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DETERMINATION OF MORPHOLOGICAL, CHEMICAL AND PHYSIOCHEMICAL PROPERTIES AND ANTIOXIDANT CAPACITIES OF PODS FROM LEBANESE CAROB *(Ceratonia siliqua L.)* VARIETIES AND IDENTIFICATION OF HIGH-ANTIOXIDANT CANDIDATES FOR FOOD APPLICATIONS.

by
YARA JOSEPH CHAMATA

A thesis
submitted in partial fulfillment of the requirements
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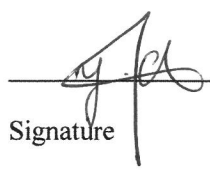

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AN ABSTRACT OF THE THESIS OF

Yara Joseph Chamata for Master of Science
Major: Food Technology

Title: Determination of morphological, chemical and physicochemical properties and antioxidant capacities of pods from Lebanese carob (*Ceratonia siliqua* L.) varieties and identification of high-antioxidant candidates for food applications.

Twenty-three carob varieties were collected from different regions of Lebanon and from different locations at the campus of the American University of Beirut. The morphological and chemical parameters including width, thickness, weight, number of seeds, moisture, ash, fat, protein, total dietary fiber, macrominerals (Na, P, Mg, Ca, K), microminerals (Cu, Zn, Mn, Fe), sucrose, glucose, fructose, and total phenols contents were determined. Further, the antioxidant capacities of the samples were determined by the 2,2-diphenyl1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays.

The ranges of the samples were 11.8-26.5 g/pod for weight, 8.2-18.5 cm/pod for length, 1.5-3 cm/pod for width, 0.7-1.0 cm/pod for thickness, 7-13 seeds per pod for number of seeds, 11.0-13.2% for moisture content, 2.4-4.7% for ash content, 0.1 – 1.4 g/100 g for fat content and 3.4 - 6.5 g/100 g for protein content. Potassium was present at the highest concentration amongst the surveyed macrominerals and Fe was the most abundant micromineral. More specifically, the ranges for the microminerals were 0.13-0.58 mg/100g for Cu, 0.18-0.73 mg/100g for Mn, 0.23-0.56 mg/100g for Zn,1.77-15.34 mg/100g for Fe and the macrominerals' ranges were 5.46-21.26 mg/100g for Na, 20.17-103.10 mg/100g for P, 25.92-100.10 mg/100g for Mg, 110.45-442.43 mg/100g for Ca and 1046.75-3992.50 mg/100g for K. The dietary fiber content of the samples varied between 4.7 and 8.2 g/100 g. Further, the samples displayed wide variations in their sugar contents with ranges of 50.3-140.9 g/kg for fructose, 21.3-96.3 g/kg for glucose and 109.9-358.3 g/kg for sucrose. The phenolic content of the samples ranged from 1.03 to 3.30 g Gallic Acid Equivalents/ 100g. The antioxidant capacity of the samples as determined with FRAP was in the range of 1.0 and 3.38 μM Fe (II)/100g of carob; values for DPPH ranged from 0.03 to 0.55 mg/L and ABTS from 0.32 to 1.03 mg/L expressed as the carob extract concentration providing 50% inhibition (IC_{50}).

All antioxidant tests correlated highly with the total phenolic content and with each other ($P < 0.01$) thereby suggesting that the phenolic compounds are responsible for the antioxidant activity of carob. Varieties V4, V5, V6 and V16 exhibited high dietary fiber

and total phenolic contents, whereas varieties V2, V11, V15, V17, V19 and V20 displayed high sucrose contents. This study provides useful information on the composition and antioxidant capacities of Lebanese carob varieties and the relationships amongst the different constituents/nutrients of carob. The findings also indicate that varieties with high phenolic contents and antioxidant capacities have lower sugar levels and vice-e-versa.

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DEDICATION

To my father, the man who inspired me to become a scientist and taught me to always strive for knowledge. I will forever cherish his memory and carry him in my heart

To my mother, who always believed in me and influenced me to be a strong and independent woman

To my sister, who has always been my source of inspiration and support

To my grandfather and aunt, who have always provided me with endless love and encouragement

CHAPTER
INTRODUCTION

The carob tree (*Ceratonia siliqua* L.) is an ever-green and pod-bearing tree indigenous to the Mediterranean countries and has been widely cultivated around the world since ancient times (Batlle & Tous, 1997; Zografakis & Dasenakis, 2002). The ancient Greeks were the first to propagate the tree in Greece and Italy long before the Arabs distributed it further along the coast of Northern Africa and consequently to Spain, Portugal and France (Battle *et al*., 1997 & Zografakis *et al*., 2002). The carob tree later spread to India, South Africa, Australia, Argentina, Mexico, Chile, Arizona and California (Sahin *et al*., 2009; Zografakis *et al*., 2002). Carob trees in Lebanon are found on the coastal areas and up to 1000 m on the inferior slopes of coastal mountains (Estephan *et al.*, 2002) and their annual production was estimated at 2,051 tons in 2016 (FAO, 2016). The annual Lebanese production was estimated to be 50kg/tree (FAO, 2001), and the number of carob trees in Lebanon was reported to be between 15,000 and 20,000 trees in the sixties, and their number has dropped to almost 12,000 trees recently (ILS LEDA, 2008).

Carob trees require minimal maintenance due to their ability to adapt to harsh environmental conditions in warm temperate areas and their tolerance to temperatures as low as minus 6° C (Zografakis *et al*., 2002). Carob trees have a relatively-long life span of up to 100-150 years and produce a large amount of pods (90-115 Kg per year) that have good nutritional value for both human and animal consumption (Marakis, 1996; Sahin *et al*., 2009).

The use of carob pods dates back to ancient times where they were consumed in their raw form as a candy (Owen et al., 2003). Over the years, their use in the human diet has evolved and are currently incorporated into a variety of food products including beverages, molasses, baked goods and traditional Arab confectionary (Bravo et al., 1994). Carob pods are also used in a variety of processed food products as a substitute, flavorant or extender for cocoa, when roasted at ~150°C (Biner *et al*., 2007; Marakis *et al.*, 1996).

Carob pods are naturally sweet and their sugar content is as high as 60% (mainly sucrose) (Biner *et al*., 2007). Carob is also high in dietary fiber (up to 40%) and polyphenolic compounds (up to 20%). Further, carobs contain substantial amounts of protein (up to 7.6%), vitamins and minerals (Makris *et al.*, 2004; USDA, 2006). The carob contains low amounts of fat and sodium thereby rendering it a healthy food source (Marakis et al., 2004).

However, despite its nutritional potential, the use of carob in the food industry is limited and its economic value is low (Avallone et al., 1997). Its application in the food industry is mainly focused on the extraction of carob bean gum (locust bean gum), which is added to a variety of products as a stabilizer, thickener or flavorant (Bouzouita et al., 2006). Even in places where carob trees are abundant, most of this highly nutritious product goes to waste every year; therefore, more effort should be exerted towards the use of carob pods as a valuable food source and as an ingredient in a variety of low-technology food products (Iipumbu *et al*., 2008).

Despite the recent interest in carob production in Lebanon reflected by the planting of carob trees under a reforestation program (Estephan *et al*., 2002), few studies are available on the Lebanese carob varieties (Haddarah et al., 2013).

Therefore, the main objective of this study was to determine the composition of Lebanese carob to assess the potential of using carob pods as an alternative nutritious food source. Another aim of the study was to investigate relationships amongst the different chemical constituents and, therefore, the nutritional potential of carob to allow for informed selection of varieties for different applications in foods. The different physical and chemical parameters and antioxidant capacities of 23 carob varieties, collected from the different regions in Lebanon and from the American University of Beirut campus, were determined and the underlying relationships amongst them were investigated. To this end, samples from the different varieties were analyzed for their pod weight, length, thickness, width and number of seeds. The samples were also assayed for their contents of moisture, ash, protein, fat, sugars, (glucose, fructose and sucrose), total dietary fiber, macrominerals (Na, P, Mg, Ca, K), microminerals (Cu, Zn, Mn, Fe), and phenolic compounds. Furthremore, the antioxidant capacities of the samples were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and 2,2'azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS). Principal component analysis and hierarchical cluster analysis were performed to interpret and evaluate relationships amongst the different chemical and morphological characteristics and identify similarities between the varieties in an attempt to identify candidates for different applications.

CHAPTER II

LITERATURE REVIEW

A. Background on carob

The carob tree has been widely cultivated around the world, including the Mediterranean region, since antiquity for its edible fruits (Battle *et al*., 1997; Zografakis *et al*., 2002). Its scientific name *Ceratonia siliqua* was derived from the Greek word "keras" which means horns and "silique" which is attributed to the shape of the pod and its hardness (Sahin *et al*., 2009). The species *Ceratonia siliqua* is part of the subfamily *Caesalpinioideae* of the *Leguminosae* family (Baumgartner *et al.*, 1986 & Biner *et al*., 2007). Carob seeds were used as a gauge to measure the "carat" value of diamonds by jewellers due to the consistence in their size and weight (Battle *et al*., 1997). Other common names for carob pods are "St John's bread" and "locust beans" (Kumazawa *et al.*, 2002).

The carob tree has been reported to originate from Syria, Turkey, or Yemen. Further, some researchers believe it might have originated from the carob-related species found in the North of Somalia and Oman, while others linked its origin to a xerotropical Indo-Malesian flora. It is believed that the Greeks were the first to recognize its numerous nutritional values and to propagate the tree in Greece and Italy long before the Arabs spread it along the coast of Northern Africa and subsequently to Spain, Portugal and France (Battle *et al*., 1997 & Zografakis *et al*., 2002). The carob tree was later spread to India, South Africa, Australia, Argentina, Mexico, Chile,

Arizona and California (Sahin *et al*., 2009; Zografakis *et al*., 2002). Carob trees in Lebanon are found on the coastal areas and up to 1000 m on the inferior slopes of coastal mountains (Estephan *et al.*, 2002).

The carob is a long-lived evergreen tree with pinnately compound leaves. It is a long producing tree (100-150 years) that can grow to more than 20 m when the environmental conditions are favourable (Kumazawa *et al.*, 2002; Owen *et al*., 2003). Carob trees can adapt to harsh environmental conditions in warm temperate areas and are also tolerant to temperatures as low as minus 6° C thereby requiring minimal maintenance. Although its optimum growth temperature requirements are similar to those of other tropical fruit trees, carob can thrive in poor calcareous soils and require much less water (Zografakis *et al*., 2002). Even in areas with just 250 mm rainfall per year, carob production is possible due to the deep tap root system of the tree (Curtis *et al.*, 1998). Furthermore, unlike many tropical fruit trees, carob orchards hardly require any fertilizers, irrigation or annual pruning; however, their yield will improve with their implementation (Battle *et al*., 1997). The carob tree is characterized by its sturdy branches, thick trunk and semispherical shape. The carob pod is composed of two main constituents: the seed which accounts for 10% of the weight, and the pulp which makes up the balance of 90% (Battle *et al*., 1997). The pods with low seed percentage contribution to total pod mass are considered to be the better quality pods for processing purposes (Marakis *et al.*, 1992; Marakis *et al*., 2004; Petit *et al.*, 1995). The pod is first green in color and soft in texture then it gets light to dark brown and hard in texture as it ripens. It can be elongated or short, flattened or narrow, thick or thin, straight or slightly curved in shape. Its length, width, weight, and thickness range from 10 to 20 cm and from 1.5 to 2 cm, 5 and 30 g, and up to 1.3 cm, respectively. The green unripe pod is

very astringent and moist, whereas the ripe pod is sweet. Isobutyric acid is largely responsible for the broken pod's characteristic odor (Calixto *et al*., 1982; Zografakis *et al*., 2002). Carob can be used as a cheap food source (Markis *et al*., 2004). Carob pods were reported to have a high sugar content (more than 50%), which makes them a naturally sweet food (Biner *et al*., 2007). Their dietary fiber content can be up to 40%, and their protein content ranges between 1 and 7.6% (Marakis *et al.*, 1996; USDA, 2006). Carob also contains a substantial amount of vitamins, minerals, and up to 20% phenolic compounds (Binder *et al.*, 1959; Makris *et al.*, 2004; USDA, 2006). Their nutritional value can therefore be compared to that of cereal grains such as wheat and barley (Battle *et al*., 1997).

There exists around 50 named cultivars of carob pods in the literature. Along the centuries, the different varieties of carob pods have spread by budding and seeds. New ones have originated from unintentional breeding among local carob pods and were later established in commercial orchards. The carob varieties differ in their morphological characteristics such as size, shape, quality, seeds' yield and color, and in agronomic characteristics such as productivity, resistance to diseases and pests and habitat. The varieties also differ in their flavor, quality and sugar and gum contents as well as in the technological, agronomical and morphological features; however, low polymorphism between cultivars of same and different origin was detected in DNA analyses (Battle *et al*., 1997). The pods from the 5 common carob varieties in Lebanon "Al Safadi', "Al Kobrosi', "Al Sandali", "Al maqdisi", and "Al Khishebeh" and other wild carob pod varieties were reported to differ in width, color, thickness, shape, length and number of seeds in the pod (Abu Al Naser *et al*., 1963).

