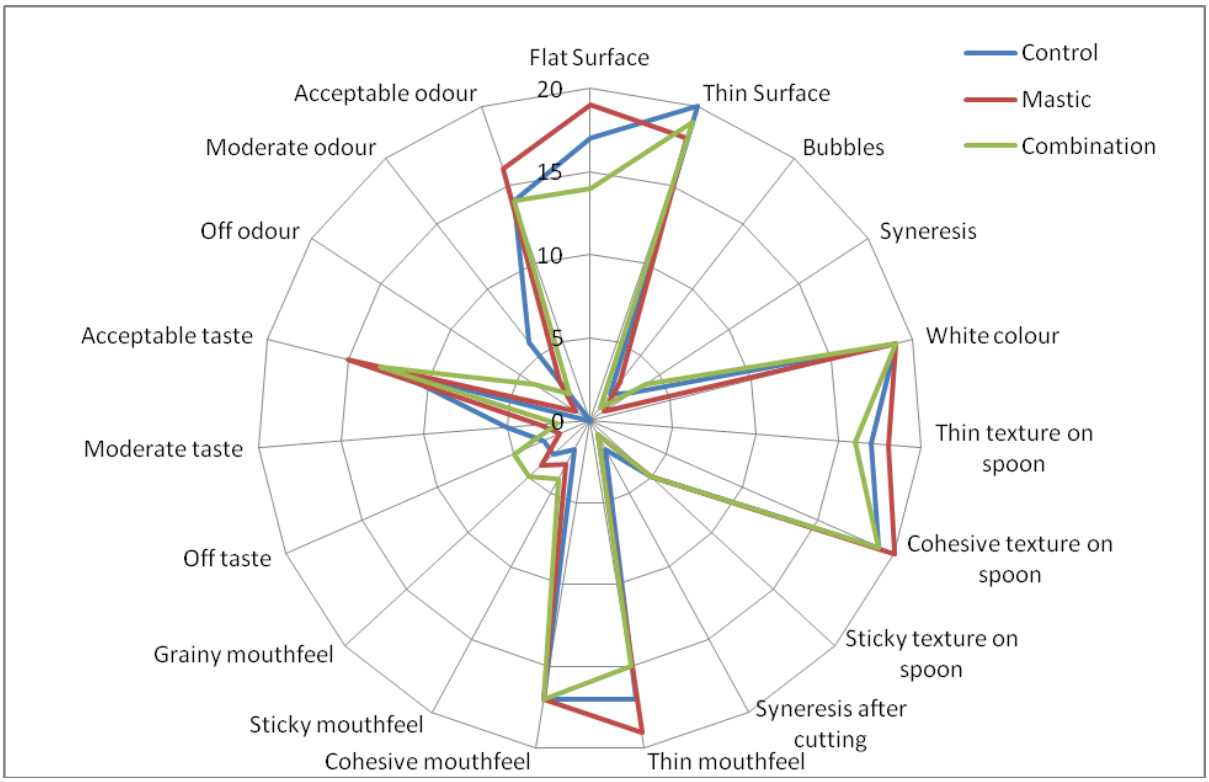

Figure 17 Spider diagram presenting the results of the group organoleptic assessment for the organoleptic properties of Yoghurts 1, 4 and 5.

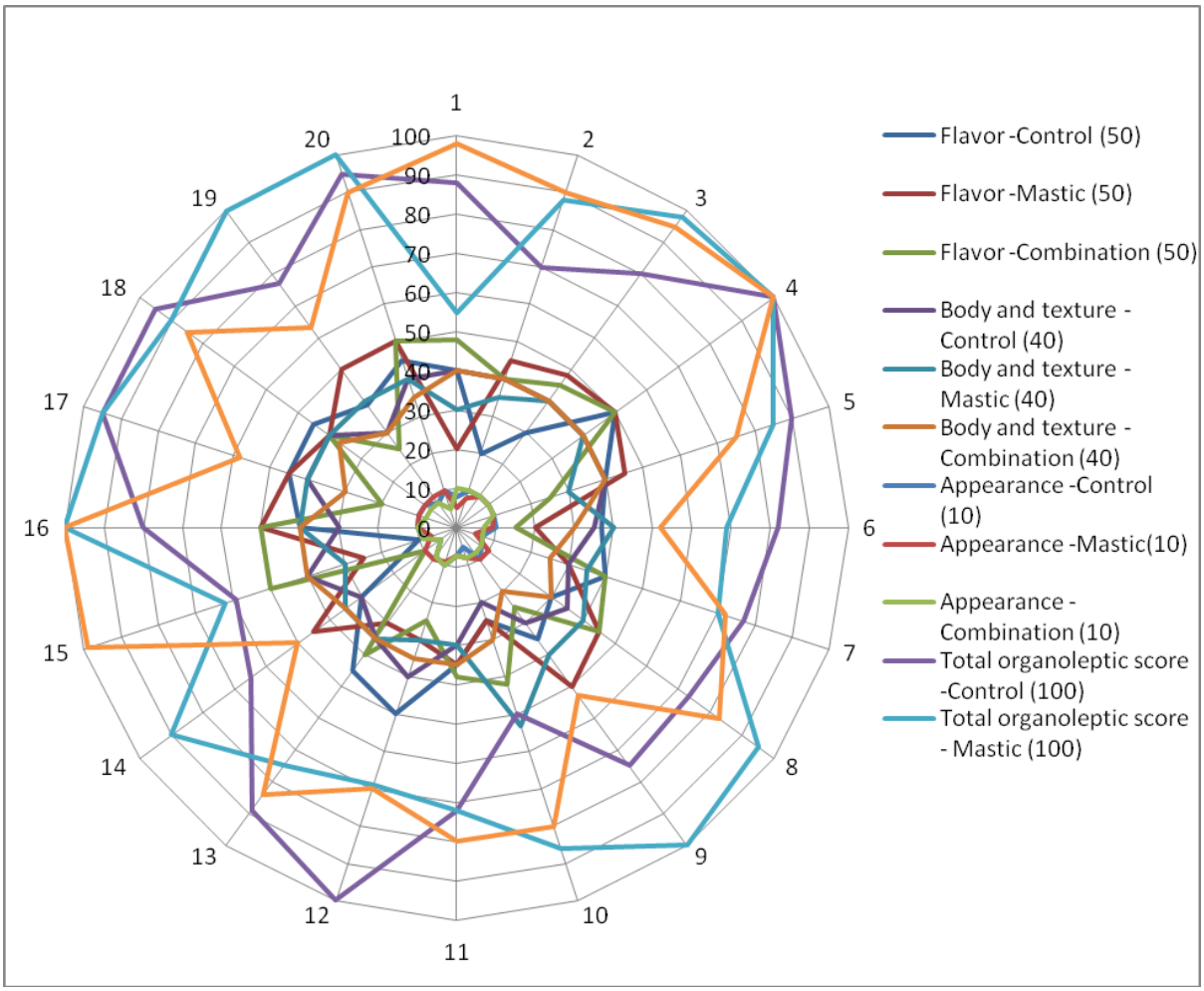

Figure 18 Spider diagram presenting the results of the organoleptic score from the twenty panel testers. Parameters tested : Flavor, Body and texture, Appearance, Total organoleptic score

15 Lipid profiles of Yoghurts 2, 3, 4 and 5

In all yoghurts four fatty acids were identified by comparison of their ^{1}H NMR data (appendix 2). The chemical shifts at 2.76 ppm, with a triplet, indicates the presence of linoleic acid and at 2.79 ppm, with a singlet, indicates the presence of linolenic acid (Alexandri et al. 2017), (Figure 19). The signal at 4.98 ppm with doublet of doublets indicated the presence of caproleic acid (Alexandri et al. 2017), (Figure 20). Finally the spectrum of conjugated linoleic acid (CLA) consists of a triplet at 5.93 and a triplet 6.2ppm (Tsiafoulis et al. 2014), (Figure 21). All these fatty acids were presented at all spectrums of yoghurts from day 0 to day 25. Yoghurt during shelf life keeps intact all lipids.

Regarding quantities it seems that in all yoghurts and during shelf life CLA remains in the same initial quantity. Regarding Yoghurts 3 and 5 (Neutral and Combined) all lipids remain constant until the last day. Regarding Yoghurt 4 – Total in linoleic acid there is a 9.6% reduction during the middle of shelf life, but no further reduction at the end. Linolenic acid remains constant until the middle of shelf life and decrease at the last day by 16.7%, while caproleic acid decreases by 7% during the 14^{th} day and by 17.7% at the end. With regard to Yoghurt 2 – Acidic all lipids remain constant except caproleic acid which it is constant until 14^{th} day and decreases by 60% at the end of shelf life.

At the end of shelf life of yoghurts the larger quantities in linoleic acid are presented in Yoghurt 2 – Acidic, in linolenic acid in Yoghurt 5 – Combined and in caproleic in Yoghurt 3 – Neutral.

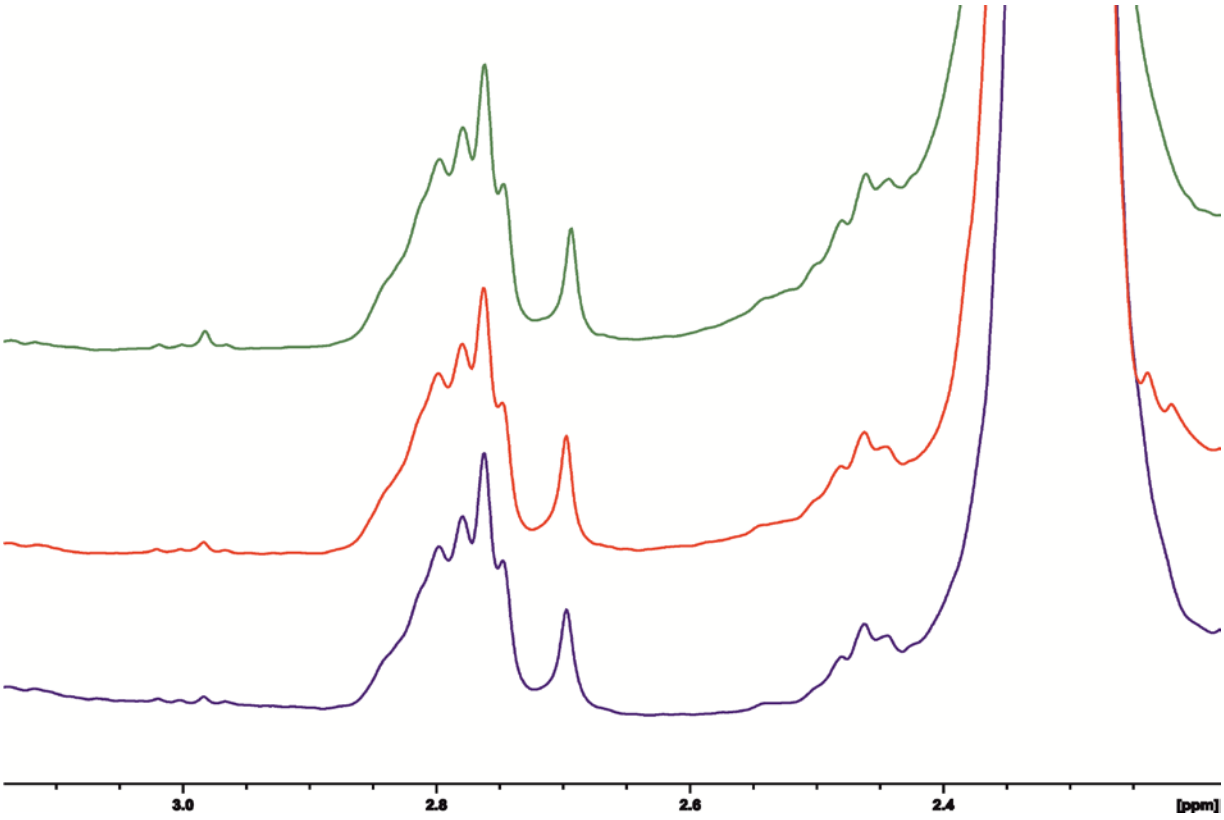

Figure 19 linoleic and linolenic acid. Yoghurt 4 – Total, where green color : day 0, red color : day 14, blue color : day 25. Exactly the same spectrums for Yoghurts 2, 3 and 5.

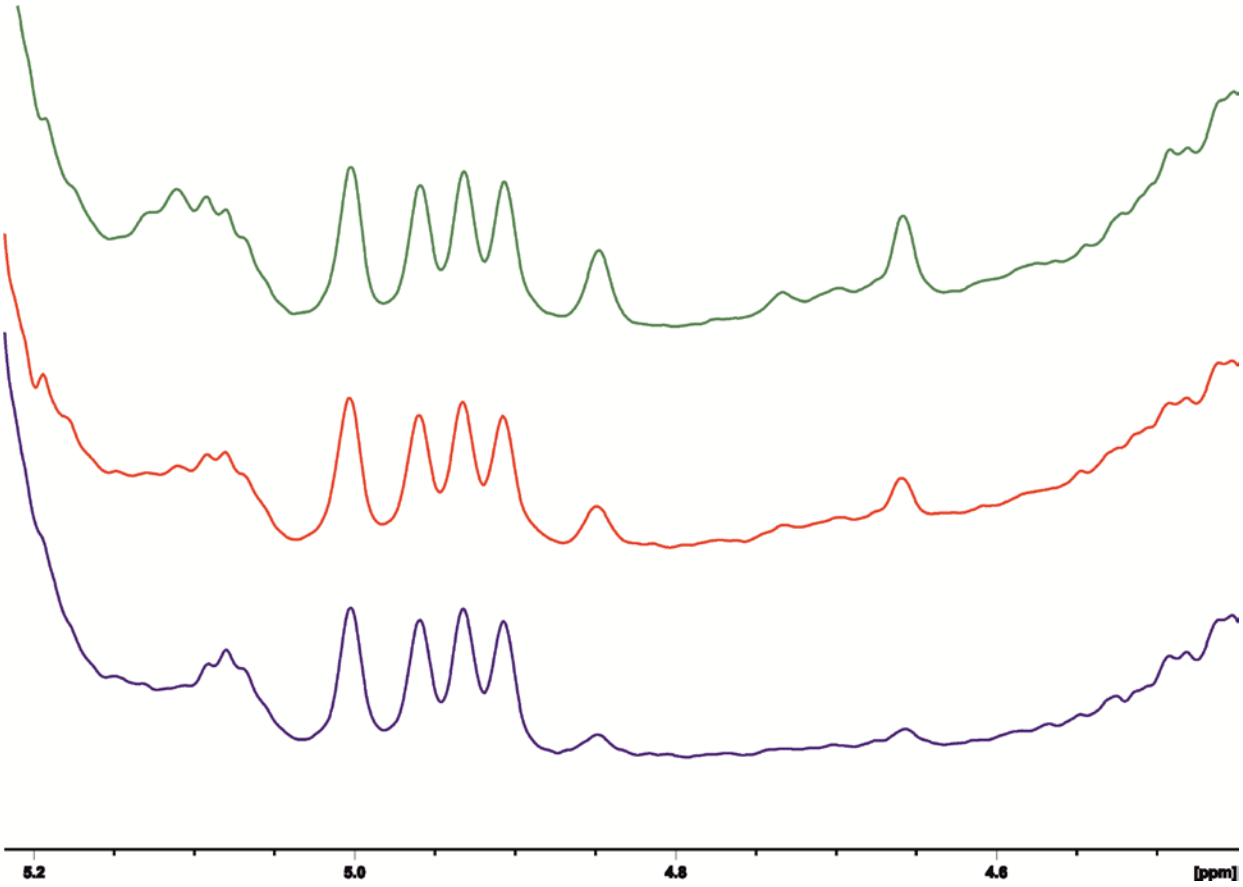

Figure 20 Caproleic acid. Yoghurt 4 – Total, where green color : day 0, red color : day 14, blue color : day 25. Exactly the same spectrums for Yoghurts 2, 3 and 5

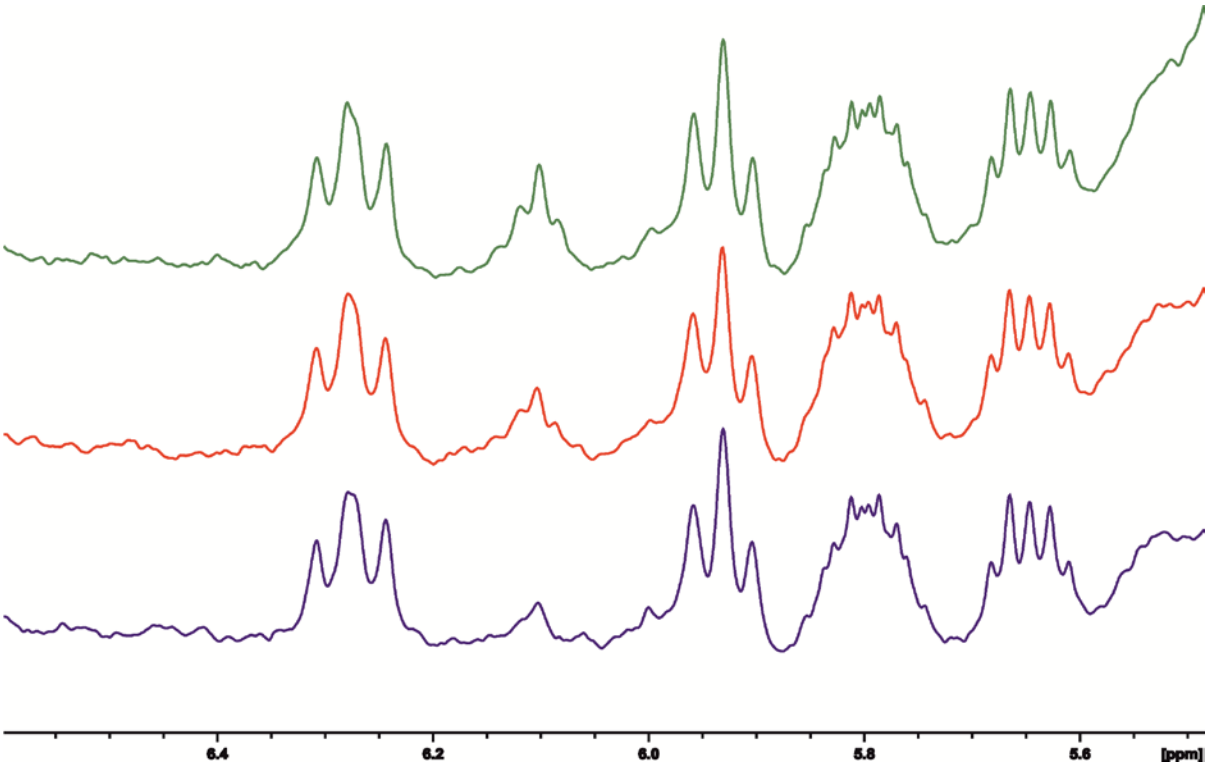

Figure 21 CLA. Yoghurt 4 – Total, where green color : day 0, red color : day 14, blue color : day 25. Exactly the same spectrums for Yoghurts 2, 3 and 5.

16 Bioactive compounds in yoghurt 2, 3, 4 and 5

Bioactive compounds in yoghurts samples during self life were identified using the spectrum of mastic extracts and by comparison of their ^{1}H NMR data (appendix 3). Two signals at 9.4 ppm and at 9.3 ppm indicate the presence of aldehydes (oleanonic aldehyde, oleanolic aldehyde) (Kim et al. 2004). Signals at the area of 6 ppm indicates the possible presence of masticadienonic acid, isomasticadienonic acid and isomasticadienolic acid (Paraschos et al. 2007, Nicholson et al. 2011, Makino et al 2004). Regarding Yoghurt 2 – Acidic and Yoghurt 5 – Combined, it seems that over time all the bioactive compounds are maintained during shelf life (Figures 22, 25). Regarding Yoghurt 4 – Total, it seems that over time there is a reduction at aldehydes components and at day 25 the signals are disappeared. On the other

hand signal at 6.1 ppm is detected in all samples (Figure 24). Finally with regard to Yoghurt 3 – Neutral, the bioactive compounds are disappeared from day 14 (Figure 23).

Regarding quantities of bioactive compounds there are no reductions in Yoghurts 2 and 5 (Acidic and Combined). Regarding Yoghurt 4 – Total triterpenic aldehydes decrease by 100% at the end of shelf life, while there is a 50% reduction for oleanolic aldehyde at 14th day and 16.7% reduction for oleanonic aldehyde. The triterpenic acid shows a decrease of 17% in the middle and by 75.6% in the end of shelf life. Regarding Yoghurt 3 – Neutral there is a large reduction in triterpenic aldehydes from the middle of shelf life (80% for oleanolic aldehyde and 89.3% for oleanonic aldehyde and at the last day the reductions are 92% and 96.4% respectively. There are no any reductions in triterpenic acid.

At the end of shelf life of yoghurts the higher quantities in all bioactive compounds are presented in Combined Yoghurt followed by Acidic Yoghurt.

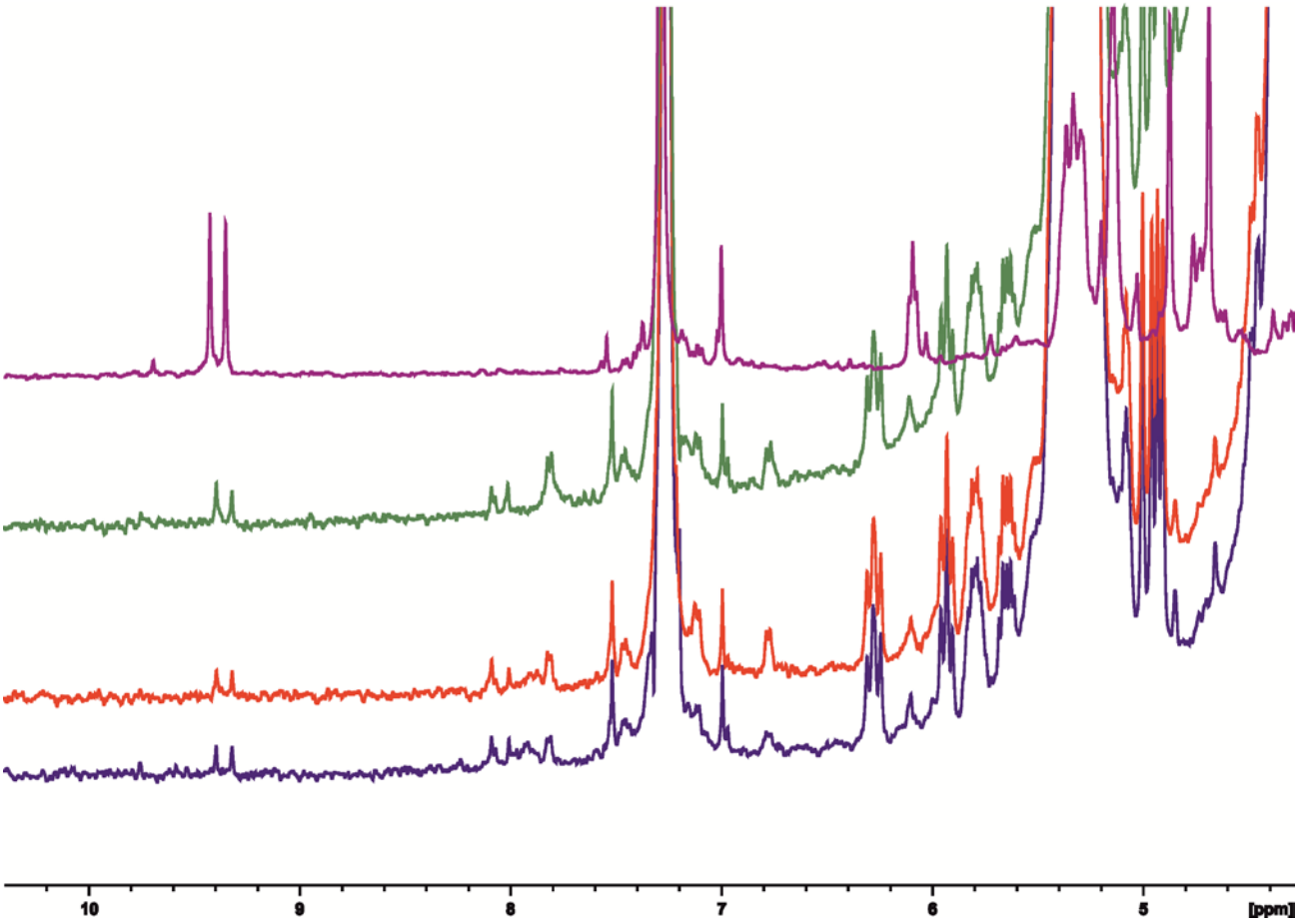

Figure 22 Bioactive compounds in Yoghurt 2 – Acidic. Blue color: day 0, red color: day 14, green color: day 25, purple color: acid fraction of mastic extract

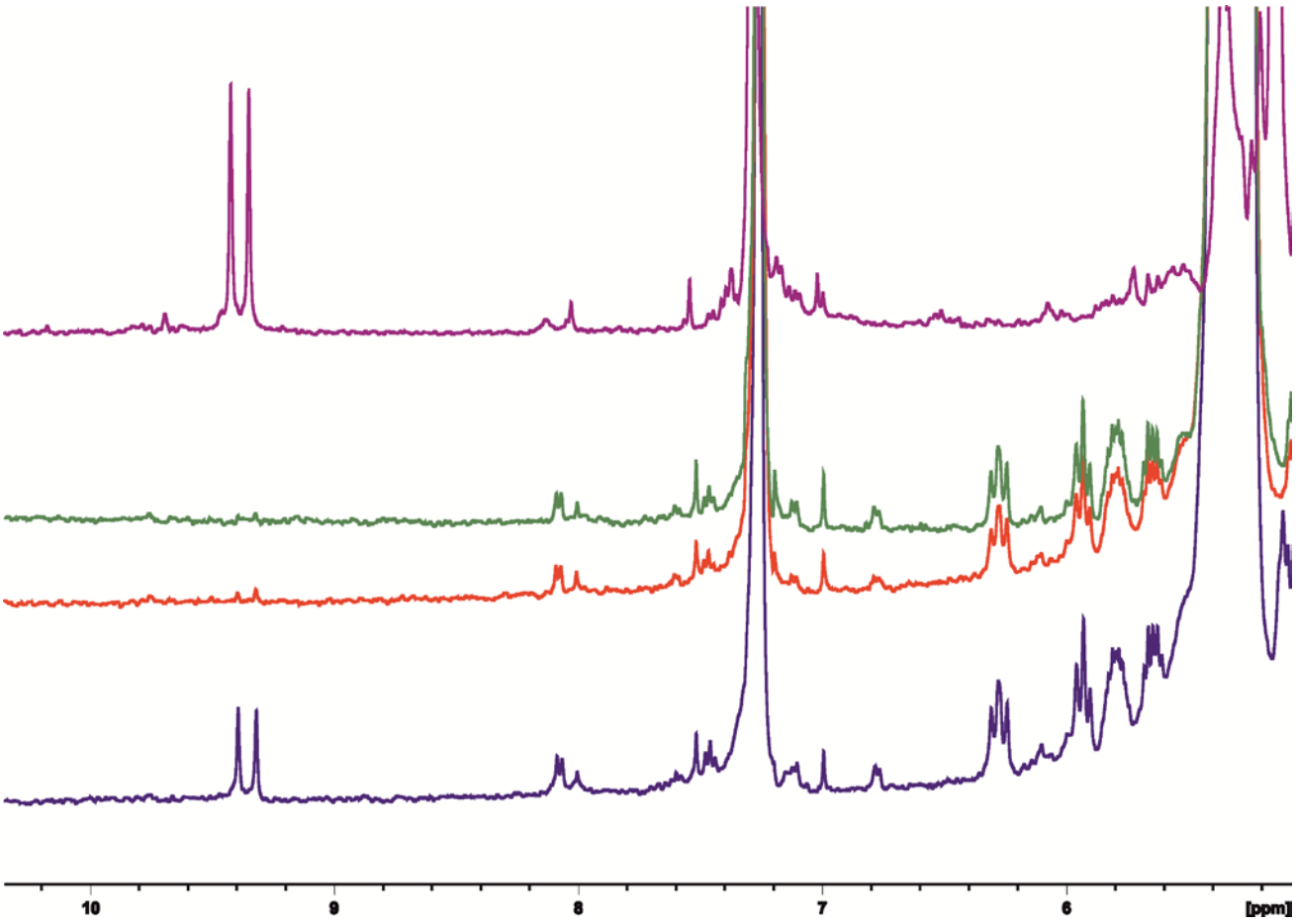

Figure 23 Bioactive compounds in Yoghurt 3 – Neutral. Blue color: day 0, red color: day 14, green color: day 25, purple color: neutral fraction of mastic extract

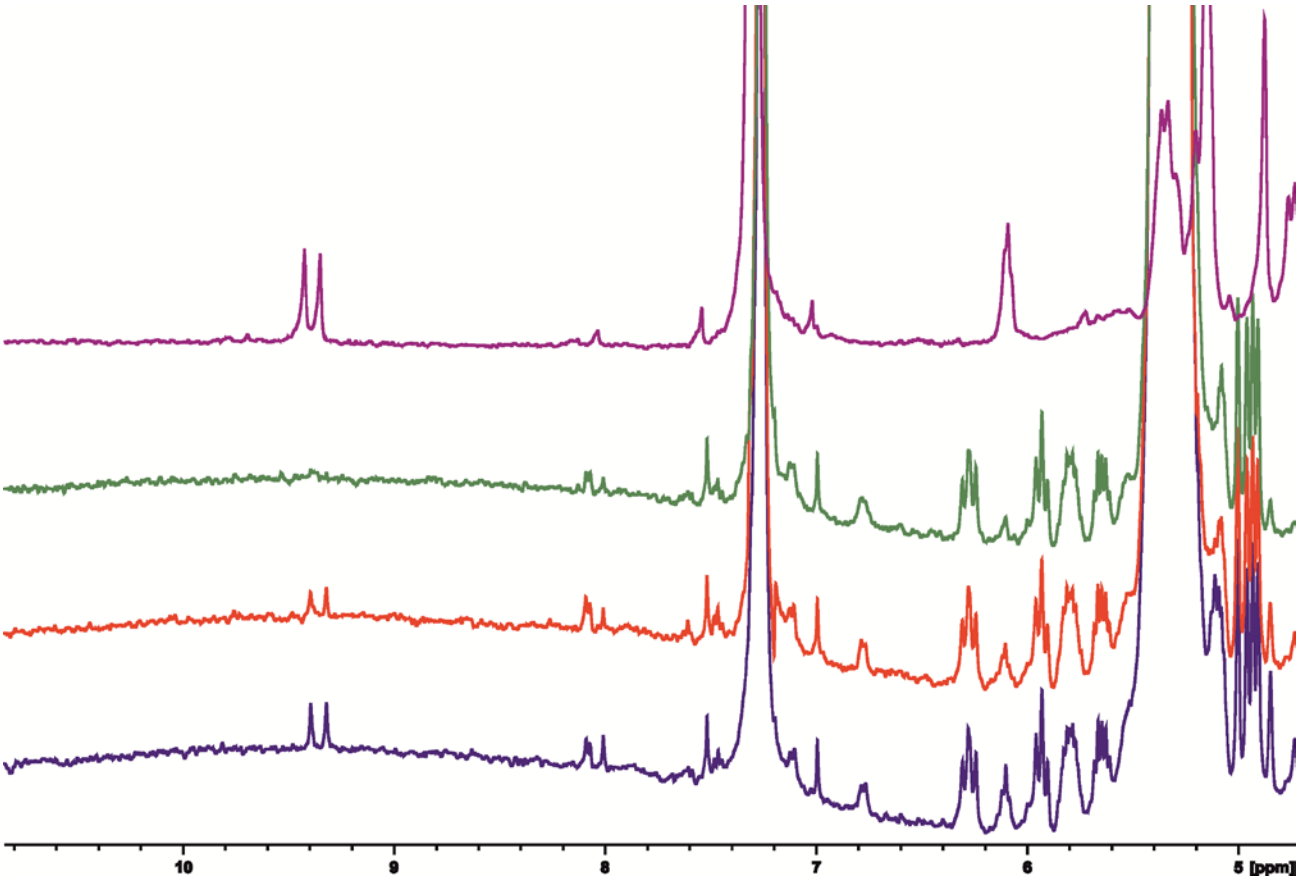

Figure 24 Bioactive compounds in Yoghurt 4 – Total. Blue color: day 0, red color: day 14, green color: day 25, purple color: total mastic extract

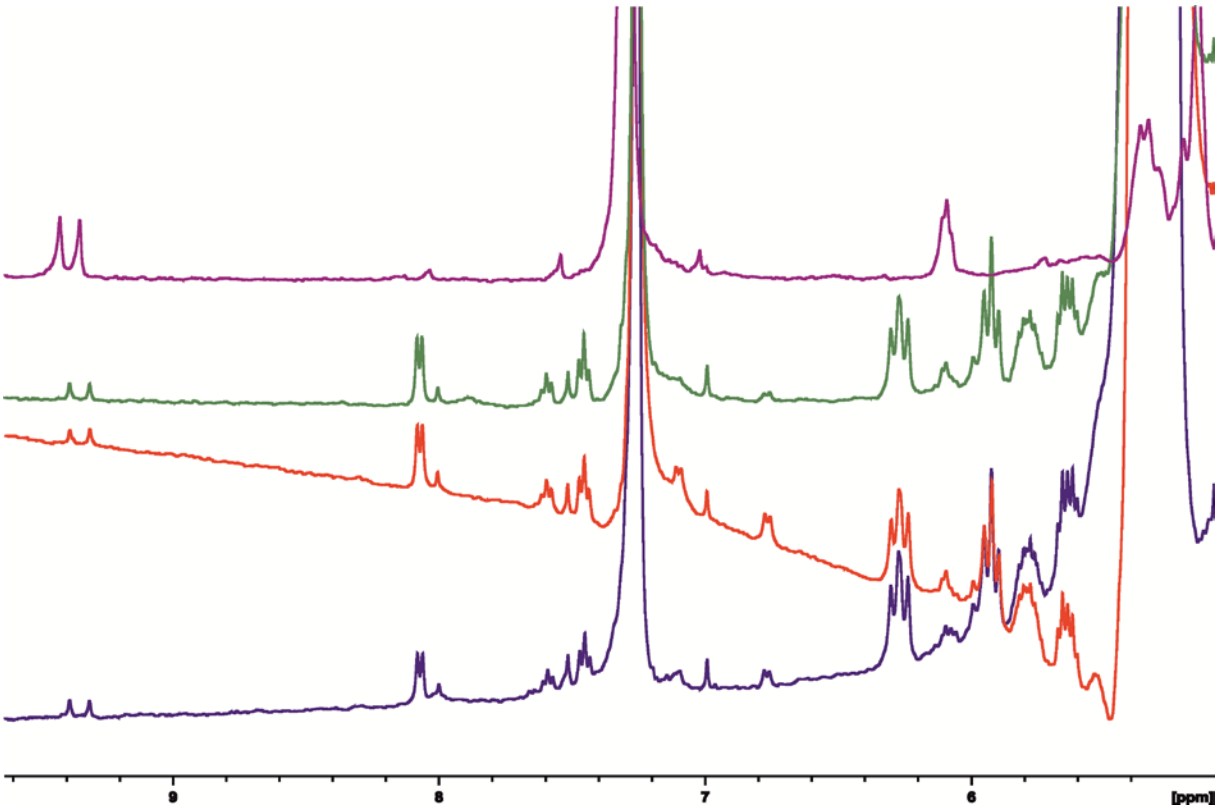

Figure 25 Bioactive compounds in Yoghurt 5 – Combined. Blue color: day 0, red color: day 14, green color: day 25, purple color: total mastic extract

17 Thermal Action

Total LAB were enumerated for all three different batches of yoghurt, stored at 6^{o} C, 10^{o} C and 14^{o} C respectively from day 0 to day 28. The initial number was 8.76 log10cfu/g for the three batches.

