AMERICAN UNIVERSITY OF BEIRUT

QUANTIFICATION OF ANTIOXIDANTS AND ANTIOXIDANT CAPACITIES OF LEBANESE COMPOSITE DISHES

by
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A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Nutrition and Food Sciences
of the Faculty of Agricultural and Food Sciences
at the American University of Beirut

Beirut, Lebanon
April 2019

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ACKNOWLEDGMENTS

First and foremost, I would like to thank God for giving me the grace, privilege, patience and determination to successfully complete my Master's Degree.

Above all, I would like to thank my dad and mom for the tremendous sacrifices and support to achieve excellent education. For this and much more, I owe this achievement to them.

My recognition and gratitude are addressed to my advisor and mentor, Dr. Imad Toufeili, for offering me this valuable opportunity and believing in my abilities. Thank you for your guidance and patience.

I would like to deeply acknowledge my co-advisors, Dr. Lara Nasreddine and Dr. Nada El Darra, for their insightful comments, assistance and overall contribution to my thesis.

Last but not least, I would like to thank my friends and colleagues for their support, motivation and faith throughout my journey.

AN ABSTRACT OF THE THESIS OF

Marwa Abdul Rahman Itani for Master of Science
Major: Food Technology

Title: Quantification of Antioxidants in Lebanese Composite Dishes and Assessment of Antioxidant Intakes by the Lebanese Population

Thirty-two Lebanese composite dishes were prepared according to procedures commonly followed in the Middle East and their contents of extractable polyphenols (EPP) were quantified by the Folin-Ciocalteu procedure on the aqueous-organic extracts. The non-extractable proanthocyanidins (NEPA), in the residues after extraction, were measured colorimetrically. Moreover, the antioxidant capacities (AC) of the dishes were assessed by the trolox equivalent antioxidant capacity (TEAC) and the ferric reducing ability of plasma (FRAP) assays.

The total EPP contents of the analyzed dishes ranged between 10.96 and 455.80 gallic acid equivalents/100g fresh weight (FW). The values of non-extractable proanthocyanidins (NEPA) ranged from 15.20 to 2103.29 mg/100g FW. It was noted that NEPA was found at higher levels than EPP in the analyzed dishes. The antioxidant capacity of the samples as determined by FRAP was in the range of 0.04 and 3.19 mmol Fe (II)/kg FW and the TEAC from 0.15 to 14.8 mmol/kg FW. The vegetable-based dishes (List the dishes here) contributed most to the dietary antioxidant capacity (DAC) using ABTS assay (50.45%) while the stuffed vegetable-based dishes showed the highest contribution (34.60 %), among the tested groups, using the FRAP assay.

Significant correlation was found between the FRAP value and the EPP content thereby suggesting phenolic compounds could be one of the major constituents responsible for the reducing ability of these dishes. Furthermore, a weak correlation was found between FRAP and ABTS signifying that the compounds capable of reducing oxidants could be different from those scavenging free radicals in the studied dishes. This study has shown that the NEPA fraction contributes significantly to the total antioxidant intake. The generated data will have a pivotal role in assessing the antioxidant quality of Lebanese, and related Mediterranean, diets and in identifying dietary items with high AC for improving the dietary antioxidant intakes of populations.

CONTENTS

ACKNOWLEDGEMENTS v

ABSTRACT..... vi

LIST OF ILLUSTRATIONS..... ix

LIST OF TABLES..... x

LIST OF ABBREVIATIONS..... xi

Chapter

I. INTRODUCTION..... 1

II. LITERATURE REVIEW 3

A. Background on the Lebanese Diet..... 3

B. Types and distribution of polyphenols in foods..... 6

C. Quantification of total phenolic content in foods 11

D. Total antioxidant capacities assays 15

1.FRAP 18

2. ABTS..... 19

E. Factors affecting antioxidant contents and their activities in food..... 20

1. Differences between varieties..... 21
2. Environmental factors and Degree of ripeness..... 21
3. Storage 22
4. Culinary Practices 23

III. MATERIALS AND METHODS 26

A. Selection of Food Products and Sampling..... 26

B. Chemical Analyses..... 29

1. Determination of extractable polyphenols ……..…….. 30
2. Non-extractable Proanthocyanidins …… ……..…….... 31
3. Antioxidant Assays…….... 31
a. Ferric reducing antioxidant 31
b. 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic 32
4.Estimation of % contribution to dietary antioxidant capacity…..… 33
5.Statistical Analysis…….. 33

IV. RESULTS AND DISCUSSION……..... 34

A. Extractable polyphenol content in Lebanese composite dishes…..... 34

B. Non-extractable Proanthocyanidins content in Lebanese composite dishes…… 35
C. Antioxidant capacities of Lebanese composite dishes……... 39

D. Estimation of % contribution to dietary antioxidant capacity (DAC)…..... 44

V. CONCLUSION AND RECOMMENDATIONS …….. 47

Appendix

I. MOISTURE CONTENT VALUES..... 50

II. CALIBRATION CURVES..... 52

BIBLIOGRAPHY..... 54

ILLUSTRATIONS

Figure		Page
1.	Chemical structures of different classes of polyphenols.....	7
2.	The concept of antioxidant/oxidant imbalance and the development of oxidative stress.....	16
3.	Redox reaction for ferric complex in the FRAP assay.....	18
4.	Redox reaction for ABTS+• in the ABTS assay.....	19
5.	Diagram of determinations performed for quantification of EPP and NEPA.....	29
6.	Percent contribution of the various food groups to the dietary antioxidant capacity of the Lebanese diet.....	46

TABLES

Table		Page
1.	Annual and daily per person of selected dishes from the National Food Consumption Database.....	27
2.	Extractable polyphenols and non-extractable proanthocyanidins contents in the most frequently consumed composite dishes in Lebanon and breads.....	38
3.	Mean and percentage antioxidant capacity from FRAP and ABTS assays of the most frequently consumed composite dishes and breads in Lebanon.....	41
4.	Correlation coefficients between the extractable polyphenols and the antioxidant capacities measured by the FRAP and TEAC assays……..	43
5.	Percent contribution of the different Lebanese dishes to the dietary antioxidant capacity (DAC) of the Lebanese diet based on data obtained by FRAP and ABTS assays……..	45

ABBREVIATIONS

Et al	Et allii (and others)
v/v	Volume/Volume
˚C	Degree Celsius
Min	Minutes
h	hour
g	Grams
GAE	Gallic Acid Equivalents
mmol	Millimole
mL	Milliliters
µL	Microliters
µM	Micromolar
mm	millimeter
nm	nanometer
kg	kilogram
HCl	Hydrochloric acid
$FeCl_3$	Ferric chloride
EPP	Extractable polyphenols
NEPP	Non-extractable polyphenols
NEPA	Non-extractable proanthocyanidins
HPP	Hydrolysable polyphenols
rpm	revolutions per minute
FW	fresh weight of cooked food
TE	trolox equivalents
i.e.	in example
S.D.	Standard deviation

CHAPTER I

INTRODUCTION

The Lebanese diet is comprised predominantly of a group of cooked vegetable-based composite dishes along with fresh fruits and vegetables and processed cereals chiefly in the form of bread. People in Lebanon recognize their cuisine as distinctive and expressive of their identity. The Lebanese traditional diet includes different varieties and proportions of plant food, olive oil, fresh fruits, legumes, whole dairy products, wild edible plants, fish, poultry and meat (Farah Naja et al., 2014). Therefore, it is considered as a variant of the typical Mediterranean Diet that is actively promoted for its positive health effects.

Recent reports suggest that the Lebanese cuisine is witnessing a transformation to a more westernized-type of diet characterized by high consumption of fast food sandwiches, pizzas and pies, carbonated beverages, desserts, fried foods and mayonnaise (Hwalla, Naja, & El Labban, 2018). Nevertheless, the traditional Lebanese diet still retains many of its Mediterranean-type-diets attributes with their positive impact on longevity, obesity, non-communicable diseases, diabetes, and many others (Nasreddine et al., 2014). The positive health effects of this diet are attributed to its

relatively-high levels of the polyphenolic antioxidants (Obrenovich, Nair, Beyaz, Aliev, & Reddy, 2010).

The Mediterranean-style diet has been reported to benefit human health by reducing morbidity caused by cardiovascular diseases and colorectal cancer (Farinetti, Zurlo, Manenti, Coppi, & Mattioli, 2017). In addition to their content of vitamins and minerals, plant-based diets are rich in polyphenols with antioxidative capacities capable of reducing oxidative stress in cells (Fraga, Croft, Kennedy, & Tomás-Barberán, 2019). Consumption of Mediterranean diet mitigates the harmful effects of oxidative stress on glands (Shively et al., 2018). Further, adherence to diets rich in antioxidants and mono-saturated and poly-saturated fatty acids may decrease the risk of developing neurodegenerative diseases (Dohrmann et al., 2018) and are useful in complementing antidepressants in depression treatments (Masana et al., 2018).

In view of the beneficial health effects of plant-based diets it is imperative to generate data on the antioxidant capacity of the commonly-consumed plant-based items in the Mediterranean-type Lebanese diet. No data exists on the content of phenolic antioxidants and antioxidant capacity of the composite dishes consumed in Lebanon. Accordingly, the purpose of this study was to quantify the amounts of extractable and non-extractable antioxidants in the most consumed Lebanese composite dishes. Another aim was to determine the antioxidant capacity of the Lebanese composite dishes and their percentage contribution to the dietary antioxidant capacity of the Lebanese diet.

CHAPTER II

LITERATURE REVIEW

A. Background on the Lebanese Diet

Lebanon, a highly urbanized Mediterranean country, is renowned for its healthy traditional cuisine. The Lebanese diet comprises an intangible cultural heritage that expresses the culinary knowledge gathered by the Lebanese people over centuries. In addition, this cuisine reflects a unique interaction with the Babylonians, Phoenicians, Egyptians, Greeks, Romans, Persians, Byzantines, and Turks (Hwalla & El Khoury, 2008).

The Lebanese diet is a set of minimally processed vegetarian recipes and a profusion of vegetables, fruits, legumes, cereals, and nuts. A number of workers (Cowan, 1965; Cowan, Chopra, & Houry, 1964; Sabry, 1961) characterize the Lebanese diet as: a plenty of plant food (e.g. fruit, vegetables, breads, other forms of cereals, potatoes, legumes, nuts and seeds); minimally processed fresh fruit as the typical daily dessert with sweets containing concentrated sugars or honey consumed several times per week; olive oil as the major source of fat, replacing other oils such as butter and margarines; low to moderate consumption, on daily basis, of dairy products (e.g., cheese and yogurt); wild edible plants, such as akkoub (tumbleweed), babounij (camomile),

farfahin (green purslane), hindbeh barrieh (chicory), khubbaizeh (mallow), qursaaneh (cryngo), sumac (sumach), and zaatar bari (wild thyme)) used in many of the traditional dishes; low intake of fish, poultry and red meat; and low to moderate consumption of wine. Thus, this traditional cuisine offers many items typical to the Mediterranean Middle Eastern Diet, known by its positive correlation with health.