The carob tree production has declined from 650 000 tonnes in 1945 to 310 000 in 1997. This was mainly due to low home consumption and low pod prices. Additionally, the costal land is being used for housing projects, industrial properties and infrastructure (Zografakis *et al*., 2002).

However, there has been a recent interest in planting carob trees due to their commercial value and the multipurpose use of their fruit (Sidina *et al*., 2009). Carob, as a nutritious product, didn't receive much attention in terms of research and product development in the past, but it has been recently garnering interest as an alternative in agricultural development and reforestation, especially in tropical regions (Biner *et al*., 2007; Zografakis *et al*., 2002). Moreover, recent interest is due to the nutritional potential of the pods and the host of industrial and agricultural uses associated with them. This increased interest is made evident by the recent distribution of carob trees into countries such as Australia and South Africa (Battle *et al*., 1997).

In Turkey, due to the increase in demand for carob based products and the development of the food industry, the interest in planting carob trees has increased in recent years (Gubbuk *et al*., 2010). In Lebanon, the recent interest in carob production has been reflected by the planting of carob trees under a reforestation program aimed at preserving this local traditional food source and improving its economic value through processing. The Lebanese government, with the help of numerous local and international organizations such as the International Plant Genetic Resources Institute, encouraged carob production by supplying the farmers with higher yielding varieties and cleaning their forestlands. This measure has the potential of improving the seeds' quality, thus increasing the monetary returns from exporting them. The increase of carob production can potentially assist the production of juicier carob pods that would

ultimately improve the quality of carob bean gum as natural suspending agents in food products and the quality of carob molasses, frequently used in traditional Lebanese pastries and sweets. The main Lebanese carob extraction units are located in Batroun, Jbeil, Chouf and Tyr (Estephan *et al*., 2002). A factory to process organic and traditional Lebanese products, among which will be carob molasses, was also proposed for launching (Estephan *et al*., 2002; FAO, 2001).

The world annual production of carob pods is estimated to range from 374,800 to 441,000 tons, depending on the growing region and farming practices (Vekiari *et al*., 2011). Spain and Italy are the leading carob producers holding 23 and 20%, respectively, of the world production in 2014 followed by Morocco (14%), Portugal (14%), Turkey (9%), Greece (8,5%) and Cyprus (7%) (FAO, 2014). The Lebanon carob production quantity was at level of 2,051 tonnes in 2016 (FAO, 2016).

B. Uses of carob

Carob trees have always been an important component of the Mediterranean vegetation due to their adaptation to the marginal soils of the Mediterranean regions both environmentally and economically (Battle *et al*., 1997; Sahin *et al*., 2009; Zografakis *et al*., 2002). They are useful in orchards, parks, or even in backyards for providing shade and evergreen beauty. In Spain, they are also grown close to villages as barriers to fields and wind (Curtis *et al.*, 1998). Moreover, carob has a range of industrial uses in chemicals, pharmaceuticals, textiles, cosmetics, explosives, stationeries, mining, and carpentry building materials (Albanell *et al.*, 1991; Battle et al., 1997; Calixto *et al*., 1982; Zografakis *et al*., 2002).

Due to its high sugar content, carob has historically been collected and consumed as a food product (Owen *et al*., 2003). For hundreds of years, many low-income groups in the world have consumed aqueous carob extracts and baked carob beans as part of their diet (Marakis *et al.*, 1996). In ancient time, it was consumed in war and famine and as a candy for children (Berna *et al.*, 1997).

In modern times, carob pods are used in a variety of processed food products as a substitute, flavorant or extender for cocoa (Biner *et al*., 2007; Marakis *et al.*, 1996). Carob powder has a very low fat content (maximum 2.3%) and contains neither caffeine nor oxalic acid, which can be toxic to humans if consumed in large amounts, hence the preference of carob over cocoa (Biner *et al*., 2007; Yousif *et al*., 2000). Yousif & Alghazwi (2000) also reported that carob pods have higher amounts of dietary fiber when compared to cocoa. Carob is thus relatively healthier than cocoa and is an excellent alternative for individuals who are sensitive to oxalic acid or caffeine, or simply for those who prefer to have a healthy diet and consume food with low fat-content but with a nutty chocolate-like flavour (Blenford *et al.*, 1988). Carob is also used in a wide variety of food products ranging from sweet bars and confectionaries to beverages and ice creams (Biner *et al*., 2007; Yousif *et al*., 2000).

Furthermore, as compared to cocoa, carob reduces the need for sweeteners in some food products due to its high sugar content (Kumazawa *et al.*, 2002; Owen *et al*., 2003; USDA, 2006; Yousif *et al*., 2000). In fact, carob was described as a sweetener with an appearance and flavour similar to that of chocolate (Yousif *et al*., 2000). Other workers reported that carob syrup, which is extracted from carob pods with water, as one of the popular drinks in countries like Egypt (Zografakis *et al*., 2002).

Examples of potential carob products are flavoured milks, carob-based beverages, and high fiber products such as bakery and confectionary products (Biner *et al*., 2007; Blenford *et al.*, 1988; Gruendel *et al.*, 2006; Yousif *et al*., 2000). Therefore, the addition of carob as a food ingredient to a variety of new and modified food products can be explored to improve its economic revenues for the producers.

Due to the high industrial demand for its seeds for locust bean gum production, carob is considered a high value cash crop, which is one of the major attributes to its economic value (Albanell *et al.*, 1991; Biner *et al*., 2007). The carob gum is located in the endosperm of the carob seeds. Because of its ability to act as a thickener, dispersing, stabilizer, binder and gelling agent in food products, it has been used extensively as a natural food additive (E 410) (Biner *et al*., 2007; Battle *et al*., 1997; Naghmouchi *et al*., 2009; Sahin *et al*., 2009; Sidina *et al*., 2009; Zografakis *et al*., 2002). Locust bean gum, the commercial name of carob bean gum, has been used as an ingredient in the manufacturing of many food products such as bakery products, ice creams, cheese, fruit pies, soups, sauces, confectionary and canned meats. It has also been incorporated in industrial items such as cosmetics, pharmaceuticals, paper and textiles (Battle *et al*., 1997; Biner *et al*., 2007; Gubbuk *et al*., 2007; Zografakis *et al*., 2002).

The carob kibbles, in their regular or milled form, have been used as animal feed for centuries, mainly due to their high sugar content. Moreover, the carob tree is highly recommended as feed supplement for animal farming in drought-stricken conditions due to its ability to adapt to dry environments (Battle *et al*., 1997). After being soaked and extracted with water, carob kibbles yield molasses which may be consumed as a sweetener or utilized in the production of a range of confectionary or carob drinks (Battle *et al*., 1997; Sidina *et al*., 2009; Yousif *et al*., 2000; Zografakis *et*

al., 2002). In addition to its food uses, carob kibbles are fermented for the production of biofuels (Battle *et al*., 1997).

C. Processing of carob pods

Carob pods are usually harvested in late summer or early autumn, depending on the type of cultivar and the region where the pods are grown (Battle *et al*., 1997). The processing of carob pods is illustrated in Figure 1.

The pods are sorted to remove damaged or unhealthy looking units and then washed with water to remove dirt and other extraneous matter. The wet pods are dried, either mechanically or under the sun, to avoid any microbial growth and are then stored in ventilated places to a final moisture content of 8% (Battle *et al*., 1997; Iipumbu *et al*., 2008; Zografakis *et al*., 2002). The carob pods are subjected to kibbling which entails crushing by a mechanical kibbler to separate the seeds from the pulp (Battle *et al*., 1997; Iipumbu *et al*., 2008). Afterwards, they can either be left unroasted and ground then sieved resulting in fine unroasted carob powder or they can be roasted and later sieved resulting in fine roasted carob powder (Iipumbu *et al*., 2008).

Roasting the kibbles has been reported to increase the amount of compounds which impart chocolate-like pleasant odors such as esters, furans and pyrroles (Cantalejo *et al.*, 1997), and decrease the amount of isobutyric acid which imparts an undesirable smell to the kibbled pods (Berna *et al.*, 1997; Cantalejo *et al.*, 1997).

Selecting the correct temperature and time combination is of utmost importance to obtain roasted carob powder with a good chemical profile and high acceptability ratings as different time-temperature combinations result in different

sensory and chemical profiles of carob powder. The best time-temperature combination for roasting carob kibbles was reported to be around 150˚C for 60 min (Naghmouchi *et al.*, 2009). The kibbling process can also result in kernels that either get treated with acid or get roasted to peel off the seed coat. The peeled seeds are then forced through a splitting machine and the brittle embryos turn out as a fine powder, or germ meal. The locust bean gum is obtained from the seeds' ground endosperm (Battle *et al*., 1997).

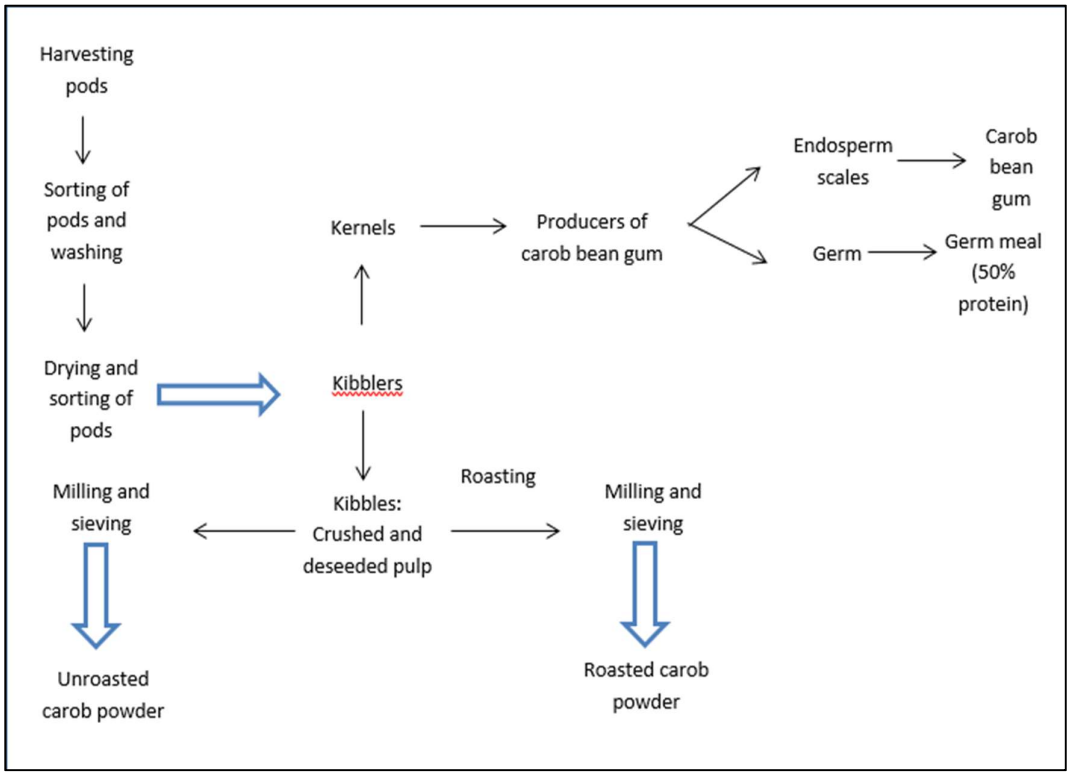

Figure 1. Processing of the carob pods.

D. Physical properties of carob pods

The evaluation of the physical characteristics of carob pods is of great importance for their industrial use. In order to achieve high yields of kernels and carob gum, the correlation of different measurements indicated that narrow and thin pods and or/or kernels which are fat, short and heavy need to be harvested (Albanell et al., 2012). Moreover, the physical properties of carob pods provide indicators of their quality: the higher the pod to seed ratio and the thicker the pod, the better is the pod quality (Yousif *et al*., 2000). The morphological characteristics of carob pods vary depending on the genotypes of the carob seeds, the geographical zone in which they are grown, and the agricultural techniques applied during cultivation (Gubbuk *et al*., 2010; Naghmouchi *et al.*, 2009; Sidina *et al*., 2009). Physical parameters of carob pods of different cultivars, as reported in the literature were as follows: 2.9 – 31.9 g/pod for weight, 1.4 – 27.3 cm/pod for length, 0.2 – 2.9 cm/pod for width and 0.05 – 1.1 cm/pod for thickness (Gubbuk *et al*., 2010; Naghmouchi *et al.*, 2009; Rabah *et al*., 2017; Sidina *et al*., 2009).