Total LAB numbers remained significantly higher and steady at about 8 log10cuf/g in yoghurt stored at 6^{o} C for 14 days compared with the other two batches. Similarly total LAB numbers remained significantly higher and steady at about 7 log10cfu/g in yoghurt stored at 10^{o} C for 14 days compared with yoghurt stored at 14^{o} C.

At the last day of storage the values of total LAB were decreased in all three batches. However it seems that values did not decrease below a critical value of 3.5 log10cfu/g for all three different batches. Although the decrease of total LAB numbers in yoghurts stored at 14^{o} C was faster until the 14 days, this trend did not last until the 28 days, where the decrease in values was very small.

The results are depicted graphically in Fig. 26 below

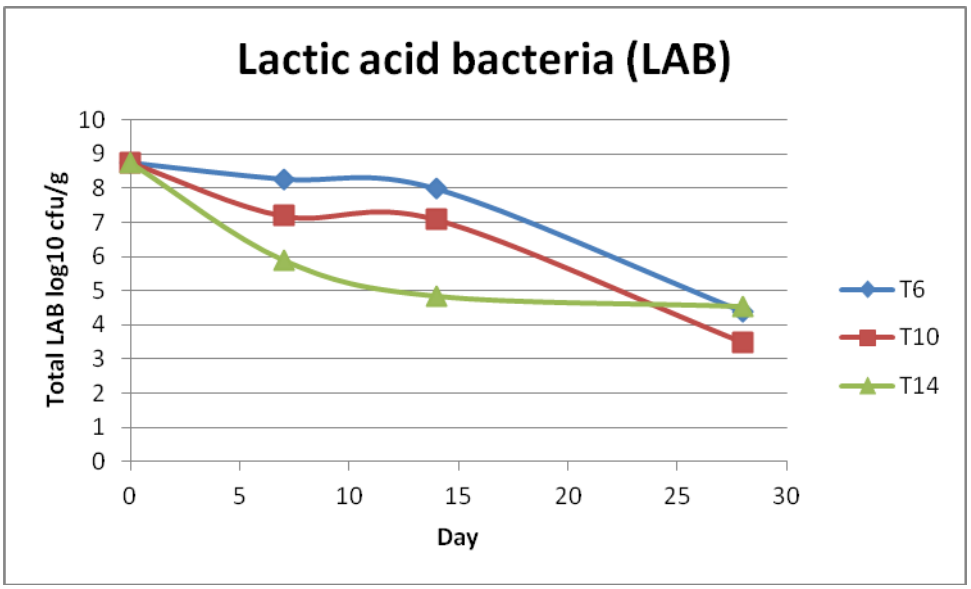

Figure 26 LAB population vs Storage time for three different temperatures 6, 10 and 14^{o}C

The data in Fig.26 were replotted as Total LAB population vs Thermal Action, Fig.27 and the linearity of the curve was estimated:

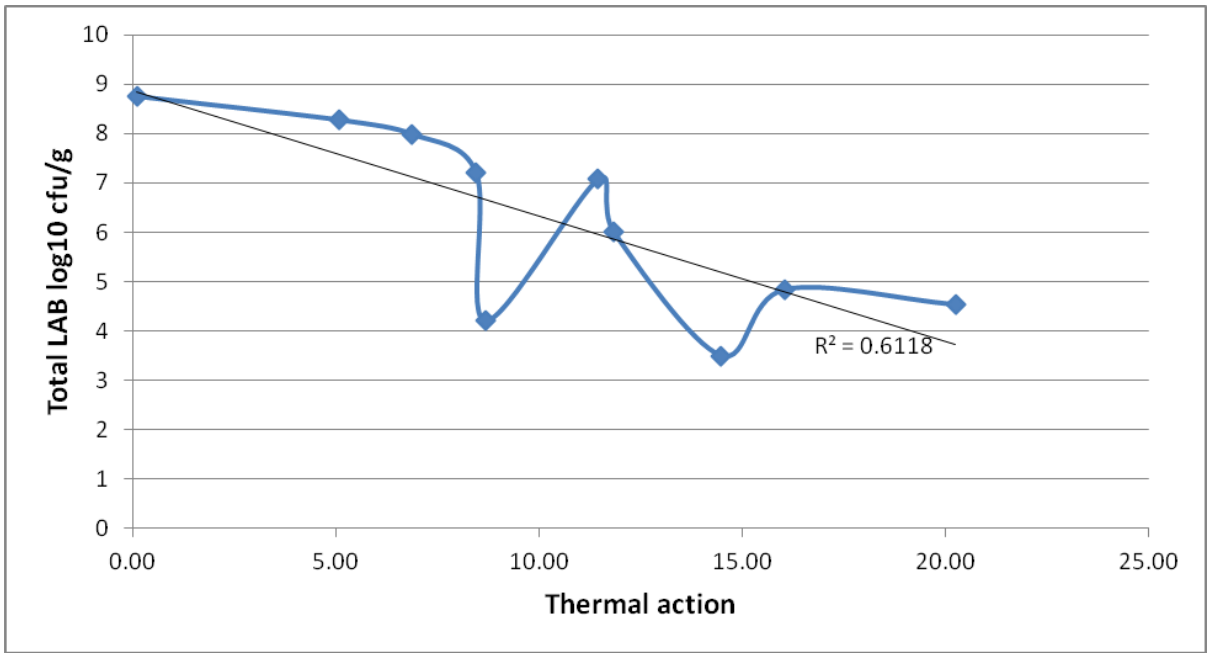

Figure 27 LAB population plotted vs Thermal Action

The two obvious outliers correspond to the higher 14^{o}C temperature for times 14 days and 28 days, storage time for which the yoghurt, as more or less was expected was rather deteriorated. Despite that the coefficient R^2 was 0.6118, which is not too bad for a complex biological system to show a tendency to linearity. If the two outliers are removed an even better coefficient is obtained (Fig. 28)

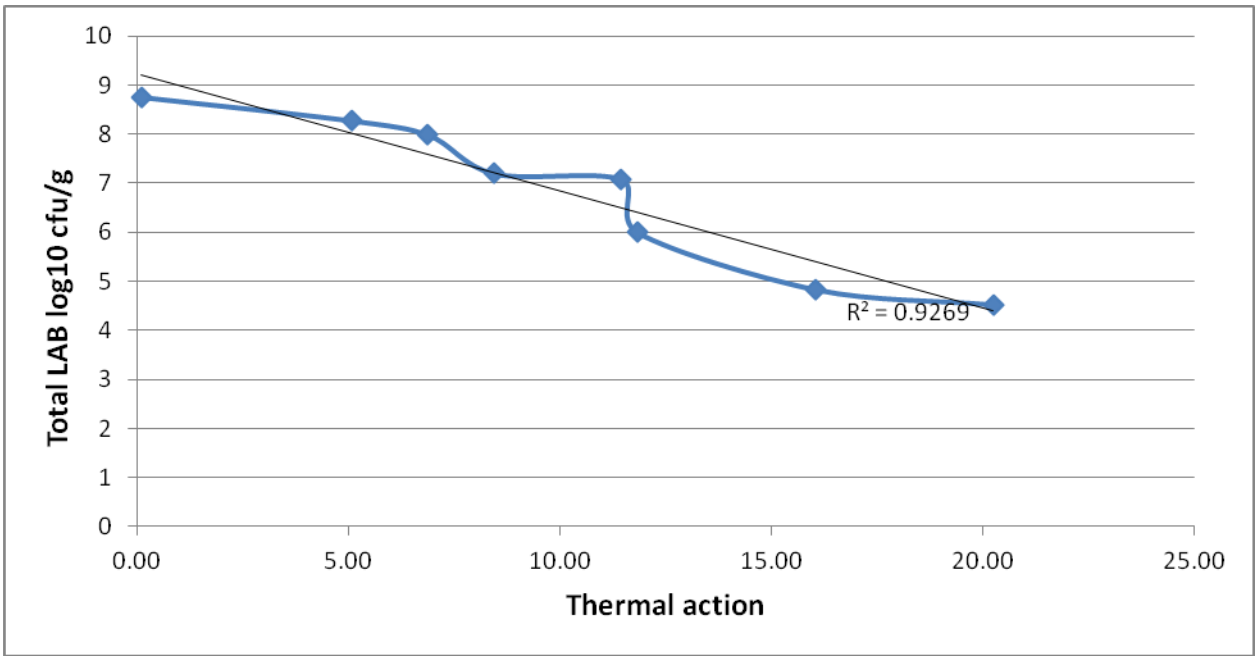

Figure 28 The diagram LAB population vs Thermal Action omitting the two outliers

The R^2 obtained is a 0.9269 which is a very good one to show that the above studied complex system responds to positively to the thermal action approach.

18 Bayes approach

We were able to obtain an estimation of the Bayes factor for the various treatments. Our hypothesis is that a high and stable LAB population should occur during the 25 first days of storage. Because of the shape of the curves a compromise for taking both properties, high values, stable values was followed by assuming the probabilities posterior and prior are proportional to the areas under the curves to the axis of X.

If the hypothesis applies to the total treatment then

$p(H/E) = c.\ A_{total}$

and

$p(H) = c.\ A_{control}$

and

Bayes factor = $p(H/E) / p(H) = A_{total} / A_{control} = 1.12$

If the hypothesis applies to the combined treatment i.e. samples containing total extract plus the S. boulardii yeast, following a similar procedure as above we obtain the Bayes factor for the boulardii sample as

Bayes factor = $p(H/E) / p(H) = A_{boulardii} / A_{control} = 1.33$

DISCUSSION

Since the transition from adolescence to young adulthood is a critical period during which young people adopt and establish lasting health behavior patterns it is important to examine the dietary habits of this unique age group.

The majority of our participants (64.8%) had a normal BMI. Our results are in line with a previous study conducted in Cyprus in children and adolescents where 62.8% of subjects were also shown to have normal weight (Tornaritis et al., 2014). Moreover Savva et al. (2014) indicated that the prevalence of obesity (8.1%) and overweight (20.1%) in children and adolescents in Cyprus has increased substantially over a decade, mainly in rural areas and in school-aged boys. Our results showed that among young adults the prevalence of obesity was lower (4.1%) and the prevalence of overweight a little higher (24.9%).

The majority of young females were classified as normal weight (75.5%). It is concerning that about 9.4% of women were underweight. On the other hand, 41.4% of young males were classified as overweight. However, given that young men of that age exercise more than women, as also supported by our data (Table 3), BMI may not be a very good index for them because of the increased muscle mass (Szabo and Tolnay, 2014) and therefore our results should be interpreted with caution. It is useful to note that 12.4% of the study sample was taking supplements at the time of the survey (Table 3) and of those 21.8% were males further supporting the above.

In the present study, only 26.9% of young adults had an optimal Mediterranean diet. Previous studies conducted in children and adolescents, reported similar results to ours, namely that there is a low to moderate adherence to the principles of the Mediterranean diet (Kontogianni et al. 2008, Lazarou et al. 2009, Chatzi et al. 2007). However, we did see a rather normal distribution among the groups, with about half in the average, middle category and the other

half split in the two extremes. A key finding of the study was that although median KIDMED score did not differ significantly between men and women, young women were less likely to be in the "high adherers" category compared to men (19.8% Vs 35.6% for women and men respectively; p=0.015). Previous studies had suggested that women have a better dietary profile than men (Leblanc et al. 2015). However these studies were conducting in older populations and may not be directly comparable to our study population. When looking at individual index items, women in the study ate less fruit/vegetables and nuts and more sweets, which may explain the difference in the overall score.

Overall, the daily intake of fruits and vegetables was low in our study population. Only about 30% of young adults consumed a second serving of fruit and vegetables more than once a day. Previous studies conducted in similar young populations showed even lower consumption of fruits and vegetables (McLean-Meyinsse et al. 2013, Avram et Oravitan, 2013) Put together, these findings suggest that young adults eat much less than the recommended amounts of fruits and vegetables. Although, the Cypriot young adults studied here are not reaching the target of five portions of mixed fruit and vegetables a day (five-a-day) as per the Department of Health's and other health agencies recommendations (Ashfield-Watt et al. 2004), it is encouraging that we at least report a higher consumption of fruit and vegetables than in other similar, non-Mediterranean, populations.

Our results indicate that a high percentage (76.2%) of Cypriot students consume at least two dairy products daily, complying with current dietary recommendations for dairy products (2-3 servings daily) (Weaver 2014). Our findings are therefore, encouraging and support the hypothesis that at least some aspects of the Mediterranean diet are still pursued in Mediterranean countries such as Cyprus, including dairy and pulses, which ~50% of study participants consume more than once per week. Furthermore the fact that our young

population consumes dairy products may have even more clinical implications as yoghurt consumption may be beneficial for prevention of diabetes (O'Connor et al. 2014).

About a third of participating college students (30.6%) consumes sweets and candy several times a day. The high intakes of sweets and candy reported here are in line with similar findings from other studies in young populations (Yannakoulia et al. 2004, Slining and Popkin 2013).

Another finding from our study was that a fourth of study participants (25.9%) visit a fast food restaurant more than once a week.

Our study sample demonstrated a lower than recommended consumption of fruit and vegetables but a recommended daily consumption of dairy product. This is in accordance with –and perhaps explained by- very recently published data in a large sample of college students in Canada (Matthews et al. 2016), showing that students believed they needed fewer vegetables and fruit and more milk than recommended quantities. Their findings further highlight the need for simpler age- and sex- specific recommendations and targeted campaigns.

While we report on the dietary habits and adherence to the Mediterranean diet of Cypriot young adults, it is also important to put our results in context with relevant findings from children in Cyprus, as reported from the CYKIDS study. The CYKIDS study (Lazarou et al. 2009) was a similar study conducted among school children (aged 9-13) in Cyprus during the school year 2004 -2005, with a representative sample of school-aged children. Our study was conducted among young adults (aged 18-25) in 2014, making it very likely that the study sample described here comes from at least the same generation, after a time-span of 10 years. Although the sample of the CYKIDS study was representative of the total school-aged children's population, and our study was based on convenience sampling and therefore may

not be representative of the total population of college students, we feel that comparisons are perhaps justified with regards to possible changes in dietary habits, prevalence of overweight and obesity and adherence to the Mediterranean diet during the transition from childhood to young adulthood, especially as adherence to the Mediterranean diet was assessed using the same tool, the KIDMED index.

At childhood only 6.7% of the participants in the CYKIDS study were classified as high adherers to the Mediterranean diet, whereas 37% had a poor KIDMED index (Lazarou et al. 2009). We report a lower prevalence of low adherence in adulthood (21.8%), and a much higher prevalence of high adherence to the Mediterranean diet (26.0% Vs 6.7%) compared to the CYKIDS population (Lazarou et al. 2009). Other differences between reports in the CYKIDS study and ours include a decrease in having breakfast and in the consumption of fruit and vegetables. Although, no direct inferences can be made, given that our population is not necessarily representative of the Cypriot young adult population, it would be reasonable to assume that young college students may indeed skip breakfast more often now than as kids or eat less fruit and vegetables. Importantly though, we also show a relatively high daily consumption of dairy products in this young adult population as was also shown for the CYKIDS population. This finding may have further implications, as it may represent one food group that maintains a high consumption pattern through the transition from childhood to adulthood in Mediterranean populations. A recent report has highlighted the role of low-fat fermented dairy intake, and especially yogurt which had a high reported consumption in our population, with a reduction in the risk of developing type 2 diabetes, further implicating specific food groups in public health interventions (O'Connor et al. 2014).

Our study sample demonstrated a moderate coffee consumption (2-3 times per day) overall, with no big differences in consumption between genders, with more women though being in

the lowest category (few times/year/never). A review regarding caffeine consumption concluded that for the healthy adult population, moderate daily caffeine intake at a dose level up to 400mg/day is not associated with adverse effects (Heckman et al. 2010). A standard 8 oz (240 ml) cup of coffee is thought to have an average of 100 mg of caffeine (Bae and Kim 2009).

With regards to alcohol consumption, we show that our study participants are occasional drinkers, in line with other studies, (Gotia et al. 2013, Loxton et al. 2015).

Smoking prevalence among young adults and especially among young males in our study was high but in agreement with previews reports from Cypriot school children (Christophi et al. 2008).

About one third of college students in the study did not consume breakfast on a regular basis. This is in agreement with previous reports, albeit in non-Mediterranean countries, that many young adults have the habit of skipping breakfast (Merten et al. 2009, Ackuaku-Dogbe and Abaidoo, 2014).

In the present study about half of young adults exercised regularly and in line with other studies (Sabau 2014, Olchowska-Kotala and Chromik, 2013) young men in our study population were more active than young women. This could be explained by cultural norms and trends in Cyprus, where joining a gym on a regular basis has increased in popularity among young adults.

About a fifth of the young adults surveyed used vitamins/minerals supplements, with one fifth of males also taking protein supplements (another popular trend among young ages in Cyprus). On the other hand, there is no evidence to suggest that supplements are required for optimal muscle growth or strength gain and even strength-trained athletes should consume

protein consistent with general population guidelines, or 12% to 15% of energy from protein (Phillips 2004). Protein supplementation in this young population deserves further attention.

With regards to body image, about half of young adults reported not being happy with their body weight, with females being unhappy at a higher percentage than males (58.5% Vs 37.9%, p=0.004). Factors associated with body image included breakfast consumption and exercise.

Therefore as yoghurt is widely consumed by Cypriot young adults, seems to be a good vehicle for providing functional ingredients. In the present study we investigated how Pistacia resins alone or combined with *Saccharomyces boulardii* affect both the LAB normally present in the yoghurt and the taste of the product. The stability of the resins has been investigated in order to ensure functionality of the product throughout its commercial life period. We aimed to the development of a novel, functional, local product that does not exist in the market in order to promote good health in young adults.

We have chosen goat milk for making yoghurt because nowadays goat's milk consumption considered having potential benefits to human health. It seems to have anti-inflammatory and anti-allergic properties (Jirillo and Magrone, 2014). Also goat milk has better digestibility and higher amounts in vitamins and minerals than cow milk (Park et al. 2007).

Total LAB numbers were steady higher in Yoghurt 5 with total mastic extract and the probiotic yeast *S. boulardii* at least until the 25th day. However this yoghurt lacks flavor compared to the other yoghurts. These findings are in line with a previous study conducted by Lourens-Hattingh and Viljoen (2001), which indicated that *S. boulardii* stimulate the growth and survival of LAB but excessive gas and alcohol production by the yeast species proved to be major constraints for incorporating this yeast into bio-yoghurt. *S. boulardii* was also enumerated and its numbers remained steady at about 6.7 $\log_{10}$cfu/g. Karaolis et al. (2013)

also demonstrated that *S. boulardii* can survive in high steady numbers during the life time of yoghurt.

After evaluation of LAB numbers and organoleptic assessment and concerning to have all bioactive compounds in our product we chose yoghurt with total mastic extract. Therefore we continued with further analysis only in this yoghurt.

Four fatty acids with an important nutritional value were identified: linoleic acid, linolenic acid, caproleic acid and CLA. It is noteworthy that all these fatty acids were presented at all spectrums of yoghurts from day 0 to day 25 and therefore our product keeps intact all lipids during shelf life. This was very important finding as, in a previous study conducted by Serafeimidou et al. (2012), in order to determine for first time chemical and fatty acid composition in various Greek yoghurts, the analysis was conducted at the third day only, after the manufacture of the product.

CLA is predominantly found in milk and milk products. At present there is a great interest in CLA because it appears to exhibit many health benefits related to anti-obesitic, anti-carcinogenic, anti-atherogenic, anti-diabetagenic, immunomodulatory, apoptotic and osteosynthetic effects (Benjamin and Spener, 2009).

Linolenic acid (ω-3) and linoleic acid (ω-6) belong to polyunsaturated fatty acids and are essential fatty acids. In diet is important to use a ratio of ω-6 to ω-3 polyunsaturated fatty acids of about 1-2:1 instead of a radio 20-30:1 that appears in Western diets. ω-3 fatty acids have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties. These beneficial effects may contribute to the prevention of many chronic diseases, such as, coronary heart disease, hypertension, type 2 diabetes, renal disease, rheumatoid arthritis and inflammatory bowel diseases, as reviewed by Simopoulos 1997.

An important finding is that the bioactive compounds of total mastic extract were identified in yoghurts samples during self life.

CONCLUSION

Our results support the proposed shift from traditional healthy diets to more unhealthy eating patterns in Mediterranean countries with at least one fifth of young adults having adopted poor dietary habits. Specifically, study participants consumed low quantities of fruits and vegetables, visit fast food restaurants often and consume sweets and candy several times a day. Also about half of the young adults do not exercise and one quarter of them smoke. On the other hand, the majority of young adults in Cyprus still consumes at least two dairy products daily and use olive oil at home, while also eating pulses more than once per week.

Moreover behavioral factors were associated with adherence to the Mediterranean diet and more particularly, a higher adherence to the Mediterranean diet was associated with regular breakfast consumption, exercise, positive body image, a higher meal frequency, higher water consumption, lower fried food consumption and lower consumption of meals away from home.

Based on these findings, tailored-made public health strategies targeting the young adult population would be warranted, focusing on interventions to increase fruit and vegetable consumption, maintain dairy consumption and generally to shape health behavior patterns in order to increase adherence to the Mediterranean diet, with positive effects carried into later adulthood.

Therefore, focusing on the promotion of good health in young adults and reducing future burden of chronic diseases, the manufacture of a new, innovating functional food was completed with success. Yoghurts fortified with *Pistacia* resins alone or in combination with *S. boulardii* were produced. When *Pistacia* resins have been added in goats' milk yoghurt promoted the growth of LAB and demonstrated very good organoleptic properties. This new local product could be marketed as a potential innovative functional food, offering an

alternative way of disease prevention and promoting good health. However, further testing is needed.

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APPENDICES

Appendix 1: Eating habits questionnaire

EATING HABITS QUESTIONNAIRE

This questionnaire was designed to investigate the eating habits of young adults, aged 18-25. The questionnaire is anonymous and will be used strictly for the needs of this research work. We would very much appreciate your honest, complete and accurate answers. Please answer all the questions below.

A. Choose the correct answer by putting √ in the appropriate box:

1. SEX: MALE ☐ FEMALE ☐
2. Are you student?: Yes ☐ No ☐
3. Are you working ; Yes ☐ No ☐
4. Region of main residence: Nicosia ☐ Larnaca ☐ Limassol ☐ Paphos ☐ Famagusta ☐
5. Type of education: Public university ☐ Private university ☐

B. Complete the following data:

1. Age: ..... 2. Height: ..... 3. Weight: .....

C. Answer with yes or not the following questions:

1. Do you have a fruit or fruit juice daily? .....
2. Do you have a second serving of fruit daily? .....
3. Do you eat fresh or cooked vegetables daily? .....
4. Do you eat fresh or cooked vegetables more than once a day? .....
5. Do you consume fish regularly (at least 2-3 times per week)? .....
6. Do you go more than once a week to a fast food (hamburger) restaurant? .....
7. Do you consume pulses more than once a week? .....
8. Do you consume pasta or rice or other starchy food almost every day (5 or more times per week)? .....
9. Do you have cereals or grains (bread, etc) for breakfast? .....
10. Do you eat nuts regularly (at least 2-3 times per week)? .....
11. Do you use olive oil at home? .....
12. Do you skip breakfast? .....
13. Do you have a dairy product for breakfast (yoghurt, milk, etc)? .....
14. Do you have commercially baked goods or pastries for breakfast? .....

15. Do you consume two dairy products (milk, yoghurt, cheese) daily? .....

16. Do you consume sweets and candy several times every day? .....

17. Do you currently take vitamins/minerals supplements? .....

18. Do you currently take protein supplements? .....

19. Do you currently exercise? .....
    If yes, what do you do and how often? .....

20. Do you currently smoke? .....
    If yes, how many cigarettes per day? .....

21. Do you feel happy with your body weight? .....

22. Are you currently on a diet? .....

D. Answer briefly the following questions:

1. Who prepares meals in your home;

   .....
2. How many meals do you eat (main and snacks) during the day?

   .....
3. What kind of fat do you use for frying or sautéing at home?

   .....
4. How many glasses of water do you drink every day?

   .....

5. How often do you eat lunch or dinner away from home

Every day	4-6 times/week	1-3 times/week	1-3 times/month	Few times/year or never

6. How often do you eat fried food?

Every day	4-6 times/week	1-3 times/week	1-3 times/month	Few times/year or never

7. How much extra salt do you put in your dish;

Much	Little	Not at all

8. Medical history (Report of disease, e.g. diabetes, inflammatory bowel disease etc).

   .....

9. Medication

   .....

E. Fill how often you eat the following foods on a daily, weekly or monthly basis, by putting √ in the appropriate box.

Foods and quantities	6+ times/ day	4-6 times/ day	2-3 times/ day	1 time/ day	5-6 times/ week	2-4 times/ week	1 time/ week	1-3 times/ month	Few times per year or never
Dairy foods									
Whole milk (1 glass)									
Skim or low fat milk (1 glass)									
Full fat yoghurt (1 cup)									
Skim or low fat yoghurt (1 cup)									
Ice cream (2 scoops)									
Full fat cheese (e.g. kefalotyri, cheddar, feta, halloumi) (1 slice or 1 serving of 30g)									
Low fat cheese (e.g. edam light, cottage cheese) (1 slice or ½ cup)									

Foods and quantities	6+ times/ day	4-6 times/ day	2-3 times/ day	1 time/ day	5-6 times/ week	2-4 times/ week	1 time/ week	1-3 times/ month	Few times per year or never
Fruits									
Apples or pears (1)									
Oranges (1)									
Bananas (1)									
Peaches (1)									
Apricots (1)									
Watermelon (1 slice)									
Melon (1 slice)									
Other fruits, please specify:									
Juice (1 glass)									
Vegetables									
Tomatoes (1) or tomato juice (1/2 cup)									
Cucumbers (1)									
Carrots (1)									
Zucchinis (1)									
Eggplants (1)									
Onions (1)									
Salads (lettuce, rocket, cabbage, parsle , celery) (1/2 cup)									
Broccoli (1/2 cup)									
Cauliflower, Brussels sprouts (1/2 cup)									
Green beans (1/2 cup)									
Green black eyed beans (1/2 cup)									
Corn (1 or ½ cup)									
Peas (1/2 cup)									
Spinach (1/2 cup)									
Sweet peppers(green,red, yellow) (1)									
Other vegetables, please specify:									
Pulses									
Beans (1 cup)									
Black eyed beans (1 cup)									
Lentils (1 cup)									
Chickpeas (1 cup)									

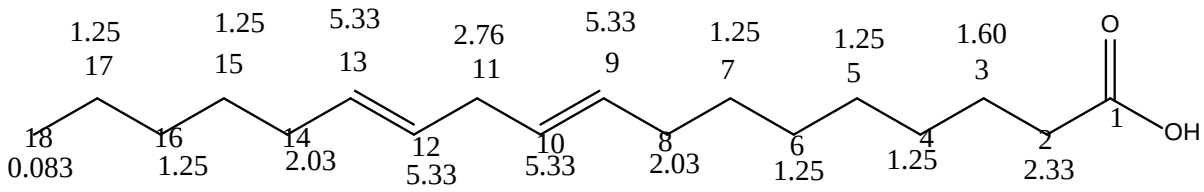
Foods and quantities	6+ times/ day	4-6 times/ day	2-3 times/ day	1 time/ day	5-6 times/ week	2-4 times/ week	1 time/ week	1-3 times/ month	Few times per year or never
Meats									
Chicken (120-180 gr.)									
Beef, pork or lamb as a main dish (steak, chop, burger, etc) (120-180 gr.)									
Beef, pork or lamb as a sandwich or mixed dish) (60-90 gr.)									
Ham or turkey (1 slice)									
Sausages, salami (1 slice or 1 piece)									
Bacon(1 slice)									
Liver or offal (90-120 gr.)									
Fish (120-180 gr.)									
Sea foods (120-180 gr.)									
Eggs (1)									
Rabbit (120-180 gr.)									
Canned tuna (90-120 gr.)									
Cereals/starch									
White bread (1 slice)									
Whole meal bread (1 slice)									
Pita bread(1 medium)									
Loaves (1 medium)									
Breakfast cereals (1/2 cup)									
Muesli(1/4 cup)									
Oats (1/2 cup)									
Potatoes boiled, baked (1) or puree (1/2 cup)									
Potatoes fried (1 portion)									
Pasta (1 cup)									
Pasta whole meal (1 cup)									
Barley (1 φλιτζ.)									
Bulgur wheat (1 cup)									
Rice (1 cup)									
Brown rice (1 cup)									

Foods and quantities	6+ times/ day	4-6 times/ day	2-3 times/ day	1 time/ day	5-6 times/ week	2-4 times/ week	1 time/ week	1-3 times/ month	Few times per year or never
Fat									
Olive oil (1 tablespoon)									
Olives (8-10)									
Nuts (1/2 cup)									
Avocado (1/2)									
Tahine (1 tablespoon)									
Butter (1 teaspoon)									
Margarine (1 teaspoon)									
Peanut butter (1 tablespoon)									
Mayonnaise (1 tablespoon)									
Fresh cream or whipped cream (1 tablespoon)									
Other foods									
Pastitsio (1 portion)									
Moussaka (1 portion)									
Stuffed grape leaves (6-8)									
Sheftalia (2-3)									
Potato chips (1 small bag)									
Chocolate (30 gr.)									
Biscuits (1)									
Cake(1 slice)									
Chocolate based sweets (1 portion)									
Traditional sweets (baklava, galaktoboureko, kataifi) (1 portion)									
Spoon sweets (e.g. walnut, citrus, watermelon) (1 portion)									
Sugar (1 teaspoon)									
Honey (1 teaspoon)									

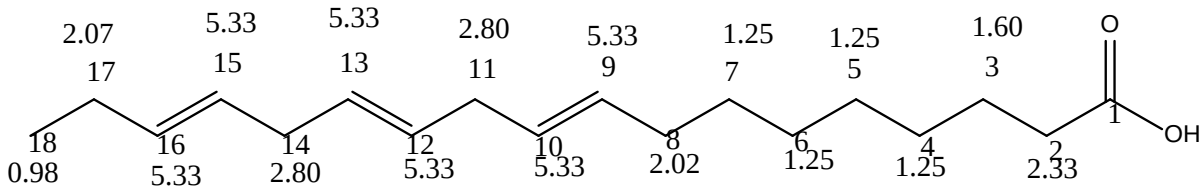
Foods and quantities	6+ times/ day	4-6 times/ day	2-3 times/ day	1 time/ day	5-6 times/ week	2-4 times/ week	1 time/ week	1-3 times/ month	Few times per year or never
Fast Food									
Hamburger (regular)									
Hot dog (1)									
Chicken nuggets (1 serving)									
Pizza (2 pieces)									
Cypriot traditional foods									
Flaouna (1 slice)									
Trahana soup (1 cup)									
Kolokasi (1 cup)									
Soutzoukos, palouzes									
Pafitiki pissa									
Carob honey (1 teaspoon)									
Pasteli									
Loukoumi Geroskipou									
Beverages/alcohol									
Coffee (1 cup)									
Tea (1 cup)									
Wine (1 cup)									
Beer (1 cup)									
Whiskey, vodka, zivania (1 drink or shot)									
Soft drinks (1 glass)									
Soft drinks light (1 glass)									
Sports drinks (1 glass)									

End of questionnaire. Thank you very much for your cooperation.

