



AMERICAN UNIVERSITY OF BEIRUT

AN ASSESSMENT OF SODIUM LEVELS IN DIFFERENT BREADS  
AND BREAD INTAKE IN THE LEBANESE POPULATION

by  
NATHALIE GABY BARAKAT

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 2015

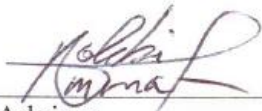
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## ACKNOWLEDGMENTS

My deepest gratitude is addressed to the Almighty who has given me the will, patience and strength to complete this research.

I would like to thank my advisor Dr Ammar Olabi for his guidance, encouragement and constant help in this project. Apart from the subject of my research, I learnt a lot from him, which I am sure will be useful in different stages of my life.

Special thanks are addressed to Dr Imad Toufeilli for his professional advice and for sharing his sincere and valuable guidance throughout the project.

I would like to thank my colleague and friend Layal Abou Jaoude for her technical assistance, strong commitment, continuous encouragement and moral support.

Last but not least, I would like to express my everlasting gratitude, devotion and appreciation to my family. I would like to thank them for their love, patience and motivation. This journey would not have been possible without their inspiration, their care and their prayers. A very special recognition needs to be given to my best friend for her non ending support, for putting up with me during my odd hours and for helping me stay sane all throughout this research.

# AN ABSTRACT OF THE THESIS OF

Nathalie Gaby Barakat for Master of Science

Major: Food Technology

Title: An assessment of sodium levels in different breads and bread intake in the Lebanese population

Bread is considered a staple food in Lebanon. It is one of the most consumed food products by the population. The objectives of this study were to assess the salt content in bread and to determine its daily intake.

Six types of bread were collected from bakeries (n= 45) across all Lebanese provinces. AACC 40-71(American Association for Cereal Chemists) was followed for sodium analysis. Food frequency questionnaires were filled by random panelists in many regions in Lebanon (in 3 provinces).

The salt content of the different types was as follows: white pita 1.3 % ( $\pm 0.66$ ), brown pita 1.5 % ( $\pm 0.77$ ), white baguette 2.2 % ( $\pm 0.9$ ), brown baguette 1.9 % ( $\pm 1.03$ ), markouk 2.8 % ( $\pm 1.16$ ) and tannour 2.2 % ( $\pm 0.96$ ). Daily bread consumption was higher among males, as expected. Consumption levels were as follows: white pita 88.6 gram per day ( $\pm 135.21$ ), brown pita 39.74 grams per day ( $\pm 71.63$ ), white baguette 14.06 grams per day ( $\pm 43.36$ ), brown baguette 5.12 grams per day ( $\pm 13.83$ ), markouk 11.83 grams per day ( $\pm 38.7$ ) and tannour 3.77 grams per day ( $\pm 13.23$ ). Consumption trends of bread were affected by several factors: gender, province, type of bread and the interaction of some variables together. This work highlights the necessity of establishing national policies with respect to salt content in foods.

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## ABBREVIATIONS

B.C.	Before Christ
et al	Et alii (and others)
g	Gram
/	Per
%	Percent
±	Plus or minus
L	Liter
<	Less than
mL	Milliliter
mmol	Millimols
HCl	Hydrochloric acid
=	Equal to
SAS	Statistical analyses software
x	By
df	Degree of freedom
UK	United Kingdom
US	United States
NaCl	Sodium Chloride
HNO <sub>3</sub>	Nitric acid
CsCl	Cesium Chloride
Cs	Cesium
SRM	Standard Reference Material
AACC	American Association of Cereal Chemists

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# CHAPTER I

## INTRODUCTION

Having very old roots, bread is considered to be a major staple food on many levels: nutritional, economic and socio-cultural. Bread varies broadly around the world in terms of production techniques and bread types. However, some basic ingredients are always present in the recipe: cereal flour, yeast (or any other leavening agents), water and sodium chloride. Bread is considered to be one of the main contributors to dietary salt intake due to its high consumption (Ni Mhuruchu, 2011). Sodium chloride plays several vital roles in bread: it provides the saltiness flavor, delays staling and strengthens the gluten network (Kilcast, 2007). Manufacturers tend to increase salt levels in their bread products due to the positive impact that salt has on the dough's rheology. Salt improves the flavor of the bread making it more acceptable (Lynch, 2009).

High salt consumption is associated with heart disease, kidney disease and obesity. Around 25 % of the salt consumed among the Lebanese population was estimated to originate from Arabic bread and bakery products, such as Manakish. The same above study indicated that 39% of Lebanese individuals have high blood pressure and that 30 % of those cases are related to a high salt intake (Nasreddine, unpublished).

As a result of all the above trends, efforts were oriented towards reducing salt intake by reducing the levels of salt used in bread production. Thus, researchers are trying to come up with low salt bread that is still as acceptable in a similar manner to its regular salt counterpart. Some countries are even setting strict limits to the amount of salt used in bread production. In Portugal for example, the limit is 1.4 % (A. Pla´cido, 2012).

The objectives of this national study were to assess the salt content of six types of bread in Lebanon (White Pita, brown Pita, white Baguette, brown Baguette, Markouk and Tannour)

and to determine the daily intake levels of these breads to establish the dietary salt intake originating from bread.

## CHAPTER II

### LITTERATURE REVIEW

#### **A. History**

Bread is one of the oldest food products. The earliest evidence in history for processed flour goes back 30,000 years ago in Europe. People accidently left a mixture of flour and water on the rocks in the sun and after sometime they found that it had baked and tasted it. During this period, the food consumed was limited to what was hunted and gathered. It was not until the Neolithic period (10,000 years ago) that cereals and bread became a staple food (Chris, 2005). There was a shift in the World from hunter gatherer survival to cereal based agricultural diets. Although cereal crops are not nutritionally similar to the hunting-gathering diet, they can be accessible to a larger portion of the population. The first sources of yeast to ferment bread were either obtained from beer foam or from grapes since beer and wine were regularly consumed by Egyptians and Greeks. Bread was also important in many cultural rituals. For example Ramses III sacrificed 200,000 loaves of bread every year to the gods. In the middle ages, bread was needed by the Church to be part of the sacred communion. Baking was considered an honorable profession and bakers enjoyed many benefits in the 13th century (the science of bread making). The demand for bread increased since this product was considered a staple food. Countries began to enact laws concerning bread.



## **B. Importance of bread**

Having ancient roots, bread is considered to be an important staple food on many levels: nutritional, economic and socio-cultural. All types of bread have a fair amount of carbohydrates and thus provide the human body with the most important source of energy. Although the fiber present in bread cannot be digested, it plays an important role in the body. Both soluble and insoluble fibers are present in bread. Soluble fibers create a substance in the digestive tract that blocks the absorption of LDL into the bloodstream (Nuria Martin-Carron, 1999). It also helps in sugar regulation in the blood. Insoluble fiber improves the digestive tract health and provides bulk to stool, which can help in preventing some problems like constipation. Whole grain bread contains higher quantities of fiber than processed grain breads (white bread). In addition, most breads contain proteins. For example, wheat bread has significant amounts of gluten, which constitutes around 80% of the protein in wheat (Manley, 2000). On average, each American consumes more than 25 kilograms of bread each year. Most European countries have an average consumption of 50 kilograms per year. As for the Arab region, bread consumption was estimated at 146.7 g per day in 2008 in Lebanon (Nasreddine, unpublished). Global bread consumption rose by 30.1 % from 2005 until 2010. Being a staple food, bread plays an important role in the economy. Wheat goes through many steps before turning into bread. Each step involves the labor of many citizens and contributes to their income. Improving harvesting conditions and wheat varieties can contribute to the solution to hunger.

Finally, bread is an important part of many cultures. As mentioned previously, producing bread was considered an honorable job in the past. Being a “bread-winner” or “putting bread on the table” are examples of some expressions used in the literature to express the importance of bread in all societies. This product is present in many traditions and rituals

nowadays. Many Mediterranean restaurants provide their customers with bread, as soon as they arrive, as a sign of welcoming them. In addition, the Christian religion uses bread in one of the most sacred ritual: the Holy Communion. Most countries have very strict laws on bread prices to make sure it is accessible by all social classes. Finally, being a simple cheap and adaptable food, bread is often used as a carrier in many cuisines. Each culinary practice modifies the recipe in accordance with the food consumed. Bread is turned into a variety of products including pizza, tortilla, brioche, sandwiches...

### **C. Product identification and types of bread**

Bread is one of the oldest and most popular foods in the World. This staple food is obtained from baking a mixture of flour and water with other ingredients. Given that there is a variety of types of flour and proportion of ingredients used, there are several types of bread. Each type has specific sensory attributes, nutritional value and price. Table 1 below provides a list of some types of bread (although there are more than 300 types of bread in the world).

Table 1. Different types of bread (Adapted from Wikipedia)

Type of bread	Origin	Description	Ingredients
Arabic/Pita	Middle East, Levant and South Eastern Europe	Ancient semi leavened	Flour, sugar, salt, yeast, water
White pan	Varies	Made from flour containing core of the grain (endosperm)	Flour, sugar, salt, yeast, butter , water
Brown bread	Varies	Contains significant amounts of whole grain and colorings	Flour, sugar, salt, yeast, water
Wholemeal	North America	Contains whole wheat grains	Flour, sugar, salt, yeast, grains ,water
Wheat germ	Varies	Germ is added for flavoring	Flour, sugar, salt, yeast, germs ,water
Whole-grain	Varies	High fiber content	Flour, sugar, salt, yeast, grains ,water
Roti	South Asia	Indian unleavened	Flour, salt, oil, water
Granary	UK	Made from flaked wheat grains and white or brown flour	White flour, granary bread flour, salt, sugar, yeast, milk, egg ,olive oil, water
Rye	Scandinavia	Higher fiber content, darker color and stronger taste	Rye flour, bleached all-purpose flour, dry yeast, sugar, butter, egg, milk, salt, caraway seeds, vegetable oil, egg, water
Matzo	Eastern Europe	Jewish unleavened	Flour, water
Sourdough	Italy	Long fermentation (lactobacilli and yeast) and sour taste	Flour, water, lactobacilli and yeast
Flatbread	Northern Ireland,Scotland	Might be leavened or unleavened	Flour ,water, salt, yeast, oil, sesame and

---

Crisp bread	Canada	Dry type of cracker	curry are optional Rye flour, salt, water Wheat flour, spices grains, yeast, milk or sesame seeds can be added
Hemp	Varies	Strongly flavored hemp flour	Hemp flour, water Flavorings may be added

---



## **D. Product development**

Several types of bread can be formulated, depending on the ingredients used. White wheat flour can be replaced by whole wheat, oat, bran and soya. Bread can be fortified as well with fibers or proteins. Consumers have a wide choice each with a specific taste and nutritional value. One of the new trends in the bread industry is “gluten free” bread. Celiac disease is becoming more common among most populations; hence the need for developing “gluten free” bakery products.

## **E. Bread**

### ***1. Composition***

Bread is known to be an ample source of grain based nutrients. Accordingly, bread is considered a good source of carbohydrates and through the use of whole grains of nutrients such as magnesium, iron, selenium, B vitamins and dietary fiber. Arabic bread (Pita) is the most common type of bread consumed in Lebanon and the Arab region. A large pita loaf (122g) contains approximately 170 calories, while a small pita loaf has about 74 calories (depending on the ingredients and on the brand). Most of the calories come from the high carbohydrate content. Pita bread is rich source of carbohydrates (78.83 %) with low dietary fiber content (5.06%). Pita bread is characterized by having a low quantity of fat (1.6%) and a fair amount of proteins (12.98%)(E. I. Moussa, 1992). This food is very low in saturated fat and cholesterol. A large whole-wheat pita loaf has about 6 g of protein, and a small loaf has almost 3 g of protein. Whole-wheat pita contains 10 mg of calcium and less than 2 mg of iron. It can be enriched in calcium (to contain about 5% of the total daily value for calcium) or with iron (to contain about 1.6 g). (Imad Toufeili, 2010)

Although people do not estimate the amount of salt ingested when consuming bread, pita bread is an important source of sodium in the diet. One large loaf contains about 250 mg of sodium (Quail, 1996). In most countries where bread is a staple food, people tend to consume large quantities of salt from bread.