Large-scale studies on the Mediterranean diet have demonstrated the positive impact of this type of diet on longevity and prevention of morbidity (Trichopoulou & Critselis, 2004; Visioli, Bogani, Grande, & Galli, 2005; Visioli, Grande, Bogani, & Galli, 2004). Interest in the Lebanese diet has risen from its healthy Mediterranean characteristic, which appears to have provided an effective measure for protection against non-communicable diseases (Noah & Truswell, 2001). According to a study done by (Ruxto, 2004), the consumption of dark green leafy vegetables (e.g., spinach and purslane), as well as nuts and seeds provide ω-3 fatty acids that contribute to the reduction in the risk of heart diseases, depression and inflammatory infections. The study of (James, Muir, Curtis, & Gibson, 2003) showed that vegetables, fruits, legumes, pulses, and nuts supply fiber which offers protection against cancer (mainly colon cancer), heart disease, diabetes, and constipation. Other studies have shown that foods such as parsley, lentils, wheat, olive oil, thyme, onions, tomato, citrus fruits, tea, beans, nuts, wheat, celery, chickpeas, lentils and other legumes provide protective effects against many inflammatory conditions, and possess antioxidant and anticancer

potentials (James et al., 2003; Liao, 2002) . (Innami, Nakamura, Tabata, Wada, & Takita, 1995) concluded that it exists a strong association between the consumption of melokhia (*Chorchorus olitorius* or Jew's mallow), a rich source of soluble dietary fiber, and the reduction in the levels of cholesterol and the increase in the excretion of bile acids and neutral sterols. As reported by (Arora, Tripathi, & Shukla, 2005), organosulfides can act as potential chemopreventive agents with antioxidant, antimicrobial and antimutagenic activities. These are found in garlic and onions, the essential flavor ingredients in the Lebanese cuisine.

Although the Lebanese cuisine has retained many of its Mediterranean properties, globalization, urbanization and mechanization have had their impacts in transforming the Lebanese diet to a more westernized-type of diet rich in saturated fat, added sugar and animal protein and low in dietary fiber, fruits, vegetables, whole wheat breads and cereals (Baba, 2000; Mehio Sibai et al., 2010) . This phenomenon, known as nutrition transition, is highly related to the rapid shifts in mortality and morbidity due to the higher incidence of cardiovascular diseases (CVDs) that represent 60% of all-cause mortality in persons aged 50 years and older (Sibai, Fletcher, Hills, & Campbell, 2001). Furthermore, a study done by (Vazzana, Santilli, Sestili, Cuccurullo, & Davi, 2011) has proven the association of obesity with increased mortality and morbidity from CVDs. This association between obesity and the "Westernized" dietary pattern is characterized by the high intake of fat fast foods, sweets and soda drinks (F. Naja et al., 2011;

Nasreddine et al., 2014). In addition, a study on Lebanese adults showed the increased risk of hypertension and associated cardiovascular disease, such as stroke and coronary heart diseases, due to excessive sodium intakes (He & MacGregor, 2010). Of more concern is the high prevalence of obesity and low adherence to a Mediterranean diet pattern that were reported in a study done on rural, poor and aged Lebanese population with low income and limited academic education (Issa et al., 2010). These findings strongly suggest that Lebanon, a middle-income developing country of the Eastern Mediterranean basin, is experiencing nutrition transition not only in urban settings, but also in rural areas (Batal & Hunter, 2007). Hence, despite maintaining some of its traditional aspects, the Lebanese diet is being influenced by modernity and consumption of more sugar, fast food, meat-based products, frozen food and industrialized products (Mouawad, 2004).

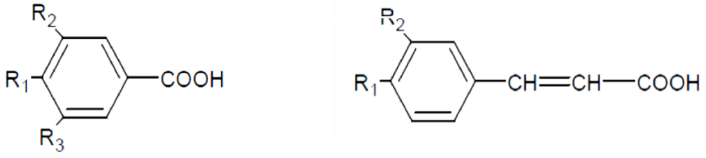
B. Types and distribution of polyphenols in foods

Natural polyphenols are largely found in fruits, vegetables, cereals, and beverages. In food, they contribute to the bitterness, astringency, flavor, color, odor and oxidative stability (Pandey & Rizvi, 2009). These compounds are broadly distributed in the plant kingdom and belong to the class of secondary metabolites synthesized during the normal development of plants. Plant polyphenols act as antioxidants which are largely responsible for the positive health effects of plant-based diets . Polyphenols also possess antiaging, antimicrobial and anti-mutagenic properties which contribute to the

protective effects of plant-rich foods against chronic diseases and the ability of their phytochemicals to act as chemo-preventive agents for lung cancer (Pandey & Rizvi, 2009) (Amararathna, Johnston, & Rupasinghe, 2016; Del Rio et al., 2013).

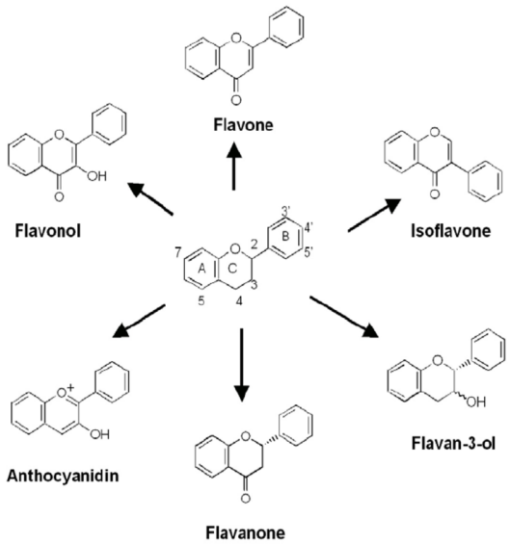
Chemically**,** phenolics are compounds made up of one or more aromatic rings with one or more hydroxyl groups. They are derived from the amino acids phenylalanine and tyrosine and exist as glycosides with different sugar molecules. Phenolics can range from simple molecules to highly polymerized compounds and are into different groups viz. phenolic acids, flavonoids, stilbenes and lignans (Dai & Mumper, 2010) (Fig. 1)

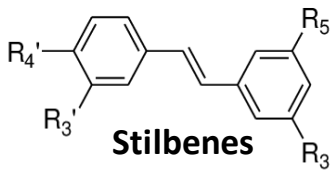
Phenolic acids



Benzoic:	$R_1=R_2=R_3=H$	Cinnamic:	$R_1=R_2=H$
p-hydroxy-benzoic:	$R_1=OH;\ R_2=R_3=H$	Caffeic:	$R_1=R_2=OH$
p-bromo-benzoic:	$R_1=Br;\ R_2=R_3=H$		
p-cyano-benzoic:	$R_1=CN;\ R_2=R_3=H$		
p-chloro-benzoic:	$R_1=Cl;\ R_2=R_3=H$		
Vanillic:	$R_1=OH;\ R_2=CH_3O;\ R_3=H$		
Gallic:	$R_1=R_2=R_3=OH$		

Flavonoids





Resveratrol: $R_4' = OH, R_3' = H, R_3 = R_5 = OH$
Piceatannol: $R_3' = R_4' = R_3 = R_5 = OH$
Pinosylvin: $R_3 = R_5 = OH$

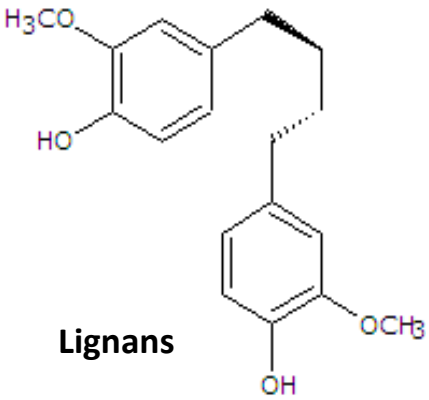


Figure 1. Chemical structures of different classes of polyphenols (Dai & Mumper, 2010).

Phenolics in plants are distributed in variable proportions at the tissue, cellular and subcellular levels. Soluble phenolics are found within the plant cell vacuoles, while the insoluble ones are present in cell walls. The outer layers of plants contain higher levels of polyphenols which are predominantly bound to the other cell components (Scalbert, Rémésy, Morand, Jiménez, & Manach, 2004) and offer these structure strength and ability to combat pathogens (Lattanzio, M T Lattanzio, & Cardinali, 2006).

Flavonoids are the most numerous polyphenols in our diets with more than 4,000 derivatives having been identified (de Groot & Rauen, 1998). Amongst thee flavonoids, catechins are found in high proportions in green tea (up to 800 mg/L), chocolate (up to 600 mg/L), red wine (up to 300 mg/L), cherry (250 mg/kg fresh weight) and apricots (250 mg/kg fresh weight) (D'Archivio et al., 2007). Quercetin conjugates were found in significant levels in broccoli, red-pigmented and green leaf lettuce. Onions are also rich sources of flavonoids with mainly quercetin in the skin and anthocyanins in the outer fleshy layer and the red onion skin (Crozier, Lean, McDonald, & Black, 1997; M Susan DuPont, Zofia Mondin, Gary Williamson, & Keith R Price, 2000). Anthocyanins are water-soluble pigments responsible for the coloring of fruits and vegetables and are widely distributed in fruits, red wine (350 mg anthocyanins/L), and in certain vegetables (radishes, beans, cabbages…)(Es-Safi, Cheynier, &

Moutounet, 2002). In tomato, flavonols are the predominant phenolics, present mainly in the skin (Stewart et al., 2000). Different varieties of flavonoids, in addition to phenolic acids, have been found in high levels in spinach; the 'Brain Food' needed to avoid memory loss and Alzheimer (Clarke, 1999; Howard & Pandjaitan, 2008). Proanthocyanidins, known as condensed tannins, are common in many fruits, such as grape, apple or cocoa, and are responsible for the astringent character of fruits and the bitterness of chocolate. These compounds are the main causes of haze in beers prepared from the barley grain (Rasmussen, Frederiksen, Struntze Krogholm, & Poulsen, 2005).

The phenolic acids are divided into two classes: hydroxybenzoic acids (such as gallic acid and protocatechuic acid) and hydroxycinnamic acids (mainly caffeic acid and ferulic acid). Gallic acid is the predominant phenolic acid in tea with levels up to 4.5 g/kg fresh weight of tea leaves (Tomás‐Barberán & Clifford, 2000) while protocatechuic acid is the chief acid in raspberries with concentrations of up to 100 mg/kg fresh weight (Shahidi & Naczk, 1995).

Caffeic acid is the most widespread phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which represents the main phenolic compound in coffee. Caffeic acid is the chief phenolic acid in kiwis with concentrations up to 1g / Kg fresh weight (D'Archivio et al., 2007) and ferulic acid is the most abundant in cereals where it undergoes esterification to give hemicelluloses, which are deposited in the cell walls. In wheat kernels, ferulic acid

accounts for 90% of the total polyphenols and is also the predominant phenolic acid in barely seeds and barley brans (Renger & Steinhart, 2000).

The occurrence of stilbenes in human diet is quite low except in grapes and their products with red grapes reportedly containing 50-100 g/kg in the fresh skin. Most stilbenes have antifungal properties and protect the plants against fungal infections (Hsieh, Wang, Hamby, & Wu, 2005). Further, certain classes of stilbenes possess strong antioxidant, anti-cancer and anti-inflammatory properties (Seyed, Jantan, Bukhari, & Vijayaraghavan, 2016).