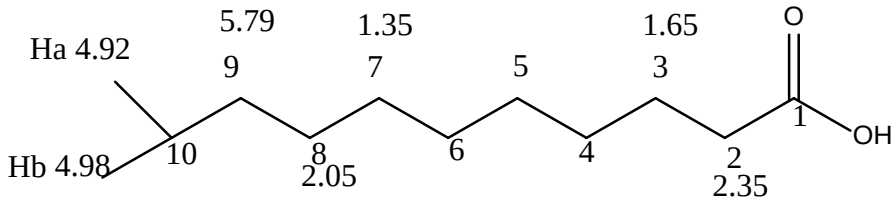
Appendix 2: Chemical structures and ^{1}H NMR shifts of linoleic acid, linolenic acid, caproleic acid and the four geometric isomers of CLA



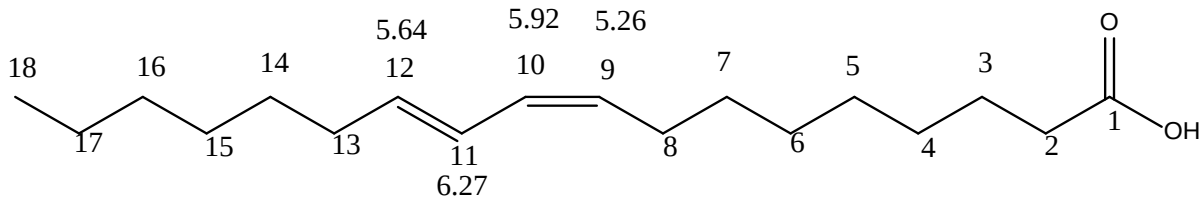
Linoleic acid



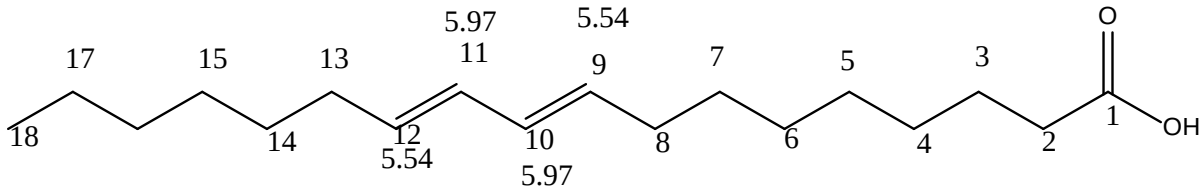
Linolenic acid



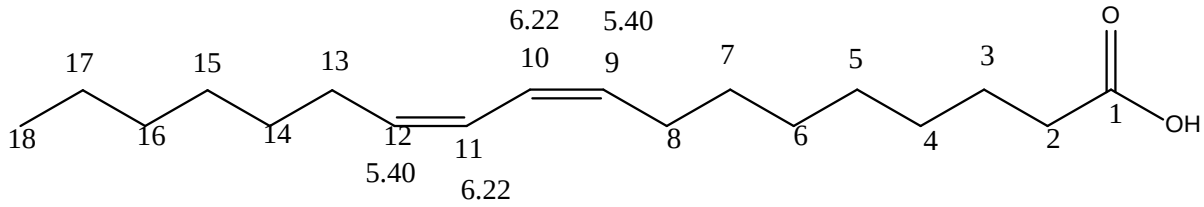
Caproleic acid



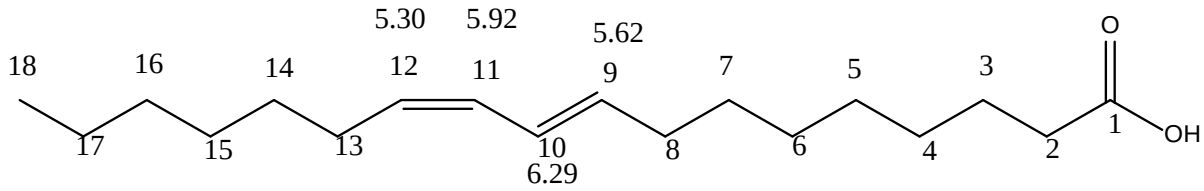
(9-cis, 11-trans) 18:2 Conjugated linolenic acid (CLA)



(9-trans, 11-trans) 18:2 Conjugated linolenic acid (CLA)

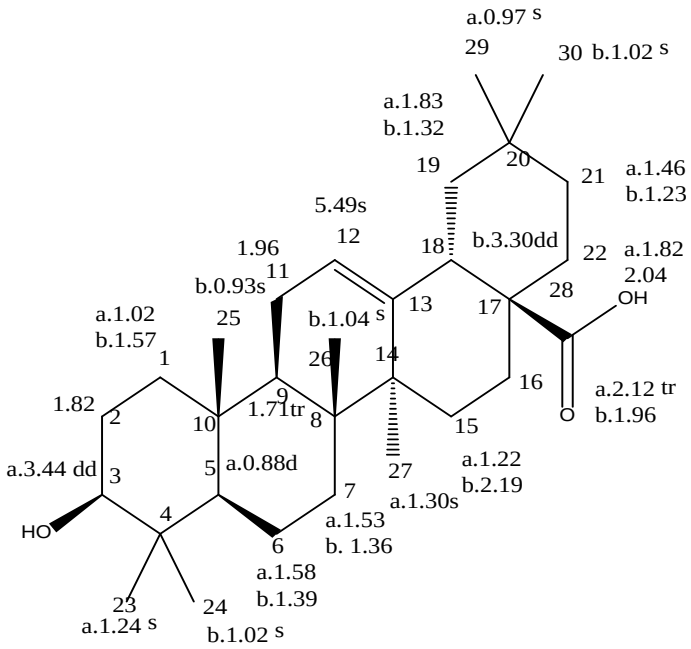


(9-cis, 11-cis) 18:2 Conjugated linolenic acid (CLA)

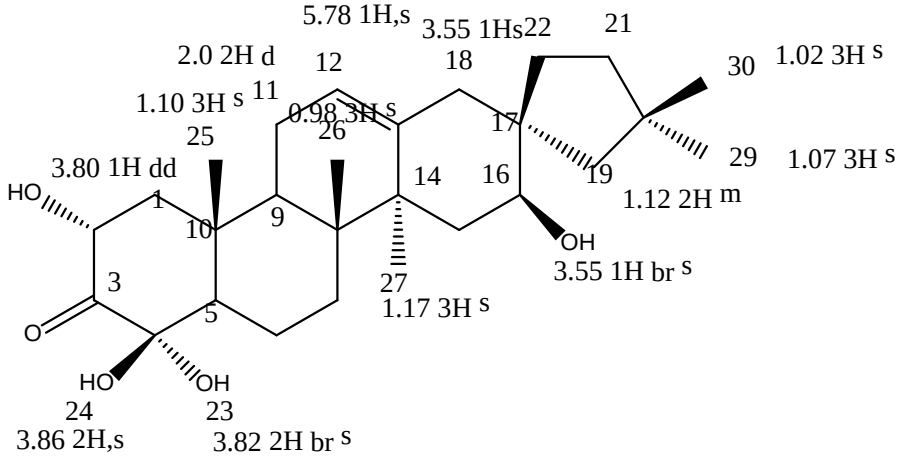


(9-trans, 11-cis) 18:2 Conjugated linolenic acid (CLA)

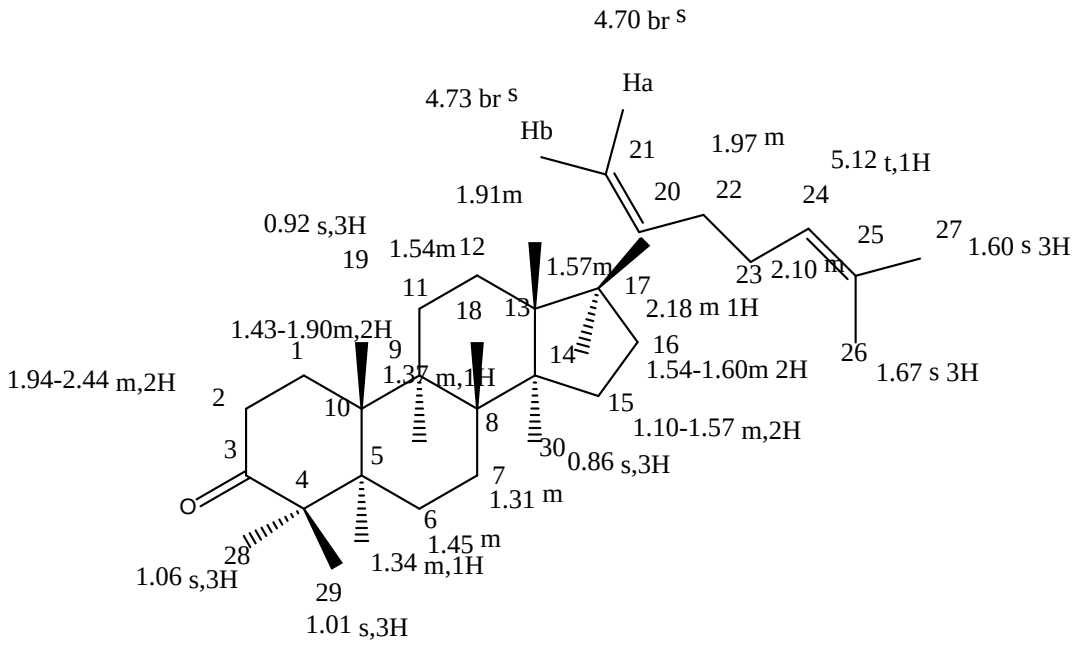
Appendix 3: Chemical structures and ^{1}H NMR shifts of triterpenic compounds



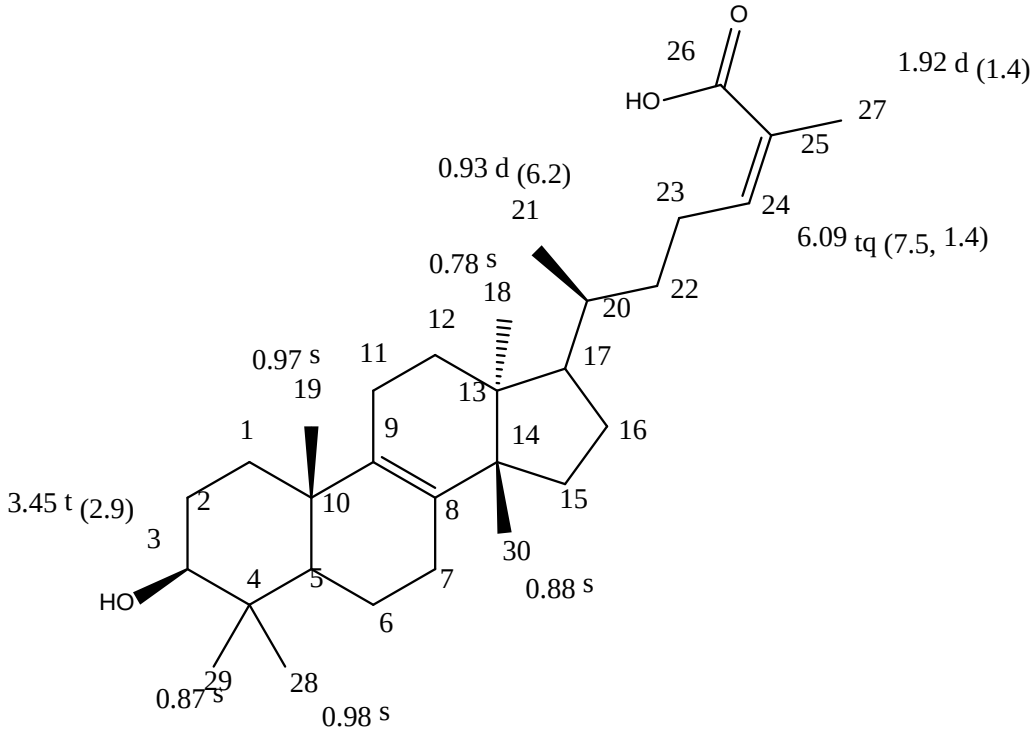
Oleanolic acid



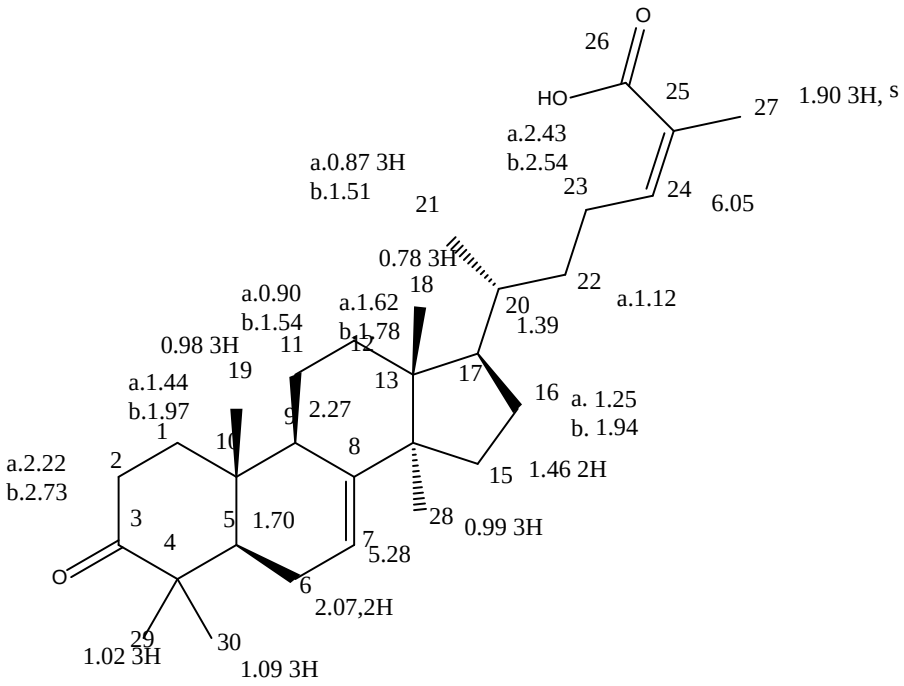
28-norolean-12-en-3-one



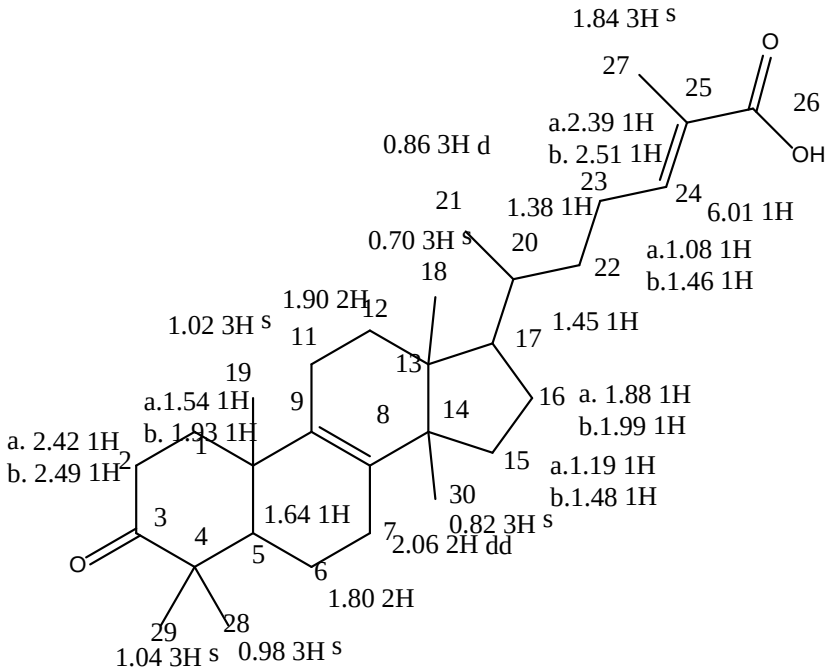
Dammaradienone



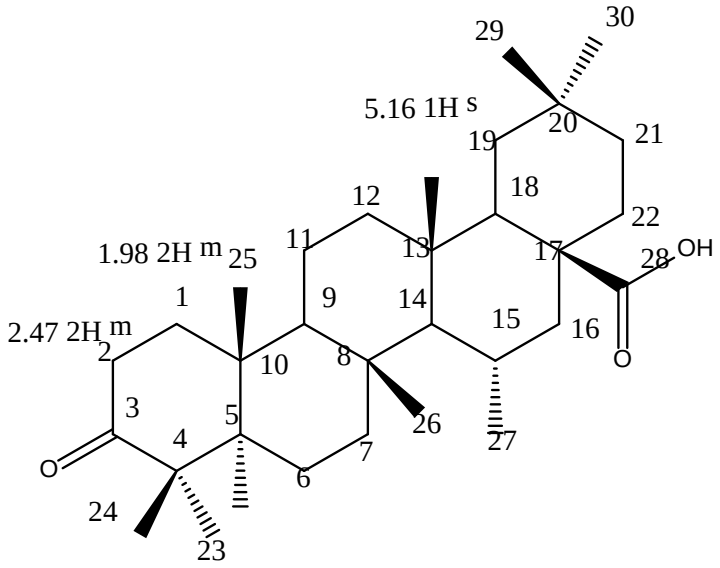
Isomasticadienolic acid



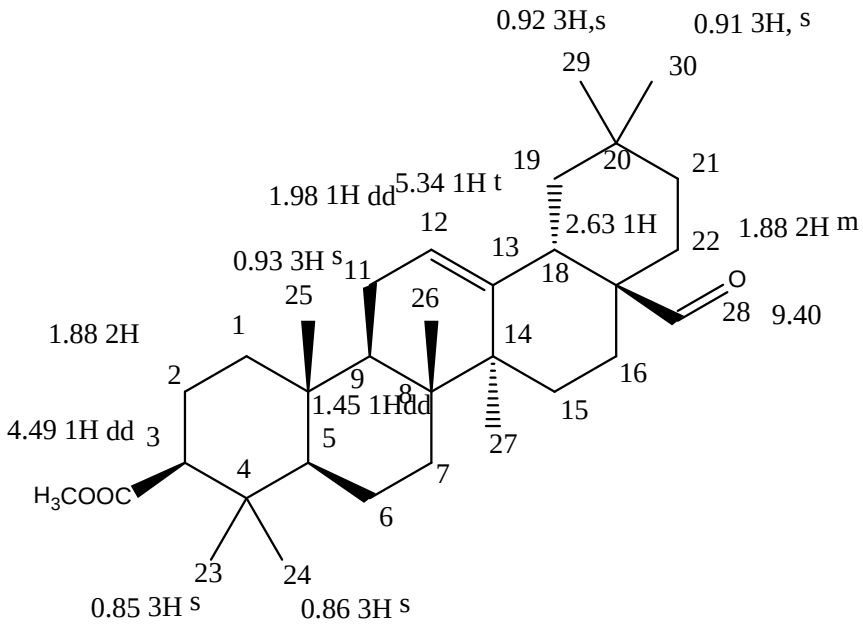
Isomasticadienonic acid



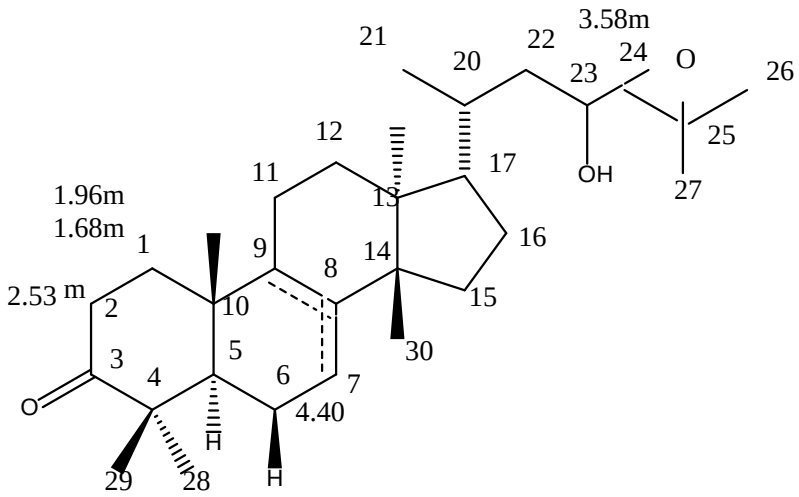
Masticadienonic acid



Moronic acid



Oleanolic aldehyde



Tirucallane triterpene

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ORIGINAL RESEARCH

Functional stability of goats' milk yoghurt supplemented with *Pistacia atlantica* resin extracts and *Saccharomyces boulardii*

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The present study describes the development of a functional goats' milk yoghurt, using Pistacia atlantica *resin extracts and* Saccharomyces boulardii, *in an attempt to combine the beneficial effects of the milk, extract and probiotic microorganisms. Results demonstrated that* Pistacia *extracts promoted the survival of lactic acid bacteria and NMR spectra additionally revealed that the functional fatty acids can be retained during the shelf life of yoghurts, alongside with the other bioactive compounds. The organoleptic assessment revealed that there were no significant differences in flavour, body, texture and appearance between the different yoghurts.*

Keywords Goats' milk, Yoghurt, Functional properties, Lactic acid bacteria, Organoleptic properties.

INTRODUCTION

Yoghurt is one of the most consumed and widely accepted fermented foods in the world with important functionality. Beneficial effects of yoghurt have been associated with the presence of calcium, phosphorus, potassium, vitamins A, B2 and B12, high biological value proteins and essential fatty acids. Yoghurt is also a well-known probiotic carrier, while *Bifidobacterium*- and *Lactobacillus*-enriched yoghurts are among the most common types of functional foods. The consumption of yoghurt has been linked with significant health benefits, including the prevention of osteoporosis (Sahni *et al.* 2013; Laird *et al.* 2017), diabetes (Tong *et al.* 2011) and cardiovascular diseases (Moreno *et al.* 2015), while promoting general gut health (Adolfsson *et al.* 2004) and modulating the immune system (Meyer *et al.* 2006).

The functional food market is constantly growing in the developed world, and yoghurts are rapidly gaining space as they provide ideal vehicles to deliver bioactive nutrients to humans (Sharma 2005). Already several attempts have been undertaken to produce yoghurts fortified with various plant extracts. Such examples are yoghurts produced from extracts from artichoke (*Cynara scolymus* L.), strawberry tree fruit (*Arbutus unedo* L.), cherry (*Prunus avium* L.) (Cossu *et al.* 2009), *Aloe barbadensis* and *Aloe arborescens* (Chiodelli *et al.* 2017), green tea (*Camellia sinensis*) (Amirdivani and Baba 2015), grape seed (*Vitis vinifera*) (Chouchouli *et al.* 2013) and seaweed (*Ascophyllum nodosum, Fucus vesiculosus*) (O'Sullivan *et al.* 2016).

Pistacia resins (*P. Atlantica, P. lentiscus* and *P. terebinthus*) are composed of a complex mixture of bioactive compounds, especially triterpenes and essential oils (Assimopoulou and Papageorgiou 2005; Koutsoudaki *et al.* 2005). There is accumulating evidence, suggesting that *Pistacia* resins may contribute to the reduction and prevention of many chronic diseases, including cardiovascular diseases, gastrointestinal disorders and various cancers, while in parallel they exhibit antimicrobial and antioxidant

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(Chadzopulu *et al.* 2011; Ansari and Siddiqui 2012; Hadjimbei *et al.* 2015). Furthermore, numerous patents have been registered for the preparation of *Pistacia* resin extracts and their formulation as herbal drugs and/or supplements (WO2018047176A1, WO2005112967A2, WO2003092712A1).

Saccharomyces boulardii is a probiotic yeast known for its anti-diarrhoeal effects. It is recommended for the prevention of antibiotic-associated diarrhoea, traveller's diarrhoea, acute diarrhoea, enteral nutrition-related diarrhoea and *Clostridium difficile*-associated diarrhoea (Surawicz *et al.* 1989; Kotowska *et al.* 2005; McFarland 2010; Ehrhardt *et al* 2016; McFarland and Goh 2019). Additionally, *S. boulardii* exhibits many probiotic properties, which include the optimum growth at 37 °C and its ability to survive through the GI tract. Furthermore, it inhibits the growth of a number of microbial pathogens and it demonstrates immunostimulatory effects on the intestinal mucosa (Czerucka *et al.* 2007). *Saccharomyces boulardii* can also be applied as a supplement for maintenance treatment of Crohn's disease (Guslandi *et al.* 2000; Garcia *et al.* 2008). Finally, it is widely accepted that functional foods not only need to be safe for consumption throughout the commercial life, but also must remain functional. Previously, it has been suggested that the combined use of probiotic yeasts such as *Saccharomyces boulardii* in yoghurt enhances the survival of lactic acid bacteria (LAB) (Karaolis *et al.* 2013).

A number of studies showed that the majority of dairy products are characterised by the limited viability of probiotics throughout their commercial life (Tamine *et al.* 2005; Cruz *et al.* 2010). The aim of this study was to develop an innovative functional yoghurt containing *Pistacia atlantica* resins alone and in combination with *S. boulardii* in order to promote potential physiological benefits. The interactions between the LAB in the yoghurt and *S. boulardii,* alongside the survival of the probiotic yeast, for a period of 25 days, will be investigated. Moreover, ^{1}H NMR (Nuclear Magnetic Resonance) spectroscopy was used to screen the stability of *Pistacia* resin extracts and fatty acid profile. Finally, the developed yoghurts were also assessed organoleptically to reveal the acceptability by young adults, as this life stage is critical for promoting good health and preventing the risk of chronic diseases.

MATERIALS AND METHODS

Preparation of *Pistacia atlantica* resin extracts

The total resin, neutral and acid extracts were prepared according to Paraschos *et al.* (2007) with slight modifications. Briefly, a quantity of 50 g resin was diluted in 50 mL ethyl acetate, and then, 150 mL methanol was added. The mixture was stirred, and ultrasound irradiation was applied for 2 h. The solution obtained was then filtered using a Bucher funnel, followed by centrifugation at 4600 *g* for 10 min. The solution was then evaporated using a rotary evaporator ($T < 65$°C) in order to obtain total resin extract (TRE). Total resin extract was then re-dissolved using 100 mL Na_2CO_3 (5%, w/v), and 350 mL diethyl ether was added. Fractionation was performed through liquid–liquid extraction. The organic phase was re-extracted three times with 100 mL Na_2CO_3 (5 % w/v) and afforded the neutral fraction (NF) of resin. The aqueous phase was acidified with 300 mL HCl 1N, and then, the acidic solution was re-extracted with 600 mL diethyl ether and the organic phase afforded the acid fraction (AF) of resin. Finally, the two fractions were evaporated to obtain dry extract. *Pistacia atlantica* resin was kindly donated by the Aphrodite Delights company (Paphos, Cyprus).

Yoghurt manufacture process

Five different yoghurts using goats' milk were prepared: Yoghurt 1 – Control was the control one with only the starter culture added; Yoghurt 2 – Acidic, with AF of resin extract; Yoghurt 3 – Neutral, with NF of resin extract; Yoghurt 4 – Total, with total resin extract; and Yoghurt 5 – Combined, with total resin extract and yeast added simultaneously (Table 1). Yoghurt manufacture was performed using the described method by Karaolis *et al.* (2013). More specifically for the manufacture of the five different goats' yoghurt formulations, in each of five sterilised 1-L Scott Duran® bottles, 500 mL of fresh goats' milk was poured and under mild stirring with a heating magnetic stirrer was preheated at 55 °C. Then, 10 g of calcium caseinate (2% w/v) was added to the milk and thoroughly homogenised (IKA ®T25 Digital Ultra Turrax, Werke GmbH & Co. KG, Staufen, Germany) with a speed of 12 000 rpm for 3 min. A heat treatment at 90 °C for 5 min followed, and all five bottles containing the milk were cooled in a water bath to 46 °C. Subsequently, 2 g of the starter culture containing 1:1 *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (YC-381, Chr. Hansen A/S, Hoersholm, Denmark) was added into all samples. Three of the samples (Yoghurt 2, Yoghurt 3 and Yoghurt 4) were inoculated in parallel with 300 mg of total resin extract, neutral fraction of resin extract and acid fraction of resin extract, respectively, whereas one of the samples (Yoghurt 5) was inoculated in parallel with 300 mg of total resin extract and 200 mg of probiotic yeast *S. boulardii* (Bioflor®; Biocodex, Gentilly Cedex, France). All samples were incubated at 46 °C for 15 min. A volume of 50 mL milk was then poured into sterile plastic pots and sealed, resulting in ten different pots for each series of yoghurt. All pots were then incubated at 46 °C for 3 h and were then transferred and stored at 6 °C. Five different replicates were made in total, and starting from day 0, which was the yoghurt manufacture day, one sample pot from each of the five different yoghurts was analysed microbiologically and organoleptically. The analyses were performed up until day 25, the storage period tested.

Enumeration of total LAB and *Saccharomyces boulardii*

For plating and enumeration, a modification of the method described by Lourens-Hattingh and Viljoen (2001) was applied. Briefly, a sample of 10g from each of the yoghurts was weighted aseptically and transferred into sterile stomacher bags. It was then mixed with 90 mL sterile Maximum Recovery Diluent (MRD; Oxoid, UK) and homogenised in stomacher for 1 min. MRD was used to prepare serial dilutions (1:10) down to 10^{-6}. From each dilution, a volume of 100 μL was inoculated and dispersed onto de Man, Rogosa and Sharpe agar (MRS agar; Oxoid, UK) for the enumeration of LAB and Dichloran Rose Bengal Chloramphenicol agar (DRBC agar; Oxoid, UK) for *S. boulardii* in duplicates. The MRS plates were again overlaid with molten MRS agar at 48 °C, in order to create microaerophilic conditions. MRS plates were then incubated at 37 °C and DRBC plates at 25 °C for 3 days. The procedure was repeated every 3 days, for the duration of 25 days, and the total LAB and probiotic yeast *S. boulardii* were counted.

Sample preparation and ^{1}H NMR spectroscopy

Yoghurts were extracted with 100 mL ethyl acetate for 1 h. This procedure was repeated three times. The combined extracts were, then, evaporated in a rotary evaporator and sublimated in freeze dryer to obtain dry extracts. Approximately, 20 mg yoghurt extract was dissolved in 600 μL $CDCl_3$ and used for the NMR analyses. The NMR experiments were performed on a Bruker AV-400 spectrometer equipped with a BBI cryoprobe; the NMR system was controlled by the software TopSpin 2.1. ^{1}H NMR spectra were collected with a relaxation delay of 5 s and an acquisition time of 4.3 s; 64 K data points were collected, and the data were treated using a line-broadening exponential function of 1.5 Hz. Chemical shifts were reported with respect to the resonance of the solvent $CDCl_3$ at 7.26 ppm. For quantification, the coaxial arrangement was used (Tsiafoulis *et al.* 2014).

Organoleptic assessment

An organoleptic assessment was performed by a panel of twenty young potential consumers aged 18–25.

Table 1 Yoghurt types manufactured in the present study.

Yoghurt no.	*Yoghurt name*	*Contains*
1	Control	Starter culture
2	Acidic	Starter culture + acid fraction of the resin extract
3	Neutral	Starter culture + neutral fraction of the resin extract
4	Total	Starter culture + total resin extract
5	Combined	Starter culture + total resin extract + *Saccharomyces boulardii*

Yoghurts 4 and 5 were selected to be tested against the control one. The specific yoghurts were chosen because Yoghurt 4 contained all the components of resin and the survival of total LAB at least until 18 days was in high levels and Yoghurt 5 because of the presence, not only of resin extract, but also of *S. boulardii*, a combination with a synergistic action.

The panel evaluation was performed following the manufacture of fresh batches of Yoghurt 1 (Control), Yoghurt 4 (Total) and Yoghurt 5 (Combined) on the third day from manufacture. The basis for selecting the twenty panel assessors was the regular purchase and consumption of yoghurts (at least twice a week). The panel was asked to complete a questionnaire following the examination parameters proposed by Nelson and Trout (1981), in order to assess the differences and the acceptability of the resin extract alone and in combination with *S. boulardii*-containing yoghurts, against the goats' milk yoghurt currently available in the market. The maximum total score following the organoleptic score was 100 points: flavour – 50 points, body and texture – 40 points and appearance – 10 points.

Statistical analysis

Analysis of variance (ANOVA) methods were applied to evaluate differences in mean values of continuous variables as appropriate. Pairwise comparisons of the group means were performed using Duncan test. A two-sided p value of less than 0.05 was considered statistically significant. Data were analysed using SPSS v.20.0 (SPSS Inc. Chicago Ill) and Microsoft Office Excel 2007.

RESULTS

Survival of total LAB and *Saccharomyces boulardii*

Enumeration for LAB was carried out for all five yoghurts made (1, 2, 3, 4 and 5), throughout the testing period (day 0 to day 25). Initial numbers ranged between 6.20 log10 cfu/g and 8.73 log10 cfu/g. Total LAB remained significantly higher and steady at about 8 log10 cfu/g in Yoghurt 5 for 25 days (Figure 1a).

Total LAB followed similar trends in Yoghurts 1 and 2 and in Yoghurts 3 and 4, respectively. The numbers of total LAB remained in significantly higher values for Yoghurts 3 and 4 until about day 18, compared with Yoghurts 1 and 2. However, at the last day of storage, Yoghurts 1 and 2 had significantly higher values (about 5.5 log10 cfu/g) compared with Yoghurts 3 and 4 (about 4.5 log10 cfu/g). Yoghurt 5 was inoculated in parallel with 300 mg of total resin extract and with 200 mg of probiotic yeast *S. boulardii*. Along with total LAB, *S. boulardii* was also enumerated onto DRBC agar. As Figure 1(b) shows, the *S. boulardii* cfu/g were retained at about 6.7 log10 cfu/g.