## ***2. Processing***

Most varieties of bread go through the same basic stages during processing. Dry ingredients are weighed and transferred into the dough mixer; and water is added gradually. This stage is essential because it strengthens the gluten to achieve the desirable dough texture. Mixing time and speed are set according to the type of bread. The dough is then left to ferment (also known as the proofing stage). During this phase, the size/volume of the dough will increase due to yeast activity. It is important to mention that not all types of bread go through this step. Unleavened bread does not require fermentation. Once the proofing stage is over, the dough is divided evenly into round pieces. Conveyer belts transfer dough pieces into the preheated oven. Baking time and temperature vary depending on the type of bread. After cooling, the bread is packed or sold fresh.

## ***3. Ingredients***

Most types of bread are made from four essential ingredients: flour, salt, yeast and water. Sometimes additives might be added to improve the nutritional profile of bread, to increase shelf life or to obtain a better quality.

a. Flour

Being the most important ingredient in bread processing, flour cannot be replaced by any other element. Bread quality is directly affected by the source and type of flour. The most common grain used is wheat although several other types can be used as well (Goesaert, 2005). Gluten in flour gives the dough its main elasticity and mechanical properties. Gluten quality and quantity have an effect on mixing, aeration, water absorption and the final bread quality (Petrofsky, 1995) (Pascut, 2004). Several researchers studied the effect of different types of wheat on bread quality. They tried to find the most appropriate type to use in the production of specific bread (Qarooni, 1988) (Quail K. M., 1991) (Waniska, 2004) showed that pan bread and pita bread have the best quality when baked using hard grain wheat.

b. Salt

Salt (Sodium chloride, NaCl) is one of the most important ingredients in bread. It has an effect on dough and bread (texture, sensory properties, shelf life and staling). The role of salt will be further elaborated in the subsequent sections.

c. Sugar

Not all bakeries use sugar. However, sugar can enhance fermentation by providing a substrate for yeasts. In addition, sugar is responsible for the brownish color of the crust due to caramelization.



d. Yeast

Yeast is a leavening agent used to help dough rise as a result of the production of carbon dioxide (gas) and ethanol in the fermentation stage.

e. Water

Water is an essential ingredient of mostly any food. It works as solvent medium for all other components and aids in dispersion and stabilization of the food network. Water plays also a role in the interaction with other molecules in the food system (Kilara, 2006). In bread, water is used to make the dough. The dough consistency depends on the amount of water added. Several factors can influence the quantity of water added: flour moisture content, amount of damaged starch and other physicochemical properties of the flour (Gil, 1997). Moreover, water temperature has an effect on dough temperature and yeast activity. Finally, water is added as a plasticizer to hydrate both wheat protein and starch and to facilitate the formation of gluten matrix and starch swelling (Cesaro, 2004)

f. Other ingredients

Other additives such as fat, vegetable oils, eggs, ascorbic acid and bran can be added to enhance flavor, enrich dietary benefits or enhance the bread color and texture (Qarooni, 1988) (Toufeili, 1995) (Demiralp, 2000) (E. Dadzie-Mensah, 2000).

Table 2. Other ingredients used in bread and their function

<b>Ingredient</b>	<b>Amount</b>	<b>Function</b>
Fats and oil	0.6 %	Anti-staling agent
Emulsifiers	0.3 %	Crumb softening agent, anti-staling
Milk powder	1%	Improves crust color, flavor
Ascorbic acid	1 %	Increases volume and tenderness
Eggs	Depends on recipe	Increases tenderness and softness Adds nutritional value

#### ***4. Quality control***

Several tests are conducted to assess the quality of bread. Chemical tests usually include moisture content, water activity, pH and ash content. Those parameters provide useful information for preventing spoilage. Any change in ingredients or processing conditions will have an effect on the above factors. Texture analysis can be made on the dough and bread. Scientists usually focus on the following parameters to assess dough quality: Stickiness, time to reach optimum viscosity, resistance to mixing and dough development time. Understanding the dough profile can save significant time and money for companies.

## **F. Salt**

### ***1. Role in food***

Sodium chloride (NaCl), also known as table salt, is one of the oldest seasonings used in food products. It is one of the humans' basic tastes. Being exploited in different food products and culinary practices, salt can take many forms: refined salt (table salt), unrefined salt (such as

sea salt), and iodized salt. All those types provide basically the same functions in foods. One of the most well-known functions of salt is flavoring. In small quantities, salt will intensify sweetness and is that is why it is often sprinkled on fresh fruit or added to candies like caramel or chocolate bars. Salt can also counteract bitter flavors in food and is often used to "de-bitter" cruciferous vegetables and olives.

In addition, salting is one of the oldest food preservation techniques. Thousands of years ago, curing meats or some other products with salt was heavily used before refrigeration. Salt binds to water molecules thus rendering moisture unavailable for microorganisms. Besides preservation, salt has a very effective role in enhancing texture. It directly affects yeast fermentation (in bakery products) and protein gelatinization and denaturation (in processed meats and cheese). Furthermore, salt is a binding agent. Since it can help proteins form gels, it is utilized in many foods such as sausages (F. Jiminez Colmenero, 2005). Moreover, salt is partially responsible for the lively color of many processed meats (such as ham or hot dogs). Its presence helps maintain color (whether artificial or natural) and prevents it from turning grey or muddy. Finally, most of the table salt is fortified with iodine to prevent iodine deficiency, which can lead to goiter disease.

## ***2. Effect on health***

Salt consumption has increased over the years in the world, especially with the higher consumption of processed foods. Adequate sodium intake should not exceed 2300 mg per day for healthy individuals (World Health Organization, 2-7 October 2007). Ingesting high salt levels is associated with renal disease, high blood pressure, increased risks of cardiovascular diseases such as stroke, heart attack and heart failure. The positive correlation between high sodium

consumption and blood pressure was recognized a century ago. Moreover high salt intake have been shown to have risks on left ventricular hypertrophy, stomach cancer, kidney stones, and osteoporosis and obesity (He, 2012)

Every 75 to 100 mmol (1725 to 2300 mg) reduction in sodium intake showed to lower blood pressure by 1 to 2 mm Hg (Johnston, 2000). Although a noticeable decline in blood pressure is observed when salt intake is reduced, responses vary among individuals. In addition, data obtained from randomized controlled studies illustrate that plasma rennin activity, sympathetic nerve activity, insulin resistance and glucose levels are all affected by sodium intake. The World Health Organization (WHO) and Food and Agriculture Organization (FAO) link the causes of hypertension to adapting an unhealthy lifestyle including a high fat and high sodium diet, lack of physical activity and other factors (N. Campbell, 2009). Sodium, potassium, magnesium and calcium are the nutrients of most interest in the diet blood pressure relationship (Karen E. Charlton, 2007).

Pavel and Goldstein (2014) showed a correlation between adding salt and depression. It seems that salt intake from food and depression are directly related in men. This was not the case among female subjects since those variables were inversely linked (Pavel Goldstein, 2014).

Decreasing salt intake has positive effects on health. A decrease in dietary salt of 3 g/day would significantly reduce the incidence of CVD, stroke, myocardial infarction, and mortality, with an associated estimated healthcare cost saving of between \$10,000 million and \$24,000 million/year(Quilez, 2012). There is a broad agreement that reducing sodium in the food supply can reduce the population's blood pressure levels. In South Africa, cardiovascular disease is responsible for 17 % of all deaths and comes second after HIV/AIDS. Sodium (from salt) is a major contributor to coronary disease.

### ***3. Sodium intake from different foods***

Information about the major dietary sources of sodium is vital to develop public health plans to reduce salt intake. In western societies, around 75 % of sodium consumption comes from processed foods and foods eaten away from home. Many processing techniques involve adding sodium to foods for preservation, flavor and quality. (World Health Organization, 2-7 October 2007) (James W.P, 1987). Cereals and cereal products (bread, breakfast cereal, cake and biscuits) contribute 30-50 % of the estimated sodium intake in the U.S and U.K. In Asian countries, sauces (such as soy sauce) and salt added in cooking are responsible of a large proportion of dietary sodium consumption.

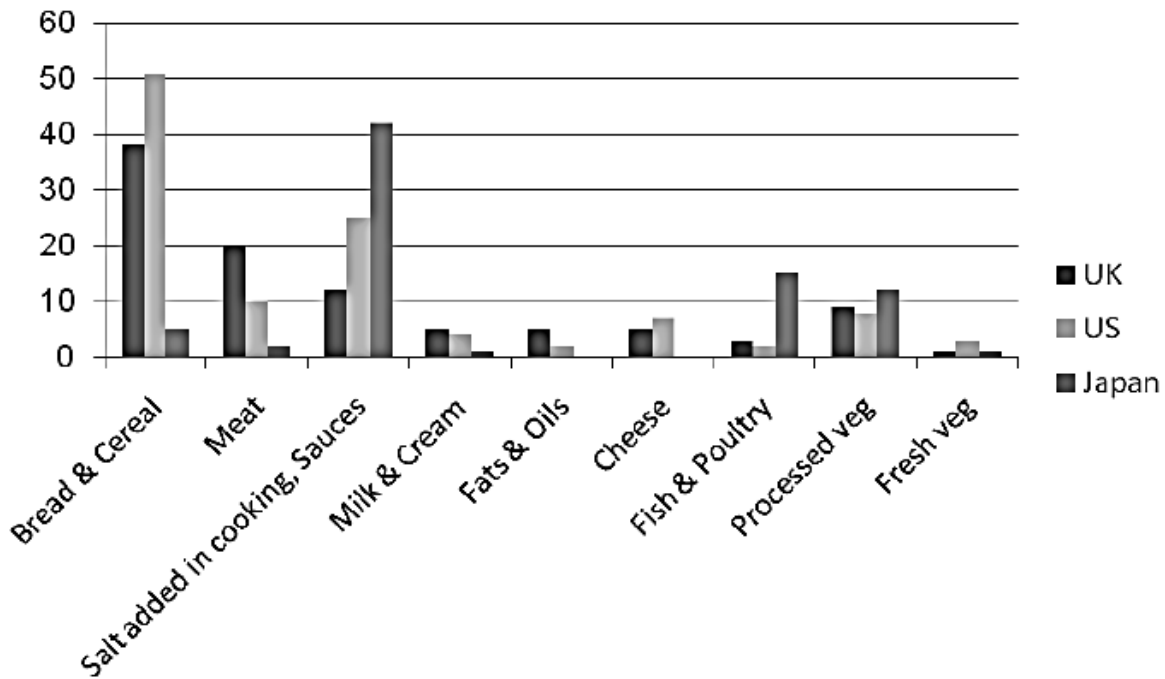


Figure 1. Contribution of food products to salt intake (James W.P ,1987)

A national dietary survey in Brazil revealed that the average daily sodium intake was 3190 mg/day. The food groups contributing to the highest intake were: rice dishes (20.8 %), beans and legumes (15.2%), meats (8.2 %), breads (11 %) , and soups (5.1 %). Sodium consumption was higher among male subjects and decreased with age among females. (Amanda de Moura Souza, 2013).A cross-sectional population based study was also conducted in Brazil to evaluate the use of 24-hour dietary records in the assessment of sodium intake. Daily sodium intake ranged from 8.5-10 g (Elaine T. Micheli, 2003).