Lignans are diphenolic compounds formed by the dimerization of two cinnamic acid residues with the richest dietary source being linseeds, which contain up to 3.7 g/kg dry weight. Minor sources of lignans include garlic, asparagus, carrots, pears and lentils (Thompson, Robb, Serraino, & Cheung, 1991) while olives and virgin olive oils contain significant amounts (Owen et al., 2004). These of polyphenols are considered as phytoestrogens (Adlercreutz & Mazur, 1997) and have shown to inhibit the growth of cancer cells in the skin, breast, colon and lung (de Torres et al., 2018).

It is noteworthy that polyphenolic compounds exhibit synergism showing more enhanced effects in reducing the risk of various degenerative diseases than the effects of the individual polyphenols (de Kok, van Breda, & Manson, 2008). The interactions between the polyphenols found in the diet with other phytochemicals, pharmaceuticals and therapies provide synergistic enhanced beneficial effects (Brglez Mojzer, Knez

Hrnčič, Škerget, Knez, & Bren, 2016). Even though several polyphenols exert anticancer activities on their own, numerous studies have shown that treatment with a combination of polyphenols is more efficacious in inhibiting cancer formation and growth than treatment with a single polyphenol (Fantini et al., 2015; Lewandowska, Kalinowska, Lewandowski, Stępkowski, & Brzoska, 2016).

C. Quantification of total phenolic content in foods

Among approaches for controlling oxidative stress and non-communicable diseases risks, the use of antioxidant-rich extracts from plants, or phenolic/polyphenolic compounds, is the most effective, appropriate and economical mean. According to (Shahidi & Zhong, 2015), antioxidants are substances that when present at low concentrations in the food or in the body, control, delay or prevent oxidative damages leading to food quality deterioration or initiation and propagation of degenerative diseases in the body. Effective antioxidants can be free radical scavengers, singlet oxygen quenchers, metal ion chelators, quenchers of secondary oxidation products, neutralizers of peroxides and other reactive oxygen species (ROS), and inhibitors of pro-oxidative enzymes. Therefore, dietary antioxidants exert their inhibitory effect against oxidation processes through different mechanisms and with varied activities (Shahidi & Zhong, 2007).

The extraction of bioactive compounds from plant materials is essential for efficient analysis and quantification of phenolic contents of foods. The extraction of

polyphenols is affected by several parameters including the particle size of the samples, type of solvent used, solute to solvent ratio, agitation rate, mass transfer efficiency and temperature (Dai & Mumper, 2010). The preparation step is crucial as it affects the accuracy and repeatability of the analyses (Zhao, Lv, Chen, & Li, 2011). For extraction of phenols, the plant samples are subjected to milling, grinding and homogenization followed by air-drying or freeze-drying. These steps disrupt the cell walls of the plant materials and yield finely-powdered samples with larger surface areas suitable for maximal extraction of polyphenols especially when freeze-drying is employed in sample drying (Abascal, Ganora, & Yarnell, 2005). Within this framework, freeze-dried marionberries, strawberries and corn consistently exhibited higher total phenolic contents than samples prepared by air drying (Asami, Hong, Barrett, & Mitchell, 2003).

The use of the right solvent for extraction is crucial for maximal recovery of phenols from food samples. To this end, the solubility of phenolic compounds is highly influenced by the chemical nature of the plant material and the polarity of the solvents used. The solvent used should be able to extract the diverse groups of simple compounds (e.g., anthocyanins, phenolic acids) and highly polymerized substances (e.g., tannins) that might be present in the samples. Moreover, the phenolics can be associated with other plant components such as carbohydrates and proteins, and/or the extracts may contain non-phenolic substances such as fats, acids and chlorophylls which require additional steps in the treatment of samples and removal of interfering

compounds (Dai & Mumper, 2010). Solvents, like methanol, acetone, propanol, ethanol, ethyl acetate and their combinations have been used, often with different proportions of water for the extraction of polyphenols. Methanol, in particular, has been found to be more effective in extracting lower molecular weight polyphenols while acetone is more efficient for the extraction of high molecular weight flavanols (Metivier, Francis, & Clydesdale, 1980). Addition of acids, commonly ~1.0 % hydrochloric acid, is recommended because acidic conditions promote denaturation of the cell membranes and dissolve and stabilizes the anthocyanins, simultaneously (Nicoue, Savard, & Belkacemi, 2007). Therefore, it is of critical importance to select efficient extraction procedures which maximize the quantity and maintain the stability of the extracted phenolic compounds.

The data on total polyphenols, reported in the literature, normally refers to the extractable polyphenols (EPP) that are present in the supernatant after centrifugation of the solvent-treated samples. However, significant amounts of bioactive compounds, referred to as the non-extractable polyphenols (NEPP), remain in the residue left after separation of the supernatant (Hellström & Mattila, 2008). The NEPP fraction includes both hydrolysable polyphenols (HPP) and non-extractable proanthocyanidins, (NEPA). The EPP fraction is comprised chiefly of low molecular weight compounds including flavanols, hydrolysable tannins, phenolic acids which are soluble in aqueous-organic mixtures and are potentially bioavailable in the small intestine. In contrast, HPP, mainly

hydrolysable tannins, are low molecular weight phenolic compounds that are strongly associated with polysaccharides or proteins while NEPA are primarily high molecular weight structures chiefly oligomeric proanthocyanidins. The HPP and NEPA are not amenable to extraction by the commonly-employed aqueous-organic solvents and require chemical or enzymatic treatment of the residues to release the polyphenols from the food matrix for spectroscopic analysis; the HPP and NEPA fractions are accessible and bioavailable only in the large intestine (Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2013; Saura-Calixto, 2012). Although studies of the health effects of NEPP are limited, there are indications that these compounds contribute positively to gastrointestinal health and protect against cardiovascular diseases. The positive health effects of the NEPP fraction spurred interest in quantifying their levels in foods and available studies indicate that the NEPP are the main phenols in certain foods accounting to 57% of the total polyphenol contents of fruits and vegetables (Arranz, Saura-Calixto, Shaha, & Kroon, 2009).

The quantification of the total phenolic compounds in plant extracts is most-often executed by the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). This method, which is based on electron transfer between the F-C reagent and the phenolic compounds, was initially used in the analysis of proteins (Folin & Ciocalteu, 1927) and later adapted to the quantification of total phenols (Singleton et al., 1999). In the assay, reduction of the F-C reagent by the phenolic compounds under

alkaline conditions generates a blue–colored chromophore with maximum absorption at 765 nm (Magalhães, Segundo, Reis, & Lima, 2008). The F-C reagent also interacts with other reducing non-phenolic compounds, thus leading to an overestimation of the total phenolic contents. Gallic acid is the most-widely used reference for the expression of total phenolic contents with levels often reported in milligram of gallic acid equivalent per kilogram or liter of extract or plant. The F-C spectrophotometric assay provides simple, convenient, fast and reproducible method for the quantification of the total phenolic contents in crude plant samples (Dai & Mumper, 2010).

D. Total antioxidant capacities assays

There has been an increasing interest in the study of the antioxidant profiles of different foods and their antioxidant potential due to their physiological effects in the human body. Antioxidants have the ability to inhibit the oxidation processes precipitated by the reactive oxygen species (ROS) which include superoxide ($O_2 \bullet^-$), hydroxyl (OH•) and peroxyl (ROO•) radicals and hydrogen peroxide (H_2O_2) produced during normal cellular metabolism (Sánchez-Moreno, 2002). Excessive levels of ROS can overwhelm protective enzymes such as superoxide, catalase and peroxidase and predispose membrane lipids, DNA, cellular proteins and enzymes to oxidation and,

ultimately, the development and progression of degenerative diseases (Michael Antolovich, Paul D. Prenzler, Emilios Patsalides, McDonald, & Robards, 2001).

Moreover, oxidation is considered the major causes of chemical spoilage that enhance the rancidity and deterioration of the nutritional quality, flavor, texture, color and safety of foods (Kong & Singh, 2016). The increased levels of ROS lead to oxidative stress that is characterized by an imbalance in the antioxidant status (Fig. 2). Accordingly, presence of adequate levels of antioxidants to counteract the harmful effects of excessive oxidations that are essential for the control of oxidative damage (Poljsak, 2011).

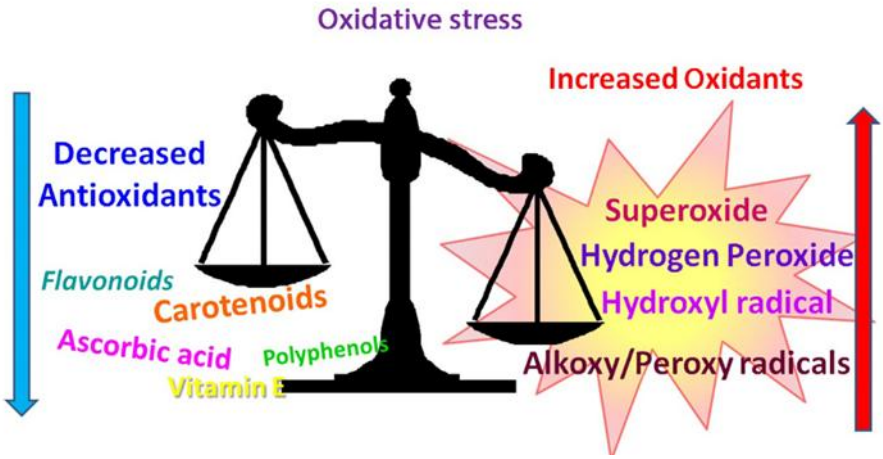


Figure 2. The concept of antioxidant/oxidant imbalance and the development of oxidative stress (F.F. Benzie & Choi, 2014).

Due to the ability of antioxidants to contain oxidative damage of biological tissues, attention has increasingly been directed towards the study of the antioxidant capacity of natural products, such as vegetables, fruits, beverages, etc. as they are inversely associated with the risk of developing some pathologies like cardiovascular diseases (Hung et al., 2004). The efficacy of antioxidants is influenced by their structural features, concentration, temperature, type of oxidation substrate, and physical state of the system, as well as the presence of pro-oxidants and synergists (Zhong & Shahidi, 2015). A positive correlation between phenolic compounds and antioxidant capacity has been demonstrated (Velioglu, Mazza, Gao, & Oomah, 1998). The activity of these compounds is due to their aility to chelate metals, inhibit lipoxygenase, and scavenge free radicals like superoxide, hydroxyl radical and other reactive species (Decker, 1997; Seifried, Anderson, Fisher, & Milner, 2007).