Organoleptic assessment

The panel organoleptic assessment was performed for Yoghurts 1, 4 and 5. Concerning the surface's appearance, the majority of panel reported for all yoghurts flat and thin surface without bubbles and without syneresis. However, more persons reported a not flat surface for Yoghurt 5 compared with Yoghurts 1 and 4. Syneresis was observed more in Yoghurts 4 and 5. The colour was reported as white for all samples tested. Texture assessment was divided into two categories: firstly on the spoon, which was assessed by the majority of all yoghurts as thin, cohesive and not sticky without syneresis after cutting, and secondly mouthfeel, which was evaluated as thin, cohesive, not sticky to the palate and not grainy. More persons reported better texture on the spoon and in the mouth for Yoghurt 5 comparing with the other two yoghurts. Regarding taste and odour, most of the testers defined it as satisfying and pleasant for all samples tested. Only a few persons mentioned a goaty aftertaste (eight for the Yoghurt 1, two for Yoghurt 4 and one for Yoghurt 5). Moreover, six tasters noted the resin flavour in Yoghurt 4 and only one taster noted a taste of yeast in Yoghurt 5. ANOVA was performed in an attempt to statistically explore the differences among the mean scores of the three yoghurts, regarding flavour, body and texture and appearance. Figure 2 presents the results of the organoleptic score. No significant differences were observed for all the parameters, flavour ($P = 0.708$), body and texture ($P = 0.292$), appearance (0.537) and the total organoleptic scores ($P = 0.422$). Regarding the acceptability, Yoghurt 1 was preferred by three tasters (15%), Yoghurt 4 was preferred by ten tasters (50%), and Yoghurt 5 was preferred by seven tasters (35%). The tasters preferred Yoghurt 1 because of a reported pleasant, sour taste, nice appearance and odour. Yoghurt 4 was preferred due to its pleasant taste and odour and also due to its characteristic resin taste. Moreover, tasters mentioned that this yoghurt was less sour. Yoghurt 5 was preferred due to its pleasant taste and odour, and increased acidity. Tasters also reported that this yoghurt was more pure and traditional.

Lipid profile of yoghurts

NMR spectroscopy was initially used to monitor fatty acid composition in yoghurts during storage. Four major fatty acids (Figure S1) were identified by comparison of their ^{1}H NMR data (Tsiafoulis *et al.* 2014; Alexandri *et al.* 2017). In particular, the triplet due to allylic protons –HC=CH–C$\underline{H}_2$–CH=CH– at 2.76 ppm indicates the presence of linoleic acid, and the broad triplet at 2.79 ppm indicates the presence of linolenic acid (Figure 3). The signals at ~4.98 ppm with doublet of doublets indicate the presence of 10a and 10b protons' caproleic acid. The spectrum of conjugated linoleic acid (CLA) consists of a triplet at 5.93 ppm and a pseudo-triplet (resulting from a doublet of a doublet) at 6.2 ppm (Figure 3). The ^{1}H NMR spectra, therefore, demonstrate the presence of four fatty acids in yoghurt during shelf life.

Regarding quantities, in both yoghurts and during shelf life, CLA remains in the same initial quantity. Regarding Yoghurt 5, all lipids remain constant until the last day. Regarding Yoghurt 4, in linoleic acid there is a 9.6% reduction during the middle of shelf life, but no further reduction at the end. Linolenic acid remains constant until the middle of shelf life and decreases at the last day by 16.7%, while caproleic acid decreases by 7% during the 14th day and by 17.7% at the end.

Pistacia resin bioactive phytochemicals in yoghurts

The stability of bioactive phytochemicals in yoghurts during shelf life was studied comparing the ^{1}H-NMR spectrum of total resin extract with the spectra of various yoghurts. Two aldehyde signals (singlets) at 9.4 and 9.3 ppm (Figure 4) indicate the presence of oleanonic and oleanolic aldehydes

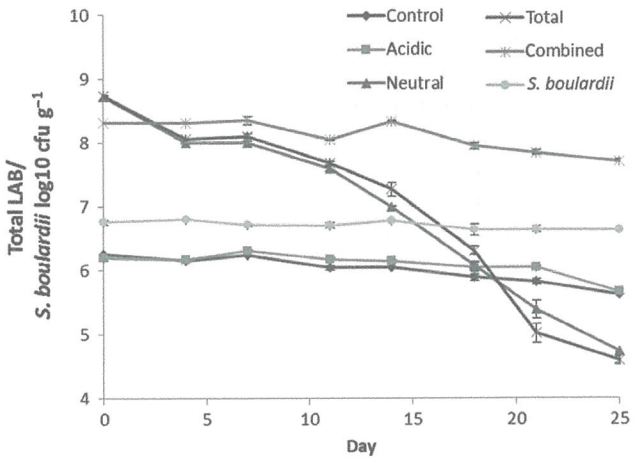

Figure 1 Survival of total lactic acid bacteria (LAB) in yogurt formulations (1–5) and Saccaromyces boulardii in Yoghurt 5. Means of duplicates. Day 0 = day of inoculation.

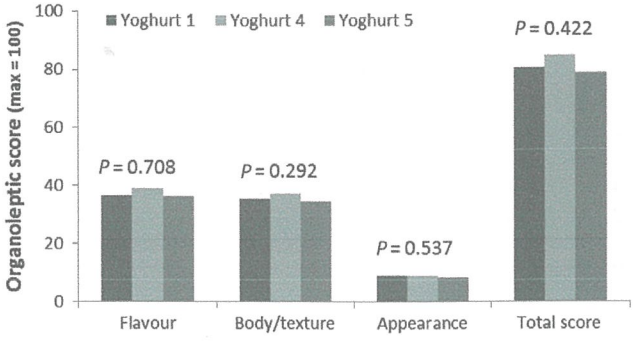

Figure 2 Results of the organoleptic score for Yoghurts 1, 4 and 5 from the twenty panel tasters. Parameters tested: flavour, body and texture, appearance and total organoleptic score.

(Figure S1). The broad triplet at 6.1 ppm should be attributed to the presence of the olefinic signal of the $-CH_2-\underline{\hat{C}}H=C(CH_3)-$ moiety of masticadienonic, isomasticadienonic and/or isomasticadienolic acids (Figure S1). A possible fine structure of the triplet, after resolution enhancement, may be attributed to further splitting due to the presence of the CH_3 group. Regarding Yoghurt 4, it seems that over time there is a reduction of the aldehyde components as the signals disappeared at day 25. On the other hand, the signal at 6.1 ppm was detected in all samples although with a progressive decrease in intensity (Figure 4). Regarding Yoghurt 5, it seems that over time all the bioactive compounds are maintained during shelf life (Figure 5).

The quantitative differences in bioactive composition during shelf life of yoghurts were studied comparing the integrals of characteristic peaks. NMR data highlight that there are no reductions in *Pistacia* phytochemicals in Yoghurt 5 during shelf life. Regarding Yoghurt 4, triterpenic aldehydes decrease by 100% at the end of shelf life, while there is a 50% reduction for oleanolic aldehyde at 14th day and 16.7% reduction for oleanonic aldehyde. The triterpenic acid content shows a decrease of 17% in the middle and by

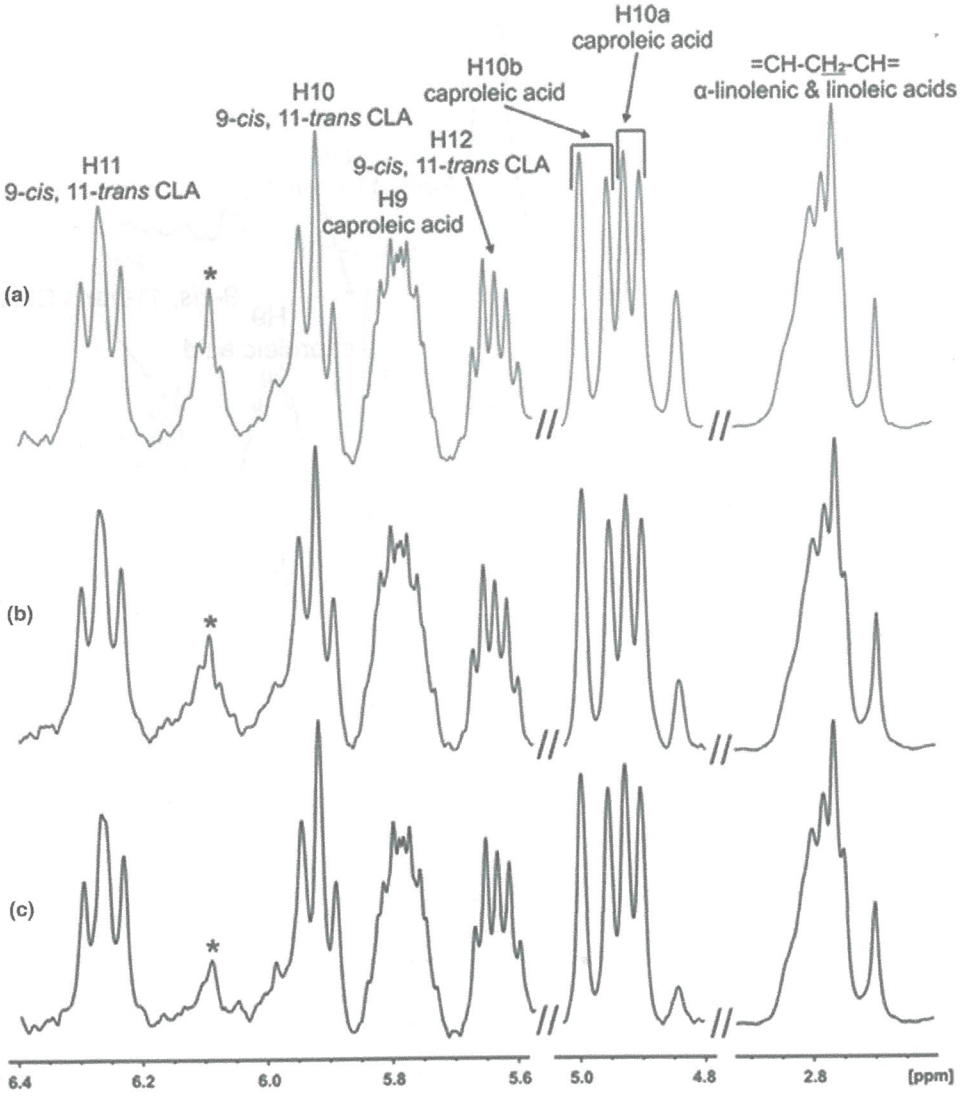

Figure 3 Selective regions of 400 MHz ^{1}H NMR spectra in $CDCl_3$ (298 K, number of scans (ns) = 32, acquisition time (AQ) = 5.1s, total experimental time = 5 min 24 sec) of the lipid extract of Yoghurt 4 – Total, where letter a: day 0; letter b: day 14; and letter c: day 25. The asterisk at 6.1 ppm denotes the olefinic signal of the $-CH_2-CH = C(CH_3)-$moiety of masticadienonic, isomasticadienonic and/or isomasticadienolic acids. Similar spectra were obtained for Yoghurts 2, 3 and 5.

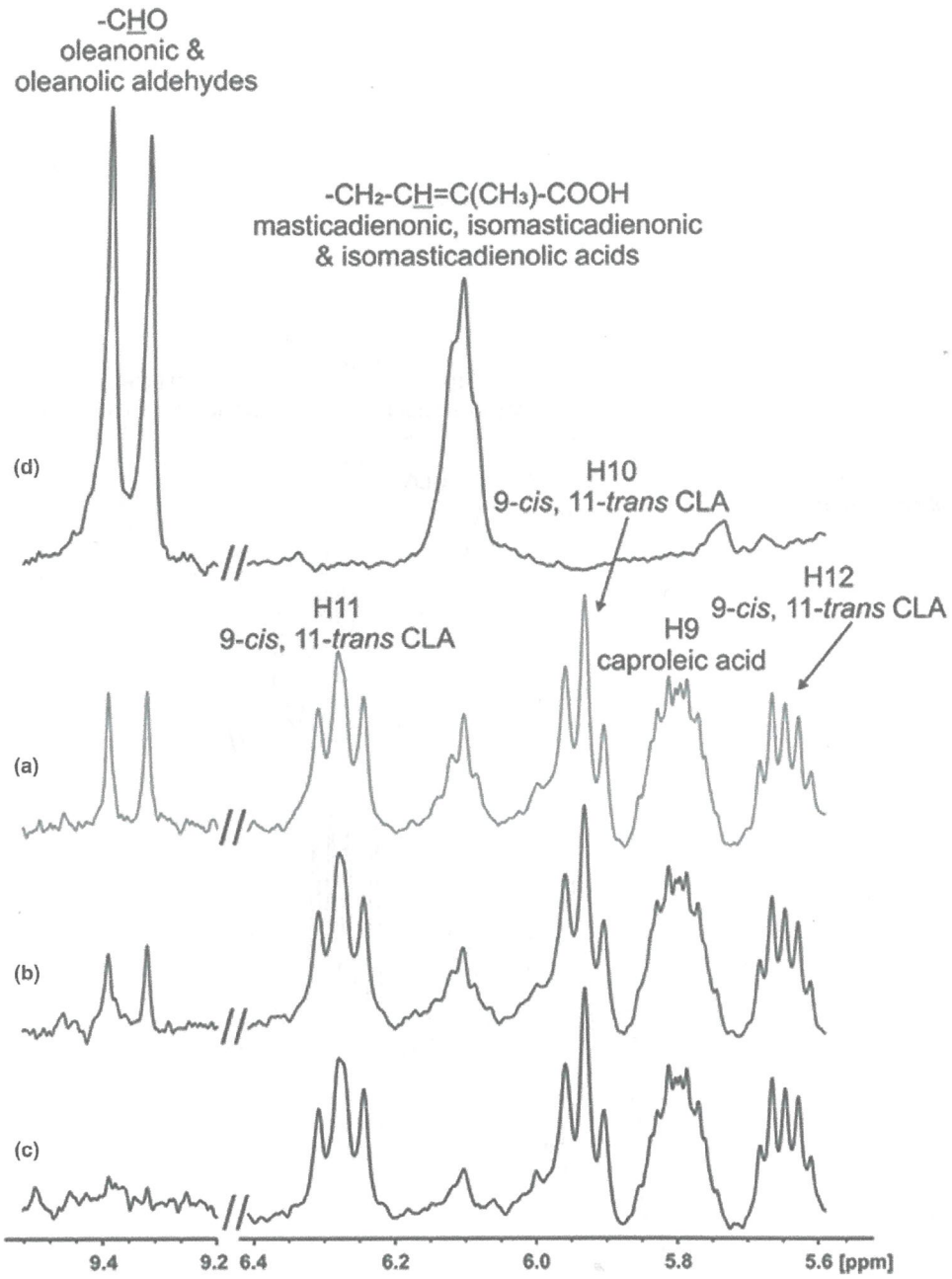

Figure 4 Selective regions of 400 MHz ^{1}H NMR spectra in $CDCl_3$ (298 K, acquisition time (AQ) = 5.15 s, total experimental time = 8 min 52 sec) of the lipid extract of Yoghurt 4 – Total, where letter a: day 0; b: day 14; c: day 25; and d: total resin extract.

75.6% in the end of shelf life. At the end of shelf life of yoghurts, the higher quantities in studied bioactive compounds are presented in Yoghurt 5.

DISCUSSION

Yoghurt provides a good vehicle for delivering functional ingredients because it is widely accepted and consumed by young adults (Hadjimbei *et al.* 2016). This study investigated how *Pistacia atlantica* resins alone or combined with *Saccharomyces boulardii* affect both the LAB and the organoleptic properties of the product. The stability of the resins has been investigated in an attempt to ensure the functionality of the yoghurt throughout its shelf life. We aimed to the design of a novel, functional, Mediterranean product that does not exist in the market in order to promote health, especially in young adults. Young adults are an important age group where intervention programs focusing

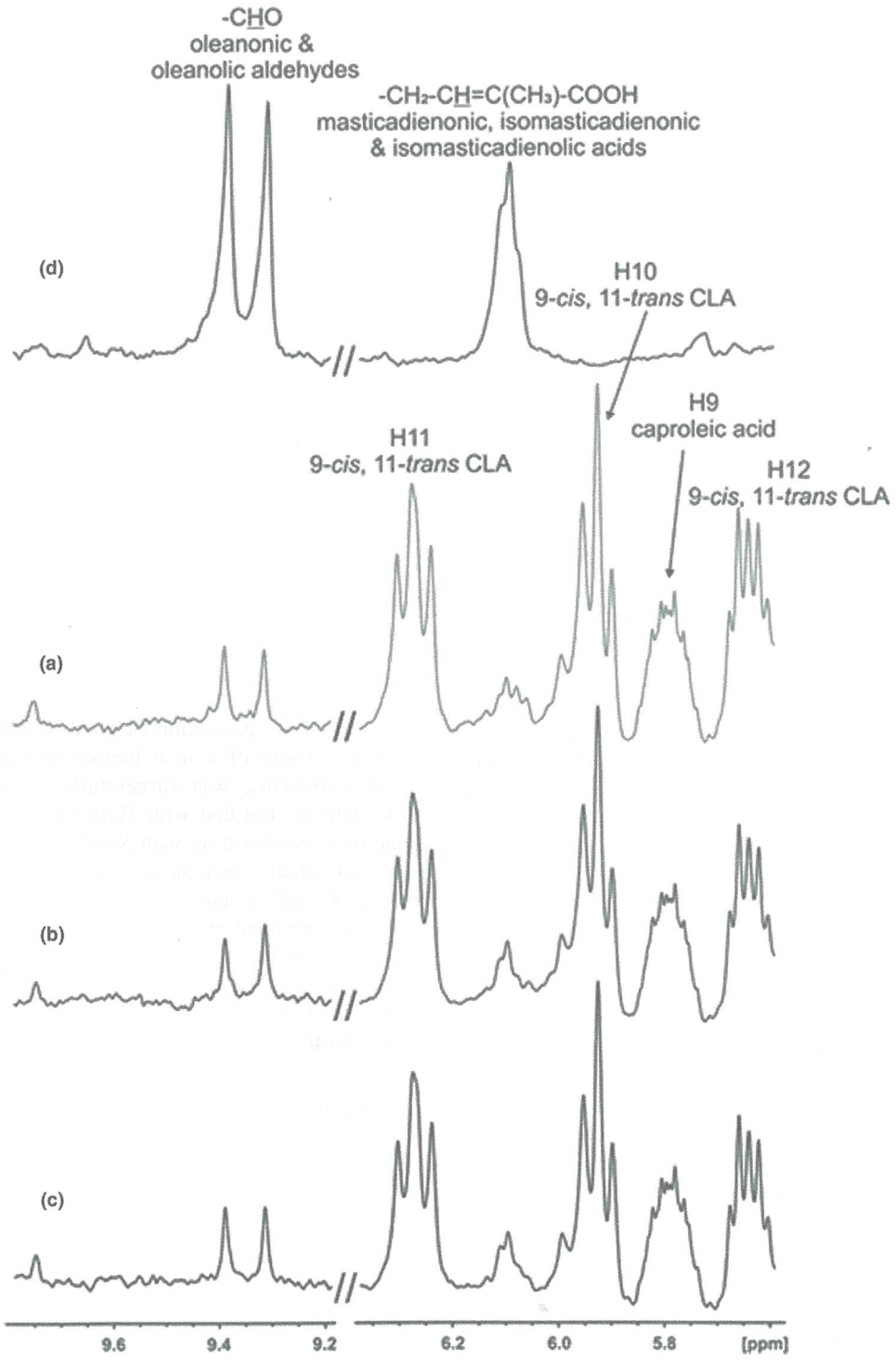

Figure 5 Selective regions of 400 MHz ^{1}H NMR spectra (298 K) in $CDCl_3$ of the lipid extract of Yoghurt 5 – Combined, where letter a: day 0; b: day 14; c: day 25; and d: total resin extract.

in their good health are important, not only for their concurrent good health, but also for reducing their future risk of a number of chronic diseases.

We have chosen goats' milk for making yoghurt because nowadays goats' milk consumption considered having potential benefits to human health. It seems to have anti-inflammatory

and anti-allergic properties (Jirillo and Magrone, 2014). Also, goats' milk has better digestibility and higher amounts of vitamins and minerals than cow's milk (Park *et al.* 2007).

On the other hand, goats' milk yoghurt is inferior with respect to preference and sensory attributes compared to that of cow's milk yoghurt, because of the characteristic goaty taste. The results revealed that by adding *Pistacia atlantica* resin extracts, this goaty taste was covered (Uysal-Pala *et al.* 2006; Eissa *et al.* 2010). Therefore, it is also an alternative way to increase acceptability of the product, while enhancing nutritional benefits.

Total LAB numbers were steadily higher in Yoghurt 5, at least until the 25th day, and therefore, it seems that *S. boulardii* stabilise better the LAB population. Moreover, based on organoleptic assessment, there were no significant differences in flavour between different yoghurts batches. However, Yoghurts 4 and 5 were preferred by more tasters compared to Yoghurt 1. This fact indicated that yoghurts containing resin alone or combined with yeast have outscored the control one.

Saccharomyces boulardii was also enumerated, and its numbers remained steady at about 6.7 $\log_{10}$ cfu/g. Karaolis *et al.* (2013) also demonstrated that *S. boulardii* can survive in numbers during the commercial life of yoghurt. *Saccharomyces boulardii*, except for enhancing the survival of LAB, has many beneficial intestinal effects.

After evaluation of LAB numbers and organoleptic assessment and concerning to have all bioactive compounds in our product, we chose Yoghurts 4 and 5. Therefore, we continued with further analysis only in these yoghurts. Four fatty acids with an important nutritional value were identified: linoleic acid, linolenic acid, caproleic acid and CLA. It is noteworthy that all these fatty acids were presented at all spectra of yoghurts from day 0 to day 25 and, therefore, our product keeps intact all lipids during shelf life. This was a very important finding as, in a previous study conducted by Serafeimidou *et al.* (2012), in order to determine for the first time fatty acid composition in various Greek yoghurts, the analysis was conducted at the third day only, after the manufacture of the product.

Conjugated linoleic acid is predominantly found in milk and milk products. At present, there is a great interest in CLA because it appears to exhibit many health benefits related to anti-obesitic, anti-carcinogenic, anti-atherogenic, anti-diabetogenic, immunomodulatory, apoptotic and osteosynthetic effects (Benjamin and Spener, 2009). Linolenic acid (ω-3) and linoleic acid (ω-6) belong to polyunsaturated fatty acids and are essential fatty acids. In diet, it is important to use a ratio of ω-6 to ω-3 polyunsaturated fatty acids of about 1–2:1 instead of a ratio of 20–30:1 that appears in Western diets. ω-3 fatty acids have anti-inflammatory, anti-thrombotic, anti-arrhythmic, hypolipidaemic and vasodilatory properties. These beneficial effects may contribute to the prevention of many chronic diseases, such as coronary heart disease, hypertension, type 2 diabetes, renal disease, rheumatoid arthritis and inflammatory bowel diseases, as reviewed by Simopoulos (1999). Our results highlighted that the bioactive compounds of total resin extract were identified in yoghurts samples and were maintained during shelf life in Yoghurt 5. Although *Pistacia* resins are used as active ingredient and or flavouring in many food such as ice cream, yoghurt, bakery products, desserts and beverages, the stability of triterpenoid fraction of resins is unexplored since it is a difficult task. The present NMR methodology allows us to monitor their stability through NMR fingerprint. Results revealed a dramatic decrease of triterpenic aldehydes in Yoghurt 4. This fact is maybe attributed to the protonation of carbonyl group at acidic medium like yoghurt. In contrast, the presence of *S. boulardii* enhances the stability of triterpenic aldehydes in Yoghurt 5 due to its ability to metabolise organic acids shifting pH at higher values (Karaolis *et al.* 2013).

CONCLUSION

Focusing on the promotion of good health in young adults, the development of a new innovative yoghurt, with functional ingredients, was successfully accomplished. Goats' milk yoghurts fortified with *Pistacia atlantica* resin extract alone or in combination with *Saccharomyces boulardii* were produced. Dual supplementation of yoghurt promoted the growth of LAB, enhanced the stability of resin phytochemicals and improved the organoleptic properties. This food formulation exploits elements of Mediterranean flora and fauna producing an innovative functional food that offers an alternative way of disease prevention and promoting good health. Further testing is needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

The following supporting information is available for this article:

Figure S1 Chemical structures of (a) caproleic acid, (b) linoleic acid, (c) linolenic acid, (d) cis-9, trans-11 CLA, (e) oleanolic aldehyde, (f) oleanonic aldehyde, (g) masticadienonic acid, (h) isomasticadienonic acid and (i) isomasticadienolic acid.

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Health-Promoting Effects of *Pistacia* Resins: Recent Advances, Challenges, and Potential Applications in the Food Industry

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Pistacia *resins are a complex mixture comprising of different bioactive compounds, with the presence of triterpenes and essential oils being characteristic for these resins. Since ancient times,* Pistacia *resins have been used in traditional medicine of Mediterranean and Middle Eastern countries as herbal remedies. There is accumulating evidence that suggests that* Pistacia *resins may contribute to the reduction of many chronic diseases, such as gastrointestinal disorders, cardiovascular diseases, and some forms of cancer, while in parallel promoting oral health and other physiological functions such as antimicrobial and antioxidant activities. This review aims at presenting and critically reviewing the health effects from the consumption of* Pistacia *resins whilst revealing future challenges and potential applications in the food industry.*

Keywords Chios mastic gum, Functional foods, Health benefits, Phytochemicals, *Pistacia lentiscus* L., Resin, Triterpenes

Introduction

Pistacia is a genus of flowering plants belonging to the Anacardiaceae family and is widely distributed in the Mediterranean basin.[1] *Pistacia* plants yield resins by making longitudinal incisions at close intervals from the base of the trunk up to the thicker branches and allowing the sap to drip onto the specially prepared ground below.[2] *Pistacia lentiscus* L. variety chia is growing almost exclusively at the southern area of Chios Island (at the eastern Aegean Sea in Greece) and produces the well-known resin called mastic gum, which is globular, with pyriform or elongated tears, 4–8 mm in diameter, pale yellow, and clear and glassy when fresh and becoming dull and brittle during keeping. Mastic gum is characterized by an aromatic odor and distinct taste.[3] Related species such as *P. atlantica, P. palaestina*, and *P. terebinthus*, native to Asia and the Mediterranean, can also produce a resin similar to mastic.[4]

Pistacia resins have been extensively used in Mediterranean and Middle Eastern countries, both as a dietary supplement and as an herbal remedy, since ancient times. Mastic gum is the most cited *Pistacia* resin in traditional medicine. Ancient Greek physicians as Hippocrates, Theophrastus, Dioscorides, and Galenus recommended its use

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for the treatment of various gastrointestinal malfunctions.[5] According to Dioscorides, *P. terebinthus* resin has also been used as antidotal, aphrodisiac, stimulant, and diuretic and is suitable to treat leprosy.[6] Iatrosophikon, a collection of prescriptions from a monastery in Cyprus written down during the island's Ottoman period (1571–1878), also described the use of *Pistacia* resins as an analgesic against heart, belly, and headaches, to treat problems with ears, eyes, teeth, and bad breath, and to heal diseases related to skin, gastrointestinal tract, and rheumatics. The health benefits of *Pistacia* resins were exploited with different modes of applications, such as oral, external topical application, and fume.[7,8] The health-promoting effects and their phytochemical composition of *Pistacia* resins have been explored thoroughly in order to correlate individual phytochemicals with possible health claims.[7,9]

This review aims at presenting the reported beneficial effects of *Pistacia* resins from scientific literature. In an attempt to review critically the health-promoting effects of *Pistacia* resins, first the bioactive composition is presented, followed by specific effects in human health. Important parameters such as antimicrobial activity, oral health effects, gastrointestinal and cardiovascular disorders, and cancer are reviewed separately, focusing on the influences of *Pistacia* resins onto each separate pathological condition. Finally, the last section of the review is devoted to the possible future potential applications in the food industry towards the development and production of novel and/or functional foods using *Pistacia* resins and exploits opportunities and serious considerations that must be taken into account prior to any effort to develop novel functional products.

Bioactive Composition

Pistacia resins are a complex mixture of different bioactive groups, with the presence of triterpenes and essential oils being characteristic for these resins, followed by phenolics. The bioactive compounds are classified in Figure 1 based on their chemical structures.

Essential oils constitute the most studied group of *Pistacia* species components, and significant qualitative and quantitative differences were found in terms of chemical composition. Essential oils can be divided into two groups of chemical constituents, the terpene hydrocarbons (monoterpenes and sequiterpenes) and the oxygenated compounds, which are mainly phenols, alcohols (monoterpene and sequiterpene alcohols), aldehydes, ketones,

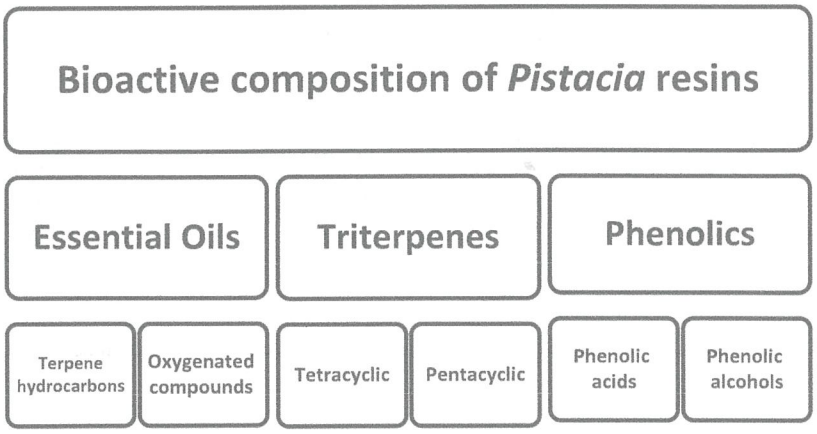

Figure 1. Main groups of bioactive compounds found in *Pistacia* resins.

esters, lactones, coumarins, ethers, and oxides. Monoterpene content of mastic gum is high, whereas α-pinene (~80%) is the predominant essential oil of the gum of *Pistacia lentiscus* var. chia. β-Pinene, camphene, myrcene, and limonene have been also detected in their essential oils. The major sequiterpene present in mastic gum is β-caryophyllene, and the major monoterpene alcohol is linalool.[7,9,10]

Several health claims have been associated with the presence of penta- or tetracyclic triterpenes from the oleana(e)ne, dammarane, lupa(e)ne, and tirucalla(e)ne skeletons.[11] A comprehensive gas chromatography–mass spectrometry (GC-MS) analysis of resin reported the identification of 36 triterpenes.[12] The main triterpenes in resins were in the following order: isomasticadienonic acid, masticadienonic acid, and 28-norolean-17-en-3-one. In particular, the acidic fraction included the major triterpenic acids: masticadienonic acid, masticadienolic acid, morolic acid, oleanonic acid, and ursonic acid, and their derivatives have been detected in acidic fractions of *P. lentiscus, P. terebinthus*, and *P. atlantica resins*. Triterpenoid compounds have been also isolated from neutral fraction of *P. lentiscus* and *P. terebinthus* resins, such as tirucallol, dammaradienone, β-amyrin, lupeol, oleanolic aldehyde, and 28-norolean-12-en-3-one.[5]

Contrary to fruit and leaves, *Pistacia* resins are poor in phenolic compounds. Phenolic acids are the main phenolic compounds in the resin. In particular, *p*-hydroxyphenylacetic, vanillic, gallic, and *trans*-cinnamic acids are detected in mastic gum. Regarding phenolic alcohols, tyrosol has been reported as the major alcohol in mastic gum.[13]

"Omics" Approaches Meet *Pistacia* Resins

Pistacia resins are complex mixtures of bioactive compounds in which each component contributes to their overall bioactivity. Food processing and storage conditions can dramatically affect food composition and bioactivity. Metabolomics approach is extremely useful for monitoring the stability of individual bioactive compounds during processing and storage. These novel data will allow designing new processing procedures, ensuring high quality of foods in terms of bioactivity as foodomics approach requires.[14]

Phytochemomics approach is also an emerging working field to understand the health-promoting properties of *Pistacia* resins. Phytochemomics concept was recently introduced and aimed to increase the knowledge on phytochemicals bioactivity and their impact in health, aging, and diseases, which is of growing importance in food, medicine, and cosmetic sciences.[15] This "omics" approach will be expected to authorize or reject nutrition and health claims made on *Pistacia* resins.

Pistacia Resins and Human Health

Numerous studies have revealed several physiological responses to *Pistacia* resins that may be relevant to the promotion of health and the prevention or treatment of some chronic diseases. Figure 2 summarizes the health claims that are correlated with *Pistacia* resins.

Antimicrobial Effects

Many studies reported that the mastic gum exhibits antimicrobial activity. A strong antimicrobial effect of mastic gum oil has been found against *Staphylococcus aureus, Lactobacillus plantarum, Pseudomonas fragi, Salmonella enteritidis, Escherichia coli, Bacillus subtilis,* and *Rhizoctania solani*.[7,16,17]

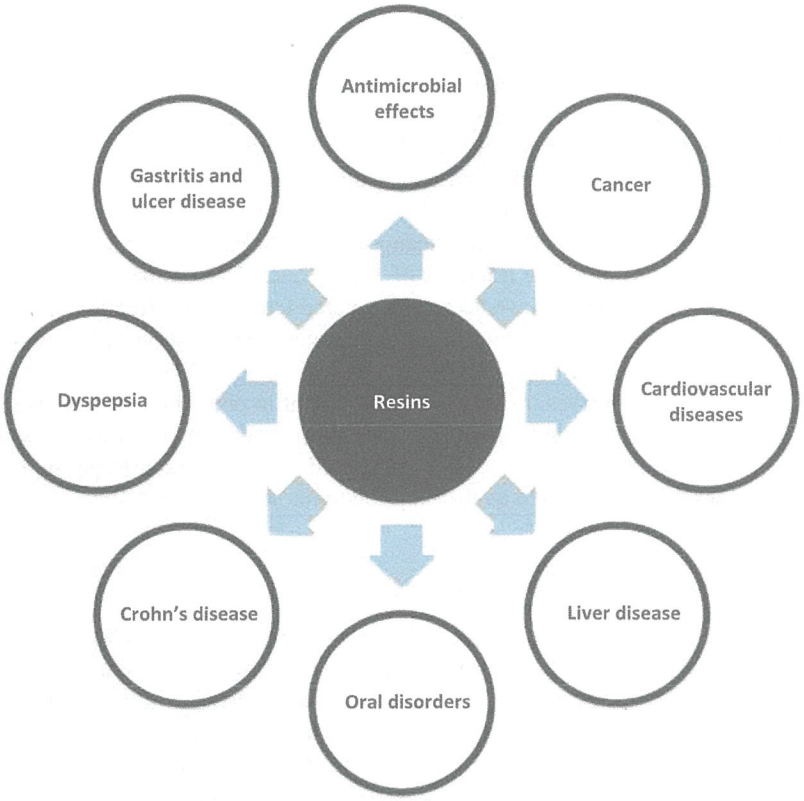

Figure 2. Diseases and disorders potentially prevented or treated by *Pistacia* resins.