E. Chemical composition of Carob Powder

The variations in the chemical composition of carob as a function of the cultivar, harvesting time and processing conditions have been reported by many workers. The main chemical constituents of carob pods are sugars, dietary fiber (soluble and insoluble), protein, ash, moisture and polyphenols. The major chemical constituents in carob pods and their amount are summarized in table 1. (Biner *et al*., 2007 ; Gubbuk *et al*., 2010 ; Iipumbu *et al*., 2008 ; Naghmouchi *et al*., 2009 ; Khlifa *et al.*, 2013).

Table 1: Chemical composition of carob powder.

Constituent	Carob powder
Sucrose (%)	17.2-63.5
Fructose (%)	1.8-17.9
Glucose (%)	0.7-17.4
Moisture (%)	3.6-18
Ash (%)	1-6
Fat (%)	0.2 – 2.3
Protein (%)	1-7.6
Dietary Fiber (g/100g)	2.6 – 39.8
Total Phenolics (GAE g/100 mg)	0.5 – 20

1. Moisture content

Carobs are picked and, where necessary, dried to low moisture levels to improve their keeping quality and facilitate their transportation. The moisture content of carob powder has been reported to range between 3.6% and 18% (Iipumbu *et al*., 2008). The difference in moisture content between carob powders has been attributed to differences in the carob cultivars, ripening duration, rainfall, humidity and other environmental conditions, and harvesting and storage time (Albanell *et al.*, 1991; Avallone *et al.*, 1997; Iipumbu *et al*., 2008). The pods generally have moisture contents between 10 and 20% when they're still in the fresh state (Battle *et al*., 1997). Drying to a moisture contents below 10% has been suggested to avoid rotting of the pods prior to processing (Battle *et al*., 1997; Marakis *et al.*, 1996; Wursch *et al.*, 1984).

2. Ash content

The ash of a foodstuff is the inorganic residue that remains after the complete oxidation or ignition of the organic matter. Ash is an indicator of the mineral content in foods (Nielsen, 1998).

Carob powder was found to have an ash content ranging between 1% and 6% presumably reflecting differences in the type of carob and the processing conditions (Albanell *et al.*, 1991; Avallone *et al.*, 1997; Iipumbu *et al*., 2008).

3. Minerals

Calcium and phosphorus are minerals abundant in the human skeleton. Some physical deformations and malfunctions can be observed when the body isn't provided with adequate amounts of calcium and phosphorus. Other minerals participate in the regulation of the metabolic and circulatory systems. Less than 10 µg quantities of the trace minerals (Cu, Cr, Fe, Fr, Mn, Se, Si, I, and Zn) are required daily, while more than 100 µg of the macro minerals (Ca, Cl, K, Mg, Na, P, and S) are required on a daily basis in the human diet (Nielsen *et al.*, 1994). P, Mn, K, Na, Zn, S, N, Cl, Mg, B, Co, Ca, P, Fe, Cu have been reported to be present in carobs with levels being shaped with differences in the cultivars and the processing conditions (Table 2). (Ayaz *et al.,* 2007 ; Fidan *et al.,* 2015 ; Khlifa *et al.*, 2013 ; Özcan *et al*., 2007).

Table 2: Mineral contents in carob pods.

Mineral	Concentration (mg/100g of carob)
Potassium	970 – 2777
Calcium	266.6 – 628.29
Magnesium	34.6 – 132.57
Sodium	8.47 – 23.44
Copper	0.24 – 0.85
Iron	1.78 – 7.66
Manganese	0.072 – 1.29
Zinc	0.16 – 1.19
Phosporus	68.2 – 878.9

4. Dietary fibers

The amount of fiber in carob has been reported as total dietary fiber (Bravo *et al.*, 1994; Iipumbu *et al*., 2008) or hemicelluloses and cellulose and crude fiber (Sidina *et al*., 2009).

Dietary fiber can be defined as lignin and plant polysaccharides that can't be digested by enzymes in the human body (Nielsen *et al.*, 1994). Fiber aids in the digestion in the gastrointestinal track, and, thus, may protect against GIT cancer and reduces the risk of cardiovascular disease by contributing to the normalization of blood lipids (Nielsen *et al.*, 1994; Owen *et al*., 2003; Pérez-Olleros *et al.*, 1999; Zunft *et al.*, 2001).

The dietary fiber content in carob was found to range between 2.6 and 39.8g/100g (USDA, 2006). The different methods applied to calculate different fractions of fiber can explain the large variation in the amount of fiber reported in the literature (Marakis *et al.*, 1996; Iipumbu *et al*., 2008). The carob powder was also found to have an acid detergent fiber content ranging between 24.13% and 49.47% and was significantly affected by the carob pod variety (Albanell *et al.*, 1991; Iipumbu *et al*., 2008).

5. Proteins

Proteins are the building blocks for almost all living cells, and are vital for the normal biological functioning of the cell (Nielsen *et al.*, 1994).

The protein content of carob has been reported to range from 1% to 7.6% (Albanell *et al.*, 1991; Avallone *et al.*, 1997; Ayaz *et al.*, 2007; Bravo *et al.*, 1994; Calixto *et al*., 1982; Iipumbu *et al*., 2008; Sidina *et al*., 2009; Yousif *et al*., 2000; Youssef *et al*., 2013**)** and significantly differed between carob varieties and by farming practices (Albanell *et al.*, 1991; Avallone *et al.*, 1997; Calixto *et al*., 1982; Iipumbu *et al*., 2008; Owen *et al*., 2003).

6. Sugars

The sugars in carob pods are almost entirely sucrose, glucose and fructose, (Biner *et al*., 2007; Kumazawa *et al.*, 2002) with sucrose accounting for up to 70% of the total sugars (Zografakis *et al*., 2002). Levels up to 95% sucrose of total sugars are found in the literature (Bravo *et al.*, 1994). This makes carob pods a good source of

sucrose and can potentially contribute to commercial sucrose production along with sugar cane and sugar beet. Due to the high sucrose content, carob pulp is used in food, especially in confectioneries and other sweet-tasting products (Bravo *et al.*, 1994; Biner *et al*., 2007). The cultivated and wild carob types generally have similar ratios of individual sugars to the total sugar content (Bravo *et al.*, 1994).

The sucrose content was found to range between 27.5% and 63.5% (Albanell *et al.*, 1991; Avallone *et al.*, 1997; Battle *et al*., 1997; Biner *et al*., 2007; Gubbuk *et al*., 2010; Iipumbu *et al*., 2008; Sidina *et al*., 2009; Yousif *et al*., 2000). The sucrose content was significantly affected by the type of carob powder and the processing conditions (Albanell *et al.*, 1991; Biner *et al*., 2007; Gubbuk *et al*., 2010; Iipumbu *et al*., 2008).

The glucose content ranged between 1.79% and 17.4% and the fructose content ranged between 1.8% and 17.9% (Avallone *et al.*, 1997; Battle *et al*., 1997; **7,** Gubbuk *et al*., 2010; Iipumbu *et al*., 2008; Sidina *et al*., 2009). Many factors including the extraction and quantification methods, the differences in carob pod varieties, geographical locations and origin of the fruits contributed to the variations in the sugar profiles of carob pods (Calixto *et al*., 1982; Gubbuk *et al*., 2010).

7. Fat

Fat contents in carob reportedly ranged between 0.2 and 2.3% with the differences being largely due to genetic variation in the pods (Avallone *et al.*, 1997; Biner *et al*., 2007; Bravo *et al.*, 1994; Calixto *et al*., 1982; Marakis *et al.*, 1996; Yousif *et al*., 2000).

F. Phenolic compounds in carob powder

Phenolic compounds are biologically active secondary plant metabolites which act as antioxidants. Plant phenols generally have beneficial effects on human health because of their capacity to modulate proteins and their antioxidant properties (Harborne *et al.*, 1989; Sakakibara *et al.*, 2003). Their beneficial effects also comprise promoting anti-allergy effects, coronary heart diseases and cancer prevention, as well as vaso-relaxation (Harborne *et al.*, 1989).

Natural phenolic compounds can range from simple molecules to highly polymerized compounds. They are made of an aromatic ring which bears one or more sugar residues linked to hydroxyl groups. The associated sugars can occur in the form of monosaccharides, disaccharides, or oligosaccharides (Harborne *et al.*, 1989; Sakakibara *et al.*, 2003). Depending on their basic chemical structure, polyphenols can be divided into at least 10 different classes with the flavonoids being the most important single group (Almanasrah *et al.*, 2015). Plant polyphenols have been of interest for scientists for decades. They contribute to plant pigmentation and are involved in the reproduction, growth, and resistance of plants to predators due to their capacity of increasing food astringency and acting as phytoalexins, and thus protecting crops from plague and pre-harvest seed germination (Vinson *et al.*, 2001).

The carob pod contains considerable quantities of polyphenols especially highly condensed tannins. Tannins are complex polyphenolic compounds classified in two groups: The condensed tannins or proanthocyanidins that are flavonoid polymers and the hydrolysable tannins that are polymers of ellagic or gallic acid esterified to a core molecule such as glucose or a polyphenol such as cathechin. Tannins contribute to the astringent taste of the fruit and may also react with certain proteins or inactivate

digestive proteolytic enzymes thereby interfering with the digestive process (Bravo *et al.*, 1994).

Appreciable amounts of tannins are present in carob pods. Most of them are highly condensed tannins, or proanthocyanidins (El Bouzdoudi *et al.*, 2016). Gallic acid was reported to be the main constituent and other predominant polyphenolic compounds found in extracts of carob pods are catechin, epicatechingallate, epigallocatechingallate, and quercetin glycosides (Avallone *et al.*, 1997; Corsi *et al.*, 2002; Marakis *et al.*, 1997; Ortega *et al.*, 2009; Papagiannopoulos *et al.*, 2004). The condensed tannins in ripe carob pods consist of subunits of flavan-3-ol groups and their galloyl esters, whereas the hydrolysable tannins of green carobs are derived from gallic acid (Iipumbu *et al*., 2008; Makris *et al.*, 2004; Youssef *et al*., 2009).

Papagiannopoulos et al. (2004) reported that carob kibbles contain 448 mg/kg extractable polyphenols; the polyphenolic compounds included gallic acid (174 mg/ kg), hydrolysable tannins (26 mg/kg), condensed tannins (15 mg/kg) and derivatives of myricetin (171 mg/kg), quercetin (53 mg/kg) and kaempferol (9 mg/kg). In some studies, carob pod was reported to contain 1.9 mg of total phenols, 0.28 mg of proanthocyanidins/g and 0.1 mg/kg of hydrolysable tannins/g, mainly located in the germ, with only traces of these compounds being found in the seeds. Other workers reported total polyphenols and total flavonols of 19.2g/100g and 4.37g/100 g, respectively. A total of 6.1 % of polyphenols was also reported for carob pods in other studies (Iipumbu *et al*., 2008; Makris *et al.*, 2004; Youssef *et al*., 2009). Figure 2 shows the structures of the most common phenolic compounds present in carob.

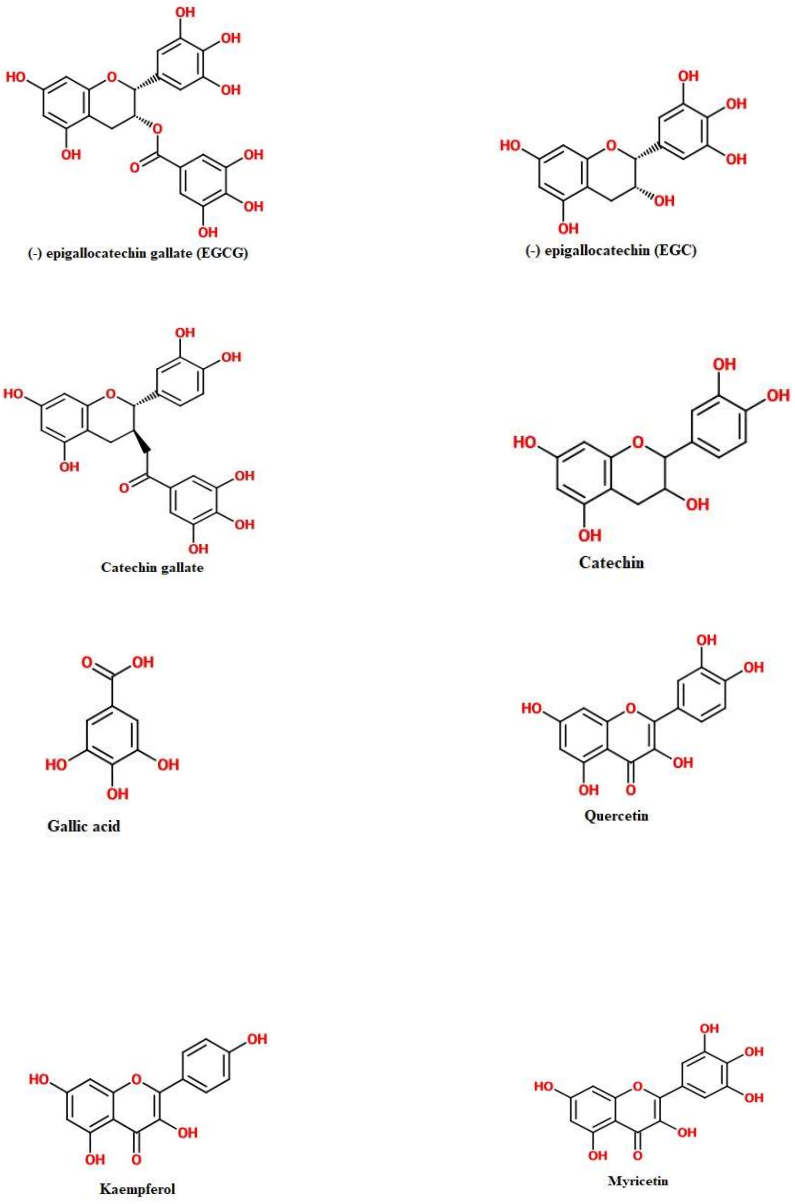

Figure 2: Structures of the most common phenolic compounds present in carob pods.