Tanase (2011) analyzed the sodium content of 154 food composites, widely representative of the foods commonly consumed in Canada. Processed foods and soups contained the largest quantities of salt. In addition, fast foods, pork and baked beans, cheeses, soy sauce and breads contributed mostly to the daily sodium intake (Corina M. Tanase, 2011).

## **G. Salt and bread**

### ***1. Role in bread***

One of the products that witnessed a rise in salt levels is bread. Lowering salt content is thought to have an impact on consumer acceptance. Manufacturers tend to increase salt levels in their bread products due to the positive impact that salt has on the dough's rheology. Salt improves the flavor of the bread making it more acceptable (Lynch, 2009).

In the western diet, up to 35 % of sodium intake is from bread and cereal products (Martijn W.J Noort, 2012). Around 25 % of the salt consumed among the Lebanese population is from Arabic bread and bakery products, such as Manakish. The same above study indicated that 39% of Lebanese individuals have high blood pressure and that 30 % of those cases are related to a high salt intake (Nasreddine et al., unpublished data)

As a result of all the above trends, efforts were oriented towards reducing salt intake by reducing the levels of salt used in bread production. Thus, researchers are trying to come up with low salt bread that is still as acceptable in a similar manner to its regular salt counterpart. Salt may be reduced by non-homogeneous distribution of salt in bread. This method reduced salt level by 28% while keeping the flavor intensity, but it cannot be used for all types of bread. Another method for salt reduction is using salt substitutes such as potassium chloride (KCl), calcium chloride (CaCl<sub>2</sub>), or magnesium chloride (MgCl<sub>2</sub>). Decreasing salt level in bread (to 0.3% salt in 100 g of bread) was achievable and only needed the Introduction of simple changes to the bread-making process. The resulting product did not differ in quality but varied in taste. When bread was made using KCl instead of NaCl, a metallic aftertaste was detected and was noticed more significantly with increasing levels of KCl (Quilez, 2012). To avoid this off-flavor, a certain ratio of NaCl to potassium salts combination may be used. When NaCl in bread was

substituted with 30% potassium salts, no difference in taste or other characteristics were noticed and the product was overall acceptable (Braschi A, 2008).

## ***2. Effect on dough characteristics***

It is well known that salt has a major influence on dough characteristics. Sodium chloride increases dough stability, decreases its stickiness and makes it easy to handle. Increasing salt content delays protein coagulation and starch gelatinization; hence thermo-mechanical properties of the dough change with salt concentration (Angioloni, 2005). The behavior of salt is complex whereby adding NaCl in low concentrations increased starch gelatinization; however, very high concentrations had the same effect. Dough development time and resistance to extension and extensibility were noticed to increase with higher salt levels, while yeast activity in the dough decreased which delayed production of gas. Salt does not affect rheology and flavor only, it also plays an important role in maintaining the desirable texture of crumb and crust and getting the most appealing color. Uncharged salts (Sodium chloride) have an effect on hydrophobic interactions by inducing conformational changes of the biopolymers in dough. (Quilez, 2012).

## ***3. Effect on bread characteristics***

Salt plays an important role in determining bread quality. It has a large influence on the crust color because it controls yeast fermentation. The rate of fermentation decreases with higher levels of salt thus less sugar will be utilized by the yeast and therefore will be available for



caramelization. It was confirmed that the absence of salt decreases the intensity of brown color of bread, so bread becomes whiter (Farahnaky, 2007).

However, fundamental rheology has been used to describe the effect of large amounts of salt on dough with contradictory outcomes (Larsson, 2002). Furthermore, various authors have reported that adding salt either increases, decreases or has no effect on specific volume measurements (Czuchajowska, 1989). An experiment conducted on wheat pan bread showed that the higher the salt content the lower the loaf volume (Linko, 1984).

#### ***4. Effect on shelf life and staling***

Traditionally, pita bread was purchased and consumed within hours of baking. However, because of changing lifestyles and urbanization, bread is distributed over long distances, hence the need for a longer shelf-life. Although Arabic bread is more shelf stable compared to fresh foods, it is subject to spoilage in a similar manner to other processed food products.

Bread staling (physical spoilage) has been a major concern in industrial bakeries due to the large economic losses reported. In a global market of approximately 20 billion pounds of bread produced annually, the return of 3% (600 million pounds) due to staling is an economic loss to both the producer and consumer. Staling leads to a loss of consumer acceptability and therefore to a decrease in sales. During storage, many changes between the bread components lead to a stale flavor. The bread crumb becomes very firm while the crust becomes less crispy. Bread loses its fresh odor. In the case of Arabic bread, the loaves lose their elasticity and the texture hardens. Researchers are actively investigating the factors governing bread staling. Staling is defined as "almost any change that occurs in bread during the post baking period,

making it less acceptable to the consumer" (Zobel, 1996). The major changes that occur after baking are moisture redistribution, starch retrogradation, increased bread firmness associated with loss of flavor and aroma (Quail K. M., 1991). Pita bread, if packaged soon after cooling, is considered fresh for up to 8 hours. After 8 hours, staling begins, however, after 24 hours, pita bread is still acceptable. Beyond 24 hours, pita bread tends to stale (Quail K. M., 1991) if left on the shelf and unpackaged. Evidence shows that bread with high protein content tends to have high specific volume, softer crumb and lower firming rate. Gluten quality is positively related to the rate of firming (Maleki, 1980). In addition, moisture plays a significant role in staling through evaporation and water redistribution (Baik, 2000); (Bollain, 2005). It is known that different moisture contents can result in different degrees of retrogradation during aging (Zeleznek, 1986); (LIU, 1998). In fact, water has a major part in the firming process, either by enhancing the molecular mobility of polymer chains or by acting as a coordination agent between them (Schiraldi, 1996). There is no clear trend about the effect of salt on bread staling. However, salt, similar to any ionic compound, is characterized by its moisture retention ability. It will logically influence the mobility of the polymers and the distribution of water in the system.

### ***5. Sodium analysis in bread***

Bread is known to be a rich source of salt in the diet. Six types of local breads were analyzed in Saudi Arabia to determine their chemical composition. Among minerals, sodium ranged between 64.4 and 301 mg/g (1.6 % and 7.5 %) depending on the bread variety. Both tames and Korsan bread seem to have a high salt percentage: 3.01 % and 2.88 % respectively. (E. I. Moussa, 1992) .

A study conducted in Egypt, focused on the mineral and trace elements contents of nineteen types of bread. The overall average sodium concentration was estimated to be 1709 micrograms/gram(0.05%). However, some samples showed elevated quantities of salt (5450 micrograms/gram; same as above) when compared to the RDA. (Felib Y. Iskander, 1992).

In many countries, authorities set strict limits to the amount of salt used in bread production. A Portuguese study wanted to compare the accuracy of three methods in determining the sodium content of bread; and whether the amount was in the norms put. The results did not differ significantly but the amount of sodium chloride exceeded the legal limit (1.4%). (A. Plácido, 2012).

Cross sectional surveys were carried in the UK to explore the salt contents of bread available in the market. In 2001, the average salt level was 1.23 %. It was reduced to 1.05 % in 2006 and 0.98 in 2011. The UK was able to reach a 20 % cutback in the quantity of salt used in bread over the last decade (Hannah C Brinsden, 2013), thereby showing the promise of well thought and planned policies.

## **H. Sodium intake**

### ***1. Methods for assessing Sodium intake***

Sodium intake can be determined using various methods. The estimation can be indirect (either from questionnaire or food consumption data) or direct (by the measurement of urinary excretion). Published reports refer to sodium intake as either mass or millimolar amounts of sodium or as mass of sodium chloride (salt).

a. Dietary survey methods

In general, dietary survey methods are used to gather data on food consumption as reported on the questionnaires. The data is then converted into nutrient intakes estimated from the standardized food tables. However, those methods are prone to a number of errors such as incomplete and inaccurate food composition tables, reporting errors and sampling bias. With regard to sodium intake, more specific sources of errors are found. It can be very difficult to estimate the amount salt added during cooking and in restaurants. In addition, a portion of the salt may be left on the plate; hence not consumed. And finally, manufactured food products may have a variable amount of sodium chloride depending on the brand. As a result of the abundant errors stated previously, dietary survey methods tend to underestimate sodium intakes when compared to urinary excretion measurements.

b. 24-hour urine collections:

Because of the problems of underestimation of sodium chloride intakes in dietary survey methods, 24-hour urinary sodium excretion has become the best method to obtain data on salt consumption. There is a marked diurnal variation in sodium; thus the need for a 24-hour period to capture the patterns of sodium excretion. In healthy individuals, electrolyte excretion reaches a maximum before midday and a minimum at night. This method does not take into consideration electrolyte loss in organs other than the kidneys. Its disadvantages include: burdening the participants, incomplete collections and inaccurate timings. To overcome this trouble, overnight and spot urine collections are proposed since they require less effort. In order to calculate an individual's sodium excretion, three values are needed: Sodium concentration (mmol per liter), total volume of urine collected (mL) and collection time. Flame absorption

spectrophotometry (or ion selective electrode potentiometry) is conducted to measure the sodium concentration. To calculate 24-hour sodium excretion, sodium concentration is multiplied by total volume and their product is multiplied 24/collection time (in hours).

c. Other markers of sodium intake:

Other methods to determine sodium intake have been investigated. Chloride titration sticks show a correlation between chloride excretion and sodium excretion. Petinen studied the relationship between the excretions of those two minerals in 1976. The correlation factor was 0.87. This technique shows however a variety of inconveniences. Sodium is not the only potential source of chloride. In addition, the stick has a very narrow calibration range and is not precise enough to estimate dietary sodium intake.

Other procedures have been explored in preliminary studies but they are not established for use in population studies. These include sodium content in human hair and salivary sodium concentrations. Hair mineral analysis is common among young babies and children. (Paul Elliott, 5-7 October 2006).

## CHAPTER III

### MATERIALS AND METHODS

#### A. Bread Sample Collection

##### 1. Bread Types

Since there are many kinds of bread available in the Lebanese market, the six most consumed types were chosen for the purpose of this study: White Arabic/Pita, brown Arabic/Pita, white Baguette, brown Baguette, Tannour and Markouk. Table 4 below provides the characteristics for each of the above bread types.

Table 3. Ingredients and brief description of bread types examined

<b>Bread type</b>	<b>Description</b>	<b>Ingredients</b>
<b>White Pita</b>	Most consumed bread in Lebanon with almost every dish	White flour, yeast, sugar, salt, water
<b>Brown Pita</b>	A developed and “healthiest” version of white pita	Whole wheat flour, yeast, sugar, salt, water
<b>White Baguette</b>	Mostly consumed with sandwiches	White flour, yeast, salt, sugar, fat, water
<b>Brown Baguette</b>	A developed and “healthiest” version of white baguette	Whole wheat flour, yeast, salt, sugar, fat, water
<b>Tannour</b>	Traditional Lebanese bread made in villages and baked in a ceramic oven	Wheat flour, whole wheat flour, wheat bran, whole corn flour, yeast, salt, water
<b>Markouk</b>	Traditional and thin Lebanese bread made in villages and baked on <i>saj</i>	Whole wheat flour, white flour, corn flour, salt, yeast, water

## 2. Bakeries

In order to cover as much as possible from the bread market in Lebanon, samples were collected from 45 bakeries in Lebanon. Those bakeries were located in all Lebanese provinces (Mouhafazat). According to previous data, those bakeries are responsible for 80% of the bread production in Lebanon. They varied between large scale bakeries, medium scale bakeries and small scale bakeries. Some had several branches in the same province, only one of them was selected to collect samples. Bread was gathered from each region depending on the availability of different types. Baguette samples were put in paper bags to minimize any moisture loss during transportation.