In general, the inhibition was greater on gram-positive bacteria compared with gram-negative bacteria.[16] Terpenes such as verbenone, α-terpineol, and linalool contribute significantly to the antimicrobial activity of mastic gum oil. In addition, the antimicrobial potential of isolated terpenes showed that the antibacterial efficacy of mastic gum oil is attributed to its mixture of components working synergistically.[7]

Thus, mastic gum oil exhibits a significant antimicrobial activity and is promising, since no toxic effects have been reported.

Oral Disorders

Oral diseases usually including dental caries, periodontal disease, and tooth loss are a common health problem. Nutrition and microbiological infection seem to play important roles in oral health.[18] Mastic gum appears promising as a potential antibacterial against oral bacteria. Chewing mastic gum decreases the salivary concentrations of cariogenic bacteria. Chewing mastic gum reduced significantly the bacteria colonies during the 4 hours of study, whereas the mastic group showed a significant reduced plaque and gingival index compared with the placebo group.[19] Furthermore, studies reported that chewing mastic gum had significant antibacterial activity against *Streptococcus mutans* and mutans streptococci, *Porphyromonas gingivalis*, and *Prevotella melaninogenica*.[20,21] Aksoy et al.[22] confirmed these results, showing that chewing mastic gum decreased the total number of

viable bacteria, *Streptococcus mutans*, and lactobacilli in saliva in orthodondically treated patients with fixed appliances. Moreover, their findings demonstrated that mastic stimulates the survival of oral polymorphonuclear leukocytes by preventing their apoptosis and thus may contribute to the potentiation of natural immunity, such as elimination of bacteria from oral cavity. Finally, mastic may have protective role of oral malodor.[23]

Dyspepsia

Dyspepsia or indigestion is a general term that is frequently used to describe discomfort in the upper digestive tract. Symptoms may include abdominal pain, bloating, nausea, vomiting, and belching. Symptoms of prolonged dyspepsia may be related to underlying problems such as gastrointestinal reflux, gastritis, peptic ulcer disease, delayed gastric emptying, gallbladder disease, or cancer. Dyspepsia in the absence of any specific pathology is referred to as functional dyspepsia. Chios mastic gum is traditionally used in dyspepsia. Recently, a double-blind placebo-controlled trial assessed the effects of this natural remedy in patients with functional dyspepsia. It is worth noting that 77% of the patients receiving 350 mg Chios mastic gum three times daily for 3 weeks reported significant improvement of symptoms. Therefore, mastic gum appears to be effective in the treatment of functional dyspepsia.[24]

Gastritis, Ulcers, and Liver Disease

Gastritis and peptic ulceration may result when microbial, neural, or chemical abnormalities disrupt the factors that normally maintain mucosal integrity.[25] The most common cause is *Helicobacter pylori* infection. *H. pylori* is a gram-negative, microaerophilic, spiral bacterium that colonizes the stomach. Infection with *H. pylori* is associated with gastritis, peptic ulceration, gastric lymphoma, and adenocarcinoma.[26]

The in vitro antibacterial activity of mastic gum against *H. pylori* has been described.[27,28] However, other in vivo experiments showed that mastic gum has no effect on *H. pylori*.[29,30] The explanation came later when researchers prepared a total mastic extract without polymer (TMEWP). The crude resin contains an insoluble polymer that reduces the bioavailability of the bioactive mastic constituents. Administration of TMEWP over a period of 3 months in infected mice was effective in reducing *H. pylori* colonization levels by 30-fold. The anti-*H. pylori* activity of mastic gum is mainly attributed to the presence of triterpenic acids such as oleanonic acid, moronic acid, 24Z-masticadienonic acid, 24Z-isomasticadienonic acid, 24Z-masticadienolic acid, and 24Z-isomasticadienolic acid, but a synergistic effect was found.[31] Recently, Dabos and coworkers[32] confirmed that mastic gum possesses in vivo antibacterial activity against *H. pylori* and is able to eradicate it from patients.

Two early studies have shown some effect of mastic gum on gastric and duodenal ulcers. The first study was conducted on experimentally induced gastric and duodenal ulcers in rats. Mastic administration (500 mg/kg) produced a significant reduction in the intensity of gastric mucosal damage and a significant decrease of free acidity.[33] A double-blind clinical trial carried out on patients with symptomatic and endoscopically proven duodenal ulcer. The results showed increased symptomatic relief in patients receiving 1 g mastic daily over a period of 2 weeks, compared with placebo group, whereas endoscopically proven healing occurred in 70% of the patients on mastic.[34]

Mastic gum has been reported to have hepatoprotective effect. Chios mastic powder in dose of 5 g daily for an 18-month follow-up period resulted in a decrease in serum

glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and γ-glutamyltransferase (γ-GT) levels in human population. Thus, Chios mastic powder could have a hepatoprotective role in vivo in humans.[35]

Crohn's Disease

Crohn's disease (CD) is a chronic granulomatous inflammatory disease of unknown etiology involving any part of gastrointestinal tract from mouth to anus.[36] The gastrointestinal tract is a major immune organ in that it is lined with large numbers of immune cells, such as macrophages and T lympocytes, that are capable of triggering nonspecific and specific immune responses that result in the release of potent proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), eicosanoids, and destructive oxygen radicals.[25]

Kaliora et al.[37] examined the effectiveness of mastic supplementation on the clinical course and plasma inflammatory mediators of patients with active CD. Despite the relatively small sample size, they achieved statistically significant results. Four-week mastic administration (6 mastic caps/day, 0.37 g/capsule) improved the clinical features of the disease and regulated the inflammation and antioxidant status in CD patients. It significantly decreased the activity index (an index that evaluates the severity of disease) and the plasma levels of IL-6 and C-reactive protein (CRP), whereas it increased plasma total antioxidant potential (TAP). Also, nutritional risk index (NRI), one of the clinically useful measures of nutritional status in CD, which is calculated based on serum albumin levels and body weight, was improved. Moreover, in the same subjects another study was conducted. This time the effectiveness of mastic administration on cytokine production of circulating mononuclear cells of patients with active Crohn's disease was assessed. The results showed that mastic act as an immunomodulator on peripheral blood mononuclear cells, acting as a TNF-α inhibitor and as a macrophage migration inhibitory factor (MIF) stimulator. Therefore, mastic gum might serve well in the regulation of immunity in CD patients.[38]

Cardiovascular Diseases

It is well known that cardiovascular diseases due to atherosclerosis are one of the main causes of death worldwide. They include heart attack, stroke, and gangrene of the extremities. Oxidative modification of low-density lipoprotein (LDL) by free radicals and other factors is an important determinant in the development of atherosclerosis. Oxidative modification accelerates the uptake of LDL by macrophages, which is the beginning of formation of foam cells and eventually of fatty streaks. Mastic gum is believed to offer cardiovascular protection.

Chios mastic gum consumption led to a significant reduction of total cholesterol, LDL, total cholesterol/high-density lipoprotein (HDL) ratio, lipoprotein (a), and apolipoprotein B in human subjects.[35] Moreover, mastic gum may affect the cardiovascular function through mechanisms of action related to LDL-cholesterol oxidation. Andrikopoulos et al.[6] demonstrated that Chios mastic gum inhibits LDL oxidation in vitro. It has also been shown that the saliva secreted during chewing of mastic gum exhibits protective effect against LDL oxidation.[39] The anti-inflammatory effect of Chios mastic gum on endothelium has been also investigated.[40] Mastic extract (25–200 μg/mL) inhibited the expression of endothelial adhesion molecules (vascular cell adhesion molecule [VCAM]-1 and intercellular adhesion molecule [ICAM]-1). Adhesion of monocytes to endothelium is a crucial step

in the pathogenesis of atherosclerosis where this procedure requires the expression of adhesion molecules. Thereafter, monocytes are converted into macrophages. In another study, mastic gum down-regulated the expression of CD36, which it is a scavenger receptor of oxidized LDL. Since macrophages attract oxidized LDL to scavenger receptors and through endocytosis they convert into foam cells, this study shows that mastic exert antiatherogenic effect.[41]

In summary, Chios mastic gum has been associated with cardiovascular protection because of its hypocholesterolemic, antioxidant, and anti-inflammatory effects.

Cancer

The role of mastic gum in protection against cancer has been supported by ample evidence from studies in cell culture and animal models. Mastic gum constituents that appear to be mainly responsible for its anticancer potential are triterpenoids.[5] Studies regarding to *Pistacia* resin showed their effect on different types of cancer. Chios mastic gum may exert anticancer activity in several types of cancer, including leukemia and prostate, lung, and colon cancers.

Balan et al.[42] indicated that the hexane extract of Chios mastic gum can "kill" human colon cancer HCT116 cells in vitro by a mechanism that includes several features of apoptosis. Hydroalcoholic extract (1:1, water-ethanol) of Chios mastic gum also inhibited in vitro proliferation and induced death of HCT116 human colon cancer cells.

Treatment with the ethanolic extract induced G1 arrest of the cells, detachment of the cells from the substrate, and activation of procaspase-8, -9, and -3 and further caused morphological changes typical of apoptosis in cell organelles.[43] The previous studies were extended to investigate the in vivo anticancer activity of the hexane extract of mastic gum against human colon tumor, by the use of a colon cancer/immunodeficient mouse model. The hexane extract of mastic gum administered at a dose of 200 mg/kg daily for 4 consecutive days (followed by 3 days without treatment) suppressed tumor growth about 35% without any side effects after 35 days. Thus, the hexane extract of mastic gum demonstrated a capability to delay the growth of colorectal tumors developed from HCT116 cells xenografted into severe combined immunodeficiency (SCID) mice.[44]

Mastic oil was also demonstrated to inhibit cell proliferation and survival of human leukemia K562 cells and decrease the release of vascular endothelial growth factor (VEGF) from K562 and B16 mouse melanoma cells. Moreover, mastic oil caused inhibition of endothelial cell proliferation without affecting cell survival and a significant decrease of microvessel formation both in vitro and in vivo. Investigation of mechanisms of chemopreventive effects reported that in K562 cells, mastic oil reduced the activation of extracellular signal-regulated kinases 1/2 (Erk1/2), known to control leukemia cell proliferation, survival, and VEGF secretion. Mastic oil also reduced the activation of RhoA, an essential regulator of neovessel organization in endothelial cells. These data suggest that mastic oil is a tumor and angiogenesis inhibitor, targeting components of critical signaling cascades in both leukemia and endothelial cells.[45]

In addition to the above findings, there is increased evidence showing that mastic gum possesses antiprostate cancer properties. He et al.[46] reported that mastic gum inhibits the expression and function of the androgen receptor (AR) in prostate cancer cells. It seems that androgens and therefore androgen receptor play an important role in the development of prostate cancer. These authors indicated that mastic gum constituents inhibited the expression of androgen receptor at the transcriptional level, resulting in the down-regulation of both androgen receptor mRNA and protein levels. The suppression of the androgen receptor

function was verified by the reduced expression of all three androgen-regulated genes, namely NKX3.1, hK2, and prostate-specific antigen. Another study by the same research group examined the effect of mastic gum on androgen-independent prostate cancer. They used prostate cancer PC-3 cells where AR expression is absent. Mastic gum inhibited PC-3 cells growth and blocked cell cycle in the G1 phase by suppressing nuclear factor kappa B (NF-κB) activity and NF-kB signal pathway. Therefore, mastic gum inhibits prostate cancer development in both androgen-dependent and androgen-independent prostate cancer cells.[47]

Finally, He et al.[48] also indicated that mastic gum increases maspin expression in prostate cancer cells. Maspin is a tumor-suppressing gene for prostate cancer. Mastic gum at a dose of 8 μg/mL increased maspin expression about 1.5-fold. It was further demonstrated that maspin mRNA and protein expression was significantly increased by mastic gum. Thus, the authors concluded that the increased effects of mastic gum on maspin expression mainly occur at the transcriptional level.

Mastic oil is also effective chemopreventive agent to lung cancer. Magkouta et al.[49] examined the effects of mastic oil on the experimental growth of an aggressive lung cancer type, Lewis lung carcinoma (LLC). Treatment of immunocompetent mice bearing LLC tumors with mastic oil (45 mg/kg body weight, intraperitoneally, 3 times/week for 3 weeks) significantly inhibited tumor growth without toxicity. This effect was associated with increased apoptosis, reduced neovascularization, and inhibition of chemokine expression. Recently, a novel study also addressed the antimetastatic actions of mastic oil. The authors indicated that treatment of LLC cells with mastic oil significantly limited tumor cell invasiveness and migration capabilities in transwell assays, reduced the levels of secreted matrix metalloproteinase 2 (MMP-2), restricted phorbol ester–induced actin remodeling, and limited the length of neovessel networks in tumor microenvironment.[50]

Combination of gemcitabine, an antitumor agent used for solid tumors such as ovarian, non-small cell lung, and pancreatic cancers, and mastic gum was also supported to be an effective strategy for pancreatic cancer. Huang et al.[51] found that gemcitabine combined with mastic gum causes potent growth inhibition and apoptosis of pancreatic cancer cells. BxPC-3 and COLO 357 cells were treated with grated concentrations of gemcitabine (0.01–100 μg/mL) or mastic gum (10–50 μg/mL) for 72 hours. Overall, 10 μg/mL of gencitabine or 40 μg/mL mastic gum produced maximal growth inhibition of 55.55% and 49.75%, respectively, in BxPC-3 cells. After co-treatment with these two agents, cell proliferation was inhibited to a much greater extent that with either agent alone. Treatment of COLO 357 cells was also effective. Regarding apoptosis, the results were similar, showing that compared with single-agent treatment, the combination resulted in apoptosis in a much higher percentage. Remarkably, when cells were treated with gemcitabine in combination with mastic gum, the inhibitor of NF-κB (IκBa) level was increased, whereas NF-κB activation was blocked. Moreover, the expression of Bax protein was substantially increased, and Bcl-2 protein was down-regulated.

Future Potential Uses of *Pistacia* Resins in the Food Industry

The characteristic taste in combination with health-promoting properties of *Pistacia* resins has attracted the interest of food industries. *Pistacia* resins are the main ingredient or used as additives in several foods. In particular, the resin and its by-products are exploited in a great variety of products, such as bakery, traditional and gourmet sweets, snacks, chewing gums, liquor, flavored wines, and filter coffees.

Pistacia lentiscus resin has potent antioxidative effect on oil substrates (lard, corn oil, olive oil, and sunflower oil)[52] and strong antimicrobial effect against foodborne pathogens and spoilage bacteria belonging to *Pseudomonas, Salmonella*, and *Escherichia* genera. Therefore, these resins and/or their extracts could be used as a natural preservative for the maintenance or extension of a product's shelf life, as many chemical preservatives are used by food manufacturers. Nowadays, the food industry is interested in natural, safe, and low-cost antioxidant and antimicrobial agents in an attempt to replace synthetic additives, because of (i) the concerns regarding the safety of the chronic consumption of synthetic compounds traditionally used as preservatives in foods and beverages and (ii) the public's conviction that natural antioxidants are safer than their synthetic analogues.[53] *Pistacia* resins also can be used as active ingredients in edible coatings and active packaging.

Pistacia resins can be used to produce innovative functional foods, by fortifying certain widely consumed food products, such as breakfast cereals, nutrition bars, and spreads, designed to promote beneficial physiological effects (e.g., reduce cholesterol, oral health, control *H. pylori*).

Mastic water, a commercial flavoring obtained during the steam distillation of mastic resin for the production of mastic oil, also has potential applications in the fields of functional foods. This by-product has antimicrobial properties and is less expensive than mastic oil.[54] Any attempt to fortify foods with functional *Pistacia* resin must take into account several factors that can affect organoleptic properties, stability, and interactions with the food matrix. In addition, the added amount of the resin added into the food and the stage that this will be added are of critical importance. New products must be products that the consumer is well informed about, and their intake must not be limited due to undesirable side effects. Low fat and fat-free yogurts are a good vehicle for providing functional ingredients, as they are widely consumed. Such products can be used initially to deliver the *Pistacia* resins or derived ingredients, as the technology of manufacture is flexible and allows interventions. The resins can be added following starter culture coagulation to ensure the absence of undesirable effects over the manufacturing process. The stability of the resin and its interactions with the product must be explored and thoroughly investigated in order to ensure functionality of the product throughout its commercial life period.

Conclusions

Clearly the studies reveal the beneficial effects of *Pistacia* resins in several pathological conditions as well as in foods as additive (preservative). There is accumulating evidence that needs to act as leverage to the food industry towards the development of novel food product formulations that could potentially be used as nutraceuticals, offering alternative ways of disease prevention, symptoms moderation, and eventually treatment of several disorders. The increasing evidence from research is very promising and can lead to the production of functional foods, which could be the future applications of *Pistacia* resins towards the development of novel nutraceuticals.

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Research Article

Adherence to the Mediterranean Diet and Lifestyle Characteristics of University Students in Cyprus: A Cross-Sectional Survey

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Objective. To assess dietary-related habits among young adults. *Design and Setting.* Dietary habits were assessed cross-sectionally, using a self-completed questionnaire in 193 students enrolled in public and private universities in Cyprus. Adherence to the Mediterranean diet was evaluated using the validated KIDMED index. BMI was estimated based on weight and height measurements. *Results.* The mean BMI was 23.31 (±3.98). The mean adherence score to the Mediterranean diet was 6.0 (IQR 4 to 8), with 26.9% of students being classified as high adherers and 21.8% as low adherers to the Mediterranean diet. About 32% of students consumed a second serving of fruit and vegetables more than once a day, whereas 26% reported going more than once a week to a fast-food restaurant and 31% consumed sweets and candy several times a day. On the other hand, 76% of participants reported consumption of at least two dairy products daily and 88% use olive oil at home. The majority consume coffee 2-3 times per day. *Conclusions.* Results support a shift from traditional healthy diets to more unhealthy eating patterns. However, we also report a high dairy intake and use of olive oil. Tailored-made strategies targeting the young adult population could be warranted.

1. Introduction

Young adulthood, defined as 18–25 years of age, is an important transitional period from adolescence to adulthood, during which long-term health behavior patterns are formed and established. This life stage is critical as many changes occur during it, such as the development of self-identity, leaving home, and increased autonomy in decision-making [1].

Young adults should have good dietary habits with adequate nutrient intakes, not only for overall good health, but also because the skeletal development continues during that period. Although approximately 90% of peak bone mass is attained by the age of 18 years, bone mass keeps growing until around age 30 [2]. However young adults tend to follow a poor diet, marked by low consumption of fruits and vegetables and high consumption of fast food and sugar-sweetened beverages [3]. This can influence not only their concurrent health but also their future risk for a number of chronic diseases at a later age. The Mediterranean diet pattern, originating from the traditional Mediterranean diet, has been consistently linked to a lower risk for a number of chronic diseases such as cardiovascular disease [4] and cancer [5].

Adherence to the Mediterranean pattern is therefore important in improving overall health, whereas young adulthood may be an important time for intervening and establishing long-term health behaviors. Given that a large proportion of young adults enroll in universities, university campuses represent a prime setting for data-driven health promotion intervention efforts.

Nonetheless, data on the diet of young adults, especially in Mediterranean countries such as Cyprus, are lacking. Therefore the aim of the study was to investigate the level of adherence to the traditional Mediterranean diet pattern overall and to specific dietary components, among Cypriot

young adults identified through universities; thus providing much needed evidence for intervention programs targeting this often overlooked age group.

2. Methods

2.1. Study Participants. A total of 193 young adults aged 18–25 years participated in the present study from October to December 2014 through a convenience sampling. During this period a study researcher (dietitian, who provided help with the questions and who measured height and weight) visited two university campuses, one public (Cyprus University of Technology, Limassol) and one private (University of Central Lancashire in Cyprus, Larnaca) asking students to self-complete an anonymous questionnaire on baseline characteristics, including the following questions: "are you currently on a diet" (yes/no), "do you currently exercise" (yes/no), "do you currently take protein supplements" (yes/no), "do you currently smoke" (yes/no), and "how often do you drink coffee, tea, wine, beer, whiskey-vodka" (4–6 times/day; 2-3 times/day; 1 time/day; 1–4 times/week; 1–3 times/month; few times/year or never). All data were collected anonymously and completion of the questionnaire was considered informed consent.

Adherence to the Mediterranean diet was evaluated by the KIDMED index (Mediterranean Diet Quality Index for children and adolescents) [6] which includes 16 questions based on the principles of the Mediterranean diet, where those denoting a positive aspect with regard to the Mediterranean diet are assigned a value of +1 and those with a negative aspect −1. A total score ≤ 3 implies a very low diet quality, a score between 4 and 7 implies a diet that needs improvement to adjust intake to Mediterranean patterns, and a score ≥ 8 indicates optimal adherence to the Mediterranean diet.

Weight and height were also measured with the use of a portable digital scale and stadiometer at the standing position without shoes by the same study researcher. Body mass index (BMI) was calculated as weight/height^2 (Kg/m^2) and used in the assessment of overweight and obesity among young adults according to the International Obesity Task Force (IOTF) age- and sex-specific BMI cutoffs [7].

2.2. Statistical Methods. Continuous variables are presented as mean ± SD, whereas categorical variables are presented as frequencies. The normality of variables distribution was tested with the Kolmogorov-Smirnov test. The chi-square test was used to evaluate associations between the categorical variables and the Student's independent t-test and ANOVA methods were applied to evaluate differences in mean values of continuous variables as appropriate. A two-sided p value of less than 0.05 was considered statistically significant. Data were analyzed using SPSS v.20.0 (SPSS Inc., Chicago, IL) and Microsoft Office Excel 2007.

3. Results

3.1. Demographic Characteristics of the Participants. Out of 193 participants, 87 (45.1%) were men and 106 (54.9%) were women with a mean age of 20.56 (±1.85) years. The mean height was 169.06 (±9.53) cm and the mean weight was 67.09 (±14.9) Kg. The majority of participants came from the districts of Larnaca and Limassol, reflecting the location of the universities sampled. Overall characteristics of the subjects and for men and women separately are presented in Table 1.

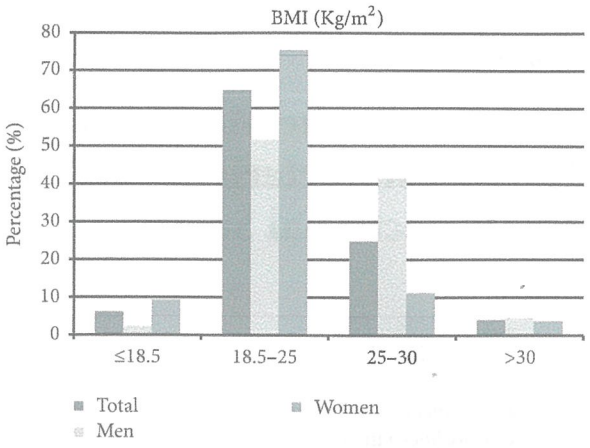

FIGURE 1: Distribution of BMI categories in all study participants and in men and women separately.

3.2. Body Mass Index. The mean body mass index (BMI) of study participants was 23.31 (±3.98) Kg/m^2 with 6.2% of them being classified as underweight, 64.8% as normal weight, 24.9% as overweight, and 4.1% as obese (Figure 1). Differences between sexes were significant for all categories with more women being in the "underweight" category (9.4%) compared to men (2.3%) ($p < 0.001$) and more men in the "overweight" category (41.4%) compared to women (11.3%) ($p < 0.001$). BMI groups for all participants and by sex are shown in Table 1.

Participants who were on a diet at the time of the study had significantly higher mean BMI values (25.54 ± 5.18 Kg/m^2) compared to those who were not (22.94 ± 3.62 Kg/m^2) ($p = 0.001$).

When looking at other dietary variables that could be associated with an individual's BMI, such as breakfast consumption, fruit and vegetable consumption, fast-food consumption, smoking, exercise, and taking protein supplements, none of them was found to be significantly associated with mean BMI and results were similar for men and women, although more men were current smokers or exercised. Data are shown in Table 2.

3.3. Adherence to the Mediterranean Diet (KIDMED Score). The median KIDMED score in the study participants was 6.00 (IQR: 4 to 8), with men having a slightly but statistically nonsignificant higher score compared to women (5.95 versus 5.46, resp., $p = 0.25$). When looking at categories of adherence in all, about half (51.3%) were in the "intermediate adherence" to the Mediterranean diet category (total score between 4 and 7), 21.8% were in the "low adherence" category (total score of ≤3), and 26.9% were in the "high adherence"

Table 1: Baseline characteristics of study participants.

Variables	All 193	Males 87 (45.1%)	Females 106 (54.9%)	p value for difference between sexes
Age (yr)	20.56 ± 1.85	21.20 ± 1.59	20.05 ± 1.89	<0.001
Height (cm)	169.06 ± 9.53	176.66 ± 6.25	162.82 ± 6.86	<0.001
Weight (Kg)	67.09 ± 14.90	78.18 ± 12.37	57.98 ± 9.75	<0.001
BMI (Kg/m^2)	23.31 ± 3.98	25.05 ± 3.68	21.89 ± 3.65	<0.001
BMI categories				
≤18.5 (Kg/m^2)	6.2%	2.3%	9.4%	
18.5–25 (Kg/m^2)	64.8%	51.7%	75.5%	
25–30 (Kg/m^2)	24.9%	41.4%	11.3%	<0.001*
>30 (Kg/m^2)	4.1%	4.6%	3.8%	
Type of education				
Public university	94 (48.7%)	44 (50.6%)	50 (47.2%)	
Private university	99 (51.3%)	43 (49.4%)	56 (52.8%)	0.638
Currently employed				
Yes	47 (24.6)	27 (31.4%)	20 (19%)	
No	144 (75.4%)	59 (68.6%)	85 (81%)	0.049
Region of main residence				0.121*
Nicosia	34 (17.6%)	20 (23%)	14 (13.2%)	
Larnaca	75 (38.9%)	34 (39.1%)	41 (38.7%)	
Limassol	65 (33.7%)	22 (25.3%)	43 (40.6%)	
Paphos	4 (2.1%)	2 (2.3%)	2 (1.9%)	
Famagusta	15 (7.8%)	9 (10.3%)	6 (5.7%)	
Currently exercising	107 (55.4%)	60 (69%)	47 (44.3%)	0.001
Currently smoking	46 (24%)	27 (31%)	19 (18.1%)	0.037
Protein supplements	24 (12.4%)	19 (21.8%)	5 (4.7%)	<0.001

Data are presented as mean ± SD and categorical variables as percentages in parentheses.
* p value for trend.

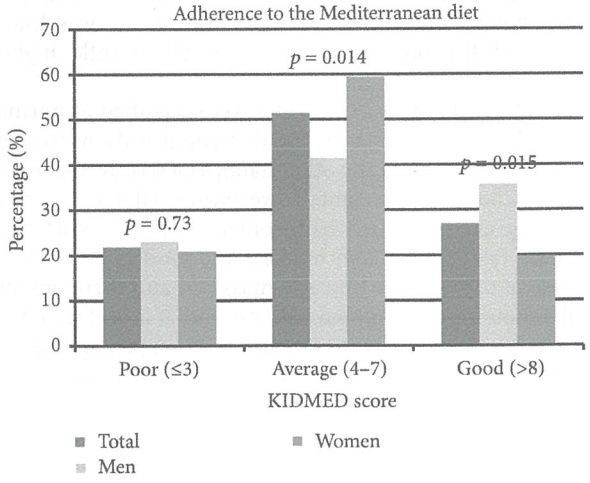

Figure 2: Distribution of KIDMED score categories in all and men and women separately.

category (total score ≥ 8) (Figure 2). Again, there were differences between men and women, with more men in this age group being in the "high adherence" category compared to women (35.6% versus 19.8%, resp., $p = 0.015$). KIDMED score categories for all and men and women separately are shown graphically in Figure 2.

In addition to overall adherence to the Mediterranean diet pattern, specific dietary categories included in the KIDMED index and their possible differences between men and women were also studied. With regard to the main nutritional categories of the Mediterranean diet, 73.1% of all study participants consumed a fruit/fruit juice daily, 56.5% consumed fresh or cooked vegetables daily, and 47.7% consumed pulses >1 per week. Additionally, 76.2% ate two yogurts and/or 40 g cheese daily, with 88.1% consuming a dairy product for breakfast, whereas 87.6% use olive oil at home. As perhaps expected in university students, ~70.0% ate pasta or rice almost daily (≥5 times per week), ~40% skipped breakfast, and 26% ate at a fast-food restaurants >1 per week. Consumption of the abovementioned categories did not differ between men and women. Statistically significant differences in consumption between men and women were observed only in having a second serving of fruit daily ($p = 0.020$) and regular nut consumption (at least 2-3 per week) ($p < 0.000$) with higher values in men for both categories,

Table 2: Association between dietary/personal habits and mean BMI in all study participants.

Question asked	BMI (mean ± SD)	p^*
Do you have a fruit or fruit juice daily?		
Yes (73.1%)	23.65 ± 3.94	
No (26.9%)	22.40 ± 3.98	0.054
Do you have a second serving of fruit daily?		
Yes (31.6%)	23.76 ± 3.07	
No (68.4%)	23.10 ± 4.33	0.292
Do you eat fresh or cooked vegetables daily?		
Yes (56.5%)	23.33 ± 3.70	
No (43.5%)	23.30 ± 4.34	0.961
Do you eat fresh or cooked vegetables > 1/day?		
Yes (29.5%)	24.14 ± 4.70	
No (70.5%)	22.97 ± 3.60	0.061
Do you skip breakfast?		
Yes (36.8%)	23.13 ± 4.40	
No (63.2%)	23.42 ± 3.73	0.626
Doyou eat > 1/week at a fast-food restaurant?		
Yes (25.9%)	23.85 ± 4.70	
No (74.1%)	23.13 ± 3.70	0.269
Do you currently smoke?		
Yes (24%)	22.69 ± 3.41	
No (76%)	23.53 ± 4.14	0.215
Do you currently exercise?		
Yes (55.4%)	23.30 ± 3.34	
No (44.6%)	23.32 ± 4.67	0.974
Are you currently on a diet?		
Yes (14.5%)	25.54 ± 5.18	
No (85.5%)	22.94 ± 3.62	0.001
Do you currently take protein supplements?		
Yes (12.4%)	23.74 ± 3.05	
No (87.6%)	23.25 ± 4.10	0.578

* p for difference in mean BMI in those who answered "Yes" versus those who answered "No" in the above questions regarding dietary/personal habits.

whereas women tended to consume sweets more often (p = 0.04). Results are shown in detail in Table 3.

When looking at other personal characteristics that could be associated with adherence to the Mediterranean diet, not smoking (p = 0.005) and currently exercising (p < 0.001) were significantly associated with a higher adherence score (KIDMED score for not smoking = 6.01 ± 2.93 versus 4.61 ± 2.82 for smoking and 6.54 ± 2.87 versus 4.62 ± 2.70 for not exercising), indicating an overall healthier lifestyle. "Currently being on a diet" was not associated with adherence (5.96 ± 2.08 versus 5.64 ± 3.08; p = 0.59).

3.4. Beverage Consumption. The majority of participants (32.5%) consume coffee 2-3 times per day, with another 23% consuming coffee once per day. With regard to tea, about half of study participants (49.2%) drink tea 1–3 times per month or even more rarely. Regarding alcohol consumption, the majority of participants (38.7%) drink wine a few times per year with another 26.7% drinking wine 1–3 times per month and 27.2% drinking wine 1 to 4 times per week; the majority (37.2%) drinks rarely beer with another 27.7% drinking beer 1–3 times per month and 26.2% drinking beer 1 to 4 times per week; about half (50.3%) of all study participants drink whiskey or vodka a few times per year or never with another 25.1% drinking these beverages 1–3 times per month.

Statistically significant differences in beverage consumption between men and women were observed for beer (p = 0.000) and whiskey-vodka consumption (p = 0.012) with higher values in men for both categories (Table 4).

4. Discussion

Since the transition from adolescence to young adulthood is a critical period during which young people adopt and establish lasting health behavior patterns it is important to examine the dietary habits of this unique age group. In the present study we investigated dietary-related habits among Cypriot young adults.

The majority of our participants (64.8%) had a normal BMI. Our results are in line with a previous study conducted in Cyprus in children and adolescents where 62.8% of subjects were also shown to have normal weight [8]. Moreover Savva et al. [9] indicated that the prevalence of obesity (8.1%) and overweight (20.1%) in children and adolescents in Cyprus has increased substantially over a decade, mainly in rural areas and in school-aged boys. Our results showed that among young adults the prevalence of obesity was lower (4.1%) and the prevalence of overweight a little higher (24.9%).

The majority of young females were classified as normal weight (75.5%). It is concerning that about 9.4% of women were underweight. On the other hand, 41.4% of young males were classified as overweight. However, given that young men of that age exercise more than women, as also supported by our data (Table 1), BMI may not be a very good index for them because of the increased muscle mass [10] and therefore our results should be interpreted with caution. It is useful to note that 12.4% of the study sample was taking supplements at the time of the survey (Table 1) and of those 21.8% were males further supporting the above.