Several assays have been used to estimate the antioxidant capacities in foods and beverages. They measure the reducing ability of antioxidants by means of electron transfer (ET) and/or by means of hydrogen atom transfer (HAT). The ET mechanism of antioxidant action with a biologically relevant radical is as follows (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016a):

$$ROO^{\bullet} + AH/ArOH \longrightarrow ROO^{-} + AH^{\bullet+}/ArOH^{\bullet+} \quad (1)$$

$$AH^{\bullet+}/ArOH^{\bullet+} + H_2O \longleftrightarrow A^{\bullet}/ ArO^{\bullet} + H_3O^{+} \quad (2)$$

$$ROO^{-} + H_3O^{+} \longleftrightarrow ROOH + H_2O \quad (3)$$

Hydrogen atom transfer (HAT) assay measures the capacity of the antioxidant to quench free radicals by donating H atom. Folin-Ciocalteu and Ferric-Reducing Antioxidant Power (FRAP) methods are ET- based methods that measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced (Cömert & Gökmen, 2018). 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay is usually classified as ET reaction, however, ABTS radical can be deactivated either by radical quenching via HAT or by direct reduction through ET mechanism (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016b). The degree of color change is recorded by spectrophotometer. Each of the mentioned assays uses different chromogenic redox reagents with different standard potentials (Apak et al., 2016a)The absorbance gets plotted against the antioxidant concentration in order to construct a linear curve. The slope of this linear curve reflects the reducing capacity (Alvarez-Suarez, Tulipani, Romandini, Vidal, & Battino, 2009).

1. FRAP

The FRAP test measures the reducing power of an antioxidant in terms of its ability to convert the Fe^{3+} complex of tripyridyltriazine [$Fe(TPTZ)^{3+}$] to the intensely blue-colored Fe^{2+} complex [$Fe(TPTZ)^{2+}$] , under acidic conditions (Tai, Sawano, Yazama, & Ito, 2011) (Figure 3). The amount of iron reduced is correlated with the amount of antioxidants present in the sample which is expressed in micromolar Fe^{2+} equivalents.

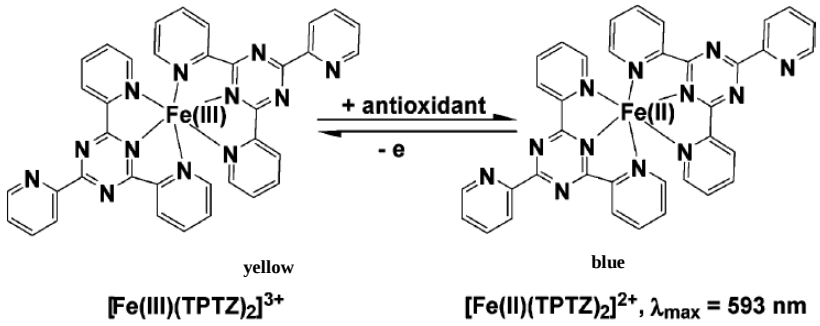


Figure 3. Redox reaction for ferric complex in the FRAP assay (Huang, Ou, & Prior, 2005).

The FRAP method is simple, rapid, inexpensive and robust, does not require specialized equipment and can be performed using automated, semiautomatic or manual methods (Michael Antolovich et al., 2001; Prior, Wu, & Schaich, 2005; Valgimigli, 2015). However, false results may be obtained in several cases; for example, compounds with

redox potential lower than that of the pair Fe(III)/Fe(II), which have no antioxidant activity, contribute falsely to a high FRAP value. Moreover, OH• can be generated from H_2O_2 into the reaction medium due to the continuous production of Fe (II), which may result in interferences (Benzie & Strain, 1999).

2. ABTS

In this assay, ABTS is oxidized by potassium persulfate to the intensely colored cationic form, ABTS+• (Pellegrini et al., 2003). This test measures the ability of hydrogen or electron donating antioxidants to scavenge ABTS+• thereby leading to a reduction in the color intensity.

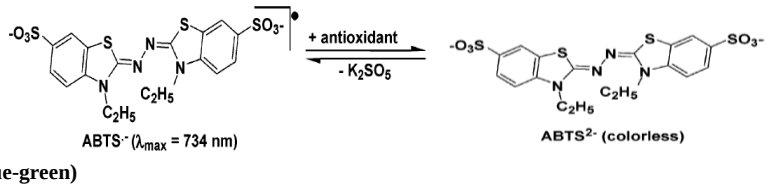


Figure 4. Redox reaction for ABTS+• in the ABTS assay (Huang et al., 2005).

Spectrophotometric measurements are done at the wavelength 734 nm with minimal interferences from other absorbing components and/or due to sample turbidity and results are expressed in terms of Trolox equivalents (Re et al., 1999). Many phenolic compounds can react with ABTS+• due to their low redox potentials and many

food products have been reported to interact rapidly with ABTS+•, typically within 30 min and over a wide range of pH. Moreover, ABTS+• is not influenced by ionic strength and soluble in both organic and aqueous solvents and, accordingly, it can be used in various media to determine both hydrophilic and lipophilic antioxidant capacities of food extracts (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003).

E. Factors affecting antioxidant contents and their activities in food

In the three last decades, interest in the study of polyphenols has risen due to their potent antioxidant properties, their relative abundance in our diets and their ability to ameliorate the risks of various non-communicable diseases. The health effects of polyphenols from food sources depend on the amount consumed and their activity within the human's body (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004).

Distribution of polyphenols is not uniform among plants and shows significant variations within the same plant. The polyphenol content of foods is significantly affected by numerous factors including:

1. Differences between varieties

Certain polyphenols are found in all plant products (fruits, vegetables, cereals, beverages…), such as quercetin, whereas others are only found in specific foods such as flavanones in citrus foods and isoflavone in soya. While the profiles of phenols tend to similar amongst varieties of apples, the polyphenol contents in cider apples have been

reported to range from 0.1-5g total polyphenols/kg fresh wt and may reach up to 10 g/kg in some varieties (D'Archivio et al., 2007; Manach et al., 2004).

2. Environmental factors and Degree of ripeness

The polyphenolic content of foods is affected markedly by environmental factors. These factors can be pedoclimatic (soil type, sun exposure, rainfall, etc.) or agronomic (different types of culture, fruit yield per tree, etc.). In particular, the exposure to sunlight has a considerable effect on most flavonoids. According to (Pandey & Rizvi, 2009), the response of plants to stress is mediated primarily via the phenolic acids which possess antimicrobial potentials and increase in concentration after infections and they further contribute to healing by lignification of damaged areas. Further, strawberries, blackberries and corn have higher polyphenolic content when produced by organic or sustainable agriculture than under the relatively-less stressful conventional and hydroponic conditions. This finding is due to the fact that organically and sustainably grown products are produced by cultural methods utilizing no or little amounts of pesticides, thus leading to the release of higher levels of phenolic compounds under pathogenic pressures (Asami et al., 2003). The degree of ripeness affects the polyphenolic compositions of plant products with decreases in concentration of phenolic acids and increases in the levels of anthocyanins being reported for fruits (Pandey & Rizvi, 2009).

3. Storage

The levels of polyphenols in foods decrease upon storage due to oxidative reactions. These reactions play a key role in altering the color and the organoleptic characteristics of plant products due to the formation of either more or less polymerized substances. Such changes may be desirable, as in black tea, or detrimental to consumer acceptability, as in the case of browning of fruits and vegetables (M. Susan DuPont, Zofia Mondin, Gary Williamson, & Keith R. Price, 2000; Sluis, Dekker, Jager, & Jongen, 2001). The changes in polyphenols contents are sensitive to storage temperatures; thus while cold storage had no effect on the polyphenols in apples, pears and onions (Price, Bacon, & Rhodes, 1997; Spanos & Wrolstac, 1992), a 60% loss of quercetin and a total loss of procyanidins were observed upon storage of apple juice for 9 months at 25 ˚C (Miller, Diplock, & Rice-Evans, 1995). A 70% decrease in phenolic acids was observed upon storage of wheat flour for 6 months as compared to that of fresh samples (Sosulski, Krygier, Krygier, & Hogge, 1982).

4. Culinary Practices

Most vegetables are cooked before consumption. Although cooking kills the microorganisms and makes the food safer, some cooking practices lead to losses in vitamins, micronutrients and phytochemicals (Chang, Prasad, & Amin, 2013). Within this framework, the type of vegetable and the cooking method are the main factors that

contribute most to the changes in the nutritional values, total phenolic contents and antioxidant capacities of the cooked plant (Bernhardt & Schlich, 2006).

In addition to the evident changes changes in the physical characteristics of the cooked material, cooking brings about a host of changes in the chemical makeup of foods influencing the concentration and bioavailability of bioactive compounds in vegetables . A study on cooking of selected vegetables (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008) reported reductions in total phenols from 69.6 mg/100g of dry weight in raw carrots to 39.6 and 48.0 mg/100g of dry weight of steamed and fried carrots, respectively. The most detrimental effect on carrot polyphenols takes place upon boiling where almost all polyphenols were destroyed. These losses are believed to be caused by the diffusion of the polyphenols into the boiling water subsequent to the thermal destruction of the cell walls and subcellular compartments, covalent binding of oxidized phenols and proteins or amino acids and the polymerization of oxidized phenols. Similar losses in polyphenol take place upon exposing courgette (zucchini) and broccoli to different heat treatments. In contrast to the indicated losses in polyphenols, broccoli, carrots and courgette showed significant increases in their antioxidant activities; more specifically, the FRAP value of courgette increased from 2.79 mmol of Fe^{2+}/100g to 7.97 mmol of Fe^{2+}/100g upon frying and the TEAC exhibited similar trends upon cooking the 3 vegetables. The increased antioxidant capacity observed upon cooking has been attributed to the possibility of formation of new molecules, most

notably through the Maillard reaction, with high antioxidant capacities (Miglio *et al*., 2008). In another study, certain vegetables, such as garlic, spinach and zucchini were reported to have exhibited losses in their ABTS radical anion scavenging activity upon boiling of 59.3%, 11.1% and 28.5 %, respectively (Jimenez-Monreal, Garcia-Diz, Martinez-Tome, Mariscal, & Murcia, 2009). The same study reported significant losses in the antioxidant activities of maize, zucchini and Swiss chard upon pressure cooking, and microwaving of maize, and increases in the ABTS radical anion scavenging activity in carrots, celery and green bean upon various heat treatments. Other vegetables, such as beetroot, artichoke, onion, pepper and cauliflower showed no changes after cooking.

According to Jimenez-Monreal *et al*. (2009), the observed increase in antioxidant activity after cooking could be attributed to the production of stronger radical-scavenging antioxidants by thermal chemical reactions, while the suppression of the antioxidative capacity of antioxidants, in some vegetable products, could be due to the thermal inactivation of of oxidative enzymes and/or production of new non-nutrient antioxidants. (Faller & Fialho, 2009) reported significant reduction in the soluble polyphenol contents in boiled onions and potatoes as well as microwaved potatoes, as compared to their raw counterparts, and a decrease in hydrolysable polyphenols during boiling and microwaving of broccoli and white cabbage. Based on this study, unlike soluble polyphenols, the hydrolysable fractions of onions and boiled potatoes maintained or resulted in higher recovery rates compared to the raw vegetables. The

reason behind this incident may be due to the alteration in the chemical composition of the phenolic compounds making them more extractable and easily detectable in the supernatant of the extractable polyphenols (Cohen, Sakihama, & Yamasaki, 2001).

Interestingly, the antioxidant content and activity of tomatoes are retained, or even increased, when cooked. To this end, the total phenolics content of the raw tomato at 142.4 µg/g of tomato increased to 148.7 µg/g of tomato when heated at 88 °C and this was accompanied by a concomitant increase in the antioxidant activity (Dewanto, Wu, Adom, & Liu, 2002). These increases are possibly due to the release of more bio-accessible lycopene after thermal processing and the additive and synergistic effects of other phytochemicals such as the flavonoids (Eberhardt, Lee, & Liu, 2000).