The phenolic composition of carob pulp depends on the carob variety, weather conditions, harvesting and storage, and geographical origin. The structural diversity of carob phenols affects their solubility in the extractants commonly used in the study of their properties and uses (Naczk *et al.*, 2004). Although mixed results regarding the effect of the type of the carob pod on the total phenolic content have been reported

(Avallone *et al.*, 1997; Iipumbu *et al*., 2008), the amount of polyphenols detected was greatly affected by the solvent used to extract the polyphenols as tannins were reported to be insoluble in some solvents (Makris *et al.*, 2004). Water extractable polyphenols included gallic acid, as the main detectable component, along with epigallocatechin, (+)-catechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate (Avallone *et al.*, 1997; Corsi *et al.*, 2002) whereas extraction with methanol (90%) containing acetic acid (0.5%) afforded, in addition, quercetin glycosides and ellagic acid (Sakakibara *et al.*, 2003).

Ethyl acetate is highly unsuitable for the extraction of polyphenols. Further, methanol alone extracted low amounts of total polyphenols and flavonoids and its extraction capacity did not improve with the addition of 20% water. These findings established that the carob tannins are highly insoluble in solvents such as methanol, acetyl acetate and ethanol. A highly efficient extraction was reported for 80% acetone (Makris *et al.*, 2004; Papagiannopoulos *et al.*, 2004).

A strategy to enable the recovery of both fermentable sugars and phenolic compounds by means of a water-based extraction only was developed in a recent study. Twenty percent of the phenolic compounds, corresponding to an extraction yield of 0.6 g Gallic acid equivalents (GAE)/100 g dry mass of carob kibbles, were recovered in a one-step extraction. Higher compound selectivity, along with a 70% increase in the yield of phenolic compounds, which corresponds to 1.9 g GAE/100g carob, were obtained in a two-step extraction process. This two-step extraction method was highly effective in yielding separate polyphenol and carbohydrates-rich streams, which can be further processed in food industries and biorefineries. The two-step extraction method was also reported to be easily scaled up (Almanasrah *et al.*, 2015).

The Folin Ciocalteu procedure is the most commonly used for measuring the total phenolics, flavonols and tannins in carob extracts. The phenolic compounds react with the F-C reagent to form a blue complex which absorbs strongly at 765 nm (Kumazawa *et al.*, 2002; Markis *et al*., 2004; Owen *et al*., 2003; Singleton *et al.*, 1999). Phenolics are also being increasingly analyzed by high-performance liquid chromatography. The HPLC procedures tend to be more accurate than the spectrophotometric assays for measuring the total phenolic content because they quantify the compounds having the phenolic structure whereas the spectrophotometric assay quantifies a range of compounds along with phenols thereby leading to the overestimation of the total phenolic content (Papagiannopoulos *et al.*, 2004).

G. Total antioxidant activity of carob extracts

For several years, scientists have been interested in the reactive oxygen species (ROS), which include superoxide (O_2 •-), hydroxyl (OH•), peroxyl (ROO•) and H_2O_2, mainly due to their implication in many human diseases (Lobo *et al.*, 2010; Sanchez-Moreno *et al.*, 2002). Hydrogen peroxide, superoxide and hydroxyl radicals are mutagens formed during normal metabolism (Sies *et al.*, 1986).

The increased amount of ROS leads to oxidative stress that results in a degenerative signaling cascade caused by the oxidation of vital cellular components which ultimately lead to cell death (Farrugia *et al.*, 2012). The oxidative stress state is characterized by the depletion of endogenous antioxidants from the intracellular stores or the rapid alteration in antioxidant enzymes such as catalase (CAT), glutathione

peroxidase (GPx), superoxide dismutase (SOD), which results in increased lipid peroxidation (Ito *et al.*, 2004).

Antioxidants are compounds that protect against these harmful species (Jacobo Velázquez *et al.*; 2009; Nabavi *et al.*, 2012) due to their ability to counteract the damage caused to tissues by scavenging ROS and upregulating the defenses of endogenous antioxidants (Migdal *et al.*, 2011). To this end, frequent consumption of natural antioxidants has been associated with reduced risk of cancer and cardiovascular disease (Renaud *et al.*, 1998; Temple *et al.*, 2000).

Antioxidant activity is a fundamental property vital for life as anticarcinogenicity, antimutagenicity and antiaging, among many others, originate from this property (Cook *et al.*, 1996; Huang *et al.*, 1992). A positive correlation between phenolic compounds and antioxidant capacity has been demonstrated (Velioglu *et al.*, 1998). The bioactivity of phenolic compounds is related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals such as superoxide ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), and other reactive oxygen species (Decker *et al.*, 1997; Rodrigo *et al.*, 2006; Seifried *et al.*, 2007). The antioxidant capacity of carob extracts is mainly related to their high content of phenolic compounds (Abu Al Naser *et al.*, 1963; Kumazawa *et al.*, 2002; Makris *et al.*, 2004). Furthermore, carob is reportedly a more efficient antioxidant source than some of the most popular antioxidant sources such as red wine (Makris *et al.*, 2004). The reducing power of carob extracts can also be four-fold that of gallic acid, catechin and caffeic acid in their pure forms. However, it was noted by some researchers that carob pods might not be very suitable for either human or animal consumption without prior processing due to the condensed tannins which may exhibit some negative nutritional properties such as reduced protein digestibility

once consumed (Bravo *et al.*, 1994). A recent study recommended carob kibbles for the production of antioxidant- rich extracts for a variety of purposes including their use as alternatives to artificial antioxidants in bio-food products, instead of being discarded from gum factories as by-products (Huma *et al.*, 2017).

To estimate the antioxidant capacities in fruits and vegetables and foods, several assays have been frequently used. These assays include 2,2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) (Leong *et al.*, 2002; Rice-Evans *et al.*, 1997), 2,2- diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995; Gil et al., 2002), and ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1999; Guo et al., 2003; Jimenez-Escrig et al., 2001). DPPH, FRAP, and ABTS measure the reducing ability of antioxidants by means of electron transfer. The antioxidant activity determined by these assays correlate highly with total phenolics and also among themselves (Jiménez-Escrig *et al.*, 2001). The mechanisms of these reactions are summarized in table 3.

Table 3. In vitro antioxidant capacity assays and their mechanisms of action.

Mechanism	**Reaction**	**Antioxidant Capacity Assay**
Electron transfer (ET)	*ROO*• + AH/ArOH → ROO^- + $AH^{\bullet+}$/$ArOH^{\bullet+}$ $AH^{\bullet+}$/$ArOH^{\bullet+}$ + H_2O ↔ A•/ArO• + H_3O^+ ROO^- + H_3O^+ ↔ ROOH + H_2O	DPPH, FRAP, ABTS, Folin Ciocalteu

Source: Apak et al. (2016)

Some assays are commonly classified as HAT- and ET- based according to the mechanism. HAT-based assays measure the capability of an antioxidant to quench free radicals by H atom donation. ET-based assays include the DPPH, FRAP, Folin-Ciocalteu and ABTS methods and measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. Each of them uses different chromogenic redox reagents with different standard potentials (Apak et al., 2016). A spectrophotometer is then used to record the degree of color change. 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 *et al.*, 2009).

1. DPPH

Due to the delocalization of the spare electron on the whole molecule, DPPH• (2,2-diphenyl-1-picrylhydrazyl) is a stable radical which does not dimerize, as happens with most of the free radicals. It acts as an oxidant with an odd electron that absorbs maximally at 515 nm. The delocalization of the spare electron is characterized by a purple color. When the unpaired electron couples with a hydrogen from the antioxidant, a yellow color is formed as 1,1-diphenyl-2-picrylhydrazyl converts into its reduced form 1,1, - diphenyl-2-picryl hydrazine, at a very rapid rate. The absorption diminution thus depends linearly on the antioxidant concentration. This spectrophotometric method is applied in the determination of antioxidant capacity (Brand-Williams *et al.*, 2005; Molyneux, 2004; Pisoschi *et al.*, 2011; Pyrzynska *et al.*, 2013). The mechanism of the DPPH assay is presented in Figure 3.

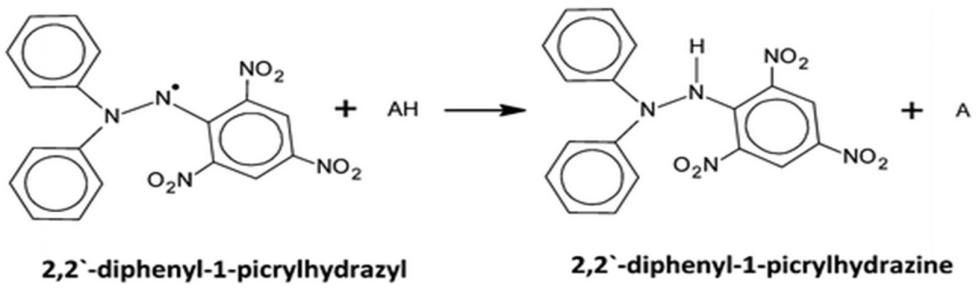

Figure 3: DPPH• radical's chemical structure and its reaction with a scavenger indicated by AH.

2. ABTS

The ABTS assay is based on the reduction of ABTS•+ by hydrogen-donating compounds. The ABTS cation radical ABTS•+ absorbs at 743 nm giving a bluish-green color. The radical is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). In the presence of a hydrogen donating antioxidant, the solution gets decolorized as the hydrogen atom gets quenched by the nitrogen atom of ABTS ABTS can be oxidized by manganese dioxide (Su *et al.*, 2007) or potassium persulfate (Pellegrini *et al.*, 2003; Thaipong *et al.*, 2006).

In comparing the ABTS assay to that based on DPPH, data showed that ABTS•+ possesses a stronger scavenging activity than that observed for the DPPH• assay (Roseiro *et al.*, 2013). This refers to the fact that ABTS radical is shown to be scavenged by antioxidative compounds at a higher level compared to DPPH radical, proposing that the reactions' kinetics differ in these two systems depending on the time of analysis. Moreover, when applied to a variety of plant foods, it was suggested that the ABTS•+ assay is more suitable than DPPH• assay as the antioxidant capacity detected by ABTS•+ assay was observed to be significantly higher for fruits and vegetables, compared to that by the DPPH• assay (Floegel *et al.*, 2011). However, a high correlation

between them is observed, indicating their similar trends (Roseiro *et al.*, 2013). the structures of the ABTS assay principle are presented in Figure 4.

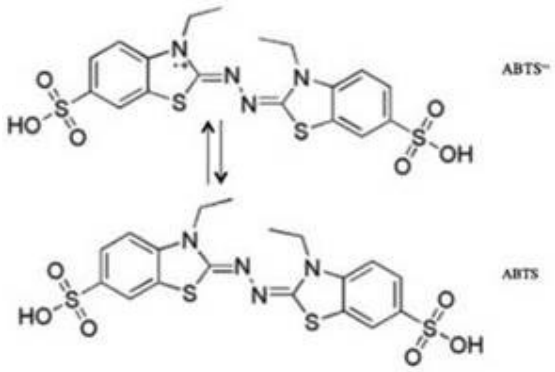

Figure 4: Chemical structures of ABTS and cation radical ABTS*+ (Diaz-Uribe *et al.*, 2016).