In the present study, only 26.9% of young adults had an optimal Mediterranean diet. Previous studies conducted on children and adolescents reported similar results to ours, namely, that there is a low to moderate adherence to the principles of the Mediterranean diet [11–13]. However, we did see a rather normal distribution among the groups, with about half in the average, middle category and the other half split in the two extremes. A key finding of the study was that although median KIDMED score did not differ significantly between men and women, young women were less likely to be in the "high adherers" category compared to men (19.8% versus 35.6% for women and men resp., p = 0.015). Previous studies had suggested that women have a better dietary profile than

Table 3: KIDMED index questions in all and men and women separately. Positive answers are given as percentages (%).

KIDMED index questions	All (%)	Males (%)	Females (%)	p^*
Fruit or fruit juice daily	73.1	77	69.8	0.26
Second serving of fruit daily	31.6	40.2	24.5	0.02
Fresh or cooked vegetables daily	56.5	55.2	57.5	0.74
Fresh or cooked vegetables > 1/day	29.5	32.2	27.4	0.47
Regular fish consumption (at least 2-3/week)	28	33.3	23.6	0.13
>1/week fast-food (hamburger) restaurant	25.9	31	21.7	0.14
Pulses > 1/week	47.7	50.6	45.3	0.46
Pasta or rice almost daily (≥5/week)	69.9	67.8	71.7	0.56
Cereal or cereal product for breakfast	73.1	70.1	75.5	0.40
Regular nut consumption (at least 2-3/week)	31.1	47.1	17.9	<0.001
Use of olive oil at home	87.6	87.4	87.7	0.94
No breakfast	36.8	36.8	36.8	0.99
Dairy product for breakfast	88.1	88.5	87.7	0.87
Commercially baked goods or pastries for breakfast	30.6	34.5	27.4	0.29
Two yoghurts and/or 40 gr cheese daily	76.2	72.4	79.2	0.27
Sweet and candy several times a day	30.6	24.1	35.8	0.04

* p value for comparison between sexes from chi-square test.

men [14]. However these studies were conducted on older populations and may not be directly comparable to our study population. When looking at individual index items, women in the study ate less fruit/vegetables and nuts and more sweets, which may explain the difference in the overall score.

Overall, the daily intake of fruits and vegetables was low in our study population. Only about 30% of young adults consumed a second serving of fruit and vegetables more than once a day. Previous studies conducted on similar young populations showed even lower consumption of fruits and vegetables. A survey conducted by McLean-Meyinsse et al. [15] on 305 college students reported that only 13% of students consumed fruits and vegetables at least two times per day, with 50% of the students consuming no fruits and 52% consuming no vegetables daily. Another recent study conducted on university students reported that two-thirds of students are not eating fruits and vegetables at all daily [16]. Put together, these findings suggest that young adults eat much less than the recommended amounts of fruits and vegetables. Although the Cypriot young adults studied here are not reaching the target of five portions of mixed fruit and vegetables a day (five-a-day) as per the Department of Health and other health agencies recommendations [17], it is encouraging that we at least report a higher consumption of fruit and vegetables than in other similar, non-Mediterranean, populations.

Our results indicate that a high percentage (76.2%) of Cypriot students consume at least two dairy products daily, complying with current dietary recommendations for dairy products (2-3 servings daily) [18]. Larson et al. [19] studied the changes in calcium and dairy intake during the transition from middle adolescence to young adulthood in a five-year follow-up study. According to their results, daily mean total intakes of dairy products were reduced by approximately 0.5 servings in both genders between baseline and follow-up. Also mean daily calcium intakes of females and males decreased by an average 153 mg and 194 mg, respectively. Reports in the literature further suggest that consumption of dairy products by children and adolescents in many countries has waned in recent decades and declines further with age [20–22]. Our findings are therefore encouraging and support the hypothesis that at least some aspects of the Mediterranean diet are still pursued in Mediterranean countries such as Cyprus, including dairy and pulses, which ~50% of study participants consume more than once per week. Furthermore the fact that our young population consumes dairy products may have even more clinical implications as yoghurt consumption may be beneficial for prevention of diabetes [23].

About a third of participating college students (30.6%) consumes sweets and candy several times a day. The high intakes of sweets and candy reported here are in line with similar findings from other studies in young populations [24, 25].

Another finding from our study was that a fourth of study participants (25.9%) visit a fast-food restaurant more than once a week. Avram and Oravitan [16] report very similar frequencies (26%) among 435 students from Timisoara University in New Zealand, while Niemeier et al. [26] in a prospective study of 9919 adolescents concluded that fast-food consumption and breakfast skipping increased during transition to adulthood, and both dietary behaviors were associated with increased weight gain from adolescence to adulthood.

Our study sample demonstrated a lower than recommended consumption of fruit and vegetables but a recommended daily consumption of dairy product. This is in accordance with—and perhaps explained by—very recently published data in a large sample of college students in Canada [27], showing that students believed they needed fewer vegetables and fruit and more milk than recommended quantities. Their findings further highlight the need for simpler age- and sex-specific recommendations and targeted campaigns.

Table 4: Coffee, tea, and alcohol consumption in all and men and women separately.

	All (%)	Men (%)	Women (%)	p^*
Coffee consumption				
4–6 times/day	8.4	11.5	5.8	
2-3 times/day	32.5	32.2	32.7	
1 time/day	23	25.3	21.2	
1–4 times/week	15.2	16.1	14.4	
1–3 times/month	7.3	9.2	5.8	
Few times/year or never	13.6	5.7	20.2	0.067
Tea consumption				
4–6 times/day	2.6	3.4	1.9	
2-3 times/day	7.9	8	7.7	
1 time/day	17.3	14.9	19.2	
1–4 times/week	23	29.9	17.3	
1–3 times/month	20.9	20.7	21.2	
Few times/year or never	28.3	23	32.7	0.329
Wine consumption				
4–6 times/day	1	1.1	1	
2-3 times/day	0.5	0	1	
1 time/day	5.8	5.7	5.8	
1–4 times/week	27.2	25.3	28.8	
1–3 times/month	26.7	35.6	19.2	
Few times/year or never	38.7	32.2	44.2	0.178
Beer consumption				
4–6 times/day	2.1	4.6	0	
2-3 times/day	0.5	1.1	0	
1 time/day	6.3	11.5	1.9	
1–4 times/week	26.2	39.1	15.4	
1–3 times/month	27.7	24.1	30.8	
Few times/year or never	37.2	19.5	51.9	<0.001
Whiskey-vodka consumption				
4–6 times/day	1	2.3	0	
2-3 times/day	1	2.3	0	
1 time/day	2.6	4.6	1	
1–4 times/week	19.9	25.3	15.4	
1–3 times/month	25.1	27.6	23.1	
Few times/year or never	50.3	37.9	60.6	0.012

$^*p$ value for comparison between sexes from chi-square test.

While we report on the dietary habits and adherence to the Mediterranean diet of Cypriot young adults, it is also important to put our results in context with relevant findings from children in Cyprus, as reported from the CYKIDS study. The CYKIDS study [12] was a similar study conducted among school children (aged 9–13) in Cyprus during the school year 2004-2005, with a representative sample of school-aged children. Our study was conducted among young adults (aged 18–25) in 2014, making it very likely that the study sample described here comes from at least the same generation, after a time-span of 10 years. Although the sample of the CYKIDS study was representative of the total school-aged children's population, and our study was based on convenience sampling and therefore may not be representative of the total population of college students, we feel that comparisons are perhaps justified with regard to possible changes in dietary habits, prevalence of overweight and obesity, and adherence to the Mediterranean diet during the transition from childhood to young adulthood, especially as adherence to the Mediterranean diet was assessed using the same tool, the KIDMED index.

At childhood only 6.7% of the participants in the CYKIDS study were classified as high adherers to the Mediterranean diet, whereas 37% had a poor KIDMED index [28]. We report a lower prevalence of low adherence in adulthood (21.8%) and a much higher prevalence of high adherence to the Mediterranean diet (26.0% versus 6.7%) compared to the CYKIDS population [28]. Other differences between reports in the CYKIDS study and ours include a decrease in having breakfast and in the consumption of fruit and vegetables. Although no direct inferences can be made, given that our population is not necessarily representative of the Cypriot young adult population, it would be reasonable to assume that young college students may indeed skip breakfast more often now than as kids or eat less fruit and vegetables. Importantly though, we also show a relatively high daily consumption of dairy products in this young adult population as was also shown for the CYKIDS population. This finding may have further implications, as it may represent one food group that maintains a high consumption pattern through the transition from childhood to adulthood in Mediterranean populations. A recent report has highlighted the role of low-fat fermented dairy intake, and especially yogurt which had a high reported consumption in our population, with a reduction in the risk of developing type 2 diabetes, further implicating specific food groups in public health interventions [23].

Our study sample demonstrated a moderate coffee consumption (2-3 times per day) overall, with no big differences in consumption between genders (0.07), with more women though being in the lowest category (few times/year/never). Others have also reported a frequent consumption of coffee among young people with some gender differences [29, 30]. A review regarding caffeine consumption concluded that, for the healthy adult population, moderate daily caffeine intake at a dose level up to 400 mg/day is not associated with adverse effects [31]. A standard 8 oz (240 mL) cup of coffee is thought to have an average of 100 mg of caffeine [32]. However coffee consumption may affect diet quality in female college student. The average intakes of dietary fiber, vitamin A, beta-carotene, and folate in the noncoffee group have been reported to be significantly higher than those in the light coffee (<250 mL) and moderate coffee (≥250 mL) groups. Also the noncoffee group consumed a significantly higher amount of vegetables compared to the light coffee group [33].

With regard to alcohol consumption, we show that our study participants are occasional drinkers, in line with other studies [34, 35]. Reasons for drinking in youth include helping with their shyness, escaping their inhibitions, or as a way of being accepted by their peers. The main occasions of

alcohol consumption are reported to be participation in social events and going out with friends [34]. Moreover a recent article indicated that students of permissive parents drank more beer and this was associated with more alcohol related problems. In agreement with our results, this study suggested that young women drank significantly less beer than young men [36].

Certain limitations of the study should be taken into account, especially its cross-sectional design, which limits any causal conclusions. In addition, study participants came from a convenience sampling from two university campuses and are not necessarily representative of the Cypriot college population. Although efforts were taken to ensure participation from both a public and a private university (thus trying to include students from all socioeconomic ranges), it is possible that our sample does not capture all the variability of the Cypriot college population. Data were based on self-reports, with some questionnaires not further validated, and therefore we cannot rule out the possibility of misreporting; however such methods are commonly used with similar studies and for the assessment of the main outcome (adherence to Mediterranean diet), a previously validated and widely used index was used.

5. Conclusion

To the best of our knowledge, this is the first study reporting on the dietary habits and adherence to the Mediterranean diet of Cypriot young adults and our results support the proposed shift from traditional healthy diets to more unhealthy eating patterns in Mediterranean countries with at least one-fifth of young adults having adopted poor dietary habits. Specifically, study participants consumed low quantities of fruits and vegetables, visit fast-food restaurants often, and consume sweets and candy several times a day. On the other hand, the majority of young adults in Cyprus still consume at least two dairy products daily and use olive oil at home, while also eating pulses more than once per week. Based on these findings, tailored-made public health strategies targeting the young adult population would be warranted, focusing on interventions to increase fruit and vegetable consumption and maintain dairy consumption, thus supporting adherence to the pattern of Mediterranean diet and reducing future burden of chronic diseases.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Elena Hadjimbei carried out all data collection, analyzed the data, and drafted the paper. George Botsaris and Vassilis Gekas conceived the study and participated in the design and coordination of the study and helped draft the paper. Andrie G. Panayiotou participated in data analysis and drafted and critically revised the paper. All authors read and approved the final version of the paper.

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RESEARCH ARTICLE

Behavioral Factors Associated with Adherence to the Mediterranean Diet in Young University Students - A Cross-Sectional Study

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Abstract

Objective: To investigate healthy behaviors associated with adherence to the Mediterranean diet in young adults. Methods: Behavioral factors were assessed using a self-completed questionnaire in 193 students enrolled in one public and one private university in Cyprus. A health habit score ranging from 0 to 5 was devised based on information on: Breakfast consumption, eating fried food, eating away from home, exercise and smoking. Adherence to the Mediterranean diet was evaluated using the validated KIDMED index.

Results: Adherence to the Mediterranean diet was found to be average for the majority of young adults, with 21.8% being classified as low adherers, and 26.9% as high adherers. A higher health habit score was associated with higher adherence to the Mediterranean diet (0.614 higher average adherence (95% CI: 1.07 to 1.55) for one unit change in health habit score). About 63% of students reported consuming breakfast on a regular basis, while half consumed three or fewer meals per day. The main person responsible for preparing meals at home were the parents (63.7%). A little over half of study participants (55.4%) reported currently exercising, with only half of them feeling happy with their body weight. Tobacco use was relatively high among students (24%).

Conclusion: A higher adherence to the Mediterranean diet was associated with a healthier overall behavioral pattern, including regular breakfast consumption, exercise, positive body image, higher meal frequency and water consumption, lower fried food consumption and lower consumption of meals away from home. Enhancing such positive health behaviors is likely to have an independent and lasting effect on later adulthood behaviors and health.

Introduction

The Mediterranean diet is a dietary pattern based on the traditional diet found around the Mediterranean basin, and is considered one of the healthiest diets worldwide, with the body of evidence from both epidemiological and experimental studies continuing to grow [1-3]. It therefore represents a pattern recommended both for primary and secondary prevention of major chronic diseases [4].

Traditional Mediterranean dietary models are characterized by abundant plant foods such as fruit, vegetables, bread and cereals, pulses, nuts and seeds. Olive oil is the principal source of fat, with dairy products (especially cheese and yogurt), and fish and poultry consumed in low to moderate amounts. Eggs are consumed a few times per week and red meat is used in low amounts, while alcohol -mostly wine- is consumed in low to moderate amounts, normally with meals. An active lifestyle is an additional component of the Mediterranean diet [5].

The protective role of the Mediterranean diet against the occurrence of several diseases is well known. Focusing in just the last five years, findings from several studies suggest that the Mediterranean diet could prove beneficial in a number of diseases associated with chronic inflammation such as atherosclerosis [6], the metabolic syndrome [7], diabetes [3,8] and obesity [9]; but also cancer [10], pulmonary diseases [11] and cognition disorders [12]. It is worth noting that the Mediterranean diet consists of a holistic dietary

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approach which includes a combination of foods and nutrients and not just a single dietary component [13].

However in spite of the recognition of the health benefits of the Mediterranean diet, there has been a gradual abandonment of this dietary pattern in recent years, especially in countries traditionally associated with the Mediterranean diet and among younger people [14-18].

Given the fact that emerging adulthood (typically defined as 18-25 years of age), is an important transitional period from adolescence to adulthood, during which long-term health behavior patterns are formed, it is especially important that healthy dietary patterns are established then, thus reducing the risk for many adult onset chronic diseases [19].

Nonetheless, data on behavioral factors associated with adherence to the Mediterranean diet, especially in young adults, are lacking. Previous studies conducted on adolescents reported that lack of physical activity and high level of screen time were inversely related to adherence to the Mediterranean diet [20-22]. A relatively recent study conducted on Spanish university students indicated a positive correlation between number of meals consumed daily and diet quality [18].

The objective of this study was to investigate behavioral factors associated with adherence to the Mediterranean diet in Cypriot young adults attending college, as this is a crucial period for establishing dietary patterns.

Materials and Methods

A total of 193 young adults aged 18-25 years were recruited in the study between October 2014 and December 2014 through convenience sampling. During these months a study researcher visited two university campuses, one public (Cyprus University of Technology, Limassol) and one private (University of Central Lancashire in Cyprus, Larnaca) and asked the students attending a course to self-complete an anonymous dietary-behavioral habits questionnaire. Completion of the questionnaire was considered informed consent.

Adherence to the Mediterranean diet was evaluated by the KIDMED index (Mediterranean Diet Quality Index for children and adolescents), a validated and widely used index [23]. It includes 16 questions based on the principles of the Mediterranean diet, with those denoting a positive aspect with respect to the Mediterranean diet being assigned a value of +1, and those with a negative aspect a value of -1. A total score ≤ to 3 suggests a very low diet quality, a score between 4 and 7 suggests a diet that needs improvement with regards to the Mediterranean patterns and a score ≥ 8 reflects an optimal adherence to the Mediterranean diet.

Moreover a 'health habit' score was created based on information derived from five important behavioral characteristics that were included in the relevant questionnaire (breakfast consumption, eating fried food, eating away from home, current exercise and smoking) ranging from 0 to 5, with questions denoting a positive behavior being assigned a value of +1 and those with a negative behavior being assigned a value of 0. Specific questions used were: "Do you skip breakfast?" (yes/no); "Do you currently smoke?" (yes/no); "Do you currently exercise?" (yes/no); "How often do you consume meals away from home?" (daily, 4-6 times/week, 1-3 times/week, 1-3 times/month, a few times/year or never); "How often do you consume fried food?" (daily, 4-6 times/week, 1-3 times/week, 1-3 times/month, a few times/year or never). An additional question on body image was included in the questionnaire ("Do you feel happy with your body weight? yes/no"), to be able to look at the possible association between a positive body perception and a healthier diet as reported with the KIDMED Index.

Weight and height were also measured with the use of a portable scale and stadiometer at the standing position without shoes by the same study researcher, who was blinded to the subjects replies. Body mass index (BMI) was calculated as weight/height^2 (Kg/m^2) and used for the assessment of overweight and obesity among young adults according to the International Obesity Task Force (IOTF) age and sex-specific BMI cut offs [24].

Data Handling and Statistical Analysis

Continuous variables are presented as mean ± SD, whereas categorical variables are presented as frequencies. The normality of continuous variables was tested by the observation of curves and the Kolmogorov-Smirnov test. Differences between categorical variables were tested with the chi-square test and a t-test or ANOVA were applied to evaluate differences in mean values of continuous variables. A p value of less than 0.05 was considered statistically significant. Data were analyzed using the SPSS vs.19 statistical package and Microsoft Excel.

Results

Demographic characteristics of the participants

Study participants had a mean age of 20.56 (± 1.85) years, with 87 (45.1%) of them being male. Regarding men the mean height was 176.66 (± 6.25) cm, the mean weight was 78.18 (± 12.37) Kg and the mean BMI was 25.05 (± 3.68) Kg/m^2, while for women the corresponding values were 162.82 (± 6.86) cm, 57.98 (± 9.75) Kg and 21.89 (± 3.65) Kg/m^2 respectively and differed significantly from men ($p < 0.001$ for all). The majority of participants came from the regions of Larnaca (38.9%) and Limassol (33.7%) reflecting the location of campuses surveyed. Demographic characteristics of the study participants are presented in Table 1.

Table 1: Baseline characteristics of study participants.

Variables	All	Males	Females	P value for difference between sexes
	193	87 (45.1%)	106 (54.9%)	
Age (yr)	20.56 ± 1.85	21.20 ± 1.59	20.05 ± 1.89	< 0.001
Height (cm)	169.06 ± 9.53	176.66 ± 6.25	162.82 ± 6.86	< 0.001
Weight (Kg)	67.09 ± 14.90	78.18 ± 12.37	57.98 ± 9.75	< 0.001
BMI (Kg/m²)	23.31 ± 3.98	25.05 ± 3.68	21.89 ± 3.65	< 0.001
BMI categories				
≤ 18.5 (Kg/m²)	6.2%	2.3%	9.4%	
18.5-25 (Kg/m²)	64.8%	51.7%	75.5%	< 0.001*
25-30 (Kg/m²)	24.9%	41.4%	11.3%	
> 30 (Kg/m²)	4.1%	4.6%	3.8%	
Type of education				
Public university	94 (48.7%)	44 (50.6%)	50 (47.2%)	
Private university	99 (51.3%)	43 (49.4%)	56 (52.8%)	0.638
Currently Employed				
Yes	47 (24.6)	27 (31.4%)	20 (19%)	
No	144 (75.4%)	59 (68.6%)	85 (81%)	0.049
Region of main residence				
Nicosia	34 (17.6%)	20 (23%)	14 (13.2%)	
Larnaca	75 (38.9%)	34 (39.1%)	41 (38.7%)	
Limassol	65 (33.7%)	22 (25.3%)	43 (40.6%)	0.121*
Paphos	4 (2.1%)	2 (2.3%)	2 (1.9%)	
Famagusta	15 (7.8%)	9 (10.3%)	6 (5.7%)	

Data are presented as mean ± SD and categorical variables as percentages in parentheses.

*Shows p value for trend.

Behavioral characteristics

Breakfast consumption: The majority of participants (63.2%) reported consuming breakfast on a regular basis. However about a third of those reported eating commercially baked goods or pastries (30.6%) for breakfast, a widely used practice in Cyprus and other Mediterranean countries such as Greece and Italy and which may not represent the healthiest choice for breakfast.

Meal patterns: About half (47.2%) of young adults consumed three or fewer meals per day while the rest consume more than four meals per day. The main person responsible for preparing meals at home were the parents (63.7%), with only 29% of young adults preparing food by themselves. The majority of young adults (45.6%) consume meals away from home 1-3 times per week.

With regards to fried foods, half of study participants (50.3%) reported consuming fried food 1-3 times per week, with another 29% consuming such foods only 1-3 times per month. Most of them however use little extra salt at the table (44.6%).

As expected, given body size, men drank more water than women, with 40% of men consuming ≥ 8 glasses of water vs. 24% of women (p = 0.017).

Exercise: A little over half of study participants (55.4%) reported currently exercising, with men exercising significantly more than women (69% vs. 44.3%, p = 0.001). Noticeably, about a fifth of men surveyed (21.8%) reported taking protein supplements compared with only 4.7% of women (p < 0.001), and about 20% of study participants took vitamins/minerals supplements (p = 0.3 for men vs. women).

Smoking: Tobacco use (currently smoking) was relatively high among this group of young Cypriots (24%) and especially among males, with 31% of them reporting current smoking, vs. 18.1% of young females (p = 0.037). Among smokers, the average number of cigarettes/day was 11.39 (± 7.14).

Body image: Only half of young adults feel happy with their body weight, with males being overall happier than females (62.1% vs. 41.5%, p = 0.004). However, out of those who feel unhappy with their body weight only 23.2% were currently on a diet and 43.2% exercised. Students with a positive body image had a higher health habit score vs. students who had a negative image (2.98 and vs. 2.41, p = 0.004).

Table 2: Behavioral characteristics.

	Total (%)	Males (%)	Females (%)	p
No breakfast	36.8	36.8	36.8	0.999
Currently Exercising	55.4	69	44.3	0.001
Taking Protein Supplements	12.4	21.8	4.7	0.000
Taking Vitamins/minerals supplements	21.8	18.4	24.5	0.304
Currently Smoking	24	31	18.1	0.037
Meal preparation by:				
Self	29	29.9	28.3	0.813
Parents	63.7	63.2	64.2	
Grandparents	5.7	4.6	6.6	
Other	1.6	2.3	0.9	
Meals per day				
≤ 3	47.2	41.4	51.9	0.146
≥ 4	52.8	58.6	48.1	
Meals away from home				
Daily	9.8	8	11.3	0.949
4-6 times/week	13	13.8	12.3	
1-3 times/week	45.6	47.1	44.3	
1-3 times/month	24.4	24.1	24.5	
Few times/year or never	7.3	6.9	7.5	
Fried food consumption				
Daily	0.5	1.1	0	0.311
4-6 times/week	5.2	6.9	3.8	
1-3 times/week	50.3	55.2	40.2	
1-3 times/month	29	24.1	33	
Few times/year or never	15	12.6	17	
Extra salt at the table				
Much	16.1	14.9	17	0.716
Little	44.6	42.5	46.2	
Not at all	39.4	42.5	36.8	
Water consumption				
≤ 3 glasses	17.1	5.8	26.4	0.017
4-7	51.8	54.7	50	
> 8	30.6	39.5	23.6	

Students that consumed breakfast and exercised reported feeling happy with their body weight more often, compared to students that skipped breakfast ($p = 0.035$) and did not exercise ($p = 0.001$), while there were no statistically significant differences with regards to smoking ($p = 0.239$), eating fried food ($p = 0.531$) and eating away from home ($p = 0.221$), for both males and females.

Behavioral characteristics are presented in detail in Table 2.

Mediterranean diet: Adherence to the Mediterranean diet as assessed by KIDMED index was found to be average (4-7) for the majority (51.3%) of young adults surveyed, with 21.8% being classified as low adherers to the Mediterranean diet, and 26.9% as high. The median KIDMED score was 6.00 (IQR: 4 to 8).

Students who skip breakfast and smoke had a lower KIDMED index value compared to students who consume breakfast and do not currently smoke (3.46 vs. 6.98; $p = 0.000$ and 4.61 vs. 6.0; $p = 0.005$).

Students who feel happy with their body weight had a higher KIDMED index compared to those who didn't (6.3 vs. 5.05, $p = 0.003$).

Health habit score: Having a higher health habit score, which included combined information on breakfast consumption, eating fried food, eating away from home, exercise and smoking, was significantly associated with higher adherence to the Mediterranean diet (p for trend < 0.001), with a one unit increase in the health score being associated with a 0.614 (95% CI: 1.07 to 1.55) (38%; 95% CI: 51.7% to 60.8%) increase in the KIDMED index.

Discussion

We report an average adherence to the Mediterranean diet among university students in Cyprus, in accordance with previous studies conducted in children and adolescents [14,25]. As expected participants with a higher health habit score also had a higher adherence to the Mediterranean diet, indicating that improvements in overall health behaviors also affect diet in a holistic approach.

About one third of college students in the study did not consume breakfast on a regular basis. This is in agreement with previous reports, albeit in non-Mediterranean countries, that many young adults have the habit of skipping breakfast; with values ranging between 33% in Australian university students [26] and 57% in American young adults [27], indicating a possible relationship between a heavy study load and skipping breakfast. In another study of adolescents and young adults in Sao Paulo, those who ate breakfast were shown to have a significantly higher mean calcium, vitamin D and dairy products intake than those who did not [28].

In the present study about half of young adults reported currently exercising. A recent study conducted in Spain with a large sample size reported that whereas the majority of children (72.2%) and adolescents (56.4%) reached their recommended physical activity levels, only a 40% of young adults managed to do so. Most active were students in primary schools, with university students being the least active [29]. We report a higher percentage of college students exercising and in line with other studies [30,31], young men in our study population were more active than young women. This could be explained by cultural norms and trends in Cyprus, where joining a gym on a regular basis has increased in popularity among young adults, especially men.

About a fifth of the young adults surveyed used vitamins/minerals supplements, with one fifth of males also taking protein supplements (another popular trend among young ages in Cyprus). Lieberman, et al. [32] indicated that 42% of college students used multivitamins/multimineral and 17% protein/amino acids. College students appear more likely to use dietary supplements than the general population in order to promote general health, provide them with more energy, increase muscle strength and enhance performance [32] and this appears to be true in Cypriot students too. While the intake of vitamin supplements in recommended doses may not pose a risk, protein supplements may pose a real risk. Excess dietary protein can adversely affect bone through urinary calcium loss [33] and high protein diets can accelerate renal disease progression [34,35]. On the other hand, there is no evidence to suggest that supplements are required for optimal muscle growth or strength gain and even strength-trained athletes should consume protein consistent with general population guidelines, or 12% to 15% of energy from protein [36]. Protein supplementation in this young population deserves further attention.

Smoking prevalence among young adults and especially among young males in our study was high but in agreement with previews reports from Cypriot school children [37]. Studies suggest that more young males than females smoke cigarettes [38,39] with even further implications, as Atalay, et al. [40] have shown that nicotine dependence may be a factor that affects physical activity among young people.

With regards to body image, about half of young adults reported not being happy with their body weight, with females being unhappy at a higher percentage than males (58.5% vs. 37.9%, $p = 0.004$). Factors associated with body image included breakfast consumption and exercise.

A birth cohort study in 4100 subjects aged between 22 and 23 years in Brazil showed that the prevalence of body dissatisfaction reached 64%, with 42% of the subjects reporting feeling larger and 22% reported feeling smaller than the desired body size [41]. It would seem that body dissatisfaction is pervasive among young adults and as perhaps expected, is a particular issue for young women, with more women than men desiring a body shape slimmer than their current one [31,42]. While body dissatisfaction may start even before adolescence, longitudinal studies have shown that body dissatisfaction increases between middle and high school and increases even further during the transition to young adulthood [43], making this an important period for future perceptions and self-confidence.

Limitations of this study include the fact that only two universities were surveyed and this was a convenience sampling, therefore the study population may not be representative of the Cypriot college student population and as such our results should be extrapolated with caution. In addition, data obtained in this survey were based on a self-completed questionnaire and therefore the possibility of bias cannot be excluded. Further information on frequency of exercise could not be used as it was missing from the majority of responders (63%), thus not allowing us to further probe into the possible relationship between frequency of physical activity and diet. Finally, as in any cross-sectional study, no causal relationships can be drawn.

However, as data is lacking on the topic it is expected that our results can help shed some light on the possible relationship between positive behaviors and adherence to a healthy diet pattern and guide possible intervention promoting a holistically healthy lifestyle in youth. As young adulthood is a critical life stage with similar trends and norms among youth, some of our results could be applicable to young adults in other countries, especially Mediterranean countries that share common

cultural and diet characteristics.

Conclusion

We report an association between behavioral factors and adherence to the Mediterranean diet; specifically, a higher adherence to the Mediterranean diet was associated with regular breakfast consumption, exercise, positive body image, a higher meal frequency, higher water consumption, lower fried food consumption and lower consumption of meals away from home.

Based on these findings, tailored-made public health strategies and methods targeting the young adult population would be warranted, focusing on interventions to shape holistic health behavior patterns in order to increase adherence to the Mediterranean diet, with positive effects carried into later adulthood.

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The authors declare that they have no competing interests.

Author's Contributions

EH carried out all data collection, analyzed the data and drafted the manuscript. GB and VG conceived the study and participated in the design and coordination of the study and helped draft the manuscript. AGP participated in data analysis, drafted and critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Action and its Applications in Food Preservation

Abstract

Action, the product of Energy times Time, a very interesting physical quantity has found application in fields of Physics such as Quantomechanics, the Planck constant having dimensions of Action. Well known is also the Principle of the Least Action followed by several phenomena in Physics and Chemistry. However, in other fields action has not found the attention, such an important concept deserves. In this paper, the concept of thermal action is coined in a suitable way so that applications are enabled in the field of Thermal Treatment of Foods. The obtained isodrastic curves following the analysis, describe the death kinetics of pathogenic bacteria with a potential application also in the field of the survival of probiotic bacteria in functional food products.

Keywords: Action; Death kinetics; Thermal action; Isodrastic curves

Short Communication

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Introduction

Food preservation techniques that are applied in food technology aim generally to reduce or prevent the growth of microorganisms in food [1-4]. Pathogenic and spoilage bacteria, yeasts and molds can be destroyed or inactivated by the application of different preservation methods thermal or non-thermal [5-7]. Thermal methods involve the heat treatment of foods applying different time/temperature combinations for different foods depending also on the targeted organisms. Some microorganisms are more heat resistant than others therefore require higher heat treatments to be inactivated. Non-thermal preservation techniques that can be applied include High Pressure Processing [8]. Nowadays the food industry is constantly seeking ways to improve production efficiency and efficacy. Most of the companies in Europe and the USA follow accredited safety management systems monitoring and recording constantly time and temperatures. From heat treatment to freezing and cold storage, time and temperature are of critical importance not only to the safety of the final product but also to the quality. Consequently, it is clear that time and temperature and therefore Energy are of major importance to the food industry. In this report we propose the adaptation of a new term "Action" towards a more theoretical and fundamental approach in a field where the empiricism dominates until now. Action in science allows the use of analogies therefore having a broader applicability in a lot of nearby fields.

Action in Physics

Energy and Time play a significant role in Food Technology in general, particularly in Food Preservation, being the two main factors affecting the process (and/or unit operation), directly and indirectly, and the quality of the food product of the process indirectly. To name but a few, typical examples are the Death kinetics of the microorganisms [9-10], the relationship between freezing temperature and shelf life [11] etc. The product Energy times Time, called Action, S, is one of the most important physical quantities and has initially been defined [12] in the branch of Mechanics related with the motion of bodies, that is Dynamics. Action is defined as an integral of kinetic energy, KE, minus dynamic energy, DE, over time, from t1 to t2, as shown in equation (1).

$$S = \int \left(KE - DE \right) dt \quad (1)$$

Where, S is the action; KE is the Kinetic energy; DE is the Dynamic energy; dt is the time interval (t1-t2). Given a motion between two points, the path followed, tends to the minimization of S, leading to the famous Principle of Least Action , formulated as early as in 1756 [13]. Later on, the Planck constant, h, marking the appearance of a new evolutionary branch of Physics, namely Quantum mechanics, has been shown to have dimensions of action, whereas the dimensions of the reduced Planck constant, h bar are of angular momentum [14], as shown in equation (2).

$$\hbar = \frac{h}{2\pi} \quad \text{.....equation (2)}$$

Where h is the Planck constant; h is the reduced Planck constant.

Although Maupertois [13] has meant a pretty general applicability of its Principle, the applications of that interesting and useful quantity, with a few exceptions in Chemistry [15] and in Neuroscience [16], have never been extended to other practical fields, such as for example Food preservation which is the topic of the present paper. The classical approach followed in death kinetics is a totally empirical based in an array of concepts such as D, Z, F, L etc. This approach has been criticized by Paul Gibbs and one of us [10], proposing the water activity as a more fundamental property to describe the said above kinetics. Furthermore in the present paper a second step has been taken towards fundamental modeling that is the introduction of the concept of thermal action. Future work can combine the two important fundamental concepts, given that water activity is closely connected to the chemical potential which is also closely

connected to Energy. Thus the water activity becomes the link between the classical empirical approach and the Action approach. The objective of the present paper is to explore the potential of application of the important physical quantity of Action, adapted for use in Food Microbiology and Food Preservation in general.