Table 4. Bakeries visited in each province

<b>Mouhafaza</b>	Beirut	Bekaa	Mount Lebanon	North	South
<b>Number of bakeries visited</b>	3	14	17	6	5

The bakery distribution in the different provinces, the number of samples collected for each type of bread and the samples collected from each bakery are illustrated below in Figures 2 and 3, and Tables 4 and 5 respectively.

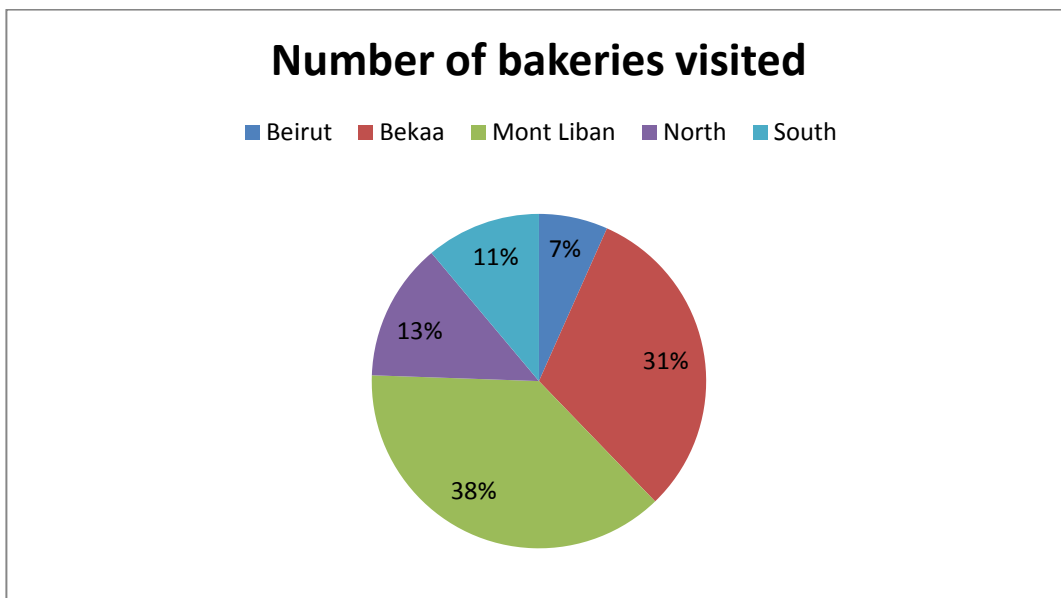


Figure 2. Bakery distribution in provinces

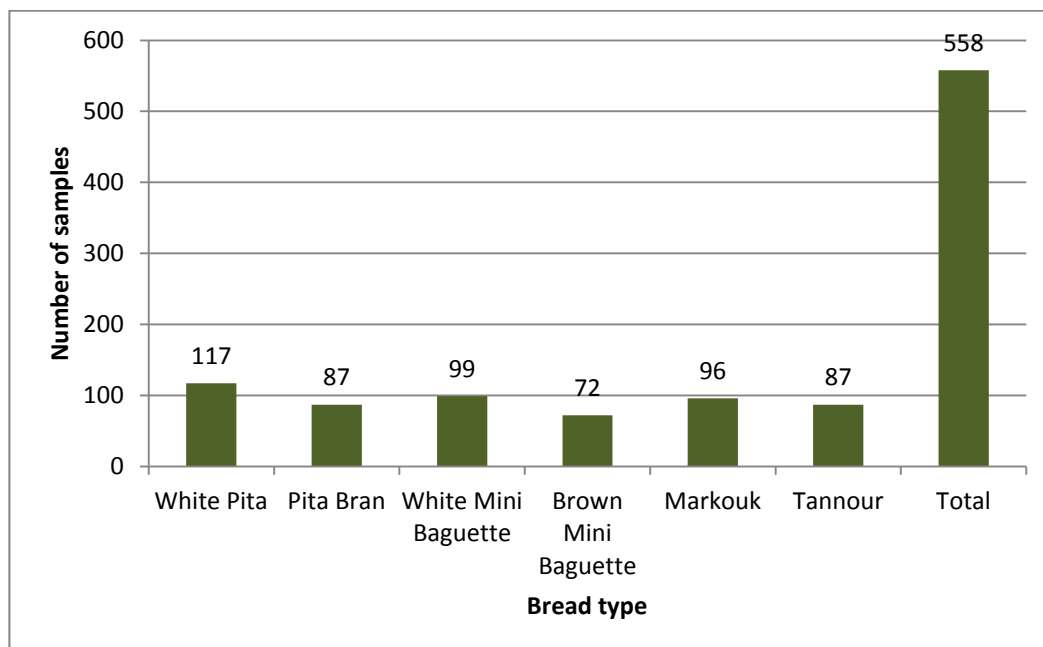


Figure 3. Number of bread samples by type



Table 5. Bread samples collected from each bakery

<b>Bakery</b>	<b>White pita</b>	<b>Brown Pita</b>	<b>White baguette</b>	<b>Brown baguette</b>	<b>Markouk</b>	<b>Tannour</b>
1	X	X	X	X		
2	X	X	X	X	X	X
3	X	X	X	X	X	X
4	X	X	X	X	X	X
5	X	X	X	X	X	X
6	X	X				
7	X	X	X	X	X	
8	X	X	X	X	X	
9	X					
10					X	
11					X	
12						X
13	X	X	X	X	X	
14	X	X	X	X	X	
15	X	X	X	X	X	X
16	X	X	X	X	X	X
17	X	X	X	X	X	X
18	X	X	X	X	X	X
19	X	X	X	X	X	X
20	X		X	X	X	
21	X	X	X	X	X	X
22	X	X	X			X
23	X	X	X			X
24	X	X	X	X	X	X
25	X	X	X	X		X
26	X					
27	X	X	X	X	X	X
28	X	X	X	X	X	X
29	X	X	X			
30	X	X	X			
31	X					
32	X	X	X	X	X	X
33	X	X	X	X	X	X
34	X	X			X	X
35	X		X	X	X	X
36	X		X	X	X	X
37	X	X	X		X	X
Local bakeries(n=8)	X		X		X	X

## B. Chemical Analyses

### 1. Equipment used

The table below includes all pieces of equipment used during the course of the experiments. The role of each one will be elaborated in the future sections.

Table 6. List of machinery, glassware and chemicals used

<b>Machinery</b>	<b>Glassware</b>	<b>Chemicals</b>
<i>Balance</i> (NETTLER TOLEDO, USA ML303/01)	Funnels	Deionized water
Oven MEMMERT WISCONSIN, USA OVEN	Beakers	Sodium stock solution
<i>Muffle Furnace</i> LINDBERG, Netherlands/ BLUE M (Box Furnace)	<i>Filter paper</i> (Whatman 3, Cat No 1003-110)	<i>Hydrochloric acid</i> (HCl) 37% AnalaR NORMAPUR, Ireland batch 10K040517
<i>Hot plate</i> (WiseStir, Germany MSH-20A)	Transfer pipette	<i>Nitric acid</i> (HNO <sub>3</sub> ) 65% AnalaR NORMAPUR, Ireland batch 12J040521
<i>Desiccators</i>	5 mL pipettes	<i>Cesium</i> (Cs) SIGMA-ALDRICH, USA Lot # MKBQ1548V
<i>Aluminum dishes</i>	15 mL plastic tubes	
<i>Crucibles</i>	50 mL plastic tubes	
<i>Atomic Absorption spectrophotometer</i> (Thermo Labsystems, SOLAAR, Netherlands)	Micropipettes	
	100 mL	
	Volumetric flasks	

### 2. Methods Sequence

For determining the amount of salt in different types of bread, the AACC method 40-71 was followed. A representative sample (around 5 grams) was dry-ashed. The residue was then dissolved in acid (50 % HCl) and diluted 400 times to a 10 mL volume. This solution, or a dilution thereof, was analyzed for sodium content by atomic

absorption spectrophotometry. Cesium chloride was added to all solutions as an ionization suppressant. The flow chart in Figure 4 below summarizes the steps followed for this method.

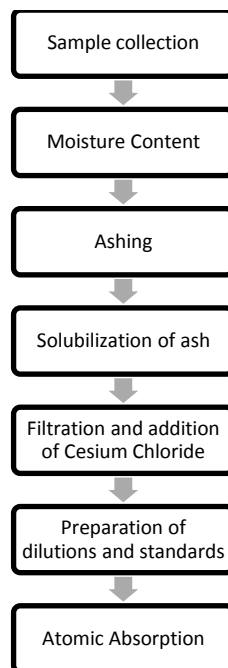


Figure 4. Flowchart of methodology followed

a. Moisture content

Three replicates from each bread type were analyzed. Samples were torn, weighed on the balance and transferred into aluminum dishes. The dishes were covered and then put in the forced air oven for 3 hours at 110 °C. After the drying process was complete, dishes were moved to a desiccator so they can cool down. Dried bread samples were weighed and moisture content was calculated according to the following formula: % moisture = [(weight of sample before drying – weight of sample after drying) / weight of sample before drying] x 100.

b. Ashing

The three dried replicates were placed inside porcelain crucibles and put into the muffle furnace. The samples were left at 500° C in the furnace for 12-15 hours. Subsequently, crucibles were allowed to cool in the desiccator. Meanwhile, chemical working solutions were prepared so they could be used in the following steps.

Preparation of chemical solutions:

Sodium stock solution: 2.452 g of commercial NaCl were dried for 2 hours at 110°C in the oven and dissolved in 1 L of water. The solution was stored in a polyethylene bottle.

Cesium stock solution (CsCl): 12.668 g of Cesium were dissolved in 2 L of deionized water and 82 mL of pure HCl.

Hydrochloric acid (1+1): 1 L of deionized water was mixed with 1 L of HCl.

20% HNO<sub>3</sub>: 400 mL of pure HNO<sub>3</sub> were made up to 2 L with deionized water.

All glassware needed to prepare bread dilutions and standards were soaked overnight in the nitric acid solution.

c. Solubilization of ash

It usually takes less than one hour for the crucibles to cool down. Crucibles were weighed on the balance before starting the digestion step. Ten mL of (1+1) HCl were added to each sample inside the hood and the crucibles were rotated on the hotplate . They were then left until the ash was dissolved.

d. Filtration and addition of Cesium Chloride

Hundred mL volumetric flasks and funnels were soaked overnight in  $\text{HNO}_3$  solution to avoid any contamination. Crucibles filled with samples ashes and HCl were emptied through filter paper. Deionized water was used to rinse the crucibles and empty their contents through the filter paper for three times to make sure there was no deposit left inside them. The cesium chloride stock solution was added until the marked line of 250 ml was reached.

e. Preparation of dilutions and standards

Dilutions

A dilution of 400 was chosen for the purpose of this experiment. It was picked after several preliminary trials were conducted to determine the dilution that yields an absorbance value in the middle region off the standard curve.

Test tubes were labeled with the sample number and 10 mL of deionized water were poured into disposable plastic tubes. 25 $\mu\text{L}$  of each bread sample solution were added to the tubes. Transfer pipettes and manual agitation were required to make sure that the bread solution was well dissolved in water.