CHAPTER III

MATERIALS AND METHODS

Materials

Selection of Food Products and Sampling

The Lebanese composite dishes analyzed are those contributing to ≥ 85% of total consumption (in grams) of plant based dishes according to the National Food Consumption Survey conducted in 2008-2009 on Lebanese adults (Chamieh et al.). These dishes are classified under six main categories, which are: vegetable-based dishes, legume-based dishes, rice-based dishes, stuffed vegetable-based dishes, wheat-based dishes and breads. Table 1 shows the annual and daily consumption data, based on 24 hr. recall, for the selected composite dishes.

The ingredients of the selected composite dishes were procured from the local market, and the dishes were prepared according to "*ألف باء الطّبخ الموسّع*", (Kamal and Osman, 2015), mimicking the cooking behavior of the Lebanese women, in regards to the texture, flavor and other details during catering. The cooked meals were freeze-dried (Freeze Dryer: LABCONCO, USA), milled to a particle size around 0.85 mm and the resulting powders were then stored at 4°C until analyzed. Freeze drying has been found to have high efficiency for better extractions of polyphenols due to the development of ice crystals that results in greater rupturing of the plant cell structure. Consequently, the solvent can have better access to reach the phenolic compounds (Asami et al., 2003).

Table 1. Annual and daily per person of selected dishes from the National Food Consumption Database developed by the NFSC Department, AUB.

Categories	Dishes	Arabic Names	Kg/person/year	g/day/person
Vegetable-based dishes	tabbouli	Tabbouli	11.25	30.81
	standard salad	Salata	10.50	28.77
	bread salad	Fattoush	4.02	11.02
	Jew's mallow	Yakhnet mulûkhîyah	3.73	10.22
	green pea stew	Yakhnet bazzelah	2.06	5.65
	grean snap beans in oil	Loubieh Rafeia b'zeit	6.43	17.63
	flat green beans beef stew	Loubieh Areeda b'lahme	1.77	4.85
	runner beans beef stew	Loubieh Bedreyeh b'lahme		
	spinach stew	Yakhnet sabanegh	1.05	2.89
Legume-based dishes	rice with lentils	Mgaddara	5.68	15.56
	lima bean beaf stew	Fassoulia Areeda b'lahme	3.73	10.22
	white bean beef stew	Fassoulia Ayshe khanum b'lahme		
	navy bean beef stew	Fasulia snubreyeh b'lahme		
	chickpea salad dip	Himmos b'tehineh	1.56	4.27
	chickpeas	Balila	1.38	3.77
	fava beans	Foul moudamas	3.62	9.92
	lentil soup with rice	Shourbat il 'adas ma' ruz	1.22	3.35
	lima bean stew in oil	Fassoulia Areeda b'zeit	1.10	3.02
	navy bean stew in oil	Fasulia snubreyeh b'zeit		
	white bean stew in oil	Fassoulia Ayshe khanum b'zeit		

Rice-based dishes	rice (basmati)	Ruz Hinde b'shaireye	16.07	44.03
	rice (egyptian)	Ruz Masri b'shaireye		
Stuffed Vegetable-based dishes	stuffed squash	Kussa Mihshi	3.41	9.35
	stuffed grape leaves	Warak inab mihshi	3.22	8.81
	stuffed cabbage	Malfouf mihshi	1.14	3.12
Wheat-based dishes	moghrabieh	Moghrabieh	20.18	55.29
Breads (Wooden Bakery, Moulin D'or and Chamsine)	white bread	Khobez Abyad	36.11	98.92
	brown bread	Khobez Asmar		

Methods

Chemical Analyses

All chemicals and solvents were of analytical grade. Gallic acid, Ciocalteau, ferric chloride, potassium persulfate, sodium acetate trihydrate, TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) and ABTS (2,2'-azino-bis(3ethylbenzthiazoline-6-sulphonic acid), hydrochloric acid, acetone, methanol and 1-butanol were purchased from Sigma-Aldrich. All spectrophotometric analyses were done using Evolution 300 UV-VIS Spectrophotometer (Thermoscientific, UK) using PMMA plastic cuvettes (Sigma Aldrich, Germany).

Extractions were performed in triplicate, using the same cooked dish. Determinations are reported on a fresh weight of cooked food basis (Appendix I, table 1). Results are expressed as mean value ± standard deviation. A diagram summarizing the general procedure is shown in Figure 5.

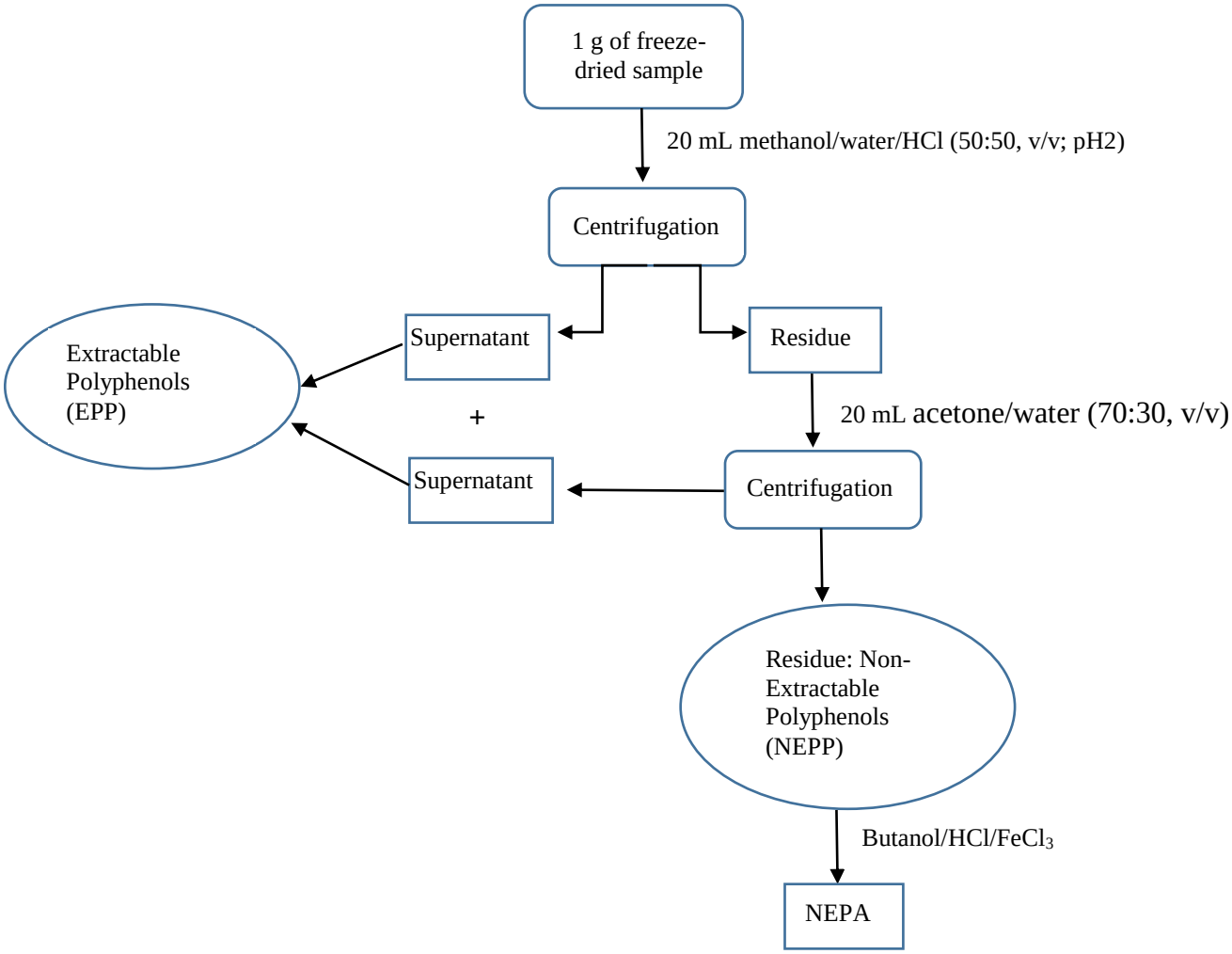


Figure 5. Diagram of determinations performed for quantification of extractable (EPP) and non-extractable polyphenols (NEPP); NEPA, non-extractable proanthocyanidins (Arranz et al., 2009).

1. Determination of extractable polyphenols

The composite dishes were subjected to a two-step aqueous extraction as described by (Arranz et al., 2009). The combination of these steps represents the

optimal conditions for higher extraction of phenolic compounds (Metivier et al., 1980). Briefly, 1g of sample was placed in a capped centrifuge tube, 20mL of acidic methanol/water/HCl (50:50, v/v; pH 2) was added and the tube was thoroughly shaken at room temperature for 1 h. The tube was then centrifuged at 3500 rpm in a Thermo IEC Centra CL3R Refrigerated Centrifuge (Thermo Scientific, USA) for 10 min, and the supernatant was recovered. Twenty mL of acetone/water (70:30, v/v) was added to the residue, and the shaking and centrifugation were repeated. The methanolic and acetonic extracts were combined to yield the EPP solutions. The residue was freeze dried and stored at 4°C for NEPA analysis.

EPP content was determined in the solutions by the Folin–Ciocalteu procedure (Singleton, Orthofer, & Lamuela-Raventos, 1998). An aliquot (1 mL) was mixed with 5 mL of Folin–Ciocalteu reagent and swirled. After 5 min, 4 mL of sodium carbonate solution (75 g/L) was added and mixed and the resulting solution was kept at room temperature (25 ˚C) for 1 h and its absorbance was recorded at 765 nm. The results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight of cooked food (mg GAE/100 g fresh weight of cooked food) by reference to a standard curve prepared with gallic acid solutions of known concentrations (0-60 μg/mL) (Appendix II, fig 6).

2. Non-extractable Proanthocyanidins (NEPA):

Fifty milligrams of the residues left after EPP extraction were treated with 10 mL of butanol/HCl (95:5 v/v) and 0.042 g of $FeCl_3$ at 100 ˚C for 1 h. The tube was then centrifuged at 3500 rpm for 10 min and the supernatant collected. After two washing with 5 mL of butanol, the supernatants were combined and the absorbance of the NEPA-containing solutions was measured at 555 and 450 nm and the concentration of NEPA determined according to the equation proposed by (Zurita, Diaz-Rubio, & Saura-Calixto, 2012).