Material and Methods

Definition of thermal action

The rationale for the definition of the quantity Thermal Action, is based on the fact that Time, t, and temperature, Θ are main factors influencing many phenomena in Food Technology and Preservation, upon thermal treatment. Temperature is dimensionally the concentration of heat that is of the thermal energy, under constant pressure, according to the dimensional analysis equation (3)

$$[Q] = [\rho.C\rho].[\Theta] \quad \text{..... equation (3)}$$

Where Q: being the heat; ρ: the product of the density; Θ: Temperature; Cp: is the specific heat capacity

Since ρ. Cp remains a constant during many processes, temperature instead of heat could be used in the definition of Thermal Action. There is an overwhelming evidence that the effect of the temperature on the result of the thermal treatment is linear, whereas the effect of time is not linear but a logarithmic one (the logarithm with the base of ten is traditionally used). Therefore we coin the concept of Thermal Action SΘ as shown in equation (4).

$$S\Theta = \Theta.\log t \quad \text{.....equation (4)}$$

In a plot of t versus Θ the obtained curves are, thus, isodrastic curves (from Δράσις, pronounced drasis, and the Greek word for Action). Furthermore in a plot of logt versus Θ the obtained isodrastic curves become parallel straight lines. Examples are given right below in the results and discussion section.

Results and Discussion

Application in death kinetics

Death kinetics is important to the Food scientist/ technologist in a two-fold purpose. There are pathogenic or spoilage microorganisms, the population of which should be kept in a minimum, and on the other hand there are beneficial microorganisms the population of which should be protected. The study of their kinetic inactivation is useful in both cases. Clostridium botulinum is a pathogenic organism belonging to the first category mentioned above. In Figure 1 the isodrastic curves are shown based on raw data taken from literature [9]. Each point in one of the curves is a pair of temperature and time values which were applied in the thermal treatment of the studied microorganism and yields the same degree of inactivation m. For example, m=12 shows the exponential degree of reduction of the microorganism's population. If we start with an initial population of the microorganism in concern of 1012, only one individual microorganism will be left active. If instead of time, the decadic logarithm of time is in the Y-axis, the family of the isodrastic curves become parallel straight lines. Also, this result is shown in Figure 2, where the isodrastic lines concern degrees of inactivation m equal to 12, 9 and 6 respectively.

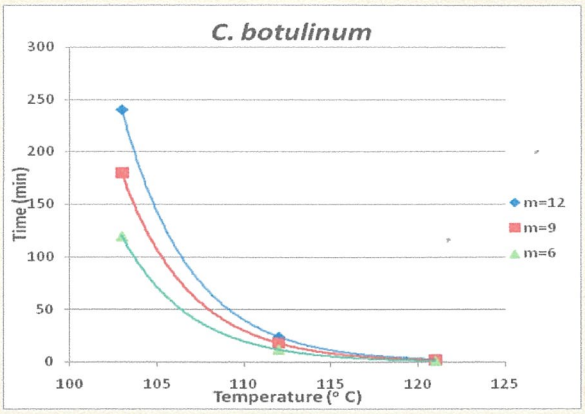

Figure 1: Isodrastic curves of Clostridium botulinum in a diagram Time-Temperature for m=6, 9 and 12 respectively.

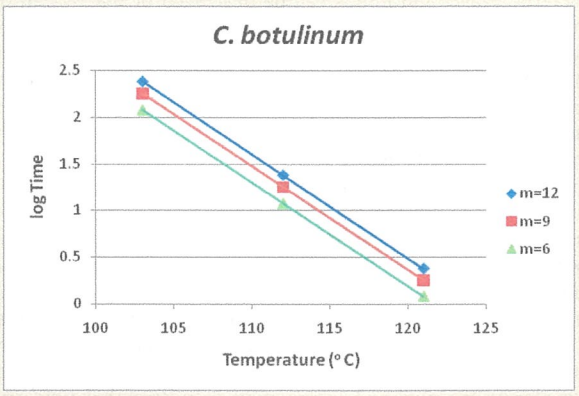

Figure 2: Isodrastic curves of Clostridium botulinum in a diagram log (time)-temperature for m=6, 9 and 12 respectively.

Analogous diagrams are shown for two more species of microorganisms, namely Bacillus stearothermophilus for low acidity foods of pH >4.5, Figure 3 and Figure 4 and Bacillus coagulans for acidic foods of pH <4.5, Figure 5 and Figure 6. The isodrastic curves are thus the loci of the points which are pairs of temperature and time values for which equivalent results are obtained in the reduction of the pathogenic microorganism. The chosen microorganisms of concern in this study are three bacteria that can cause serious effects on foods. Clostridium botulinum is a pathogenic microorganism that can cause foodborne botulism. Botulinum toxin can cause a severe flaccid paralytic disease in both humans and animals and therefore its absence from food must be verified. As a spore-forming bacterium it can survive conventional pasteurization heat treatments. To inactivate the spores pressurized heating is used to achieve temperatures above 100oC [17]. Bacillus coagulansis a lactic acid-forming bacterial species that can cause coagulation in evaporated milk [18]. Finally, Bacillus stearothermophilusis a

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thermophile and is widely distributed in soil, hot springs, ocean sediment, and is a cause of spoilage in food products [19]. Apart from the application in death kinetics of pathogenic and spoilage microorganisms thermal action can also be applied when investigating the survival of probiotic bacteria in functional food products. Probiotic yogurts are products widely consumed nowadays in developed countries since people are getting more health conscious. The monitoring and survival of probiotics in yogurts has been well investigated over the past years. Several reports have been published showing that the initial population at start does not remain the same following storage at low temperatures [20,21]. Using action we potentially aim at modeling the survival and death of the probiotic bacteria in an attempt to be in position to predict not only the conventional shelf life of the product, but most importantly its functional shelf life. The functional shelf life would be the period of time during which the numbers of the probiotic in concern will be sufficient enough to have an impact on the health of the consumer.

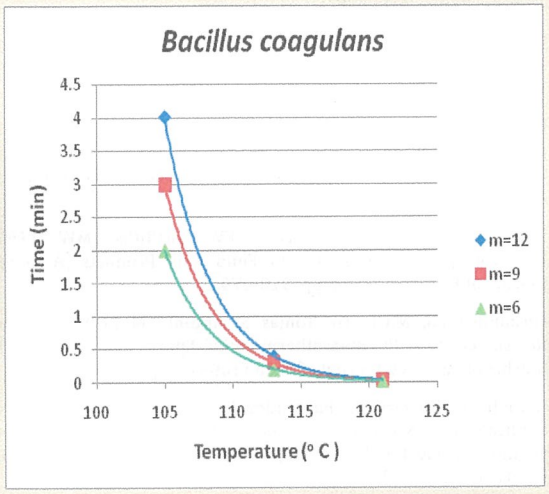

Figure 3: Isodrastic curves in a diagram time vs temperature of Bacillus stearothermophilus for m= 6, 9, 12 respectively.

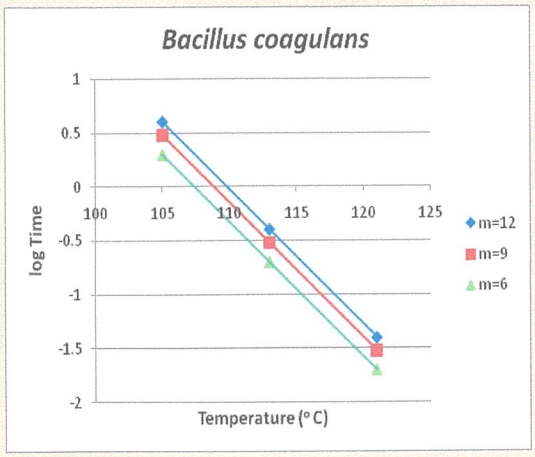

Figure 4: Isodrastic curves in a diagram log (time) vs temperature of Bacillus stearothermophilus for m= 6, 9, 12 respectively.

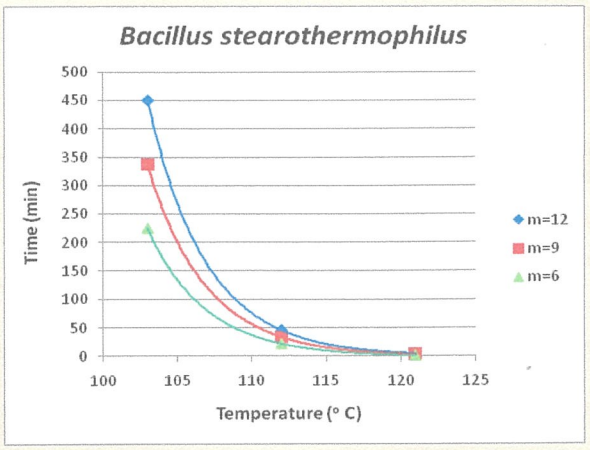

Figure 5: Isodrastic curves in a diagram time vs temperature for Bacillus Coagulants, for m=6,9,12

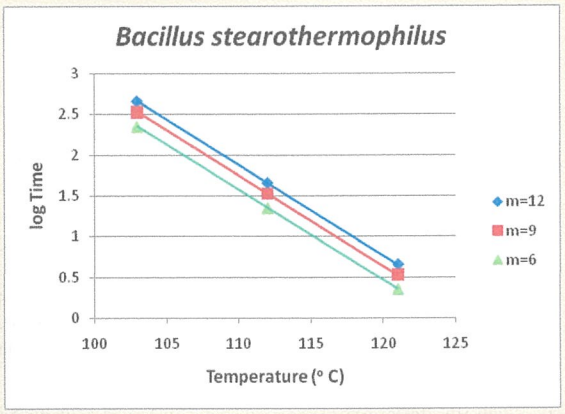

Figure 6: Isodrastic curves in a diagram log (time) vs temperature for Bacillus coagulants. m=6, 9, 12.

Conclusion

The methodology developed in this paper is an effort towards a more theoretical and fundamental approach in a field where the empiricism dominates until now. A first criticism of the empirical approach in Food microbiology, in Death kinetics in particular was the paper of Gibbs and Gekas [2] considering water activity to be the physical property that has a direct implication for microbiological safety of food. Secondly, the developed herewith approach is a useful tool to the practical Food scientist and/or engineer who does not need to deal with the array of the empirical concepts D, Z, F, L, C and so on. However the empirical those parameters have been taken implicitely into account on constructing the Time vs Temperature diagrams of this paper. Thirdly, the general concept of the physical quantity of Action in Science, it allows the use of analogies and a broader applicability in a lot of nearby fields. Next to the death kinetics of pathogenic microorganisms thought field of application of the developed approach is the field of useful microorganisms such as the lactobacilli in probiotic foods. To continue, other methods of food preservation utilizing thermal energy, like freezing, or even

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other forms of energy such as high pressure, osmotic solutions, magnetic field, multiple obstacles could be considered.

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Predicting lactic acid bacteria population in a functional yoghurt

Elena Hadjimbei, George Botsaris, and Vassilis Gekas

***Abstract*—** Lactic Acid Bacteria (LAB) are technologically necessary to manufacture yoghurt. Functional yoghurts contain not only *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, but also probiotic LAB strains which are associated with certain human health benefits. Therefore, total LAB population is an essential quality criterion, as their viability should be retained at the highest level possible, throughout the commercial life of the yoghurt. The aim of the present study was the modeling of the survival of LAB in a functional goats' milk yoghurt, enriched with *Pistacia* resin extracts and *Saccharomyces boulardii*. Novel modeling approaches were adopted, by the authors, which coined the concept of "thermal action" (Temperature x logarithm of Time). The modeling results provided a very promising approach not only in death kinetics but also in the case of moderate and low microbial population reductions as in the case of LAB survival in functional yoghurts. A modification of Bayes approach was also applied, which could provide an alternative way of modeling LAB growth and survival.

***Keywords*—**Thermal Action, Bayes approach, LAB bacteria, functional yoghurt

I. Introduction

THERE are different ways of coping with microorganisms, as there are different categories of microorganisms according to their impact on our health. Foodborne pathogens pose a significant health hazard and should be eliminated or reduced to acceptable levels, by the application of food preservation methods. In the thermal processing methods this implies the exposure to high temperatures for a specific time, depending on the kind of the microorganism and the population reduction degree is expressed logarithmically. Temperature affects the population linearly and Time affects logarithmically. Death kinetics is typically viewed in diagrams with Temperature in the X-axis whereas the logarithm of time is presented in the Y-axis.

In a previous communication we provided an alternative way through the use of the, by us coined, concept of Thermal Action, defined as the product of Temperature times the logarithm of Time [1]. In this methodology the microbial population is plotted vs. thermal action. It is understood that temperature-time combinations which produce same values of thermal action, iso-drastic values (from the Greek word δράσις, meaning action), result in same microbial population values. Examples of such diagrams are given later in the text (see Fig.1).

Beneficial (probiotic) microorganisms which are associated with documented health benefits, should be able to survive or at least retain high values, in functional food products. The functional food market is constantly growing in the developed world and yoghurts are rapidly gaining space as they provide ideal vehicles to deliver bioactive nutrients to humans [2]. The majority of yoghurts are characterized by the limited viability of probiotics towards the end of their shelf-life [3, 4]. The aim of this work was to predict LAB population in a functional goats' milk yoghurt. The yoghurt was developed in our Laboratory of Food Science and Technology at the Cyprus University of Technology, and it contained both LAB and *Saccharomyces boulardii*; a probiotic yeast. The yoghurt was manufactured from goats' milk. The yoghurt underwent different treatments, using *Pistacia atlantica* resin extracts and/or *Saccharomyces boulardii* aiming at the enhancement of its functional properties. One of the most important quality parameters is the survival of the LAB. It is a system, by far more complicated than simple thermal death of harmful bacteria but we wanted to see how our Thermal Action modeling approach could be applied in such complicate systems.

Furthermore we attempted a Bayes approach for assessing the effect of the various treatments on the initial expectation of quality preservation of the product.

II. Theory

A. Thermal Action

The rationale for the definition of the quantity Thermal Action, is based on the fact that Time, t, and temperature, Θ are main factors influencing many phenomena in Food Technology and Preservation, upon thermal treatment. Temperature is dimensionally the concentration of heat that is of the thermal energy, under constant pressure, according to the dimensional analysis equation (1)

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$$[Q] = [\rho][C\rho][\Theta] \quad (1)$$

Where Q: being the heat; ρ: the product of the density; Θ: Temperature; Cp: is the specific heat capacity

Since the product ρ times Cp remains a constant during many processes, temperature instead of heat could be used in the definition of Thermal Action. There is an overwhelming evidence that the effect of the temperature on the result of the thermal treatment is linear, whereas the effect of time is not linear but a logarithmic one (the logarithm with the base of ten is traditionally used). Therefore we coin the concept of Thermal Action S_Θ as shown in equation (2).

$$S_\Theta = \Theta \log t \quad (2)$$

In a plot of t versus Θ the obtained curves are, thus, isodrastic curves (from Δράσις, pronounced drasis, and the Greek word for Action). Furthermore in a plot of logt versus Θ the obtained isodrastic curves become parallel straight lines.

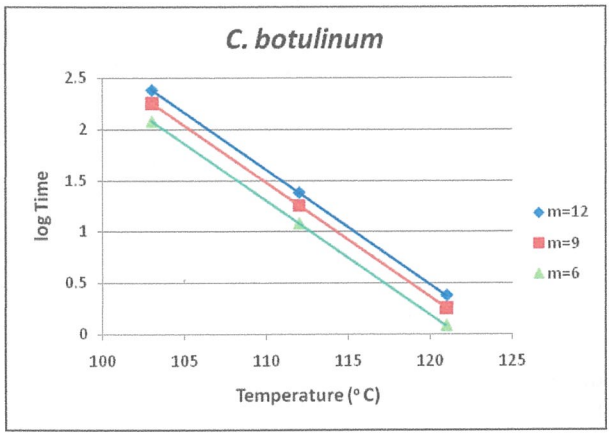

Fig.1 Isodrastic curves of *C. botulinum* for various values of m, i.e. population reduction degrees expressed logarithmically.

Consequently if we plotted the population vs thermal action the curves obtained should be straight lines, if the only parameters affecting are time and temperature.

B. Bayes approach

The Bayesian approach is one of the significant tools of prediction also in the Science and Technology (3). There are four basic elements in the Bayesian approach:

1. The prior probability of a hypothesis p(H)
2. The posterior probability given an evidence E, p (H/E)
3. The likelihood i.e. given the hypothesis is fulfilled what is the probability this to be owed to the presence of the evidence E
4. The normalization constant p(E)

Then, the well known Bayes formula connects these four elements:

$$p(H/E) = p(H).\ p(E/H)\ /\ p(E) \quad (3)$$

It is possible that we have more pieces of evidence E1, E2…and the equation can be easily generalized to take in to account all those evidences.

Another useful relation derived from the one above gives the ratio of the two probabilities the posterior and the prior as the so called Bayes factor which equals the ratio of the likelihood to the normalization constant [5].

III. EXPERIMENTAL

A. Yogurt manufacture process

Fresh batches of yoghurt were made using goats' milk and total resin extract. For the manufacture of the yoghurt, a volume of 500 ml of fresh goat milk was poured in a sterilized 1 L Scott Duran® bottle and preheated at 55 ° C. Calcium caseinate (2% w/w) was then added to the milk and thoroughly homogenized (IKA ®T25 digital Ultra Turrax) with a rotating speed of 12000 rpm for 3 min. A heat treatment at 90 ° C for 5 min was carried out, and then the bottle containing the milk was cooled to 46 ° C in a water bath. 2 gr of the starter culture and 300 mg of total resin extract were then added. The bottle was incubated at 46 ° C for 15 min. The milk was then poured into twelve sterile plastic pots and sealed. All pots were then incubated at 46 ° C for 3 h and were then transferred and stored at three different temperatures. Four pots stored at 6 ° C, four pots stored at 10 ° C and four pots stored at 14 ° C. A total of three replicates were made, and starting from Day 0 (Yoghurt manufacture day), one pot from each of the three different temperatures was analyzed microbiologically every one week, until day 28, which was the maximum storage period tested.

B. Enumeration of total lactic acid bacteria

Plating and enumeration were performed using a modification of the methods described by Lourens-Hattingh and Viljoen (2001), [6]. From each pot of yoghurt of the three different temperatures, 10 g was aseptically weighted and transferred into sterile stomacher bags, where mixed with 90 ml sterile Maximun Recovery Diluent (MRD; Oxoid, UK) and homogenized in stomacher for 1 min. Serial dilutions were prepared (1:10) using MRD down to 10^{-6}. A volume of 100μL from each dilution was inoculated and dispersed onto MRS agar (Oxoid, UK) for the enumeration of LAB in duplicates. To create microaerophilic conditions, the MRS agar was overlaid with molten MRS agar at 48 ° C. MRS plates were incubated at 37 ° C for 3 days. This procedure was repeated every 7 days, for 28 days, and the total LAB were enumerated.

C. Statistical analysis

ANOVA methods were applied to evaluate differences in mean values of continuous variables as appropriate. Pairwise comparisons of the group means performed using Duncan test. A two-sided p value of less than 0.05 was considered statistically significant.

IV. RESULTS

Total LAB were enumerated for all three different batches of yoghurt, stored at 6 ° C, 10 ° C and 14 ° C respectively from day 0 to day 28. The initial number was 8.76 $\log_{10}$cuf/g for the three batches.

Total LAB numbers remained significantly higher and steady at about 8 $\log_{10}$cuf/g in yoghurt stored at 6 ° C for 14 days compared with the other two batches. Similarly total LAB numbers remained significantly higher and steady at about 7 $\log_{10}$cuf/g in yoghurt stored at 10 ° C for 14 days compared with yoghurt stored at 14 ° C.

At the last day of storage the values of total LAB were decreased in all three batches. However it seems that values did not decrease below a critical value of 3.5 $\log_{10}$cuf/g for all three different batches. Although the decrease of total LAB numbers in yoghurts stored at 14 ° C was faster until the 14 days, this trend did not last until the 28 days, where the decrease in values was very small.

The results are depicted graphically in Fig.2 below:

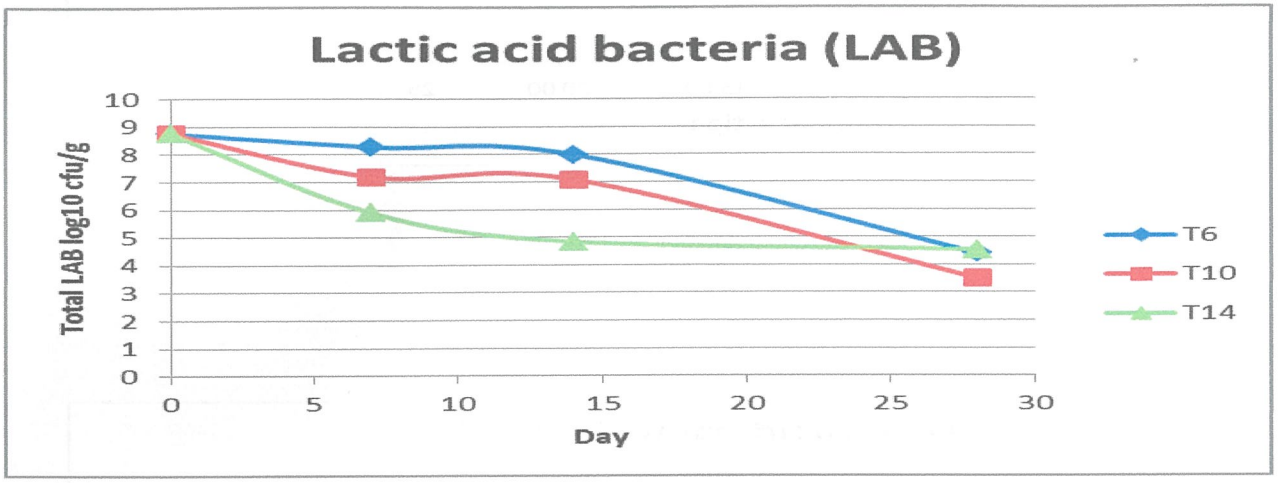

Fig.2 LAB population vs. Storage time for three different temperatures 6, 10 and 14°C

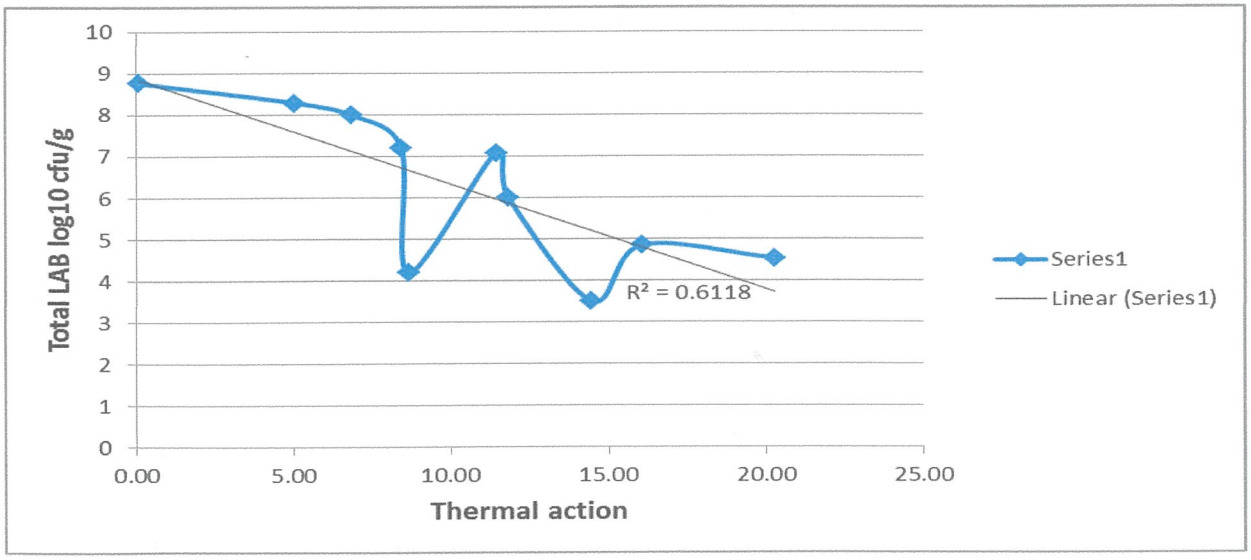

Fig.3 LAB population plotted vs. Thermal Action

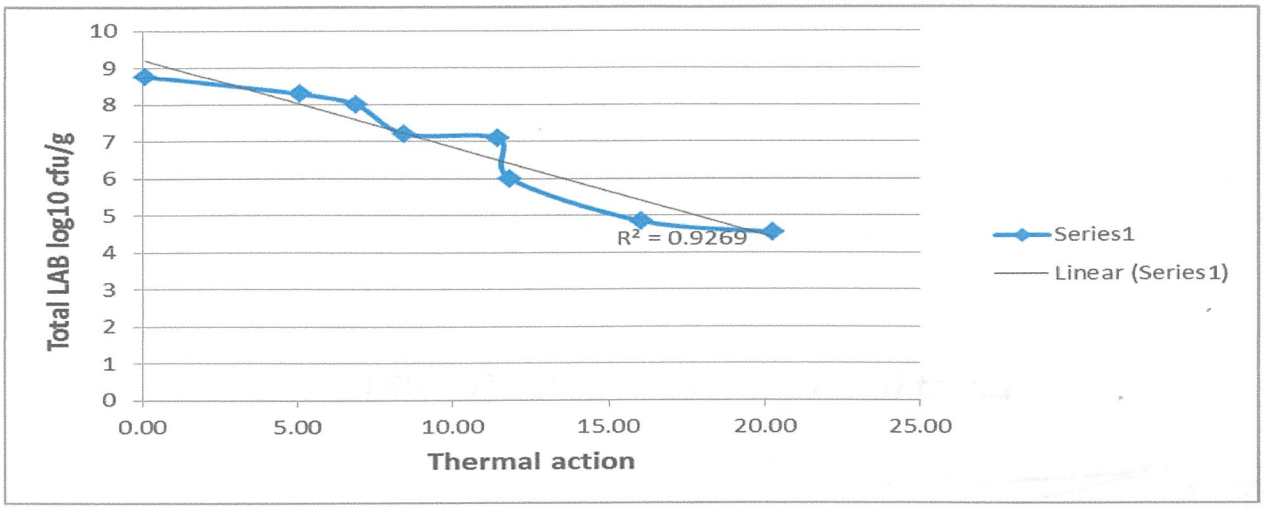

Fig.4 The diagram LAB population vs. Thermal Action omitting the two outliers

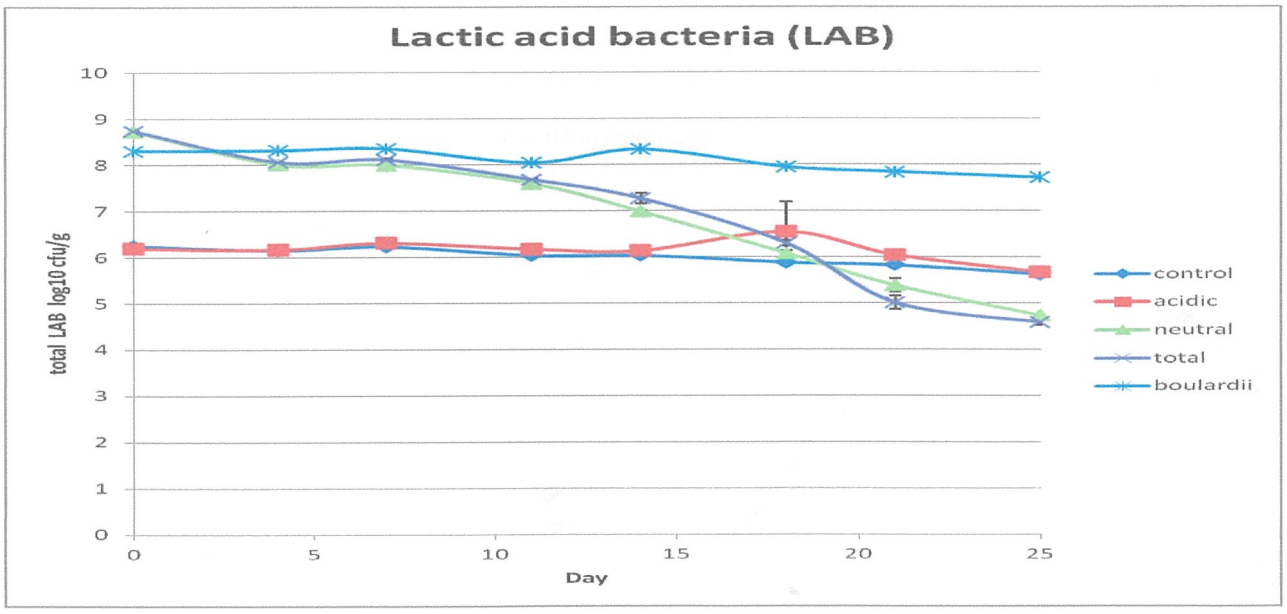

Fig.5 Survival of total lactic acid bacteria (LAB) in yoghurt formulations. Means of duplicates. Day 0 = day of inoculation.

V. DISCUSSION

A. Thermal Action

The data in Fig.2 were replotted as Total LAB population vs. Thermal Action (Fig.3) and the linearity of the curve was estimated.

The two obvious outliers correspond to the higher 14°C temperature for times 14 days and 28 days, storage time for which the yoghurt, as more or less was expected was rather deteriorated. Despite that the coefficient R^2 was 0.6118, which is not too bad for a complex biological system to show a tendency to linearity. If the two outliers are removed an even better coefficient is obtained (Fig. 4)

The R^2 obtained is a 0.9269 which is a very good one to show that the above studied complex system responds to positively to the thermal action approach.

B. Bayes approach

Figure 5 refers to the first series of experiments described elsewhere [7]. A description however of these experiments is given below with the caption of Figure 5:

Here the temperature was 6° C for all samples. Control is the sample of yoghurt without any treatment. The acidic sample was the yoghurt containing the acidic portion of the mastic extract. Neutral, respectively, the neutral portion of the extract, total the total acidic plus the neutral portions of the extract and boulardii refers to the sample in which also the *Saccharomyces boulardii* yeast was added together with the total extract.

We were able to obtain an estimation of the Bayes factor for the various treatments. Our hypothesis is that a high and stable LAB population should occur during the 25 first days of storage. Because of the shape of the curves a compromise for taking both properties, high values, stable values was followed by assuming the probabilities posterior and prior as proportional to the areas under the curves to the axis of X.

If the hypothesis applies to the total treatment then

$p(H/E) = c.\ A_{total}$

and

$p(H) = c.\ A_{control}$

and

$$\text{Bayes factor} = p(H/E)\ //p(H).= {}^{A}{}_{total/}A_{contro}l = 1.21$$

If the hypothesis applies to the combined treatment i.e. samples containing total extract plus the *Saccharomyces boulardii* yeast , following a similar procedure as above we obtain the Bayes factor for the boulardii sample as

$$\text{Bayes factor} = p(H/E)\ //p(H).= A_{boulardii}\ /A_{control}=1.33$$

VI Conclusion

In the frame of the development of a new functional product based on yoghurt, one of the most important quality parameters is the microbial population of the Lactic Acid Bacteria (LAB), which belong to the human friendly and beneficial microorganisms. *Pistacia* resins, treated in different ways were added, to the yoghurt, with or without the probiotic yeast, *Saccharomyces boulardii.* It was of essential importance to monitor the LAB population which needed to be retained at high values and for long periods of time, up to 3-4 weeks.

To such a complex system, such the functional yoghurt product developed, two novel modeling ideas were applied. The model of Thermal Action, has found to show a good agreement with the experiments i.e. the microbial population shows a linear correlation with the thermal action values.

The Bayes approach modified suitably by one of us [8], with the novel ideal of modifying the initial Bayes equation, enabled us to obtain experimentally the Bayes factors for the treatments for two kinds of yoghurt the total extract one and the combined treatment with the total extract and the *S. boulardii* yeast.

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Development of a novel functional goats' milk yoghurt enriched with *Pistacia atlantica* resin extracts and *Saccharomyces boulardii*: stability and organoleptic effects

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INTRODUCTION

Functional yoghurts are rapidly gaining space in the functional food market as they provide ideal vehicles to deliver bioactive nutrients to humans. Already several attempts have been undertaken to produce yoghurts fortified with various plant extracts. Furthermore, there is accumulating evidence suggesting that *Pistacia* resins may contribute to the reduction of many chronic diseases, such as gastrointestinal disorders, cardiovascular diseases and some forms of cancer (Hadjimbei et al. 2015, Ansari et al. 2012).

An important consideration in the yoghurt probiotic market is the survival of the health-promoting microorganisms throughout the commercial life of the product. However, many studies showed that the majority of dairy products are characterized by the limited viability of probiotics towards the end of their shelf-life (Cruz et al. 2010). It has been suggested that the combined use of probiotic yeasts such as *S. boulardii*, enhances the survival of Lactic acid bacteria - LAB (Karaolis et al. 2013).

The aim of the study was to develop a novel functional goats' milk yoghurt, using *Pistacia atlantica* extracts and *S. boulardii*, in an attempt to combine and expose the beneficial effects of the milk, extract and probiotic microorganisms.

METHODOLOGY

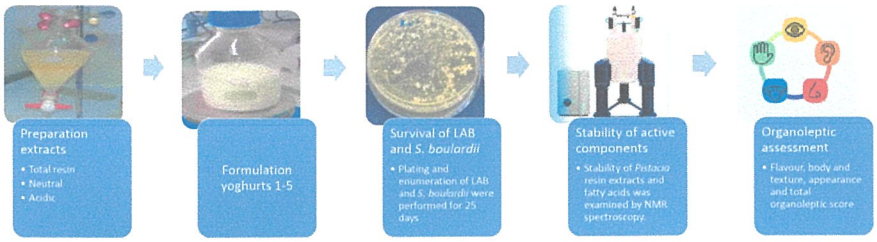

Table 1 Yoghurt types manufactured in the present study

Yoghurt No	Yoghurt Name	Ingredients
1	Control	Starter culture
2	Acidic	Starter culture + acid fraction of the resin extract
3	Neutral	Starter culture + neutral fraction of the resin extract
4	Total	Starter culture + total resin extract
5	Combined	Starter culture + total resin extract + *S. boulardii*

RESULTS

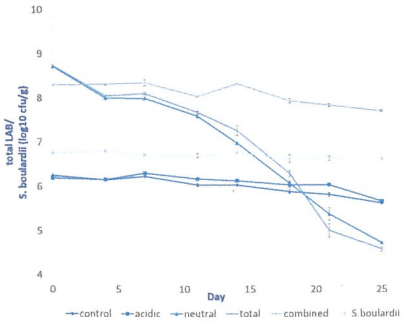

Figure 1. (a) Survival of LAB in yoghurt formulations and (b) *S. boulardii* in yoghurt 5.