Standards

Standards with concentrations of 0.2, 0.4, 0.8, 1.6, 2 and 2.5 $\mu\text{g Na/ml}$  were prepared by diluting the stock solution (1000  $\mu\text{g Na/ml}$ ) as outlined in AACC method 40-71. The components of each standard solution and their respective concentrations are presented in Table 8. Cesium chloride to a concentration of 0.1% in the final solution was added to suppress ionization.

Table 7 below summarizes the components of each standard solution and their respective concentrations.

Table 7. Concentration of different components used in the preparation of standard solutions

<b>Concentration</b>	<b>De-ionized water</b>	<b>Na stock solution</b>	<b>Cesium Chloride (0.1%)</b>
0 (Blank)	50 mL	0 $\mu$ L	5 $\mu$ L
0.2	50 mL	10 $\mu$ L	5 $\mu$ L
0.4	50 mL	20 $\mu$ L	5 $\mu$ L
0.8	50 mL	40 $\mu$ L	5 $\mu$ L
1.6	50 mL	80 $\mu$ L	5 $\mu$ L
2	50 mL	100 $\mu$ L	5 $\mu$ L
2.5	50 mL	125 $\mu$ L	5 $\mu$ L

All test tubes containing the standards were manually shaken so all components are properly dissolved in water.

f. Atomic Absorption

Bread samples were divided into several batches. Before each batch, standards solutions were read. The spectrophotometer parameters were established in the beginning of every lot. The calibration curve from the standards was prepared before and after each sample group. After the procedure ended, the software provides a plot of absorption versus concentration. The results were exported in the form of an Excel

Microsoft file that provides the concentration of each bread sample. It is important to note that in some trials, standards had to be repeated because the r-squared coefficient was relatively low ( $r^2 < 0.95$ ).

g. Standard reference material (SRM)

The standard reference material was used to calibrate instrumentation and evaluate the reliability of the above analytical method. The National Institute of Standards and Technology provides with each material a certificate of analysis that includes certified concentrations of the constituent elements. The values are based on results obtained by definitive methods of known accuracy; or alternatively, from concordant results by two or more independent analytical methods. Bovine liver (1577c) and non-fat milk powder (1549) were analyzed. The certificate of analysis provides instructions on the handling of these products. The same method followed in the chemical analysis of bread (AACC method 40-71) was applied to the SRM. The amount of sodium was then compared to the value found on the certificate to see if the results obtained were consistent. The results obtained for the standards are included in Appendix I.

h. Recovery

To assess the loss of salt during the determination, the recovery of sodium chloride should be determined. The bread samples with lowest salt concentration were chosen. Five replicates of each of 2, 4 and 6 mg of stock solution were used. After adding the stock solution to salt free bread replicates (tested earlier), AACC method 40-

71 was applied. The salt concentration was determined to account for the recovery of sodium during this whole process.

i. Statistical Analyses

- Calculations for salt content

The AACC method followed provides a formula that converts bread sample concentrations into sodium weight. The formula used is:

$$\text{mg} / 100 \text{ g} = \frac{(C_s - C_b) V \times D}{S \times 10}$$

where:

$C_s$  = concentration of sample ( $\mu\text{g}/\text{ml}$ )

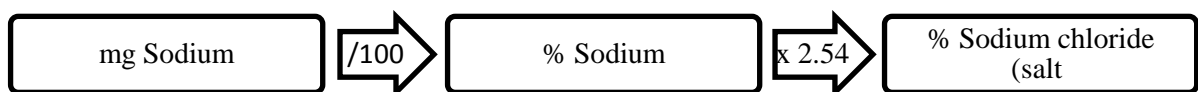
$C_b$  = concentration of blank ( $\mu\text{g}/\text{ml}$ )

$V$  = original volume (ml)

$D$  = dilution volume (ml)/aliquot for dilution (ml) if original solution is diluted

$S$  = sample weight (g)

To express the results in the form of salt percentage, two steps should be followed.



*Step 1. Conversion of sodium weight to sodium percentage*

The formula provides the results on a weight basis. To convert them into a percentage, the weight should be divided by 100.

*Step 2. Conversion of sodium percentage to sodium chloride percentage*

To convert sodium to salt (Sodium chloride), the sodium percentage above is simply multiplied by 2.54. Chemically, sodium chloride is made of 1 ion of sodium and



1 ion of chloride. However, sodium constitutes about 39.3% of the total weight of the salt molecule and that is why it should be multiplied by 2.54.

- Statistical Model

Analysis of variance using the GLM procedure of SAS<sup>®</sup> (version 8.02, SAS Institute Inc., Cary, NC) was performed to assess the significance of the salt content differences among the experimental samples. In the statistical model (Appendix II), the response variable was the salt (sodium chloride) of the samples. The factors in the model were province (governate or mouhafaza in Arabic; there were five for Beirut, Mount Lebanon, Bekaa, South and North), product for type of bread(6 types; White Arabic bread, brown Arabic bread, white French Baguette, brown French Baguette, Markouk and Tannour). Mouhafaza and product were fixed effects in the model. In addition, the mouhafaza x product interaction was included in the model. Significant means for the analyses were separated by Tukey's honestly significant difference test. Significance was established at  $\alpha < 0.05$

**C. Bread Intake**

The data collected aimed at estimating the salt intake from bread consumption in the Lebanese population. The subjects included in the data above were randomly selected. Eligible participants met the inclusion criteria. The criteria included: identification of nationality (must be Lebanese), age group (above the age of 18), consumption of bread and knowledge of bread brands. The design of the study was approved by the Institutional Review Board (IRB) of the American University of Beirut and it is conducted in compliance with the protocol approved by the IRB. The informed

consent (Appendix III) describes the purpose of the study and the procedures to be followed. A copy of the consent form was offered to the participants. Written permissions to conduct the study in the targeted supermarkets were obtained from the study sites.

### ***1. Data Collection***

Data was obtained from specific supermarkets in the different regions of Lebanon, specifically Hamra, Ashrafieh (Beirut), Jeita and Kornet Chehwan (Mount Lebanon), and Akkar (North), thereby covering 3 of the 5 governates/mouhafazat in Lebanon. The interviews were conducted at the entrance of the supermarkets in the morning and in the afternoon following a selection of every fourth person who enters. The face to face interviews, which lasted for approximately 10 to 15 minutes, were conducted to collect data. Quality control measures including data collection procedure and field monitoring of data collection were applied (Appendix IV). Socio-demographic data were collected from the participants using a multicomponent food frequency questionnaire. The questionnaires were filled through an oral, voluntary, anonymous and confidential basis.

### ***2. Tools and questionnaire***

The questionnaire was divided into four main parts (Appendix V). The first part included the participant ID and the interview date reflecting the anonymous feature of this protocol. The second part had close-ended questions about the participants' characteristics, namely gender, age, educational level, family status, work status, residence rooms (total rooms excluding the kitchen, bathrooms, corridors, balconies and

parking), residence/co-residence persons, weight and height. The third part consisted of the food frequency questionnaire main part that was divided into different sub-categories based on open-ended questions. The first sub-category included the different types of local bread consisting of: white Arabic bread, brown Arabic bread, Markouk bread, Tannour bread, white and brown Baguettes. The second sub-category was comprised of the different portion sizes whereby small, medium and large reflected different amounts of grams per bread type. These were 28g, 56g and 122g for small, medium and large for Arabic bread, 68g for baguette, 94 g for markouk and 64 g for tannour. The third sub-category included the bread intake pattern which was divided into daily, weekly, monthly or rarely/never. The fourth sub-category had the bread brands that are purchased by the subjects of the study. The fourth main part of the questionnaire consisted of close-ended questions regarding low salt consumption of different types and brands of bread. To assist subjects in assessing the portion/amount of bread consumed, quantification tools, such as images of different bread sample sizes, were used.

### ***3. Statistical Analyses***

#### ***a. Data Calculations***

The work of the whole study was divided amongst four groups in which each field personnel (student) was responsible for conducting 50 questionnaires (for undergraduate and 100 for graduate) for a total of 450 questionnaires in different sites. The obtained information from the questionnaires was filled in a Microsoft Excel worksheet. The numerical data was assigned to different variables in the spreadsheet (province, participant ID, gender, age, educational level, family status, work status,

residence rooms, residence persons, weight and height). Furthermore, the Excel worksheet included the different types of bread with the frequency of consumption for each type and the brand names, if provided by the interviewed subjects. The intake values for each type of bread were calculated by inserting formulas in the cells of the Excel worksheet. For example, if a person consumes daily two medium sized loaves of white Arabic bread then the total amount consumed is calculated by multiplying the average portion size by the number of loaves consumed. In this case, one medium white Arabic bread loaf weighs 58 grams and accordingly this gives a total number of 116 grams of white Arabic bread per day. If the product was consumed on a weekly or monthly basis, the resulting quantity was divided by seven or 30, respectively.

b. Statistical Model

Analysis of variance using the GLM procedure of SAS<sup>®</sup> (version 8.02, SAS Institute Inc., Cary, NC) was performed for the salt intake data among the different subjects. In the statistical model (Appendix II), the response variable was the daily bread intake in grams of the subjects. The analyses were conducted separately for each type of bread. For each type of bread, the factors in the model were province (or mouhafaza in Arabic; 3 levels for Beirut, Mount Lebanon and North), gender, education level (5 levels). BMI and age. All of these variables were fixed effects in the model and age and BMI were included as continuous variables while other variables were discrete ones. In addition, the province x gender, province x education level and gender x education level interactions were included in the model. Significant means for the analyses were separated by Tukey's honestly significant difference test. Significance was established at  $\alpha < 0.05$ .

## CHAPTER IV

### RESULTS AND DISCUSSION

#### **A. Moisture content**

The average moisture content of all samples was found to be 23.6%. The values ranged from 13.6% and 34.1 %. The lowest value was attributed to tannour and the highest one was for a brown mini baguette.

The results per type of bread are summarized in Table 8. The highest average moisture content was found in brown mini baguette samples (23.6%) while brown Pita had the lowest moisture content (23.5 %). The observed differences between the different types of bread were expected and can be attributed to the different water content and ingredients used to produce each type and to the storage techniques used in the bakeries. The moisture content fluctuated also within a certain type of bread. The bakeries visited varied between large scale ones and small scale bakeries. Therefore, small differences in processing methods and the level of mechanization in bread making could have an influence on moisture content. All of the breads in the table have a relatively high coefficient of variation, and a sizeable difference between the highest and lowest values. The above results presented are lower than some of those found in the literature. Brown Pita bread contained 32 % of water (Mahmoud Abu-Ghoush, 2008), and baguette ranged between 22 % and 26 % (Laura Bravo, 1998)

Table 8. Descriptive statistics of bread

<b>Bread type</b>	<b>Average</b>	<b>Max</b>	<b>Min</b>	<b>SD</b>	<b>CV</b>	<b>Range</b>
<b>White Pita</b>	23.58	19.63	33.99	3.14	13.31	19.63-33.99
<b>Brown Pita</b>	23.55	19.45	28.38	3.12	13.23	19.45-28.75
<b>White Baguette</b>	23.60	14.38	32.25	3.15	13.36	14.38-32.25
<b>Brown Baguette</b>	23.64	15.75	34.12	3.12	13.19	15.75-34.12
<b>Markouk</b>	23.56	14.57	32.21	3.15	13.37	14.54-32.21
<b>Tannour</b>	23.59	13.59	27.63	3.20	13.55	13.58- 27.63

## **B. Sodium determination:**

### *1. Standard reference material*

The results for standard reference materials came as follows. In the first trial, the lowest value for non-fat milk powder was 484 mg of sodium; and the highest value was 508 mg of sodium. The amount of sodium in bovine liver ranged between 196 mg and 208 mg. In the second trial, sodium was found to be between 488 mg and 506 mg in non-fat milk powder; and between 190 mg and 210 mg in bovine liver.