3. Antioxidant Assays

a. Ferric reducing antioxidant power (FRAP)

The reducing power of the composite dishes was analyzed according to (Benzie & Strain, 1996) with some modifications. This method is based on the antioxidants' potential to reduce Fe^{3+} into Fe^{2+} that is blue in color. The FRAP reagent was prepared using 10:1:1 acetate buffer (pH 3.6; 0.3 M), TPTZ (10 mM using 40 mM HCl) and 20 mM $FeCl_3$, respectively. This solution was prepared daily and stored in the dark at all times. An aliquot of the alcoholic extracts (100 µL) was mixed with 900 µL distilled water and 2 mL of FRAP reagent and then incubated at 37 ˚C for 30 min. The absorbance was measured at 593 nm against a blank (1 mL water + 2 ml FRAP

reagent). Aqueous solutions of ferrous sulfate (0-100 µM) were used for plotting the standard curve (Appendix II, fig 7). The reducing power was expressed as mmol Fe (II)/kg of food sample.

b. 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS)

The ABTS assay was carried out as outlined by (Fu et al., 2011). The method is based on the decolorization of the ABTS (2,2-azinobis-(3-ethylbenzoethylbenzothiazoline-6-sulfonic acid)) radical cation (ABTS•+). The ABTS•+ solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulphate in a volume ratio of 1:1.After 16 h of incubation in the dark, at room temperature, the ABTS•+ solution was diluted with methanol to an absorbance of 0.7 ± 0.005 at 734 nm. An aliquot of the alcoholic extracts (100 µL) was added to 3.8 mL of the ABTS•+ solution and kept in the dark for 30 min. The absorbance was measured at 734 nm against a blank containing methanol and the trolox equivalent antioxidant capacity (TEAC) was expressed in mmol trolox/kg of food by reference to a standard curve prepared using different concentrations of trolox (Appendix II, fig 8). The TEAC of the food samples was calculated using the following equation: (Tomasina, Carabio, Celano, & Thomson, 2012)

TE (µmol TE/ g) =Trolox (µmol/L)/sample (g/L)

4. Estimation of % contribution of various food groups to dietary antioxidant capacity

The estimation of % contribution to dietary antioxidant capacity was performed by multiplying the AC values, obtained by FRAP and ABTS assays, by the annual per person consumption values form the national survey database. Results are expressed using the following equation (Torres & Farah, 2017):

$$\% \text{ DAC} = \frac{C_f AC_f}{\sum C_f AC_f}$$

where % DAC = % contribution to dietary antioxidant capacity; C_f = per person consumption of food (kg); and AC_f = Antioxidant capacity of food.

5. Statistical Analysis:

IBM SPSS Statistics 24 was used for the statistical analysis. TP, FRAP and ABTS data were subjected to two-tailed bivariate correlation. Correlations were calculated on a sample mean basis, according to Pearson's test.

Dietary AC values based on TEAC and FRAP assay results were compared by independent samples t-test at confidence interval of 95%.

CHAPTER IV

RESULTS AND DISCUSSION

A. Extractable polyphenol content in Lebanese composite dishes

The extractable polyphenols (EPP) were determined spectrophotometrically using the Folin-Ciocalteu method which is based on electron transfer from the phenolic compounds to the Folin-Ciocalteu reagent under basic conditions. The organic solvents used for extraction, acetone and methanol in acidic water, have been reported to be optimum for efficient extraction of polyphenols from foods (Metivier et al., 1980).

As shown in Table 3, the total phenol (TP) contents ranged between 455.80±13.31mg GAE/100g FW in Stuffed Grape leaves and 10.96±0.33 mg GAE/100g FW in White Bean Beef stew. In view of the wide variations in the total phenols, the Lebanese composite dishes were divided into three groups namely: high (>200 mg/100 g), medium (100 - 199 mg/100 g) and low (<100 mg/100 g) (Kaur & Kapoor, 2002). This variation occurs generally due to several factors including agronomic conditions, processing and culinary practices, iand the duration and temperature at which the ingredients are cooked (Amarowicz et al., 2010). Only Stuffed Grape leaves belonged to the group with high TP, followed by standard salad and white and brown breads that belonged to the group with medium TP. The high value obtained for stuffed grape

leaves is in line with the literature data which report high content of polyphenols in grape leaves (Anđelković, Radovanović, Anđelković, & Radovanović, 2015; Atak, Altindisli, & Goksel, 2011; Dani et al., 2010). The low group comprised most of the dishes including spinach stew, Tabbouli, navy bean stew in oil, rice dishes, fava beans, stuffed squash, stuffed cabbage and other dishes.. These low values could be attributed to the fact that cooking practices lead to losses in vitamins and phytochemicals, in addition to changes in the chemical makeup of foods. Also, some polyphenols might be lost into the boiling water while preparing the composite dishes.

Data obtained for the analysis of phenolic contents in the Lebanese composite dishes are the first ones to be reported for a large number of samples in the Middle East. It is noteworthy that the data generated in this study may exhibit significant variations due to differences in the types of vegetables used and the cooking procedures in the different households.

B. Non-extractable proanthocyanidins content in Lebanese composite dishes

The most common procedure for estimating the amount of non-extractable proanthocyanidins (NEPA) is based on spectrophotometric analysis of anthocyanidin solutions obtained by butanolysis of the residues which remain after extraction of EPP. This hydrolysis treatment is needed to achieve the depolymerization of high-molecular size proanthocyanidins that may be bound to polysaccharides and proteins in the plant

cell walls. As a result, two major soluble products are formed: free anthocyanidins that absorb at 555 nm, and xanthylium compounds that absorb maximally at 450nm (Jurd & Somers, 1970). Carob pod tannin concentrates were selected as representative of a natural sample rich in high molecular weight proanthocyanidins as standards of this analysis (Saura-Calixto, Serrano, & Goñi, 2007).

The composite dishes were classified into three main groups: high (>200 mg/100 g), medium (100 - 199 mg/100 g) and low (<100 mg/100 g). According to the results presented in Table 3, the wheat-based dish "moghrabieh" contained the highest content of NEPA at 2103.29±223.86 mg/100g FW. In addition, stuffed grape leaves, Wooden Bakery and Moulin D'or white breads showed high amounts of NEPA. Many composite dishes were of medium NEPA content such as lentil soup with rice, Jew's mallow, lima bean beef stew. Standard salad, white bean stew in oil, stuffed cabbage belonged to the low group.

Most of the Lebanese composite dishes contained higher amounts of NEPA as compared to the EPP contents, except for some dishes such as lima bean stew in oil, white bean stew in oil, stuffed grape leaves and the three analyzed brown breads (Table 2). This is probably due to some losses of of soluble antioxidants during preparation and cooking stages (Chang et al., 2013). White bread showed higher values of NEPA compared to those of EPP. This might be due to the added sugars that significantly

favor pronyl-L-lysine formation. This type of antioxidant is obtained as a result of the reaction of the protein-bound amino acid L-lysine and starch as well as reducing sugars in the presence of heat (Lindenmeier & Hofmann, 2004). Although brown bread contains more fibers compared to the white bread, brown bread showed higher values of EPP compared to NEPA. This unexpected finding might be due to adulterations during processing practices like the addition of colorants, caramels and molasses. Further, this could be due to the higher release of free soluble ferulic acid upon baking the brown bread in comparison to the white bread (Yu & Nanguet, 2013).

This finding highlights the importance of quantifying the nonextractable polyphenols in the residues, in addition to EPP, in order not to underestimate the total phenolic content of foods. In addition, it is interesting to note that NEPA have been reported to exhibit certain health-related advantages as compared to EPP because the consumption of non-extractable macro-antioxidants promote a sustained circulation of beneficial metabolites due to their longer colonic transformation (González-Sarrías, Espín, & Tomás-Barberán, 2017), Also, given their strong association with dietary fiber, they affect the colonic fermentation of the fiber and vice versa (Saura-Calixto et al., 2010). Therefore, combined consumption of EPP and NEPP may produce complementary beneficial effects.

Table 2. Extractable polyphenols **(**EPP) and non-extractable proanthocyanidins (NEPA) contents in the most frequently consumed composite dishes in Lebanon and breads

Food Product	EPP (mg GAE[a]/100g fresh weight)	SD	NEPA (mg/100g fresh weight)	SD
Vegetable-based dishes				
Tabbouli	43.18	±2.83	33.85	±1.37
Standard salad	107.61	±6.38	15.20	±4.59
Bread salad	52.55	±3.11	19.87	±2.99
Jew's mallow	92.35	±0.80	155.42	±4.41
Green pea stew	51.63	±0.94	34.87	±2.98
Flat green beans beef stew	30.26	±0.45	41.01	±3.94
Runner beans beef stew	29.05	±1.62	28.87	±3.72
Green snap beans in oil	29.35	±0.75	38.97	±7.68
Spinach stew	78.66	±2.53	142.93	±18.26
Legume-based dishes				
Rice with lentils	21.42	±0.30	100.42	±10.39
Lima bean beaf stew	41.91	±0.48	112.64	±8.19
White bean beef stew	10.96	±0.33	91.04	±6.88
Navy bean beef stew	32.27	±1.04	63.84	±3.86
Fava beans	94.25	±1.30	120.51	±0.07
Hummus	46.15	±0.84	85.70	±12.84
Chickpeas	36.83	±2.13	92.90	±5.21
Lentil soup with rice	83.55	±0.74	134.50	±10.30
Lima bean stew in oil	94.98	±5.55	82.45	±2.8
Navy bean stew in oil	17.59	±1.44	68.08	±6.22
White bean stew in oil	48.48	±0.58	34.50	±3.42
Rice-based dishes				
Rice (Basmati)	22.63	±0.46	108.14	±4.52
Rice (Egyptian)	22.10	±1.09	115.31	±3.21
Stuffed Vegetable-based dishes				
Stuffed squash	23.29	±0.64	35.59	±1.17
Stuffed grape leaves	455.80	±13.31	316.59	±56.62
Stuffed cabbage	24.06	±3.75	35.03	±3.61
Wheat-based dishes				
Moghrabieh	26.21	±0.69	2103.29	±223.86
Breads				
Wooden Bakery -white	133.74	±2.45	236.48	±1.07
Moulin D'or -white	114.05	±2.45	258.17	±21.68
Chamsine -white	109.23	±2.55	196.22	±3.54
Wooden Bakery-brown	138.71	±2.46	103.09	±2.71
Moulin D'or-brown	157.25	±3.63	109.42	±17.21
Chamsine-brown	151.83	±1.74	107.57	±8.76

[a.]: Gallic acid equivalents

C. Antioxidant capacities of Lebanese composite dishes

The antioxidant activity of the selected dishes was determined using the ferric reducing antioxidant power (FRAP) and the trolox equivalent antioxidant capacity (TEAC) assays. The use of these two methods was to capture the different types of antioxidants and mechanisms of antioxidant action. More specifically, The FRAP assay is based on single electron transfer (SET) mechanism, where a potential antioxidant has the ability to transfer a single electron to participate in the reduction of a compound (Gülçin, 2012). In the TEAC assay both the SET and hydrogen atom transfer (HAT) mechanisms are involved in the reduction of oxidants (Prior et al., 2005).

The FRAP procedure is based on the ability of antioxidant to reduce ferric ions to ferrous ions (Benzie & Strain, 1996). The range of antioxidant capacity of the analyzed composite dishes and breads, as determined by this simple and widely used method, varied from 0.04±0.00 mmol Fe (II)/kg FW for Egyptian rice and moghrabieh to 3.19±0.10 mmol Fe (II)/kg FW for Stuffed Grape leaves.

The TEAC assay that was also used to evaluate free radical scavenging capacities of the selected dishes is based on the ability of antioxidant to scavenge ABTS radicals. Furthermore, it depends on the accessibility of the phenolic compound to the radical center rather than the chemical properties of antioxidant, especially that ABTS•+ is a large nitrogen-centered and sterically hindered radical (Cai, Luo, Sun, & Corke,

2004; Schaich, Tian, & Xie, 2015; Tian & Schaich, 2013). Lentil soup with rice exhibited the highest TEAC (14.8±0.16 mmol/kg FW), while white bean beef stew had the lowest value (0.15±0.09 mmol/kg FW) (Table 3).