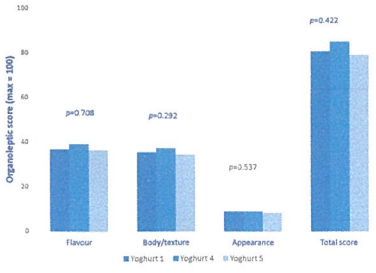

Figure 2. Organoleptic score for Yoghurts 1, 4 and 5 from the twenty panel tasters. Parameters tested: flavour, body and texture, appearance and total organoleptic score.

A. Survival of LAB and S. boulardii

- *Pistacia atlantica* resin extracts promoted the survival of LAB.
- However best results in viability of LAB were obtained in combination with *S. boulardii* (Figure 1a).
- Population of *S. boulardii* remained stable (Figure 1b).

B. Organoleptic assessment

- The organoleptic assessment revealed that were no significant differences in flavour, body, texture and appearance between different yoghurts (Figure 2).
- The majority of tasters preferred Yoghurts 4 (50%) and 5 (35%) compared to Yoghurt 1 (15%).

C. Lipid profile of yoghurts

- Four major fatty acids were identified by comparison of their ^{1}H NMR data: linoleic, linolenic, caproleic and conjugated linoleic acid (CLA).
- Functional fatty acids retained during the self-life of the yoghurts.

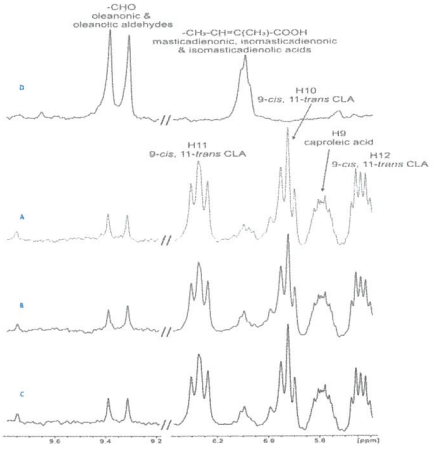

Figure 3 Selective regions of 400 MHz ^{1}H NMR spectra (298 K) in $CDCl_3$ of the lipid extract of Yoghurt 5 – Combined, where letter A: day 0, letter B: day 14, letter C: day 25, letter D: total resin extract

D. Pistacia resin bioactive phytochemicals in yoghurts

- *Two alhehyde signals (singlets) at 9.4 and 9.3 ppm indicate the presence of oleanonic and oleanolic aldehydes.*
- The broad triplet at 6.1 ppm should be attributed to the presence of the olefinic signal of the $-CH_2-CH=C(CH_3)-$ moiety of masticadienonic, isomasticadienonic and/or isomasticadienolic acids.
- All the bioactive compounds were maintained in Yoghurt 5 during shelf life (Figure 3).

CONCLUSION

The incorporation of *Pistacia atlantica* resin extracts, alone or combined with *S. boulardii*, in the yoghurt promoted LAB survival and improved organoleptic properties.

- NMR spectra revealed that the functional fatty acids and bioactive compounds were retained.
- Yoghurt formulation No 5 had the best overall results and could be marked as a potential innovative functional food.

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Adherence to the Mediterranean Diet and Lifestyle Characteristics of University Students in Cyprus: A Cross-Sectional Survey

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Introduction

- Young adults tend to follow a poor diet, marked by low consumption of fruits and vegetables and high consumption of fast food and sugar-sweetened beverages.
- This can influence not only their concurrent health but also their future risk for chronic diseases at a later age.
- Adherence to the Mediterranean pattern is important in improving overall health, whereas young adulthood may be an important time for intervening and establishing long-term health behaviors.

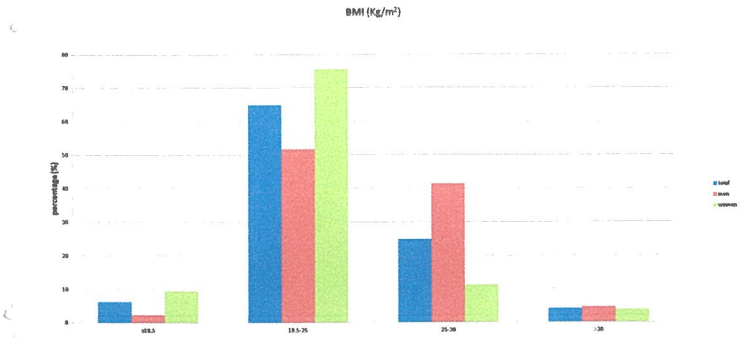

Figure 1: *Distribution of BMI categories in all study participants and in men and women separately.*

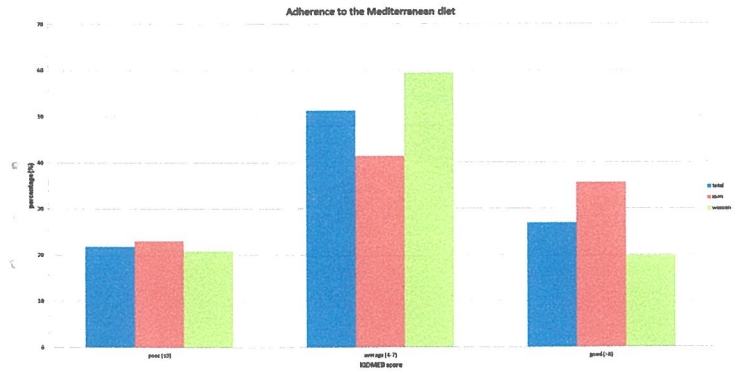

Figure 2: *Adherence to the Mediterranean diet*

Table 1: *KIDMED index questions in all men and women separately. Positive answers are given as percentages (%).*

KIDMED index questions	Total (%)	Males (%)	Females (%)	p*
Fruit or fruit juice daily	73.1	77	69.8	0.26
Second serving of fruit daily	31.6	40.2	24.5	0.02
Fresh or cooked vegetables daily	56.5	55.2	57.5	0.74
Fresh or cooked vegetables >1/day	29.5	32.2	27.4	0.47
Regular fish consumption (at least 2-3/week)	28	33.3	23.6	0.13
>1/week fast-food (hamburger) restaurant	25.9	31	21.7	0.14
Pulses >1/week	47.7	50.6	45.3	0.46
Pasta or rice almost daily (≥5/week)	69.9	67.8	71.7	0.56
Cereal or cereal product for breakfast	73.1	70.1	75.5	0.40
Regular nut consumption (at least 2-3/week)	31.1	47.1	17.9	<0.001
Use of olive oil at home	87.6	87.4	87.7	0.94
No breakfast	36.8	36.8	36.8	0.99
Dairy product for breakfast	88.1	88.5	87.7	0.87
Commercially baked goods or pastries for breakfast	30.6	34.5	27.4	0.29
Two yoghurts and/or 40 gr cheese daily	76.2	72.4	79.2	0.27
Sweets and candy several times a day	30.6	24.1	35.8	0.04

*p value for comparison between sexes from chi-square test.

Objectives

The aim of the study was:

(a) to investigate the level of adherence to the traditiona Mediterranean diet pattern overall and to specific dietary components, among Cypriot young adults.

(b) to provide evidence for intervention programs targeting this often overlooked age group.

Methods

- 193 young adults aged 18-25 years participated in the study .
- Adherence to the Mediterranean diet was evaluated by the KIDMED index , which includes 16 questions based on the principles of the Mediterranean diet. A total score ≤ 3 implies a very low diet quality, a score 4-7 that diet needs improvement and a score ≥ 8 indicates optimal adherence.
- Weight and height were measured with the use of a portable digital scale and stadiometer.

Results

A. Body Mass Index

The mean body mass index (BMI) was 23.31(±3.98) Kg/m^2 with 6.2% of them being classified as underweight, 64.8% as normal weight, 24.9%a s overweight and 4.1% as obese (Figure 1).

B. Adherence to the Mediterranean Diet (KIDMED Score)

The median KIDMED score was 6.00 (IQR:4-8), with 26.9% of students being classified as high adheres and 21.8% as low adherers (Figure 2).

With regard to the main nutritional categories of the Mediterranean diet, only about 30% of young adults consumed a second serving of fruit and vegetables more than once a day. About 40% skipped breakfast, and 26% ate at a fast food restaurant > 1 per week and 31% consumed sweets and candy several times a day.

On the other hand, 76% of participants reported consumption of at least two dairy products daily, with 88% consuming a dairy product for breakfast, whereas 88% use olive oil at home (Table 1).

Not being a smoker (p=0.005) and currently exercising (p<0.001) were significantly associated with a higher KIDMED score.

C. Beverage Consumption

The majority of participants (32.5%) consume coffee 2-3 times per day, with another 23% consuming coffee once per day.

Regarding alcohol consumption, study participants reported occasional drinking, with higher values in men, as expected.

Conclusions

- Results support a shift from traditional healthy diets to more unhealthy eating patterns.
- Young adults consume low quantities of fruits and vegetables
- On the other hand, the majority of young adults in Cyprus still consume at least two dairy products daily and use olive oil at home, while also eating pulses more than once per week.
- Tailored-made strategies targeting the young adult population could be warranted to reduce future risk for chronic diseases.

Συμπεράσματα: Οι ακολουθούμενες διαδικασίες διασφαλίζουν τη διανομή υγιεινών και ποιοτικών γευμάτων στο πλαίσιο του Προγράμματος και δυνητικά μπορεί να οδηγήσουν σε βελτίωση των συνθηκών παραγωγής, με επακόλουθο και την αδιάλειπτη διανομή ποιοτικών προϊόντων.

ΠΑ04

ΣΥΓΚΡΙΤΙΚΗ ΑΞΙΟΛΟΓΗΣΗ ΘΡΕΠΤΙΚΗΣ ΑΞΙΑΣ ΤΩΝ ΓΕΥΜΑΤΩΝ ΤΟΥ ΠΡΟΓΡΑΜΜΑΤΟΣ ΔΙΑΤΡΟΦΗ ΜΕ ΑΝΤΙΣΤΟΙΧΑ ΓΕΥΜΑΤΑ ΕΜΠΟΡΙΟΥ

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Εισαγωγή: Οι διατροφικές συνήθειες κατά την παιδική/εφηβική ηλικία διαδραματίζουν σημαντικό ρόλο για όλη τη διάρκεια της ζωής ενός ατόμου. Ως εκ τούτου, η προώθηση υγιεινών διατροφικών συνηθειών θα πρέπει να αποτελεί στόχο των προγραμμάτων σχολικής σίτισης, διασφαλίζοντας τη διάθεση υγιεινών προς κατανάλωση γευμάτων.

Σκοπός: Η ανάδειξη/ επιβεβαίωση της υψηλής θρεπτικής αξίας των γευμάτων του Προγράμματος Σίτισης και Προώθησης Υγιεινής Διατροφής, ΔΙΑΤΡΟΦΗ έναντι αντίστοιχων γευμάτων εμπορίου.

Υλικό: n=13 γεύματα του Προγράμματος ΔΙΑΤΡΟΦΗ συγκρίθηκαν με n=52 γεύματα εμπορίου (3-6 ανά είδος).

Μέθοδος: Σε όλα τα γεύματα αξιολογήθηκαν τα επιμέρους χρησιμοποιούμενα συστατικά και η διαθρεπτική επισήμανση (πληροφορίες ετικέτας). Ειδικά για τη διασφάλιση της θρεπτικής αξίας των γευμάτων του Προγράμματος ΔΙΑΤΡΟΦΗ, η περιεκτικότητά τους σε μακροθρεπτικά/μικροθρεπτικά συστατικά εκτιμήθηκε και από Πίνακες Σύνθεσης Τροφίμων, ενώ επιβεβαιώθηκε με εργαστηριακές αναλύσεις σε Διαπιστευμένα Εργαστήρια. Συνολικά πραγματοποιήθηκαν n=294 εργαστηριακές αναλύσεις (n=3 μετρήσεις/γεύμα/συστατικό), στις οποίες αποτιμήθηκε η περιεκτικότητα σε πρωτεΐνες, υδατάνθρακες, ολικά σάκχαρα, φυτικές ίνες, ολικό λίπος, προφίλ λιπαρών οξέων, αλάτι/νάτριο.

Αποτελέσματα: Σε επίπεδο πρώτων υλών τα γεύματα του Προγράμματος ΔΙΑΤΡΟΦΗ, εν αντιθέσει με αυτά του εμπορίου, παρασκευάζονταν αποκλειστικά με εξαιρετικά παρθένο/παρθένο ελαιόλαδο, αλεύρι ολικής άλεσης ≥60% επί του συνολικού και ήταν πλήρως απαλλαγμένα από συντηρητικά και τεχνητά πρόσθετα, επεξεργασμένο κρέας και ανακατεργασμένα τυριά. Επιπρόσθετα, όπως επιβεβαιώθηκε εργαστηριακά, είχαν χαμηλότερα ολικά και κορεσμένα λιπαρά, ενώ η πλειονότητά τους είχε χαμηλότερα επίπεδα ολικών σακχάρων και υψηλότερα πρωτεϊνών και φυτικών ινών.

Συμπεράσματα: Το Πρόγραμμα ΔΙΑΤΡΟΦΗ αποτελεί παράδειγμα καλής πρακτικής στο χώρο των προγραμμάτων σχολικής σίτισης, αναφορικά με το σχεδιασμό των γευμάτων, καθώς σχεδιάζονται με βάση τις αρχές της υγιεινής διατροφής, προσφέροντας σημαντικά πλεονεκτήματα έναντι των αντίστοιχων γευμάτων εμπορίου.

ΠΑ05

ΠΡΟΣΘΗΚΗ ΕΚΧΥΛΙΣΜΑΤΩΝ ΡΗΤΙΝΩΝ PISTACIA ΜΟΝΟ Η ΣΕ ΣΥΝΔΥΑΣΜΟ ΜΕ ΤΗΝ ΖΥΜΗ SACCHAROMYCES BOULARDII ΓΙΑ ΤΗΝ ΑΝΑΠΤΥΞΗ ΕΝΟΣ ΚΑΙΝΟΤΟΜΟΥ ΛΕΙΤΟΥΡΓΙΚΟΥ ΤΡΟΦΙΜΟΥ

Έλενα Χατζημπέη, Βλάσιος Γούλας, Βασίλης Γκέκας, Γιώργος Μπότσαρης

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Εισαγωγή: Το γιαούρτι αποτελεί ιδανικό μέσο στην παροχή λειτουργικών συστατικών καθώς καταναλώνεται ευρέως και κυρίως από νεαρούς ενήλικες και ήδη συμβάλλει στην καλή υγεία του οργανισμού, αφού περιέχει προβιοτικά, ασβέστιο καθώς και άλλα μέταλλα και βιταμίνες. Έχουν ήδη πραγματοποιηθεί αρκετές προσπάθειες για την παραγωγή γιαουρτιού εμπλουτισμένου με φυτικά εκχυλίσματα.

Σκοπός: Η δημιουργία ενός καινοτόμου λειτουργικού γαλακτοκομικού προϊόντος, χρησιμοποιώντας εκχυλίσματα ρητινών *Pistacia και Saccharomyces boulardii*.

Υλικά και μεθόδοι: Διαφορετικά κλάσματα εκχυλισμάτων ρητινών *Pistacia* μόνο ή σε συνδυασμό με *Saccharomyces boulardii* ενσωματώθηκαν σε γιαούρτι από αιγινό γάλα. Παρασκευάστηκαν πέντε διαφορετικά γιαούρτια: Γιαούρτι 1-Control, με προσθήκη μόνο της καλλιέργειας εκκίνησης; Γιαούρτι 2- Acidic, με το όξινο κλάσμα; Γιαούρτι 3- Neutral, με το ουδέτερο κλάσμα; Γιαούρτι 4- Total, με το ολικό εκχύλισμα μαστίχας; και Γιαούρτι 5- Combined, με το ολικό εκχύλισμα και την ζύμη. Έγιναν μετρήσεις των βακτηρίων του γαλακτικού οξέος κάθε τρεις ημέρες, για 25 ημέρες. Η σταθερότητα των εκχυλισμάτων ρητινών *Pistacia* και των λιπαρών οξέων εξετάστηκε με ανάλυση NMR. Τέλος, τα δείγματα αξιολογήθηκαν οργανοληπτικά.

Αποτελέσματα: Όλα τα εκχυλίσματα ρητινών *Pistacia* προώθησαν την ανάπτυξη των οξυγαλακτικών βακτηρίων τουλάχιστον μέχρι την 18η ημέρα. Ο αριθμός των βακτηρίων παρέμεινε υψηλός και σταθερός στα 8 $\log_{10}$ cuf/g στο Γιαούρτι 5 για 25 ημέρες. Ταυτοποιήθηκαν και ποσοτικοποιήθηκαν 4 λιπαρά οξέα στα Γιαούρτια: λινολεϊκό, λινολενικό, καπρολεϊκό και συζευγμένο λινολεϊκό οξύ (CLA) και παρέμειναν άθικτα και τις 25 ημέρες. Όλες οι βιοδραστικές ενώσεις παρέμειναν σταθερές μέχρι το τέλος ζωής του Γιαουρτιού. Με βάση την ομαδική οργανοληπτική εξέταση δεν υπήρξαν σημαντικές διαφορές στη γεύση μεταξύ διαφορετικών Γιαουρτιών.

Συμπεράσματα: Η προσθήκη εκχυλισμάτων ρητινών *Pistacia* μόνο ή σε συνδυασμό με *S. boulardii* σε αιγινό γιαούρτι

ενίσχυσε την ανάπτυξη των προβιοτικών, παρουσιάζοντας πολύ καλές οργανοληπτικές ιδιότητες. Το νέο αυτό προϊόν θα μπορούσε να προωθηθεί στην αγορά ως πιθανό καινοτόμο λειτουργικό τρόφιμο, προσφέροντας έναν εναλλακτικό τρόπο πρόληψης των ασθενειών και προαγωγής της καλής υγείας.

ΠΑ06

ΒΙΟΛΟΓΙΚΑ ΔΡΑΣΤΙΚΑ ΣΥΣΤΑΤΙΚΑ ΕΚΧΥΛΙΣΜΑΤΩΝ ΠΑΡΑ-ΠΡΟΪΟΝΤΩΝ ΟΙΝΟΠΟΙΗΣΗΣ

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Εισαγωγή: Στο λευκό και στο κόκκινο κρασί περιέχεται πληθώρα συστατικών με αντιοξειδωτική και αντι-αιμοπεταλιακή δράση, όπως αναφέρεται και σε προηγούμενες μελέτες μας. Τα παρα-προϊόντα οινοποίησης αποτελούν φθηνή και ανεκμετάλλευτη πηγή ανάλογων συστατικών, που μπορούν να χρησιμοποιηθούν στον εμπλουτισμό κατά τη διαδικασία παραγωγής λειτουργικών τροφίμων.

Σκοπός: Σκοπός είναι ο προσδιορισμός της αντι-αιμοπεταλιακής και αντιοξειδωτικής δράσης εκχυλισμάτων παρα-προϊόντων οινοποίησης.

Υλικό: Χρησιμοποιήθηκαν παρα-προϊόντα οινοποίησης από τέσσερις ποικιλίες σταφυλιού (Cabernet Sauvignon, Cabernet Franc, Syrah, Ξινόμαυρο).

Μέθοδος: Για την εκχύλιση των παρα-προϊόντων χρησιμοποιήθηκαν τέσσερις διαφορετικές μέθοδοι (Bligh-Dyer, αιθανόλη 80%, εξάνιο, νερό). Ακολούθησε ο προσδιορισμός του ολικού φαινολικού περιεχομένου των εκχυλισμάτων, της ικανότητας δέσμευσης ελευθέρων ριζών DPPH και της αντίστασης στην οξείδωση του ορού. Επίσης, εξετάστηκε η ικανότητά τους να αναστέλλουν in vitro τη συσσώρευση ανθρώπινων αιμοπεταλίων έναντι των συσσωρευτικών παραγόντων PAF, ADP και TRAP.

Αποτελέσματα: Όσον αφορά τη συσσώρευση των αιμοπεταλίων, τα εκχυλίσματα εξανίου (2,3-240 μg) προκάλεσαν κάποια αναστολή που ωστόσο δεν έφτασε μέχρι το 50%, ενώ τα υδατικά (0,1-170 μg), προκάλεσαν ενεργοποίηση. Ισχυρότερη και δοσο-εξαρτώμενη αναστολή παρουσίασαν τα εκχυλίσματα αιθανόλης, με τις τιμές των IC50 να κυμαίνονται μεταξύ 70-175 μg παρα-προϊόντων, ανάλογα την ποικιλία σταφυλιού και ακολούθησαν τα εκχυλίσματα της λιποειδικής φάσης της Bligh-Dyer. Τα εκχυλίσματα της αιθανόλης και της υδατικής φάσης της Bligh-Dyer κατείχαν την υψηλότερη συγκέντρωση φαινολικών συστατικών με 0,37 και 0,35 mg γαλλικού οξέος/mg παρα-προϊόντων αντίστοιχα και δέσμευσαν αποτελεσματικότερα το DPPH. Μαζί με τα εκχυλίσματα εξανίου, ανέστειλαν επίσης την οξείδωση του ορού κατά 60-240 λεπτά.

Συμπεράσματα: Τα εκχυλίσματα αιθανόλης των παρα-προϊόντων οινοποίησης φαίνεται να αποτελούν πηγή συστατικών με αντι-αιμοπεταλιακή και αντιοξειδωτική δράση. Η χρήση τους ως δραστικά συστατικά είναι πιθανό να οδηγήσει στην παραγωγή λειτουργικών τροφίμων με καρδιοπροστατευτικές ιδιότητες.

ΠΑ07

ΓΝΩΣΕΙΣ ΚΑΙ ΠΡΑΚΤΙΚΕΣ ΥΓΙΕΙΝΗΣ ΔΙΑΤΡΟΦΗΣ ΑΣΘΕΝΩΝ ΚΑΡΚΙΝΟΥ ΤΟΥ ΜΑΣΤΟΥ ΥΠΟΒΑΛΛΟΜΕΝΩΝ ΣΕ ΧΗΜΕΙΟΘΕΡΑΠΕΙΑ

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Εισαγωγή: Ο καρκίνος του μαστού αποτελεί το δεύτερο συχνότερο τύπο καρκίνου παγκοσμίως, καθώς και την 5η πιο διαδεδομένη αιτία θανάτου μεταξύ γυναικών. Όπως σε όλους τους τύπους καρκίνου, η υιοθέτηση υγιεινών διαιτητικών πρακτικών συμβάλει σημαντικά και στην πρόληψη, αλλά και θεραπεία της νόσου.

Σκοπός: Η αξιολόγηση των γνώσεων και πρακτικών ασθενών με καρκίνο του στήθους, υπό χημειοθεραπεία.

Υλικό: Συνολικά 85 γυναίκες (33-78 ετών) υποβαλλόμενες σε χημειοθεραπεία στο Θεαγένειο Αντικαρκινικό Νοσοκομείο στις αρχές του 2017, συμμετείχαν στη μελέτη. Μία ασθενής αφαιρέθηκε από το δείγμα (η μόνη σε στάδιο 4), οπότε το τελικό δείγμα αποτέλεσαν 84 γυναίκες (σε καρκινικό στάδιο 0-3).

Μέθοδος: Οι γνώσεις και οι πρακτικές σχετικά με την υγιεινή διατροφή αξιολογήθηκαν με το ερωτηματολόγιο *Eating Assessment Table* (EAT), ενώ η ποικιλία στη διατροφική πρόσληψη εξετάστηκε με τη χρήση του ερωτηματολογίου *Household Dietary Diversity Score* (HDDS). Κοινωνικοδημογραφικά χαρακτηριστικά και στοιχεία αναφορικά με τη νόσο καταγράφηκαν για το σύνολο του δείγματος.

Αποτελέσματα: Το δείγμα εμφάνισε χαμηλό συνολικό σκορ στο ερωτηματολόγιο EAT (43.2±10.1, μέγιστο σκορ 100 βαθμοί), ενδεικτικό ανεπαρκών γνώσεων και υιοθέτησης λανθασμένων διατροφικών πρακτικών. Οι προεμμηνοπαυσιακές γυναίκες εμφάνισαν αυξημένο σκορ EAT έναντι των μετεμμηνοπαυσιακών (47.3 vs. 41.4, p=0.014). Καμία διαφορά δεν παρατηρήθηκε στο EAT μεταξύ ασθενών

παρατηρείται μεγαλύτερη συναισθηματική επιρρέπεια στις γυναίκες συγκριτικά με τους άνδρες. Η παρούσα έρευνα βοήθησε στην κατανόηση των αιτιών της πρόσληψης τροφής, πέραν του αισθήματος της πείνας, γεγονός που μπορεί να βοηθήσει στην μείωση της υπερφαγίας.

ΠΑ47

ΒΑΘΜΟΣ ΠΡΟΣΚΟΛΛΗΣΗΣ ΣΤΗ ΜΕΣΟΓΕΙΑΚΗ ΔΙΑΙΤΑ ΚΑΙ ΧΑΡΑΚΤΗΡΙΣΤΙΚΑ ΤΟΥ ΤΡΟΠΟΥ ΖΩΗΣ ΦΟΙΤΗΤΩΝ ΣΤΗΝ ΚΥΠΡΟ: ΣΥΓΧΡΟΝΙΚΗ ΜΕΛΕΤΗ

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Εισαγωγή: Οι νεαροί ενήλικες ακολουθούν φτωχή διατροφή, η οποία χαρακτηρίζεται από χαμηλή πρόσληψη σε φρούτα και λαχανικά και υψηλή κατανάλωση γρήγορου φαγητού, αναψυκτικών και άλλων ζαχαρούχων ποτών. Αυτό μπορεί να επηρεάσει την υγεία τους στο παρόν στάδιο της ζωής τους, αλλά και τον κίνδυνο για διάφορες χρόνιες παθήσεις στην ενήλικη ζωή. Η προσκόλληση στη Μεσογειακή διατροφή είναι σημαντική και η ηλικιακή αυτή ομάδα κρίνεται ιδιαίτερα σημαντική για την εφαρμογή προγραμμάτων παρέμβασης και εγκαθίδρυσης μακροπρόθεσμων συμπεριφορών υγείας.

Σκοπός: Η μελέτη των διατροφικών συνηθειών των Κύπριων νεαρών ενηλίκων.

Υλικά και μεθόδοι: Οι διατροφικές συνήθειες αξιολογήθηκαν με τη χρήση ερωτηματολογίου σε 193 φοιτητές, ενός δημόσιου και ενός ιδιωτικού πανεπιστημίου στην Κύπρο. Ο βαθμός προσκόλλησης στη Μεσογειακή Διατροφή αξιολογήθηκε μέσω του επικυρωμένου διατροφικού δείκτη KIDMED ο οποίος περιλαμβάνει 16 ερωτήσεις. Ο δείκτης μάζας σώματος (ΔΜΣ) υπολογίστηκε με βάση τις μετρήσεις βάρους και ύψους.

Αποτελέσματα: Η μέση τιμή του ΔΜΣ ήταν 23.31 (±3.98). Η μέση τιμή του δείκτη KIDMED ήταν 6.0 (IQR 4-8), με το 26.9% των φοιτητών να παρουσιάζει υψηλό βαθμό προσκόλλησης στη Μεσογειακή Διατροφή και το 21.8% να παρουσιάζει χαμηλό βαθμό προσκόλλησης. Περίπου το 32% των φοιτητών ανέφερε ότι καταναλώνει μια δεύτερη μερίδα φρούτων και λαχανικών περισσότερο από μια φορά την ημέρα, ενώ το 26% δήλωσε ότι επισκέπτεται ταχυφαγείο περισσότερο από μια φορά την εβδομάδα και το 31% καταναλώνει γλυκά αρκετές φορές την ημέρα. Από την άλλη πλευρά το 76% των συμμετεχόντων ανέφερε ότι καταναλώνει τουλάχιστον δύο γαλακτοκομικά προϊόντα ημερησίως και το 88% χρησιμοποιεί ελαιόλαδο στο σπίτι. Η πλειοψηφία καταναλώνει καφέ 2-3 φορές την ημέρα.

Συμπεράσματα: Τα αποτελέσματα υποδεικνύουν τη μετάβαση από την παραδοσιακή υγιεινή διατροφή σε πιο ανθυγιεινά σχήματα διατροφής. Αποτελεί ωστόσο ενθαρρυντικό γεγονός η υψηλή πρόσληψη γαλακτοκομικών προϊόντων και η χρήση ελαιολάδου. Θα πρέπει να εκπονηθούν στρατηγικές προαγωγής της δημόσιας υγείας με στόχο την βελτίωση της διατροφής των νεαρών ενηλίκων.

ΠΑ48

Η ΕΠΙΔΡΑΣΗ ΤΟΥ ΣΥΜΠΛΗΡΩΜΑΤΟΣ ΛΙΠΟΥΣ ΣΤΗΝ ΕΜΦΑΝΙΣΗ ΤΩΝ ΣΥΜΤΩΜΑΤΩΝ ΤΟΥ ΣΥΝΔΡΟΜΟΥ DUMPING ΣΕ ΑΣΘΕΝΕΙΣ ΠΟΥ ΥΠΟΒΛΗΘΗΚΑΝ ΣΕ ΓΑΣΤΡΟ-ΔΩΔΕΚΑΔΑΚΤΥΛΙΚΕΣ ΕΠΕΜΒΑΣΕΙΣ: CASE SERIES

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Εισαγωγή: Ασθενείς που υποβάλλονται σε βαριατρικές επεμβάσεις συχνά εμφανίζουν το σύνδρομο Dumping. Κατά τη βιβλιογραφία, η αύξηση της πρόσληψης λίπους είναι υπό εξέταση, καθώς μπορεί να καθυστερήσει την κένωση του στομάχου και να προκαλέσει ανακούφιση των συμπτωμάτων .

Σκοπός: Στόχος είναι να διερευνηθεί εάν το συμπλήρωμα λίπους βοηθά στην ανακούφιση των συμπτωμάτων του συνδρόμου.

Υλικό: Έξι ασθενείς που υποβλήθηκαν σε βαριατρική επέμβαση, καμπύλη γλυκόζης, ερωτηματολόγια Sigstad και Arts, διατροφικό συμπλήρωμα, συμπλήρωμα λίπους.

Μέθοδος: Η διάγνωση έγινε με καταγραφή τιμής γλυκόζης νηστείας, έπειτα κατανάλωση 100ml υγρού διατροφικού συμπληρώματος και καταγραφή τιμών γλυκόζης ½, 1, 11/2 και 2 ώρες. Στη 1η και 2η ώρα, συμπληρώθηκαν τα ερωτηματολόγια. Οι ασθενείς που διαγνώστηκαν θετικά, επανέλαβαν τη διαδικασία σε δεύτερη συνάντηση όπου κατανάλωσαν επιπλέον συμπλήρωμα λίπους. Οι τιμές των δύο συναντήσεων συγκρίθηκαν.

Αποτελέσματα: Ο έλεγχος για στατιστική διαφορά ανάμεσα στις μετρήσεις γλυκόζης ανά δείγμα δεν έδειξε στατιστικά σημαντική διαφορά ανάμεσα στη 1η και 2η συνάντηση. Αντίστοιχα οι τιμές της σημαντικότητας για το ερωτηματολόγιο Sigstad (,257 και ,04) και το ερωτηματολόγιο Arts (,007 και ,027)) ήταν μεγαλύτερες από ,005.

Συμπεράσματα: Συγκρίνοντας την καμπύλη γλυκόζης και την βαθμολογία ερωτηματολογίων των ασθενών ξεχωριστά παρατηρούμε μεγάλη απόκλιση και μείωση των συμπτωμάτων του συνδρόμου μετά την κατανάλωση του συμπληρώματος λίπους.

ClinicalTrials.gov ID: 5411069DD0467

24-26 ΝΟΕΜΒΡΙΟΥ 2017

14ο Πανελλήνιο Συνέδριο ΔΙΑΤΡΟΦΗΣ & ΔΙΑΙΤΟΛΟΓΙΑΣ

3ο Πανελλήνιο Συνέδριο ΚΛΙΝΙΚΗΣ ΔΙΑΤΡΟΦΗΣ & ΜΕΤΑΒΟΛΙΣΜΟΥ

Πανελλήνιος Σύλλογος Διαιτολόγων - Διατροφολόγων

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ΕΠΑΙΝΟΣ

Βεβαιώνεται ότι η Ανακοίνωση με τίτλο:

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Έλενα Χατζημπέη, Βλάσιος Γούλας, Βασίλης Γκέκας, Γιώργος Μπότσαρης

διακρίθηκε στα πλαίσια του 14ου Πανελλήνιου Συνεδρίου Διατροφής & Διαιτολογίας
και του 3ου Πανελλήνιου Συνεδρίου Κλινικής Διατροφής και Μεταβολισμού, τα οποία πραγματοποιήθηκαν 24-26 Νοεμβρίου 2017,
στο «ΘΕΑΤΡΟΝ» Κέντρο Πολιτισμού «Ελληνικός Κόσμος», στην Αθήνα.

Χρήστος Παξιμαδάς	Αλεξία Κατσαρού	Δημήτριος Κούβελας	Θεοδώρα Ειρήνη Σιαλβέρα
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