Table 9. Descriptive statistics of standard reference materials

		<b>Average</b>	<b>SD</b>	<b>CV</b>	<b>Expected</b>	<b>Accuracy</b>	<b>range</b>
Non Fat milk	Trial 1	494.7	8.64	1.74%	497	99.54%	[484,508]
	Trial 2	497.3	7.65	1.54%	497	100.06%	[488,506]
Bovine liver	Trial 1	199.3	5.88	2.95%	203.3	98.03%	[196,208]
	Trial 2	198.3	7.79	3.90%	203.3	97.54%	[190,210]

\*Accuracy was calculated as: (measured value / expected value) x 100

a. Non-fat milk powder

The average amount of Sodium was 494.7 mg in the first trial and 497.3 mg in the second trial. In both trials the standard deviation was relatively small (8.64 and 7.65). This means that the difference between all readings is small. The coefficient of variation is also low. The variability relative to the mean is then considered to be small.

b. Bovine liver

The average quantity of sodium was 199.3 mg in the first trial and 198.3 mg in the second trial. The standard deviation was very small as well (5.88 and 7.79). The readings of all replicates then are close to each other. Both coefficients of variation (2.95% and 3.9 %) indicate that there is a low variability with regards to the mean.

The accuracy of a measurement represents the degree of closeness between the measured value and expected value. Both trials were highly accurate. This means that the amount of salt measured in all bread samples represents the actual amount of salt found. The method used during this research is reliable and precise.

**2. *Salt content in bread***

Table 10 below summarizes the salt content results by type of bread. A rough analysis of the data shows that the highest salt content was found in markouk (average 2.8%). However, there was a remarkable difference in the quantity of salt found within the same type of bread. A certain sample of markouk contained the maximum amount of salt (5.22%) while the lowest was 0.5%. A couple of samples of white pita and brown mini had no salt at all, as indicated on the package of baguette. Obviously, given the wide range of sodium content between the different bakeries, both the coefficient of variation and the standard deviation were high. This indicates that within each bread

type, salt levels varied. This might be explained by several factors. First, the bakery size plays an important role. Large scale bakeries tend to have automated processing techniques with standardized recipes, thus they use less salt; whereas smaller scale bakeries (and local bakeries) are prone to adding more salt. Second, the location of the bakery probably has an effect on the salt percentage. In urban areas, most bakeries follow strict guidelines and some of them even claim to have a low salt content. This does not apply to villages.

Table 10. Descriptive statistics of salt content in bread

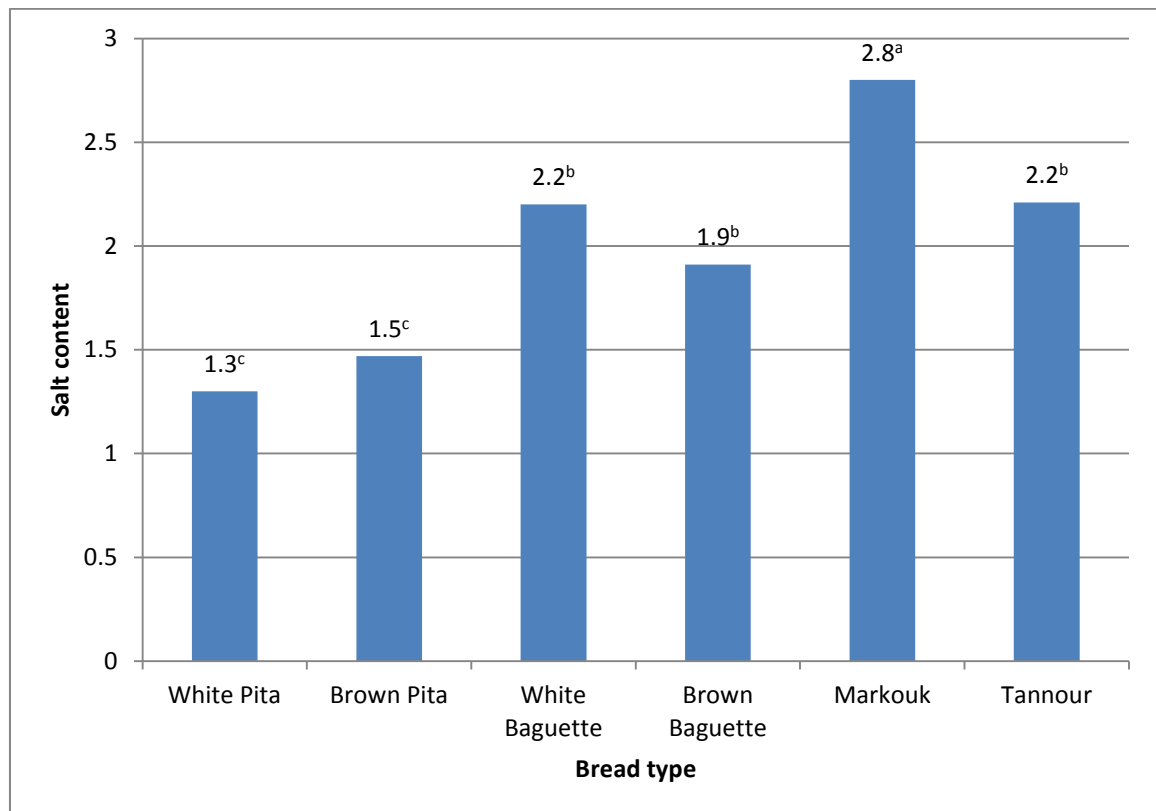
<b>Product</b>	<b>Salt %</b>	<b>SD</b>	<b>CV</b>	<b>Range</b>
<b>White pita</b>	1.3	0.66	50.77	0-3.08
<b>Brown Pita</b>	1.5	0.77	52.38	0.1-3.37
<b>White baguette</b>	2.2	0.9	40.91	0.38-3.95
<b>Brown baguette</b>	1.9	1.03	54.21	0-4.30
<b>Markouk</b>	2.8	1.16	41.43	0.50-5.21
<b>Tannour</b>	2.2	0.96	43.64	0.17-4.16

a. Salt content vs bread Type

Markouk bread (2.8%) had significantly higher salt content than other bread types. White pita and brown pita (1.3 % and 1.5 % respectively) contained the lowest amounts of salt and were significantly different from others. Both types of baguette, and tannour ranged in the middle and were significantly different from the two extremes. Those differences in salt content are probably due to the recipe followed. Each type of bread has a specific processing technique that affects the sensory attributes of the



products. It is common in Lebanon to add high amounts of salt when preparing markouk.



a,b, c Means with different superscripts are significantly different ( $P < 0.05$ )

Figure 5. Average salt content by bread type

#### b. Brief summary of the results

On average, the lowest salt content for all bread types was found in Beirut and the highest was in the South. But if one looks closer at the data different results can be noted. Three bread samples showed to contain no salt at all: White pita from a certain bakery and brown mini baguette from two different bakeries. Table 11 below summarizes the highest and lowest value of salt content of each bread type.

Table 11. Highest and lowest salt content of each bread type

<i>Bread type</i>	<b>Lowest</b>		<b>Highest</b>	
	<i>Bakery</i>	<i>Salt %</i>	<i>Bakery</i>	<i>Salt %</i>
<b>White pita</b>	A (Beirut)	0.19	F (Beirut)	2.72
<b>Brown Pita</b>	B (Mount Lebanon)	0.11	G (Mount Lebanon)	3.37
<b>White baguette</b>	C (Mount Lebanon)	0.44	H (Mount Lebanon)	3.85
<b>Brown baguette</b>	A (Mount Leb.)	0.53	I (North)	3.72
<b>Markouk</b>	D (Mount Lebanon)	0.56	J (Mount Lebanon)	5.12
<b>Tannour</b>	E (Bekaa)	0.17	K (Mount Leb.)	4.12

Quilez et al. (2012) summarized previous studies comparing the salt levels in bread in different European countries. Salt ranged between 1.36 % and 1.67 % in white pan bread in Portugal. In Spain, the amount of salt in white bread and brown bread were the same, around 1.28 %. In France, white bread contained 1.8 % of salt while it was 1.01 % in the U.K. The salt content was considerably higher in Turkey, with bread having 1.82 % salt. Some countries have set policies to reduce salt intake. In Australia and New Zealand, a study was conducted to define the effectiveness of recent efforts by the Australian Division of World Action on Salt and Health and the Heart Foundation in New Zealand to reduce sodium levels in breads (Elizabeth K Dunford, 2011). The salt percentage was reduced in whole meal bread from 1.05 % to 1.02% in Australia, while it was reduced in New Zealand from 1.3 % to 1.16 % in white bread, 1.13 % to 1.12 % in whole meal bread and 1.17 % to 1.09 % in mixed grain bread. A regional study was conducted in Iran to study salt levels in all types of bread in Shiraz city. A reduction of salt in bread during the last two decades was observed in Iran. However, 17.9% of bread sample were still higher than the standards set for salt content in bread. (MJ Zibaenezhad, 2010). Lavash, which is somehow between markouk and pita, contained about 1.47 % salt. Mashini, which is similar to tannour, had 0.88% salt.

### 3. Recovery

As expected, all samples had high levels of recovery. Sodium does not evaporate at high temperatures. Table 12 below shows values of sodium collected after ashing. Samples spiked with 2, 4 or 6 mg of Na had mean recovery rates of 96.4%, 97.4% and 96.33%, respectively. In addition, the standard deviations for the mean of each group of replicates per level were very low (1.29, 1.18 and 3.1, respectively), indicating replicate values that were very close to each other. All of the above results are indicative of the level of precision and replicability of the results obtained in this work.

Table 12. Recovery results

Sample number	Sodium stock solution added	Sodium collected after ashing	% recovery	Average	Standard Deviation
1	2 mg	1.92	96.00		
2	2 mg	1.93	96.50		
3	2 mg	1.86	98.00	96.40	1.29
4	2 mg	1.89	94.50		
5	2 mg	1.94	97.00		
6	4 mg	3.93	98.25		
7	4 mg	3.88	97.00		
8	4 mg	3.96	99.00	97.40	1.18
9	4 mg	3.85	96.25		
10	4 mg	3.86	96.50		
11	6 mg	5.98	99.67		
12	6 mg	5.82	97.00		
13	6 mg	5.54	92.33	96.33	3.10
14	6 mg	5.92	98.67		
15	6 mg	5.64	94.00		

### C. Bread Intake

The descriptive statistics for gender on the four subject covariates are summarized in Table 13 below.

Table 13. Descriptive statistics for gender

Variable	Gender	N	Mean	SE Mean	SD
Age	Female	203	37.25	0.98	14.01
	Male	190	37.49	1.07	14.76
BMI	Female	203	23.46	0.269	3.832
	Male	190	27.34	0.764	10.54
White pita	Female	203	44.33	4.9	69.77
	Male	190	135.9	12.2	168.4
Brown Pita	Female	203	36.06	4.59	65.35
	Male	190	43.67	5.64	77.76
White Baguette	Female	203	10.93	2.12	30.26
	Male	190	17.41	3.91	53.85
Brown Baguette	Female	203	4.92	0.82	11.63
	Male	190	5.34	1.15	15.88
Markouk	Female	203	8.41	1.4	19.89
	Male	190	15.49	3.74	51.56
Tannour	Female	203	2.79	0.77	11.05
	Male	190	4.81	1.1	15.18

The mean age was  $37.25 \pm 14.01$  for females and  $37.49 \pm 14.76$  for males .BMI was  $23.46 \pm 3.832$  in females and  $27.34 \pm 10.54$ , in males indicating a certain level of overweight in the interviewed male population. This outcome is not unusual given the prevalent levels of overweight and obesity in the Lebanese population (Nasreddine, 2012).