It is notable that FRAP range is narrower than the TEAC range. The remarkable variation in the ranges of the values obtained by TEAC and FRAP assays may be attributed to differences in degree of complexity of both assays, in their sensitivity, and in mechanisms and reactive species involved (Alam, Bristi, & Rafiquzzaman, 2013). Moreover, the obtained low FRAP values can be explained by the shift of the redox potential of flavonoids and other phenolic derivatives, due to inhibition of the dissociation of phenolic hydroxyls under the acidic solvent conditions used in this assay (Tomasina et al., 2012) and the preferential contribution of the catechol ring to FRAP values , (deGraft-Johnson et al., 2007). Further, phenolic acids lacking a 3-hydroxyl group had significantly lower FRAP value than compounds having this structure (Csepregi, Neugart, Schreiner, & Hideg, 2016).

The average percentage values of mean AC results from FRAP and ABTS assays were calculated. The dishes were ranked from the highest % contribution to the lowest % contribution (Table 3). Stuffed grape leaves was highest in percentage contribution to the total AC by FRAP among the analyzed dishes (41.54%) followed by fava beans and standard salad that accounted for 4.66% and 4.16%, respectively.

However, lentil soup with rice, fava beans, Jew's mallow, stuffed grape leaves and navy bean stew in oil represent high ranks in their percent contribution to the total contribution of the TEAC values among the studies dishes (8.28%, 7.44%, 7.05%, 6.19 % and 6.07%, respectively).

	FRAP (mmol Fe(II)/kg)				**ABTS (mmol trolox/kg)**			
	Mean	***SD***	***%***	***Rank***	***Mean***	***SD***	***%***	***Rank***
Vegetable-based dishes								
tabbouli	0.14	±0.00	1.83	**14**	8.64	±0.43	4.83	**11**
standard salad	0.32	±0.01	4.16	**4**	8.49	±0.19	4.75	**12**
grean snap beans in oil	0.18	±0.00	2.38	**11**	9.39	±0.24	5.25	**10**
bread salad	0.15	±0.00	1.93	**13**	10.55	±0.53	5.90	**6**
Jew's mallow	0.29	±0.03	3.72	**6**	12.60	±0.68	7.05	**3**
green pea stew	0.14	±0.01	1.78	**15**	8.04	±0.26	4.50	**13**
flat green beans beef stew	0.07	±0.00	0.88	**24**	4.22	±0.14	2.36	**16**
runner beans beef stew	0.06	±0.00	0.82	**25**	7.94	±0.09	4.44	**14**
spinach stew	0.23	±0.00	3.02	**8**	10.28	±0.38	5.75	**9**
Legume-based dishes								
rice with lentils	0.05	±0.00	0.69	**26**	1.08	±0.05	0.61	**27**
lima bean beef stew	0.10	±0.00	1.31	**19**	1.56	±0.09	0.87	**24**
white bean beef stew	0.08	±0.00	1.00	**23**	0.15	±0.09	0.08	**32**
fava beans	0.36	±0.01	4.66	**3**	13.29	±0.30	7.44	**2**
hummus	0.12	±0.01	1.53	**16**	2.24	±0.02	1.25	**18**

Table 3. Mean and percentage antioxidant capacity from FRAP and ABTS assays of the most frequently consumed composite dishes and breads in Lebanon

chickpeas	0.05	±0.00	0.66	**28**	2.19	±0.04	1.22	**19**
lentil soup with rice	0.36	±0.01	4.68	**2**	14.80	±0.16	8.28	**1**
navy bean beef stew	0.09	±0.00	1.14	**21**	2.45	±0.12	1.37	**17**
lima bean stew in oil	0.25	±0.01	3.25	**7**	6.98	±0.34	3.90	**15**
navy bean stew in oil	0.05	±0.00	0.63	**29**	10.85	±0.28	6.07	**5**
white bean stew in oil	0.11	±0.01	1.43	**18**	1.91	±0.08	1.07	**21**
Rice-based dishes								
rice (Basmati)	0.08	±0.00	1.05	**22**	10.53	±0.10	5.89	**7**
rice (Egyptian)	0.04	±0.00	0.54	**32**	0.23	±0.06	0.13	**30**
Stuffed Vegetable-based dishes								
stuffed squash	0.05	±0.00	0.59	**30**	1.65	±0.15	0.92	**22**
stuffed grape leaves	3.19	±0.10	41.54	**1**	11.05	±0.10	6.19	**4**
stuffed cabbage	0.05	±0.01	0.68	**27**	1.63	±0.52	0.91	**23**
Wheat-based dishes								
moghrabieh	0.04	±0.00	0.58	**31**	1.95	±0.07	1.09	**20**
Breads								
Wooden Bakery-white	0.29	±0.01	3.75	**5**	1.11	±0.28	0.62	**26**
Moulin D'or-white	0.12	±0.00	1.51	**17**	0.40	±0.06	0.22	**29**
Chamsine-white	0.09	±0.00	1.18	**20**	0.19	±0.06	0.10	**31**
Wooden Bakery-brown	0.16	±0.00	2.07	**12**	1.44	±0.03	0.81	**25**
Moulin D'or-brown	0.19	±0.02	2.45	**10**	0.41	±0.02	0.23	**28**
Chamsine-brown	0.20	±0.01	2.63	**9**	10.48	±0.04	5.87	**8**

The correlation coefficient between total antioxidant capacities and the EPP contents are shown in Table 4. A high significant positive correlation ($r=0.8712$, $p<0.01$) was obtained between the FRAP value and the EPP content, as determined by the Folin-Ciocalteau procedure, indicating that phenolic compounds could be one of the major constituents responsible for the reducing ability of these dishes. On the other hand, a

very weak correlation (r= 0.1074) existed between the ABTS values and the EPP content suggesting that phenolic compounds could not be main components responsible for free radicals scavenging ability of these dishes. Thus, the AC of an extract may not be predicted on the basis of its phenolic content, but also requires proper characterization of individual phenolic compounds (Kähkönen et al., 1999; Rondon, García, Cornejo, Rojas, & Terán, 2015). Furthermore, a weak correlation (r= 0.3067) has been found between total antioxidant capacities, FRAP and ABTS, thereby suggesting that the compounds capable of reducing oxidants could be different from those scavenging free radicals in the analyzed dishes (Fu et al., 2011).. It is noteworthy that proteins (compounds with –SH group) form the major contributors for high TEAC values (Rao et al., 2015) , Thus, the discrepancy in obtained trends might probably be due to the different mechanistic strategies of these two assays along with the different types of phenolic compounds present in the analyzed dishes (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002).

Table 4. Correlation coefficients between the extractable polyphenols (EPP) and the antioxidant capacities measured by the FRAP and TEAC assays

	EPP	**FRAP**	**ABTS**
EPP	1		
FRAP	0.8712**	1	
ABTS	0.1074	0.3067	1

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

D. Estimation of % contribution to dietary antioxidant capacity (DAC)

The average percent contribution of each food group to the Lebanese dietary AC, based on the AC data obtained by FRAP and ABTS assays, associated with the consumption database, is presented in Table 5 and Fig 6. By comparing the means of % DAC using the two different assays, p-value of <0.05 was considered statistically significantly different (p-value=0.998).

The vegetable-based dishes contributed most to the DAC using ABTS assay; however, the stuffed vegetable-based dishes showed the highest contribution (34.60 %) among the tested groups, using FRAP assay. These findings suggest that Lebanese people consume most the vegetable/stuffed vegetable-based dishes that contribute highly to their antioxidant intake.

Table 5. Percent contribution of the different Lebanese dishes to the dietary antioxidant capacity (DAC) of the Lebanese diet based on data obtained by FRAP and ABTS assays

Food Product	%DAC (FRAP)	%DAC (TEAC)
Vegetable-based dishes		
tabbouli	5.02	13.09
standard salad	11.07	12.02
bread salad	1.97	5.72
Jew's mallow	3.52	6.34
green pea stew	0.93	2.23
green snap beans in oil	3.89	8.14
green beans beef stew	0.38	1.45
spinach stew	0.81	1.46
Stuffed Vegetable-based dishes		
stuffed squash	0.51	0.76
stuffed grape leaves	33.89	4.79
stuffed cabbage	0.20	0.25
Breads		
Wooden Bakery, Moulin D'or , Chamsine-white	19.83	2.77
Wooden Bakery, Moulin D'or , Chamsine- Brown	2.66	2.38
Legume-based dishes		
rice with lentils	1.00	0.83
fasulia green beans beef stew	1.11	0.70
fasulia green beans in oil	0.50	0.98
fava beans	4.28	6.49
hummus	0.60	0.47
chickpeas	0.23	0.41
lentil soup with rice	1.45	2.44
Wheat-based dishes		
moghrabieh	2.96	14.63
Rice-based dishes		
rice (Basmati and Egyptian)	3.19	11.65

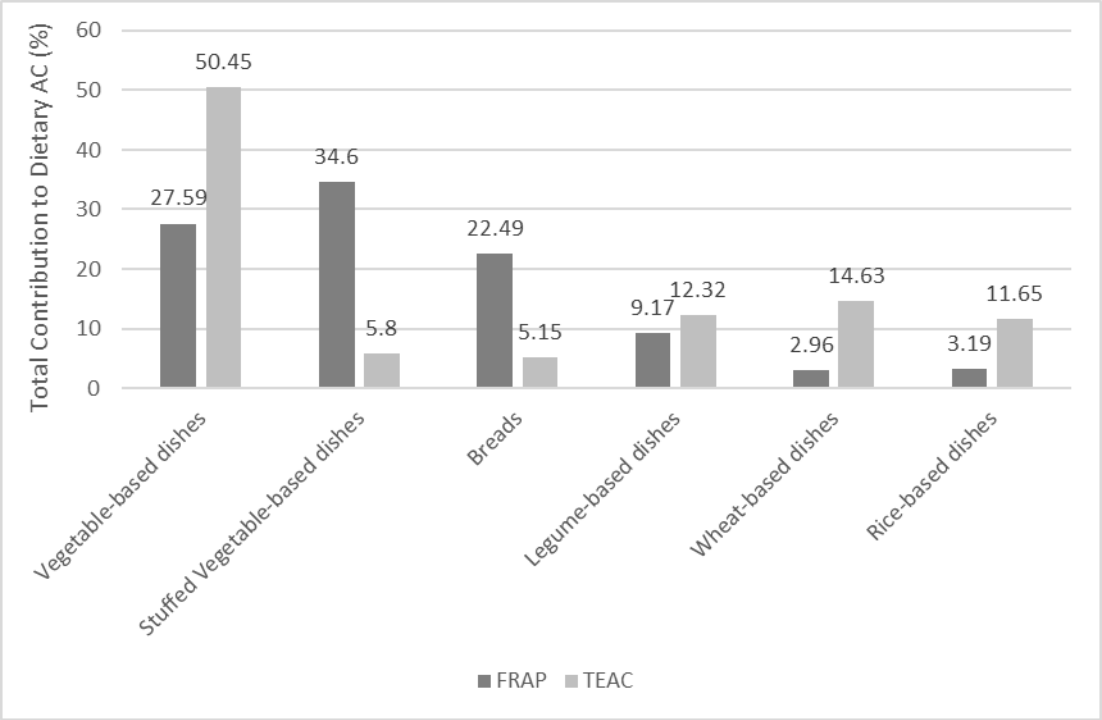


Figure 6. Percent contribution of the various food groups to the dietary antioxidant capacity of the Lebanese diet