3. FRAP

The FRAP assay is a very simple, quick and reproducible method applied to the study of antioxidant activity in food extracts and beverages (Pulido *et al.*, 2000). FRAP, or the ferric reducing antioxidant power method is based on the reduction of the complex ferric-iron TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) by the antioxidants. A very intense navy blue color is created when Fe^{2+} binds to the ligand. The absorbance is measured at 593 nm to test the amount of iron reduced, which is correlated with the amount of antioxidants (Gil *et al.*, 2002; Pellegrini *et al.*, 2003; Pulido *et al.*, 2000 Thaipong *et al.*, 2006). However, species that act by radical quenching and particularly SH group containing antioxidants cannot be detected by the FRAP procedure (Huang *et al.*, 2005). 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 and thus cause faulty results. Compounds with redox potential lower than that of the pair Fe(III)/Fe(II), which have no antioxidant activity, also contribute falsely to a high FRAP value (Benzie *et al.*, 1996). The redox reaction for ferric complex in the FRAP assay is presented in Figure 5.

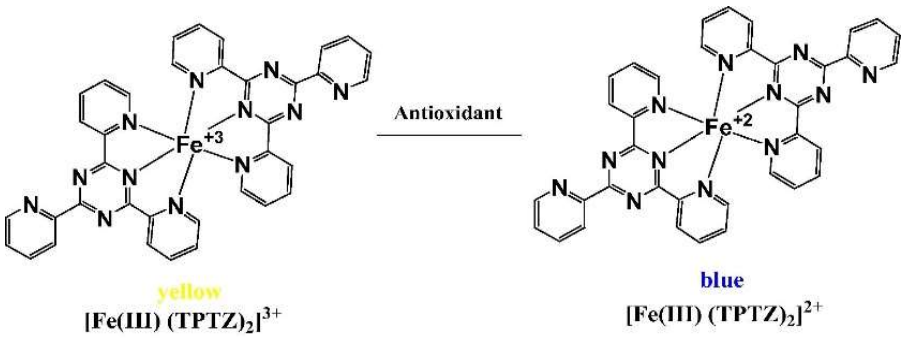

Figure 5: Redox reaction for ferric complex in the FRAP assay (Pérez-Cruz *et al.*, 2017).

MATERIALS AND METHODS

Materials

A. Carob samples

Twenty-three varieties were collected from different regions in Lebanon; "Akkari" (Akkar), "Baladi" (Selaata), "Barri" (Selaata), "Houmeiri" (Batroun) from the North of Lebanon and "Khachabi" (Bourjen), "Jnoubi Saidali" (Maaroub), and" Mkeidssi" (Marjayoun) from the South of Lebanon. The Northern varieties were provided by Salloum Carob Molasses factory (Sela'ata) and the southern varieties were obtained from (Ma'sarat Dibs L Kharroub), Choueifat. The carob pods were harvested by farmers during September and October 2016. The other varieties were collected from different locations at the campus of the American University of Beirut in October 2016.

The samples were sorted by removing damaged pods and then washed with clean water to remove the soil, dirt and other impurities. The samples were placed in cloth and kept at room temperature. The name of the different carob pod varieties, the location from which each variety was collected and their abbreviated names are presented in Table 4. The Locations of the variety collected from the American University of Beirut campus are presented in Figure 6.

Table 4*:* Name of the carob pod varieties and their locations.

Name of the carob pod variety	**Location**	**Abbreviated name**
Akkari	Akkar	V1

Architecture 1	AUB campus, near the department of architecture and design (N).	V2
Architecture 2	AUB campus, near the department of architecture and design (S).	V3
Asfari	AUB campus, near the Asfari institute (N).	V4
Baladi	Sela'ata.	V5
Barri	Sela'ata.	V6
Business 1	AUB campus, near the Suliman S. Olayan *School of Business (W).*	V7
Business 2	AUB campus, near the Suliman S. Olayan *School of Business (W).*	V8
Business 3	AUB campus, near the Suliman S. Olayan *School of Business (W).*	V9
Business 4	AUB campus, near the Suliman S. Olayan *School of Business (W).*	V10

Chemistry stairs left	AUB campus, left side of the chemistry stairs (Looking up, N).	V11
Chemistry stairs right 1	AUB campus, right side of the chemistry stairs (Looking up, N).	V12
Chemistry stairs right 2	AUB campus, right side of the chemistry stairs (Looking up, N).	V13
End of chemistry stairs	AUB campus, at the end of the chemistry stairs (Looking up, N).	V14
Facing Agri	AUB campus, facing the Faculty of Agricultural and Food Sciences (W).	V15
Green field	AUB campus, Green field (W).	V16
Houmeiri	Batroun (North)	V17
Jnoubi Saidali	Maaroub (South)	V18
Khachabi	Bourjen (South)	V19
Mkeidssi	Marjayoun (South)	V20
One AUB	AUB campus, facing the Green field (S).	V21

Physics	AUB campus, near the Department of Physics (E).	V22
Two AUB	AUB campus, facing the Green field (S).	V23

Figure 6. Locations of the carob pod varieties at the AUB campus.

Methods

B. Morphological parameters

The pod width (cm), thickness (cm), and weight (g) and number of seeds/pod were measured on 10 randomly selected pods from each variety.

The length (cm) of pod was measured using a measuring tape. The width (cm) was calculated as the mean of the widths of the top, middle, and bottom regions of the pod as measured by a Vernier caliper. Thickness (cm) was calculated as the mean thickness of the upper, middle and lower regions of the pod as measured with Iwanson gauge (1/10 mm). Weight (g) of the pod with kernels was measured using a Mettler balance. The number of seeds/pod were determined by cutting open the pod and counting the seeds.

C. Chemical analyses

All chemicals and solvents were of analytical grade. Gallic acid, Folin-Ciocalteau, ferric chloride, Ammonium persulfate, DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) were purchased from Sigma-Aldrich. The total dietary fiber kit (K-TDFR-200A) was purchased from Megazyme (Address). All spectrophotometric analyses were done with Evolution 300 UV-VIS Spectrophotometer (Thermoscientific, UK) using Suprasil quartz cuvettes.

Samples from the deseeded pods were ground on a Wiley Mill into powder to pass through a 35-mesh sieve. The resulting powder was then stored in glass containers at 4°C until analyzed. All analyses were performed in triplicate.

1. Moisture content

Moisture was determined according to the AOAC official method 934.01 (AOAC, 2006). Approximately 2 g of the carob powder were accurately weighed and placed in a previously weighed aluminum moisture dish. The dishes were partially left uncovered and placed in a forced-draft oven (Wisconsin Oven Corporation, East Troy, USA) at 100°C for 3 hours. After drying was complete, the dishes were immediately transferred to a desiccator to cool to room temperature before being weighed. The moisture content was calculated according to the following formula:

$$\% \text{ moisture} = \frac{\text{Wt (g) original sample - Wt (g) sample after drying} \times 100}{\text{Wt (g) original food sample}}$$

2. Ash

The ash content of the carob powders was determined according to the AOAC official method 972.15 (AOAC, 2006). Approximately 2g of carob powder were accurately weighed and placed in a previously ignited and weighed porcelain ashing crucible. The crucibles containing the carob powders were then ignited in a muffle furnace (Lindberg/Blue. Thermo Electron Corporation, Asheville, North California, USA). The furnace was set at 550°C for approximately 12 hours. The crucibles were then placed in a desiccator to cool down before being weighed. The ash content was expressed as percent ash retained and was calculated according to the following formula:

$$\% \text{ ash} = \frac{\text{Wt (g) crucible with ash after ignition - Wt (g) empty crucible} \times 100}{\text{Wt (g) original food sample}}$$

3. Fat

Fat content was determined according to the AOAC official method 920.39 (AOAC, 2007). Approximately 1 g of the moisture-free carob powder, which was saved from the moisture determination experiment, was weighed into an empty filter bag. The open end of the filter bag was sealed using a special heat sealer and the filter bag was then placed in the Telfon insert of the fat extractor (Ankom fat extractor). Petroleum ether (200 mL) was added directly into the extraction vessel of the fat extractor where the Teflon insert was also

The results of the carob pods' weight obtained in this study fall within the ranges of the physical parameters of Lebanese carob pods reported in the literature (Haddarah *et al.*, 2013) and with the weight of carob pods later placed. Petroleum ether (150 mL) was added into the Teflon insert before turning the heat on and extraction was carried out for 40 min. The filter bag containing the defatted carob powder was then placed in a forced-draft oven for 30 min at 100°C for drying. The filter bag was then transferred to a desiccator to cool before weighing. The fat content was calculated according to the following formula:

$$\% \text{ fat} = \frac{(\text{Wt (g) of food sample with filter bag before extraction} - \text{Wt (g) of food sample with filter bag after extraction} \times 100)}{\text{Wt (g) original food sample}}$$

4. Protein

The protein content of the carob powders was determined according to the AOAC official method 955.04 (AOAC, 2007). Approximately 1 g of carob powder was digested with concentrated H_2SO_4 in a Kjeldahl digestion tube using Kjeldahl digestion

and distillation apparatus (LABCONCO Rapidstill II, LABCONCO block-digestor, LABCONCO Corporation, Kansas City, Missouri, USA) for 2 h. The digestion tube with its contents was then placed in the distillation unit, treated with NaOH (50%) and distilled for 8 min. The resulting solution was titrated with standardized 0.1N HCl using 4% boric acid as an indicator. The percent protein was then calculated using a conversion factor of 6.25 as follows:

% Nitrogen =

$$\frac{\text{Corrected Acid Volume (ml)} \times 10\text{-}3 \times \text{N HCl} \times \text{Atomic (wt) N2} \times 6.25 \text{ x100}}{\text{Wt (g) original food sample}}$$

5. Minerals content

Carob powder (~ 0.5 g) was weighed into a microwave sample vessel, treated with concentrated HNO_3 (15 mL) and heated at 200°C for 30 min in a Microwave Oven Digestor (Brand and Company). The vessel was left to cool at room temperature for 30 min and diluted to 50 mL with deionized water.

Copper, Mn, Zn, Fe, Na, Mg, Ca and K were measured by atomic absorption spectrophotometry (SOLAAR with ASX-510 autosampler) according to AOAC (2003; Method 984.27) and P was measured calorimetrically by the method 966.01 (AOAC, 2003).

Standard solutions of Cu, Mn, Zn, Fe, Na, Mg, Ca and K were prepared from stock solutions (1000 µg/mL) of the corresponding minerals. Plots of absorbance vs concentration were constructed for each mineral at the relevant wavelength and utilized

in the quantification of the minerals in the carob samples. The levels of the minerals in the samples were expressed in mg per 100 g of carob powder.

The accuracy of the analytical determinations was assessed by analyzing a standard reference material under the same conditions. To this end, non-fat milk powder (NIST 1549), obtained from the National Institute of Standards and Technology (Maryland, U.S.A.), was digested with HNO_3 and assayed for the aforementioned minerals.

For the determination of phosphorus, the ashed samples were transferred quantitatively into a 100 ml beaker with 20% HCl (5 mL), followed by concentrated HCl (5 mL). The solution was evaporated on a steam bath, under the hood, to dryness, dissolved in 50 ml of in deionized water (50 mL) and the solution was then filtered into a 250 ml Erlenmeyer flask. A molybdovanadate reagent was prepared by dissolving 40 g of ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in 400 ml deionized H_2O and 2g of ammonium metavanadate (NH_4VO_3) in 250 ml hot deionized water and 450 ml of 70% hydrochloric acid, adding the the molybdate solution to the vanadate solution and diluting to 2 L with deionized H_2O. A stock phosphate standard solution (1000µg/ml) was prepared by dissolving dried KH_2PO_4 (1.919 g) in 1 deionized water (1 L) and a standard curve with phosphate concentrations (0-35 µg/ml) was constructed. Aliquots of the filtrate (1 mL) diluted to 50 mL with deionized water were mixed with the Molybdovanadate reagent (20 mL) and the absorbance was measured at 400 nm after 15 min against a deionized water blank. The phosphorus content was expressed in mg/100g of carob powder.

6. Sugars

Determination of glucose, fructose and sucrose in the carob samples was carried out according to Fidan et al. (2016). The carob powder (~ 1g) was weighed into a 50 ml centrifuge tube and deionized water (25 mL) was added. The solution was then transferred to an ultrasonic bath set at ultrasonic frequency of 45 KHz, power 30 W and 30°C and sonicated for 30 min. The extracts were filtered through 0.45 μm Whatman filter paper and the filtrates stored at -18°C until analyzed. The levels of sucrose, fructose, and glucose in the extracts were quantified with a high-performance liquid chromatography system Shimadzu consisting of LC 10 AD pump and equipped with a refractive index detector and a Telos NH_2 column (50 x 4.6 mm, 5 μm particle size). A guard column was employed to prevent any impurities from entering the main HPLC column. A mixture of acetonitrile: water (70:30 v/v) was used as the mobile phase with a flow rate of 1.6 ml/min and injection volumes of 20 μL were used. The sugar standard solution was made by dissolving glucose (0.2 g), fructose (0.5 g) and sucrose (1.5 g) in water: acetonitrile (1:1) in a 100 mL volumetric flask. Sugars in the carob samples were identified and quantified on the basis of retention times and peak areas by comparison with those of the pure standard.