The average daily intake values by type of bread and gender are illustrated in Figures 5 and 6. The average bread consumption differed among genders for each type of bread. Males consumed a much higher quantity of white pita daily compared to females (135.9 g and 44.33 g respectively). In fact, the average consumption for all

other bread types followed naturally the same rule; but with less disproportion between both genders. Females ate 36.06 g of brown pita whereas males ate 43.67. The disparity is higher for white mini baguette: 10.93 g for females and 17.41 g for males. The average daily intake of brown mini baguette was 4.92 g for females and 5.34 g for males. In the markouk category, the mean consumption varied between 8.41g for females and 15.49g for males. Finally, both genders had a similar trend in consuming tannour 2.79g for females and 4.81 g for males.

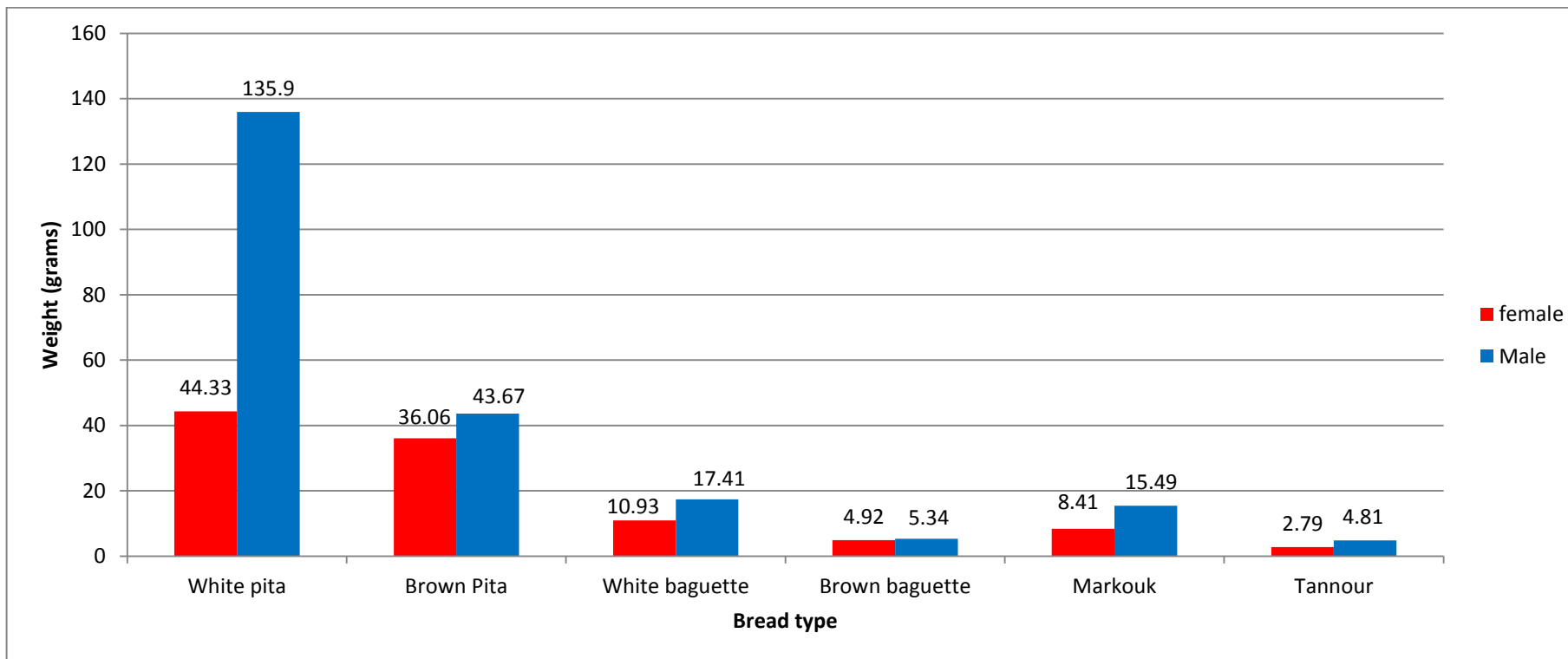


Figure 5. Daily consumption of bread among genders

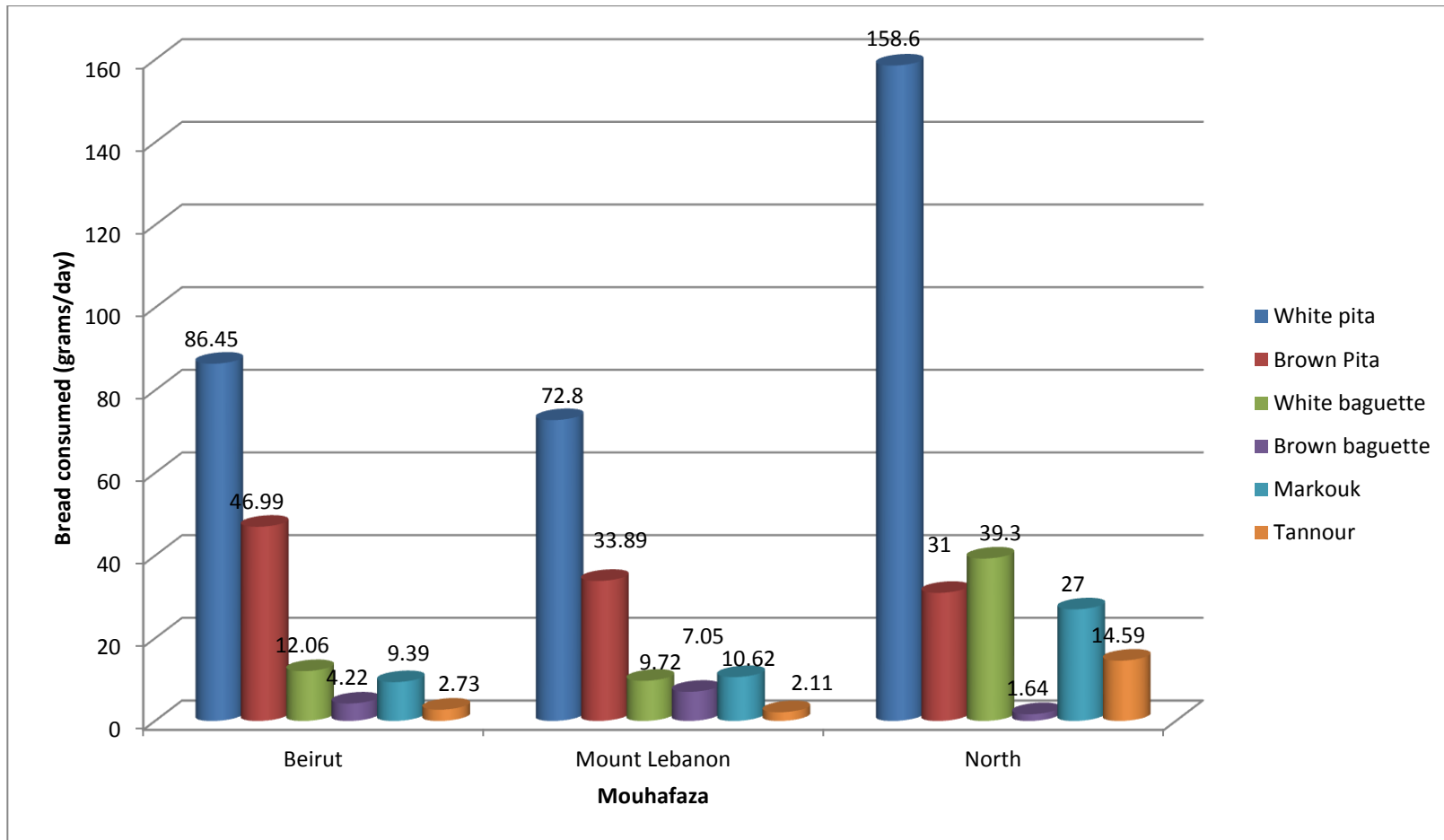


Figure 6. Daily consumption of bread by Mouhafaza

The results by Mouhafaza are illustrated in Figure 6. Mouhafaza also influenced the daily intake of bread consumed. In Beirut, white pita was the most consumed (86.45g per day) followed by brown pita (46.99 g per day), white baguette (12.06g per day), markouk (9.39g per day) , brown baguette (4.22 g per day) and tannour (2.73 g per day). In Mount Lebanon, the consumption trends were similar to Beirut. However, the quantities differed with white pita, brown pita, white baguette and tannour having lower values (72.8 g , 33.89g 9.72 g and 2.11g per day, respectively) while brown baguette and markouk were higher (7.05 g and 10.62 g per day respectively). In the North, the trends came as follows (from highest to lowest): White pita (158.6 g daily), white baguette(39.3 g daily), brown pita (31 g daily), markouk (27 g daily), tannour (14.59g daily) and brown baguette(1.64 g daily).

As seen on the graph, there are some visible disparities between provinces regarding the consumption of white pita. The highest consumption was found in the North (Akkar). This can be explained by several factors. First, Akkar is a poor region and does not represent well the North. People tend to consume more bread there because it is very cheap. In addition, bread being rich in carbohydrates, provides a rich source of energy. Second, it would be important to mention that not all bread types can be found in Akkar. In addition, males ate significantly more white pita than females (233.6g in comparison to 86.9 g) as summarized in Table 14 below.

The second highest daily consumption was attributed to males from Beirut. This value differed from that of Mount Lebanon males. Perhaps this is due to the higher intake of other bread categories as shown in Table 14.



The daily consumption of brown pita differed extensively between Beirut and the North. This might be due to the fact that such product is more available in the capital. The highest daily intake of white baguette was in the North.

Daily consumption of brown baguette varied between Mount Lebanon and the North, with Mount Lebanon as the mouhafaza with the highest intake of brown baguette.

The daily intake of markouk was the highest in the North and diverged notably from other regions. The only significant different group observed was the males from the North. They had the highest consumption of markouk (42 g per day).

According to Table 14, the quantity of tannour eaten in the North was higher than other regions. Males from the North had the highest average consumption of tannour (21.04g per day).

#### ***4. Salt from bread***

Based on the salt content and the daily intake data that are summarized in Table 15, a male consumes 3.45 g of salt from bread every day. The daily intake of salt from bread for females was 1.75 g per day. Those values seem quite high, compared to the daily recommendations of sodium (2.3 g per day which is equivalent to 6.34 g salt per day). For many individuals, more than half of the salt intake comes from bread if compared to the recommended levels. When the average for males and females is taken (2.6), it constitutes around 33 % of the estimated salt intake of the Lebanese population, which was estimated to be 7.82 g of salt (Nasreddine et al., unpublished data).

Interestingly enough, this is close to the percentage that was obtained in the aforementioned study albeit it included bread and items such as Manakish (26%) which were not included in this work.