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

The most consumed Lebanese composite dishes showed wide variations in their phenolic contents and antioxidant capacities. The extractable polyphenol (EPP) contents, of the methanolic-acetonic extracts, ranged between 455.80 and 10.96 gallic acid equivalents (GAE)/100g fresh weight (FW). Stuffed grape leaves exhibited the highest phenolic content among the analyzed dishes. Non-extractable procyanidins (NEPA), in the residues after acetone-methanol extraction, were present at higher levels than EPP in most Lebanese composite dishes suggesting that many antioxidants were lost during the preparation and cooking stages. Moghrabieh showed the highest NEPA value as compared to the other dishes. The antioxidant capacities of the selected dishes, determined using FRAP and ABTS assays, were in the range 0.04 and 3.19 mmol Fe (II)/kg FW and 14.8 to 0.15 mmol/kg FW, respectively. Stuffed vegetable-based dishes showed the highest contribution (34.60 %), to the dietary antioxidant capacity (DAC), among the tested groups by the FRAP assay. However, the vegetable-based dishes contributed most to the DAC using ABTS assay (50.45%). This finding suggests that the Lebanese consume vegetable and stuffed vegetable-based dishes that contribute significantly to their antioxidant intake.

A high significant positive correlation was found between the TP and FRAP assay; a very weak correlation existed between the ABTS values and the TP content; a weak correlation has been found between total antioxidant capacities, FRAP and ABTS. The discrepancy in obtained trends might be probably due to the different mechanistic strategies of two assays in addition to the different types of phenolic compounds in the analyzed dishes.

The information obtained for the very first time in this study is potentially useful to the medical and nutritional researches as it provides data on the polyphenolic content and the antioxidant capacities of the most consumed Lebanese composite dishes. Also, this study sheds light on the importance of contribution of the non-extractable polyphenols to the total phenolic content of foods.

Further studies are needed to identify the components that are responsible for the antioxidant properties of the analyzed dishes. It is recommended that HPLC analysis of the phenolic fractions of certain dishes with high/low antioxidant content and activities to be carried out in order to probe the differences in the phenolic profiles and relate these to the observed variations in the antioxidant activity of the dishes. Also, it is recommended that *in vivo* antioxidant assays be conducted on some dishes in order to better assess the antioxidant activity of the food and its phenolic fractions on humans. Moreover, studies addressing the antibacterial, anti-inflammatory and anti-proliferative activities of Lebanese composite dishes would further enhance the potential spread and

appeal of the Lebanese traditional diet to consumers affected by the increasing globalization of ethnic foods.

Appendix I

Table 1. Moisture content of the most frequently consumed composite dishes and breads in Lebanon

Food Product	Moisture Content Calculated (%)
Vegetable-based dishes	
Tabbouli	89.29
Standard Salad	88.76
Grean Snap Beans in oil	82.19
Bread Salad	91.91
Jew's mallow	82.30
Green Pea stew	83.25
Flat Green Beans Beef stew	84.95
Runner Beans Beef stew	88.76
Spinach stew	82.75
Legume-based dishes	
Rice with lentils	67.48
Lima Bean Beaf stew	73.90
White Bean Beef stew	64.58
Bread Bean-dip	74.65
Hummus	69.48
Chickpeas	71.35
Lentil Soup with rice	77.75
Navy Bean Beef stew	72.44
Lima Bean stew in oil	76.18
Navy Bean stew in oil	74.34
White Bean stew in oil	71.05
Rice-based dishes	
rice (Basmati)	50.68
rice (Egyptian)	61.26
Stuffed Vegetable-based dishes	

Stuffed Squash	88.60
Stuffed Grape leaves	66.39
Stuffed Cabbage	88.00
Wheat-based dishes	
Moghrabieh	73.29
Breads	
Wooden Bakery-White	24.16
Moulin D'or-White	26.34
Chamsine-White	23.44
Wooden Bakery-Brown	23.88
Moulin D'or-Brown	14.17
Chamsine-Brown	24.63

Moisture content = (wet mass-dried mass)*100/wet mass

Appendix II

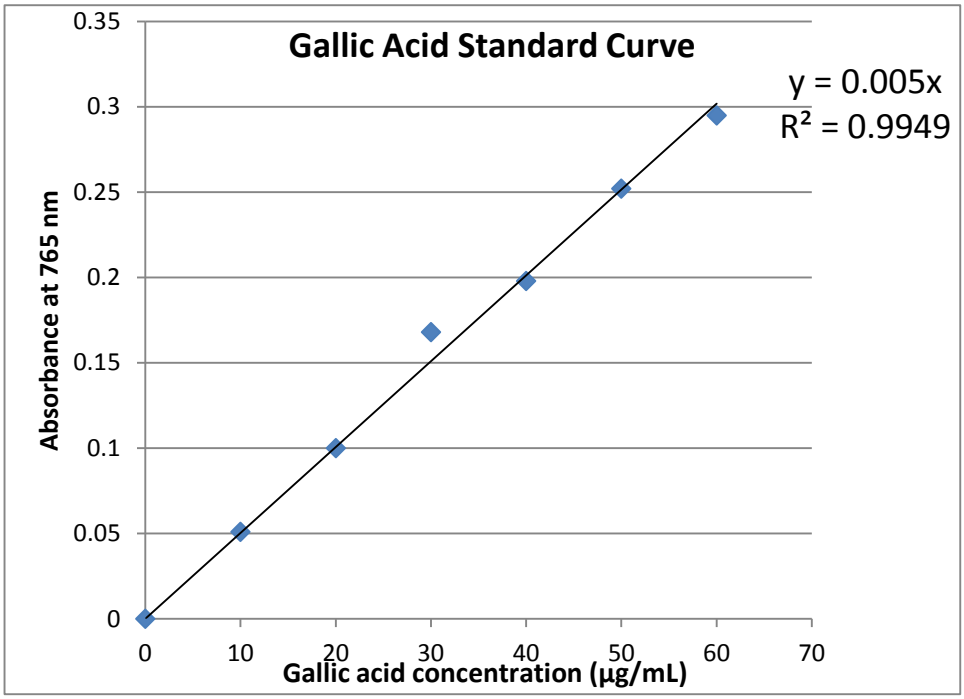


Fig 6. Gallic Acid standard curve (0-60 µg/mL) depicting the absorbance (AU) at 765 nm

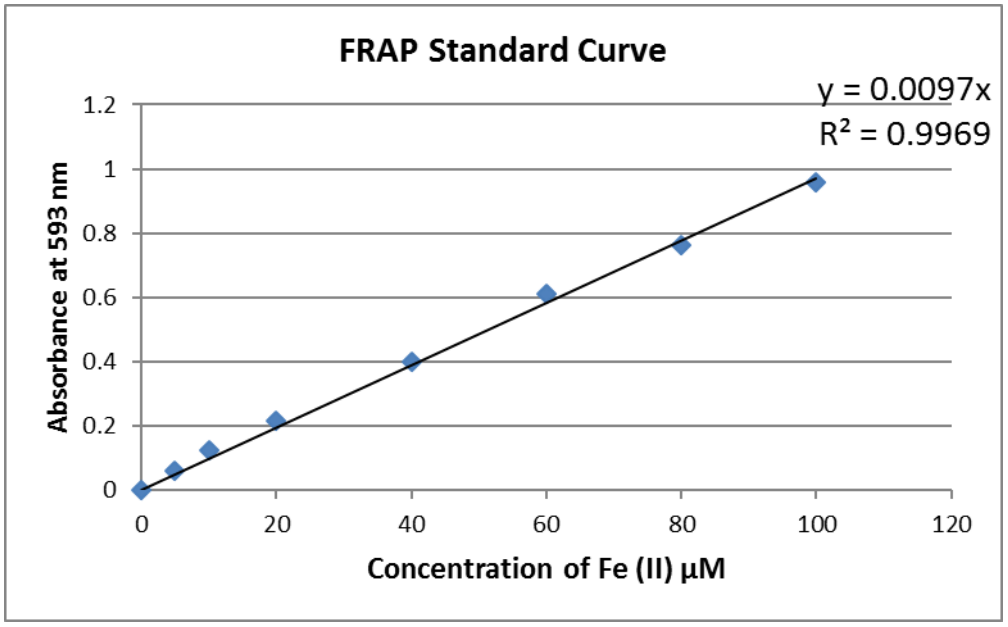


Fig 7. FRAP standard curve (0-100 µM) depicting the absorbance (AU) at 593 nm

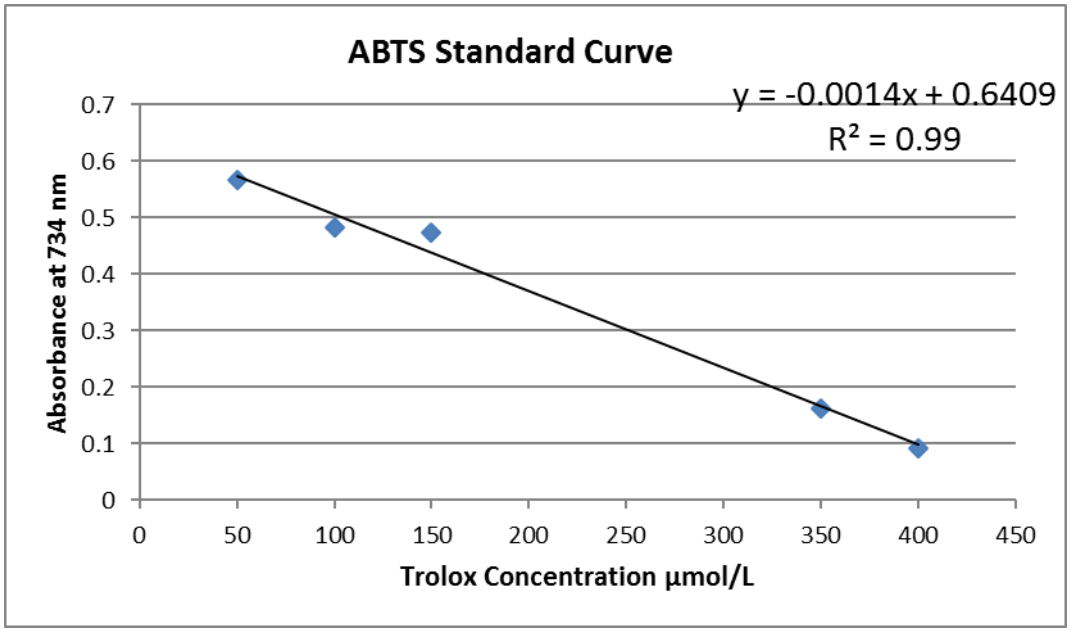


Fig 8. ABTS standard curve (50-400 µM) depicting the absorbance (AU) at 734 nm

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