7. Dietary fibers

The total dietary fiber was determined according to AOAC method 985.29 (AOAC, 2007). The phosphate buffer (0.08M) was prepared by dissolving disodium phosphate anhydrate (Na_2HPO_4) (1.4 g) and disodium phosphate monobasic monohydrate (NaH_2PO_4) (9.68 g) in 1 L of distilled water. Carob powder (~ 1 g) was

dissolved in phosphate buffer (50 mL) in a4 00 mL beaker, treated with α-amylase solution (50 µL) and kept at 98-100°C for 15 min. The pH was then adjusted to 7.5 ± 0.1 by adding 0.275 N NaOH solution (10 mL) and the mixture digested with protease solution (100 µL) at 60°C with continuous agitation for 30 min. The pH was adjusted to 4.5 ±0.2 with 0.325 N HCl solution (10 mL) and the mixture finally digested with amyloglucosidase (200 µL) at 60°C for 30 min. of preheated Ninety-five % EtOH (280 mL), preheated to 60 °C, were then added and the mixture was left at room temperature for 60 min to affect flocculation.

A crucible containing Celite was weighed to nearest 0.1 mg and the Celite bed was evenly distributed by 78% EtOH. The precipitate from the enzyme digest was then transferred to the crucible and the residue was successively washed with 78% EtOH (3 × 20 mL), 95% EtOH (2 × 10 mL), and acetone (2 × 10 mL). The crucible containing residue was then dried overnight in the forced-draft oven at 105°C.

The residue was analyzed for ash and protein contents according to the AOAC official method 972.15 (AOAC, 2006) and AOAC official method 955.04 (AOAC, 2007), respectively. The final total dietary fiber was expressed in % and was calculated according to the following formulas:

% TDF = 100 × CSR/mg sample

Corrected sample residue (CSR) = USAR – SPR – SAR- CB

Uncorrected average sample residue (USAR) = average sample residue of duplicate samples in mg

Sample protein residue (SPR) = USAR × % protein in sample/100

Sample ash residue (SAR) = USAR × % ash in sample

Corrected blank (CB) = Average blank residue of duplicate blanks (1 - % protein in blank - % ash in blank)

8. Total phenolic content

The carob samples were subjected to a two-step aqueous extraction as described by Almanasrah *et al.,* (2014) to affect removal of the sugars. Carob samples (~2 g) were suspended in distilled water (20 mL) and stirred at 30°C for 150 min. After filtering the mixture, the residue was stirred with distilled water (8 mL) at 100°C for 30 min to get a polyphenol-rich stream. The extracts were filtered through Whatman no.1 paper and kept at 4°C for immediate analysis.

The total phenolic content was determined by the Folin-Ciocalteu colorimetric method according to an improved procedure described by Hagerman et al. (2000). A diluted carob extract (1:4; 0.1 mL) was made up to 0.5 mL with distilled water and mixed with 1/1 (v/v) diluted Folin Ciocalteu reagent (0.25 mL) and 20% Na_2CO_3.10 H_2O (1.25 mL).

The solution was mixed thoroughly by a vortex and incubated for 40 min at room temperature. The absorbance was measured against a blank (water) at 765 nm. A standard curve was prepared from gallic acid solutions (0-0.5 g/L) Total phenolic content was expressed as mg gallic acid equivalents (g GAE) / 100g of carob.

9. Antioxidant assays

a. Ferric reducing antioxidant power

The reducing power of carob was analyzed according to the method of Benzie and Strain (1996) with some modifications. This method is based on the carob antioxidants' potential to reduce Fe^{3+} into the blue colored Fe^{2+}. The FRAP reagent was prepared using 10:1:1 acetate buffer, TPTZ (2,4,6-tripyridyl-striazine) and ferric chloride ($FeCl_3$), respectively by mixing acetate buffer (pH 3.6; 0.3 M) (250 mL), 10 mM TPTZ solution in 40 mM HCl (25 mL) and 0.001 M aq. $FeCl_3$ (25 mL). The solution was prepared daily before analysis and stored in the dark at all times. Aliquots of 1/100 (v/v) diluted carob solution (1 mL), were mixed with FRAP reagent (2 mL) and 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 (0-150 µM) were used for plotting the calibration curve. The reducing power was expressed as µM Fe (II) / 100g of carob.

b. 2,2-diphenyl-1-picrylhydrazyl

The antioxidant capacity as measured by the DPPH method was determined as described by Brand-Williams *et al.* (1995). A solution of DPPH• in methanol (60 µM) was prepared fresh daily and protected from light at all times. Carob solutions with concentrations (0.08- 0.75 mg/L) (50 µL) were mixed with DPPH (1950 µL) and the mixture was vortexed and incubated for 30 min at room temperature in the dark. The absorbance was measured at 515 nm against a blank containing the same amount of methanol and DPPH• solution and 50 µL of distilled water. The decrease in the

absorbance of the DPPH• solution indicated an increase of the DPPH radical-scavenging activity. Scavenging activity on the DPPH radical was calculated using the following formula:

$$\% \text{ DPPH• inhibition} = \frac{(\text{Absb} - \text{Absf})}{\text{Absb}} x100$$

Where Abs_b is the absorption of the blank sample (t = 0min) and Abs_f is the absorption of the tested extract solution (t = 30 min).

The results were expressed as the extract concentration providing 50% inhibition (IC_{50}) in mg/L calculated from the plot of absorbance vs. extract concentration.

c. 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid

The ABTS assay was carried out as outlined by Almanasrah *et al*. (2015). The method is based on the decolorization of the ABTS (2,2-azinobis-(3-ethylbenzoethylbenzothiazoline-6-sulfonic acid)) radical cation (ABTS•+). ABTS (7mM) (5 mL) was mixed with potassium persulphate (140 mM) (88 µL) leading to a 2.45 mM final concentration of the ABTS radical cation (ABTS•+) solution. After 16 hours of incubation in the dark, at room temperature, the ABTS•+ solution was diluted with 80 % (v/v) ethanol to an absorbance of 0.7 ± 0.005 at 734 nm and this absorbance was checked every 30 minutes. Aliquots of the carob extracts (30 µL), with different concentrations (0.19 – 0.83 mg/L), were mixed with 3 ml of the ABTS•+ reagent and shaken vigorously. The absorbance was measured at 734 nm after 6 minutes against a blank containing distilled water (30 µL) and the ABTS•+ reagent (3 mL). The percent absorbance reduction was determined as follows:

$$\% \text{ ABTS}^{+\bullet} \text{ inhibition} = \frac{(\text{Absb - Absf})}{\text{Absb}} x100$$

Where Abs_b is the absorption of the blank sample (t = 0min) and Abs_f is the absorption of extract solution (t = 6 min).

The results were expressed as the extract concentration providing 50% inhibition of the reagent ($IC_{50)}$ in mg/L and were calculated from the plot of absorbance vs. extract concentration.

10. Statistical analysis

IBM SPSS Statistics 19.0 was used for the statistical analysis. Total phenols, FRAP, DPPH and ABTS data were subjected to two-tailed bivariate correlation. Correlations were calculated on a carob mean basis, according to Pearson's test. Results were considered significant at $p \leq 0.05$ level.

A principal component analysis (PCA) was made to find the main variation trends between the carob pods varieties' morphological and chemicals characters. In addition, hierarchical cluster analysis (HCA) was used to investigate the similarities and dissimilarities among the varieties. For classification, the Ward's Minimum Variance Method was utilized. The squared Euclidean distance was used as the dissimilarity measure for Ward's method. *MATLAB* software was used for these statistical methods.

The RACI (Relative Antioxidant capacity index) method was used to analyze the data for the antioxidant assays. In this method, described by Sun et al. (2007), the data was calculated from several antioxidant assays, integrated and expressed as a standardized score. The antioxidant capacity values were transformed into standard

scores, since the data of each antioxidant test is expressed differently. The results of the standard scores of all the antioxidant tests were then added, and their average was calculated. The standard score was calculated to the formula found below:

The formula to calculate the standard score is found below:

$z = (x - \mu) / \sigma$

where z is the standard score for the antioxidant activity

x is the individual raw data

μ is the mean value of the data

σ is the standard deviation

The standardized data was plotted and on a bar graph (RACI versus Carob pod varieties).

RESULTS AND DISCUSSION

A. Morphological characteristics of carob pods

The results of the morphological characteristics of the 23 varieties of carob pods are summarized in Table 5 and Figures 7 and 8. The mean weight

of the carob pods ranged from 11.8 ± 3.16 g/pod for variety 22 to 26.5 ± 6.42 g/pod for variety 8. V1 pods were, on average, the longest and those of variety 10 the shortest. The average length of carob pods ranged from 8.2 ± 1.94 cm/pod to 18.5 ± 2.72 cm/pod. The shortest carob pods were those of V10 and they also had the smallest width of 1.5 ± 0.33cm/pod. The width of the pods ranged from 1.5 ± 0.33cm/pod (V10) to 3.0 ± 1.96 cm/pod (V1). The thickness of the pods ranged from 0.7 ± 0.08 cm/pod for V2 to 1.0 ± 0.14 cm/pod for V21. V2 also had the highest number of seeds at 13 ± 0.5 seeds per carob, whereas V10 had the lowest number of seeds with 7 ± 0.83 seeds per pod.

reported by Albanell et al., (1991), Naghmouchi et al. (2009) and Rabah et al. (2017). The values for carob pod length measurements were smaller than the length of Lebanese carob pods reported by Haddarah et al. (2013). However, they belonged to the carob length ranges reported by Albanell et al. (2011), Bouzouita et al. (2007) and Rabah et al. (2017). Moreover, the values for carob pod width measurements reported in this work were within the bounds reported for carob width ranges by Albanell et al. (1991) but were smaller than the width measurements of carob pods reported for Lebanese carob pods (Haddarah et al., 2013) and other studies (Gubbuk et al., 2010;

Naghmouchi et al., 2009). The values for carob pod thickness measurements reported in this study were higher than the measurements of Lebanese carob pods reported by Haddarah et al. (2013) but were in the range of thickness measurements reported in other studies (Albanell et al., 1991; Gubbuk et al., 2010; Naghmouchi et al., 2009 and Rabah et al., 2017). The number of seeds per pod also fell within the range of other reported values in the literature (Rabah et al., 2017).

The morphological parameters of Tunisian carob pods were studied by both Naghmouchi et al. (2009) and Bouzouita et al. (2007) with large differences in the two works. Accordingly, the morphological parameters of carob pods are known to differ vastly within and among geographical locations (Gubbuk et al., 2010). Difference in the genetic makeup of carob seeds and a host of environmental conditions significantly affect the morphological characteristics of carob pods (Sidina et al., 2009).

Table 5. Mean values of the morphological parameters of carob powder.