Table 14. Consumption trends by gender and province

	White pita		Brown Pita		White baguette		Brown baguette		Markouk		Tannour	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
<b>Beirut</b>	46.99	133.9	41.19	54	9.13	15.59	4.52	3.86	7.97	11.11	3.00	2.4
<b>Mount Lebanon</b>	29.26	113.8	28.93	38.56	9.07	10.34	6.44	7.62	7.87	13.26	0.98	3.18
<b>North</b>	86.9	233.6	38.4	23.22	26	53.3	1.32	1.97	12.65	42	4.02	21.04

Table 15. Salt intake from bread

Type of bread	White pita		Brown Pita		White Baguette		Brown Baguette		Markouk		Tannour		Total
	Salt content		Salt content		Salt content		Salt content		Salt content		Salt content		
Intake (g)	Bread	Salt	Bread	Salt	Bread	Salt	Bread	Salt	Bread	Salt	Bread	Salt	
Females	44.33	0.58	36.06	0.54	10.93	0.24	4.92	0.09	8.41	0.24	2.79	0.06	1.75
Males	135.90	1.77	43.67	0.66	17.41	0.38	5.34	0.10	15.49	0.43	4.81	0.11	3.45

## CHAPTER V

### CONCLUSION AND RECOMMENDATIONS

This study showed that the salt content in bread in Lebanon ranged between 0 % and 5.2 %. The amount of salt was influenced by the type of bread, the location of the bakery (Mouhafaza) and the interaction of these two variables. In terms of salt content, white pita was significantly different from white baguette, brown baguette, markouk and tannour. The same applies for brown pita. White pita and brown pita had no substantial divergences in their salt levels.

The two types of baguette had no significant differences in their salt levels. Markouk had significantly higher salt content compared to the other types. Beirut had significantly lower levels compared to the South. In general, the lowest salt content was found in a certain brand of brown pita and the highest was in a brand of Markouk. Three bread samples had no salt at all.

Consumption trends of bread were influenced by gender and mouhafaza. However, for each type of bread, the parameters affecting the intake varied.

To conclude, the average daily salt intake coming from bread was 3.45 g among males, and 1.75 g among females.

Given the results of this study, it is recommended to reduce the amount of salt used in some bread types. It would be vital if the Lebanese authorities set some standard limits about the maximum amount of sodium chloride allowed to be used in bread.

Moreover, in order to obtain a more reliable idea about the contribution of each bread type and bread to dietary sodium intake, it is advised to conduct additional analyses based on the brands purchased by consumers. Bekaa and the South should also be a part of the regions visited to fill the food frequency questionnaires.

## APPENDIX I

### SRM RESULTS

		Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Reading 6
Trial 1	Non Fat milk	492	484	508	488	500	496
	Bovine liver	200	196	192	208	204	196
Trial 2	Non Fat milk	506	490	494	504	488	502
	Bovine liver	200	192	190	210	204	194

## APPENDIX II

### STATISTICAL MODEL SALT CONTENT

#### *Salt Content*

```
Proc GLM;  
  
class Bakery Mouhafaza Product;  
  
model Salt = Mouhafaza Product Mouhafaza*Product;  
  
meansMouhafaza Product /tukey;  
  
lsmeansMouhafaza*Product /pdiff=all;  
  
run;
```

#### *Salt Intake*

```
Proc GLM;  
  
classMouhafaza Gender Education_Level;  
  
modelFQ_Arabic_White = Mouhafaza Gender BMI Age Education_Level  
Mouhafaza*Gender Mouhafaza* Education_Level Gender* Education_Level;  
  
meansMouhafaza Gender Education_Level /tukey;  
  
lsmeansMouhafaza*Gender Mouhafaza* Education_Level Gender*  
Education_Level /pdiff=all;  
  
run;
```

## APPENDIX III

### INFORMED CONSENT

Institutional Review Board  
American University of Beirut

وثيقة موافقة شفوية للمشاركة في الدراسة 15 AUG 2014

RECEIVED  
مركز البحوث في الإستهلاك اليومي للسوديوم عند عينة من الرشدنين اللبنانيين  
الجامعة الرئيسية - لارا لصورالدين

أنت مدعو للمشاركة في دراسة تجريها الجامعة الأميركية في بيروت. هذا البحث يهدف إلى دراسة لسمية مساهمة الخبز اللبناني في الإستهلاك اليومي للسوديوم عند عينة من الرشدنين اللبنانيين لأهداف بحثية فقط. ستجرى هذه الدراسة على عينة تتألف من 500 من الرشدنين اللبنانيين في مراكز التسوق الرئيسية في جميع محافظات لبنان. ستتم مقابلة المشاركين على مدخل مركز التسوق و سيتم اعطائهم معلومات حول الدراسة و أهدافها من قبل طلاب في علم التغذية من الجامعة الأميركية في بيروت.

يعني لك المشاركة في هذه الدراسة إذا كنت من الطبقة اللبنانية و عرك 18 عاماً وما فوق. و إذا كنت تعلم حاركه-  
الخبز التي تتناولها.

إذا كنت موعلاً و توافق على المشاركة بالدراسة ، يجرى الانتقال معك على المكان المناسب لتعبئة الاستبيان. سوف نسألك عن بعض الخصائص الديموغرافية، و معلومات حول استهلاكك لبعض أنواع الخبز اللبناني التقليدي. إن تعبئة الاستبيان يستغرق حوالي 10 دقائق من وقتك.

إن البيانات المستحصلة لا تتضمن معرفات شخصية. كما إن الاستبيان لا تتضمن أسماء المشاركين و المعلومات المستحصلة ستبقى سرية. كذلك سيتم الحفاظ على خصوصيتك الفردية في جميع البيانات المنشورة والمكتوبة الناتجة عن الدراسة بدون توليف المعلومات الشخصية الخاصة باسم.

مشاركتك في هذا البحث طوعية تماماً ولكنها مهمة جداً بالنسبة لنا. في حال موافقتك على المشاركة بمساعدة على الحصول على معلومات مهمة تتعلق بمساهمة الخبز اللبناني التقليدي في الإستهلاك اليومي للسوديوم عند الرشدنين في لبنان.

إن مشاركتك في هذا البحث خالية من المخاطر والمكسبات الشخصية، كما يمكنك الانسحاب من المشاركة في أي وقت دون أن يؤثر ذلك سلباً عليك. إن عدم المشاركة لن تؤثر سلباً على علاقاتك الشخصية و حالة من العواقب.

إذا كان لديك أي أسئلة لاحقاً، يمكنك الاتصال بالطاولة الرئيسية د. لارا لصورالدين على 01-350000 قسم: 4547. إذا كان لديك أي أسئلة حال جلوسك كمشارك في هذه الدراسة، يمكنك الاتصال بمجلس لجنة الأخلاقيات في الجامعة الأميركية في بيروت على الرقم التالي:

Institutional Review Board  
American University of Beirut 5445 قسم: 01-350000

01 SEP 2014 شكراً على مشاركتك في هذه الدراسة

APPROVED

## APPENDIX IV

### DATA COLLECTION PROCEDURE



Faculty of Agricultural  
and Food Sciences  
Department of Nutrition  
and Food Sciences

كلية العلوم الزراعية  
والغذائية  
والغذائية  
والغذائية

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Tel: 961 1 350 000, Ext. 4547  
Fax: 961 1 744 460  
Email: ln10@aub.edu.lb

October 13, 2014

*Attn: COOP Hamra Management Team*  
*Subject: Students' research project*

Dear Sir,

I am writing to you in reference to one of the research projects conducted by the Department of Nutrition and Food Sciences, American University of Beirut (AUB). The project aims at investigating the contribution of traditional breads to daily salt intake amongst Lebanese adults. This project is part of my students' course requirements and more specifically represents their senior year project, an experience that aims at enhancing their research, writing, data analysis and presentation skills. The project has been granted approval by the Institutional Review Board of the American University of Beirut.

To meet the objectives of the project, the students will randomly approach subjects entering the supermarket and will ask them whether they are willing to participate in the study. If interested, the subject will be asked about his/her consumption level of traditional breads. The questionnaire should not take more than 10 min to fill. The students are working on this project under my supervision.

The names of the students working on the project are:

- Joelle Al. Hadadby
- Abigail Chalhouh

I expect that their data collection will span over the period of one month. They will of course not be able to work on a daily basis given that they also have course work at AUB.

Your help in allowing them to conduct the interviews and in facilitating their research work would be highly appreciated. My phone number is the following: 03/651235.

Please let me know should you need further clarifications.

Best Regards

# APPENDIX V

## QUESTIONNAIRE

Institutional Review Board  
American University of Beirut

15 AUG 2014

RECEIVED

الجامعة الأميركية في بيروت

### A. Food Frequency Questionnaire

مساهمة الخبز اللبناني التقليدي في الإستهلاك اليومي للصدوبوم عندعينة من  
الراشدين اللبنانيين

رقم المشترك (ID): .....

التاريخ: .....

#### 1. خصائص المشاركين

1. الجنس:

a. ذكر

b. أنثى

2. العمر (بالسنوات): \_\_\_\_\_

3. ما هو مستواك التعليمي؟

a. لم تلتحق بالمدرسة

b. المرحلة ابتدائية

c. المرحلة المتوسطة

d. المرحلة الثانوية أو دبلوم تقني

e. المرحلة الجامعية (بما في ذلك درجات الدراسات العليا)

4. ما هو وضعك العائلي؟

a. أعزب

b. متزوج

c. مطلق

d. أرمل

Institutional Review Board  
American University of Beirut

01 SEP 2014

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5. ما هو وضعك الوظيفي؟

a. موظف

b. ربة منزل

c. متقاعد

d. عاطل عن العمل

e. طالب



6. كم عدد الغرف التي لديك في المنزل؟ (باستثناء المطابخ والحمامات والممرات والشرفات، والمرائب): \_\_\_\_\_

7. كم عدد الأشخاص الساكنين في المنزل؟ \_\_\_\_\_

8. الوزن (كغ): \_\_\_\_\_

9. الطول (سم): \_\_\_\_\_

## II. استبيان التردد الغذائي

الماركة	وتيرة الاستهلاك				عدد الحصص المستهلكة من المشرك	نموذج الحصة	الماكولات
	الأكثر استهلاكاً	نهاراً	في الشهر	في اليوم			
							الخبز المحطى
						1 رغيف صغير (26 غ) وسط (58 غ) كبير (122 غ)	خبز عربي أبيض
						1 رغيف صغير (24 غ) وسط (58 غ) كبير (116 غ)	خبز عربي لمر
						1 رغيف صغير (24 غ)	خبز عربي قمحة كاملة

Version date: 15/08/2014

Institutional Review Board  
American University of Beirut

Page 2 of 3

01 SEP 2014  
APPROVED

الماركة الأكثر استهلاكاً	وتيرة الاستهلاك			عدد الحصص المستهلكة من المشاركة	نموذج العينة	المأكولات
	نابراً	في الشهر	في الأسبوع			
					وسط (66 غ) كبير (115 غ)	
					1 قطعة مقفلة (94 غ)	خبز مرقوق
					1 قطعة مقفلة (64 غ)	خبز فلور
					1 Baguette = 16cm (68 غ)	خبز فرنجي baguette أبيض
					1 Baguette = 16cm (68 غ)	خبز فرنجي baguette أسمر

1. هل تتناول أصناف قليلة الملح من الخبز؟

- a. لا  
b. نعم

ii. إذا كانت الإجابة بنعم، حدد نوع الخبز: \_\_\_\_\_

iii. إذا كانت الإجابة بنعم، حدد الماركة الأكثر استهلاكاً: \_\_\_\_\_

iv. إذا كانت الإجابة بنعم، حدد وتيرة استهلاك أصناف الخبز القليلة الملح بوجاء، أسبوعياً، أو شهرياً: \_\_\_\_\_

Institutional Review Board  
American University of Beirut

01 SEP 2014

APPROVED

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