Carob pod variety	Weight (g± SD)	Length (cm± SD)	Width (cm± SD)	Thickness (cm ± SD)	Number of seeds ± SD
1	23.8 ± 3.85	18.5 ± 2.72	3.0 ± 1.96	0.9 ± 0.14	11 ± 1.40
2	12.8 ± 2.74	13.1 ± 2.09	2.1 ± 0.26	0.7 ± 0.08	13 ± 0.5
3	17.2 ± 3.68	17.2 ± 1.70	2.4 ± 0.17	0.9 ± 0.18	12 ± 1.02
4	21.1 ± 4.71	17.1 ± 1.20	2.2 ± 0.26	0.8 ± 0.95	10 ± 0.48
5	19.4 ± 5.2	13.2 ± 2.87	2.3 ± 2.07	0.8 ± 0.18	9 ± 1.14
6	19.2 ± 4.05	16.2 ± 2.85	2.5 ± 0.0.38	0.8 ± 0.09	11 ± 0.92
7	25.1 ± 3.33	15.8 ± 1.88	2.2 ± 0.27	0.9 ± 0.19	11 ± 0.48
8	26.5 ± 6.42	10.3 ± 0.60	2.7 ± 0.30	1.0 ± 0.13	9 ± 0.42

9	18.1 ± 8.06	10.1 ± 2.00	2.2 ± 0.38	1.0 ± 0.23	10 ± 0.88
10	14.3 ± 3.59	8.2 ± 1.94	1.5 ± 0.33	0.8 ± 0.20	7 ± 0.83
11	19.7 ± 2.18	13.9 ± 1.81	2.3 ± 0.26	0.9 ± 0.16	10 ± 1.07
12	19.3 ± 1.77	13.5 ± 0.50	2.2 ± 0.13	0.9 ± 0.27	10 ± 0.57
13	20.0 ± 4.38	13.5 ± 2.13	2.3 ± 1.9	0.9 ± 0.15	10 ± 1.97
14	20.3 ± 2.62	13.2 ± 4.03	2.2 ± 0.26	0.9 ± 0.36	10 ± 2.31
15	20.2 ± 5.75	12.8 ± 2.28	2.2 ± 0.19	0.9 ± 0.16	10 ± 1.05
16	20.3 ± 2.58	12.7 ± 2.13	2.2 ± 0.23	0.9 ± 0.17	10 ± 0.97
17	18.1 ± 5.58	10.9 ± 3.96	2.3 ± 0.38	1.0 ± 0.12	11 ± 1.63
18	15.7 ± 4.13	10.1 ± 1.35	1.9 ± 0.28	0.8 ± 0.10	9 ± 1.43
19	19.3 ± 3.57	11.7 ± 2.16	2.2 ± 0.33	0.9 ± 0.13	10 ± 1.26
20	18.7 ± 6.77	11.9 ± 2.40	2.1 ± 0.32	0.9 ± 0.12	10 ± 1.18
21	23.2 ± 4.77	9.9 ± 2.54	2.2 ± 1.23	1.0 ± 0.14	10 ± 0.88
22	11.8 ± 3.16	12.7 ± 1.94	1.9 ± 0.33	1.0 ± 0.17	10 ± 1.52
23	19.0 ± 4.70	11.7 ± 0.17	2.2 ± 0.59	0.9 ± 0.03	10 ± 1.34

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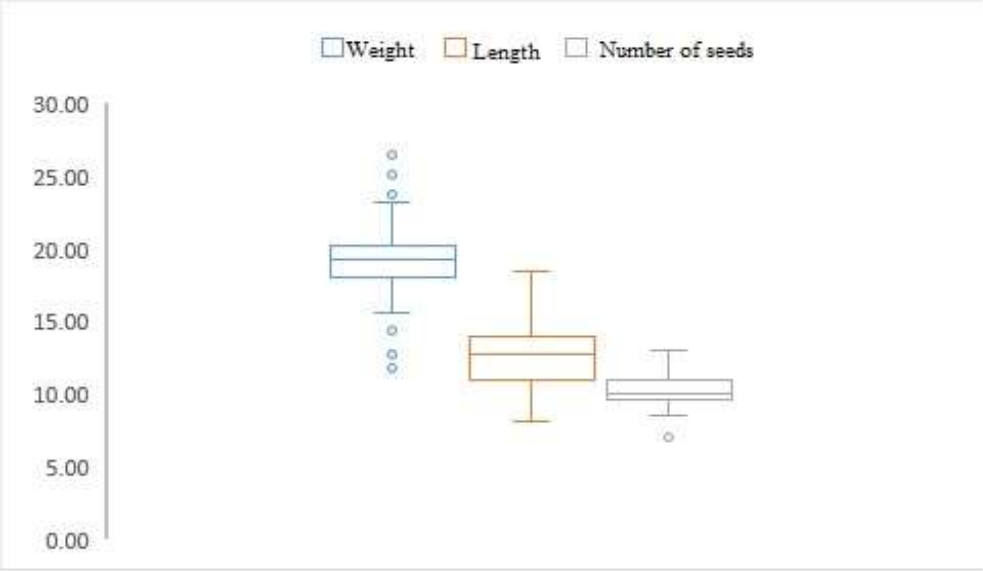

Figure 7: Boxplot of the weight (g), length (cm) and number of seeds of the carob pod varieties.

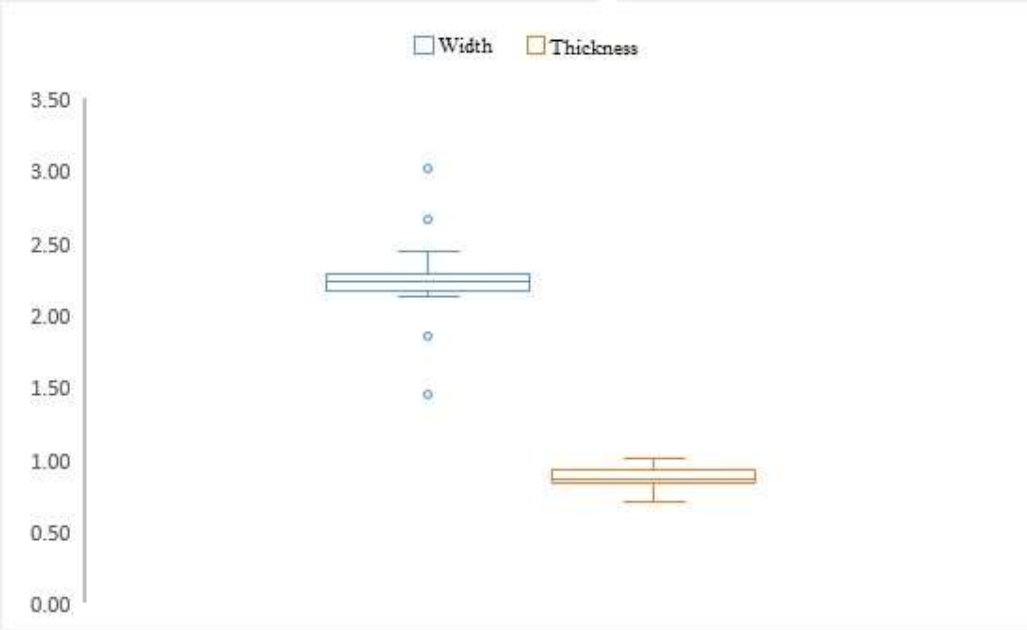

Figure 8: Box plot of the width (cm) and thickness (cm) of the carob pod varieties.

B. Moisture, protein, fat and ash

The moisture, protein, fat and ash contents are summarized in table 6 and figure 9.

1. Moisture content

The mean moisture content of carob pods ranged from 11.0 ± 0.08 % for V1 to 13.2 ± 0.03% for V10 (Table 6 & Figure 9). The moisture contents of the varieties were within the ranges of the moisture content of carob pods reported (Biner *et al*., 2007; Gubbuk *et al*., 2010; Iipumbu *et al*., 2008; Naghmouchi *et al*., 2009; Khlifa *et al.*, 2013). However, the moisture levels were considerably lower than those reported for Lebanese carob pods reported which ranged between 13.5% and 17.1% (Haddarah et al., 2013). These differences may be due to the different cultivars of the present study, the contrasting environmental conditions that the carob trees were planted in and differences in the ripening durations, harvesting time and storage periods (Batlle & Tous, 1997; Iipumbu et al., 2008).

2. Ash content

The ash content is indicative of the mineral content of biological materials. Overall, the ash contents of the varieties in the present work ranged between 2.4 ± 0.06 g/100 g (V9) to 4.7 ± 0.04 g/100g of carob powder (V22) (Table 6 & Figure 9) and were within the bracket reported by different workers (Biner *et al*., 2007; Gubbuk *et al*., 2010; Iipumbu *et al*., 2008; Naghmouchi *et al*., 2009; Khlifa *et al.*, 2013). However, the

obtained range was wider than that reported by Haddarah et al., (2013) for Lebanese carob pods spanning levels between 2.99 g/100 g and, 3.66 g/100 g.

3. Fat content

The fat content of the carob varieties < 1 g/100 g except for V17 (1.4% ± 0.21 g/100 g) and V21 (1.1% ± 0.10 g/100 g) (Table 6 & Figure 9). This is in agreement with previous reports of values ranging from 0.2 - 2.3 g/100 g (Biner *et al*., 2007; Gubbuk *et al*., 2010; Iipumbu *et al*., 2008; Naghmouchi *et al*., 2009; Khlifa *et al.*, 2013), with the exception of V19 which had a fat content of 0.1 g/100 g.

4. Protein content

The pods contained appreciable amounts of protein (3.4 - 6.5 g/100 g) (Table 6 & Figure 9). in line with the levels reported by other workers at 1 – 7.6 g/100 g (Biner *et al*., 2007; Gubbuk *et al*., 2010; Iipumbu *et al*., 2008; Naghmouchi *et al*., 2009; Khlifa *et al.*, 2013). A slightly narrower range for protein levels (3.6 – 5.6 g/100 g) was reported for Lebanese carob pods (Haddarah et al., 2013).

Table 6. Mean values of the moisture, ash, fat and protein contents of carob powder.

Carob pod variety	Moisture (% ± SD)	Ash (g/100g ± SD)	Fat g/100g ± SD)	Protein (g/100g ± SD)
1	11.0 ± 0.08	3.2 ± 0.04	0.2 ± 0.08	4.5 ± 0.13
2	11.8 ± 0.08	3.6 ± 0.08	0.2 ± 0.05	6.1± 0.26

3	11.5 ± 0.02	4.2 ± 0.11	0.3 ± 0.08	6.5 ± 0.25
4	12.6 ± 0.08	2.8 ± 0.05	0.4 ± 0.12	4.3 ± 0.20
5	12.0 ± 0.08	3.1 ± 0.01	0.6 ± 0.18	4.6 ± 0.24
6	11.6 ± 0.16	3.1 ± 0.03	0.4 ± 0.17	4.5 ± 0.19
7	11.9 ± 0.12	3.2 ± 0.07	0.7 ± 0.13	4.3 ± 0.35
8	11.8 ± 0.17	2.6 ± 0.06	0.4 ± 0.14	3.9 ± 0.23
9	12.0 ± 0.01	2.4 ± 0.06	0.3 ± 0.05	3.5 ± 0.24
10	13.2 ± 0.03	4.3 ± 0.11	0.5 ± 0.13	3.9 ± 0.11
11	12.3 ± 0.11	3.0 ± 0.02	0.5 ± 0.12	4.8 ± 0.12
12	11.8 ± 0.02	3.4 ± 0.08	0.2 ± 0.08	3.9 ± 0.07
13	12.8 ± 0.17	3.7 ± 0.04	0.9 ± 0.14	6.5 ± 0.22
14	12.5 ± 0.07	2.9 ± 0.05	0.3 ± 0.05	3.6 ± 0.04
15	12.2 ± 0.08	2.6 ± 0.02	0.4 ± 0.08	4.9 ± 0.11
16	11.3 ± 0.08	3.5 ± 0.12	0.4 ± 0.00	4.8 ± 0.19
17	12.5 ± 0.08	2.7 ± 0.06	1.4 ± 0.21	4.5 ± 0.57
18	12.4 ± 0.12	3.0 ± 0.04	0.5 ± 0.12	3.4 ± 0.33
19	11.8 ± 0.07	2.4 ± 0.09	0.1 ± 0.00	4.4 ± 0.49
20	11.5 ± 0.20	3.0 ± 0.02	1.0 ± 0.14	3.7 ± 0.25
21	11.7 ± 0.15	3.6 ± 0.03	1.1 ± 0.10	6.5 ± 0.37
22	11.0 ± 0.18	4.7 ± 0.04	0.6 ± 0.20	3.8 ± 0.52
23	11.7 ± 0.17	2.9 ± 0.05	0.7 ± 0.16	5.5 ± 0.26

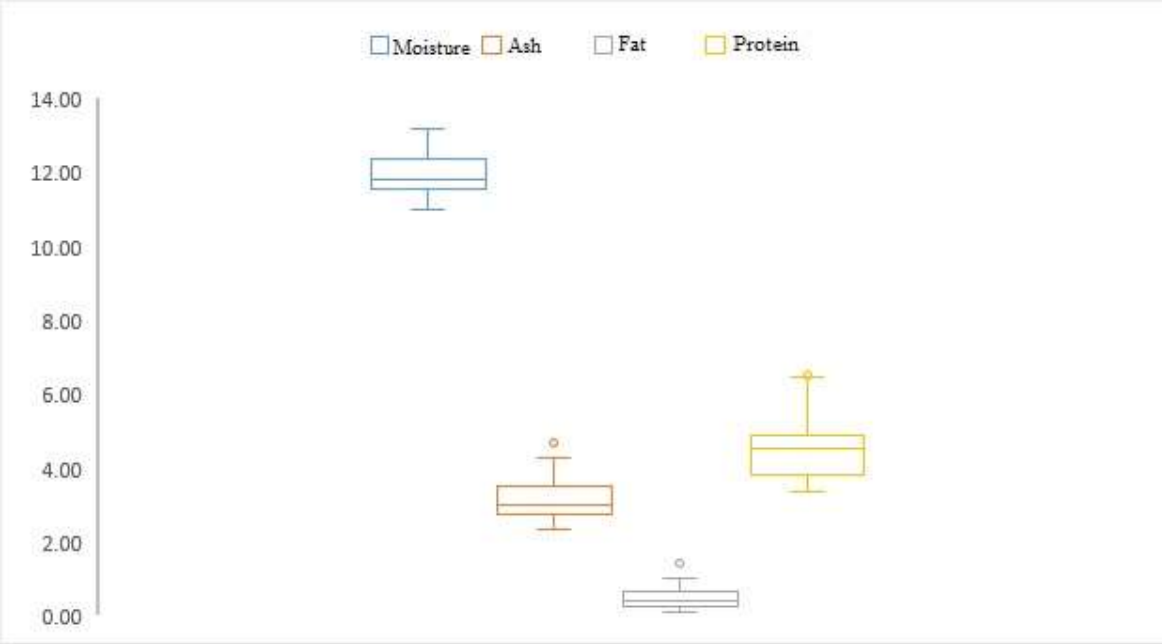

Figure 9: Box plot of the moisture (%), ash, fat and protein (g/100g) of the carob powder.

C. Minerals

Potassium was present at the highest concentration amongst the surveyed macrominerals and Fe was the most abundant micromineral (Table 7 and Figures 10 & 11). The values found for copper, manganese, zinc and sodium were within the reported ranges for carob pods (Ayaz *et al.,* 2007; Fidan *et al.,* 2015; Khlifa *et al.*, 2013; Özcan *et al*., 2007). However, the values for iron and potassium were slightly higher, and those for Phosophorus and magnesium and calcium were slightly lower than those reported by other workers (Ayaz *et al.,* 2007; Fidan *et al.,* 2015; Khlifa *et al.*, 2013; Özcan *et al*., 2007). Many factors affect the mineral content of the fruit including temperature, degree of dryness, irrigation and fertilization (Correia and Martins-Loucao, 1997) and salinity (El-Dengawy et al., 2011).

Table 7. Mean values of minerals content of carob powder.

Carob pod variety	Cu (mg/100g ± SD)	Mn (mg/100g ± SD)	Zn (mg/100g ± SD)	Fe (mg/100g ± SD)	Na (mg/100g ± SD)	P (mg/100g ± SD)	Mg (mg/100g ± SD)	Ca (mg/100g ± SD)	K (mg/100g ± SD)
1	0.32 ± 0.00	0.41 ± 0.00	0.55 ± 0.02	3.17 ± 0.01	15.63 ± 0.01	21.84 ± 2.99	40.15 ± 4.30	186.88 ± 0.56	1866.00 ± 41.00
2	0.54 ± 0.00	0.35 ± 0.00	0.35 ± 0.00	3.36 ± 0.02	8.48 ± 0.03	63.88 ± 2.86	92.98 ± 1.15	180.38 ± 1.53	3992.50 ± 49.00
3	0.58 ± 0.00	0.42 ± 0.00	0.40 ± 0.00	5.62 ± 0.03	12.19 ± 0.01	87.70 ± 2.66	38.48 ± 1.95	135.65 ± 1.35	2030.75 ± 37.50
4	0.32 ± 0.00	0.37 ± 0.00	0.42 ± 0.02	4.85 ± 0.02	6.22 ± 0.03	63.30 ± 3.89	99.55 ± 1.50	197.43 ± 1.63	1209.75 ± 13.50
5	0.46 ± 0.00	0.27 ± 0.00	0.32 ± 0.00	4.42 ± 0.02	6.05 ± 0.03	63.82 ± 4.66	88.83 ± 1.05	331.65 ± 9.75	1612.00 ± 0.00
6	0.27 ± 0.00	0.46 ± 0.00	0.30 ± 0.01	6.55 ± 0.03	5.82 ± 0.01	23.26 ± 4.48	42.38 ± 4.75	113.55 ± 2.16	1871.75 ± 15.50
7	0.57 ± 0.00	0.31 ± 0.00	0.56 ± 0.02	4.68 ± 0.03	15.34 ± 0.01	38.28 ± 3.00	53.70 ± 2.60	147.95 ± 1.55	1920.50 ± 38.00
8	0.22 ± 0.00	0.23 ± 0.00	0.55 ± 0.02	1.77 ± 0.01	11.43 ± 0.03	49.30 ± 4.48	32.6 ± 3.30	157.95 ± 1.35	1796.25 ± 32.50
9	0.45 ± 0.00	0.26 ± 0.00	0.27 ± 0.00	2.59 ± 0.03	14.77 ± 0.00	39.74 ± 1.19	34.40 ± 4.30	442.43 ± 1.88	1276.25 ± 7.50
10	0.37 ± 0.00	0.32 ± 0.00	0.50 ± 0.00	4.86 ± 0.01	21.26 ± 0.04	38.32 ± 4.89	47.65 ± 0.40	110.45 ± 1.30	1695.00 ± 23.00
11	0.49 ± 0.00	0.26 ± 0.00	0.36 ± 0.00	4.73 ± 0.02	8.23 ± 0.01	35.25 ± 1.49	94.10 ± 1.70	272.50 ± 1.00	3966.25 ± 22.50
12	0.13 ± 0.00	0.18 ± 0.00	0.23 ± 0.00	6.65 ± 0.05	15.12 ± 0.01	30.04 ± 0.75	46.15 ± 0.57	278.98± 2.43	1796.00 ± 45.00
13	0.21 ± 0.00	0.21 ± 0.00	0.45 ± 0.03	3.56 ± 0.04	9.69 ± 0.01	71.85 ± 2.19	40.45 ± 2.30	304.48 ± 0.13	1857.00 ± 12.00

14	0.24 ± 0.00	0.36 ± 0.00	0.36 ± 0.01	5.96 ± 0.07	10.75 ± 0.04	43.57 ± 2.67	100.10 ± 0.20	291.72 ± 0.97	3850.25 ± 42.50
15	0.50 ± 0.00	0.43 ± 0.00	0.43 ± 0.01	3.01 ± 0.02	15.72 ± 0.04	22.74 ± 4.48	93.78 ± 0.75	254.83 ± 0.03	3204.50 ± 16.00
16	0.34 ± 0.00	0.35 ± 0.00	0.54 ± 0.01	3.17 ± 0.00	5.62 ± 0.01	53.29 ± 0.82	46.33 ± 3.55	143.73 ± 0.68	2052.25 ± 56.50
17	0.56 ± 0.00	0.29 ± 0.00	0.39 ± 0.02	7.58 ± 0.01	6.17 ± 0.01	28.36 ± 1.79	25.92 ± 2.77	251.10 ± 1.10	2233.50 ± 47.00
18	0.33 ± 0.00	0.32 ± 0.00	0.32 ± 0.00	3.24 ± 0.03	7.55 ± 0.03	20.17 ± 1.34	68.60 ± 0.57	303.25 ± 2.75	3018.50 ± 3.00
19	0.36 ± 0.00	0.34 ± 0.00	0.25 ± 0.01	5.12 ± 0.00	5.46 ± 0.01	23.24 ± 5.71	30.02 ± 4.62	179.5 ± 1.40	1649.00 ± 46.83
20	0.53 ± 0.00	0.73 ± 0.00	0.31 ± 0.00	8.11 ± 0.04	5.51 ± 0.01	39.71 ± 1.49	48.95 ± 0.70	305.20 ± 1.70	2074.00 ± 29.00
21	0.48 ± 0.00	0.46 ± 0.00	0.46 ± 0.01	6.57 ± 0.01	18.31 ± 0.01	103.10 ± 2.54	63.77 ± 2.45	160.68 ± 0.63	1498.00 ± 49.00
22	0.16 ± 0.00	0.55 ± 0.00	0.44 ± 0.01	15.34 ± 0.08	10.84 ± 0.01	32.66 ± 5.70	98.43 ± 2.55	413.00 ± 13.10	1046.75 ± 1.50
23	0.54 ± 0.00	0.51 ± 0.00	0.37 ± 0.01	9.20 ± 0.03	16.49 ± 0.01	75.44 ± 3.13	92.35 ± 0.50	138.50 ± 0.50	3071.00 ± 45.00

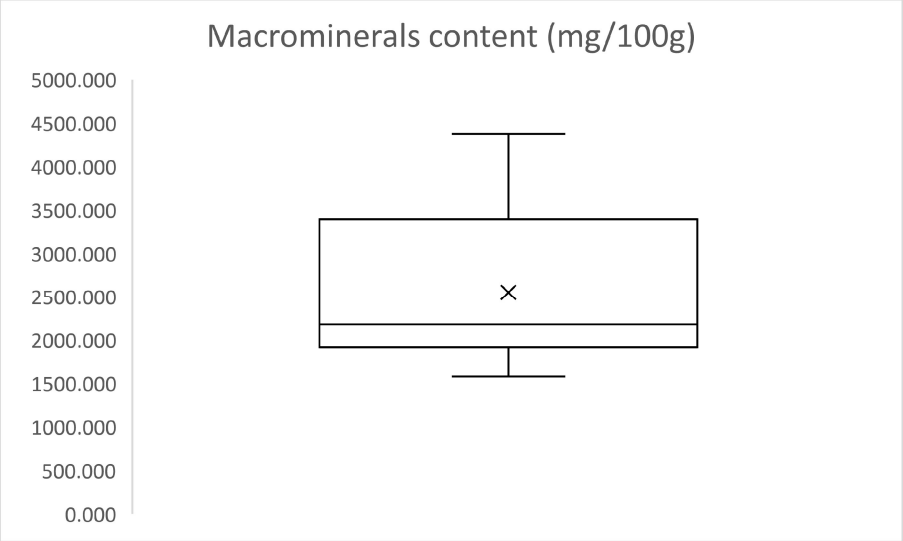

Figure 10: Box plot of the macrominerals content (mg/100g) of the carob powder.

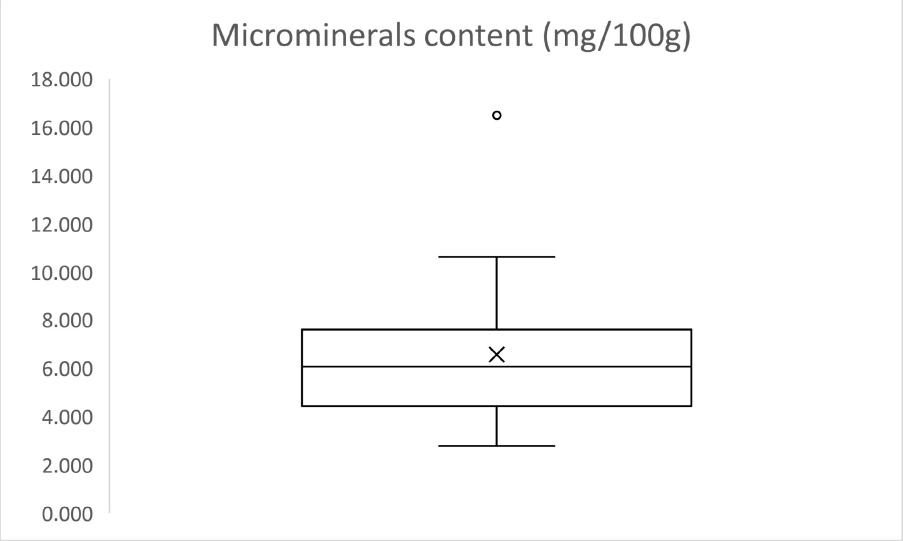

Figure 11: Box plot of the microminerals content (mg/100g) of the carob powder.

Standard reference material

The results for the non-fat milk powder standard reference material came as follows in table 8. The levels of the assayed minerals ranged from 99.76 % for K to 106.8% for Fe of the corresponding levels expected for standard non-fat milk powder (Table 8).

Table 8. Analysis of the standard reference material.

Element	Range	SD	Average (mg/Kg)	Expected (mg/Kg)	Accuracy (%)
Na	[4870, 5070]	144.68	5181.45	4970	104.25
Mg	[1170, 1230]	101.56	1197.33	1200	99.77
K	[16600, 17200]	1220.64	16859	16900	99.76
Ca	[12500, 13500]	552.19	13065	13000	100.5
Fe	[1.68, 1.88]	0.21	1.9	1.78	106.8
Mn	[0.20, 0.32]	0.02	0.27	0.26	103.85
Cu	[0.6, 0.8]	0.03	0.71	0.7	101.43
Zn	[43.9, 45.3]	1.09	47.6	46.1	103.25

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

Data shown as means ± standard deviation of three independent determinations.

D. Total dietary fibers

The dietary fiber content of the samples ranged between 4.7 g/100 g (V8) and 8.2 g/100 g (V16) (Figure 12, Table 9). These values were higher than those reported in other recent findings in the literature (Iipumbu et al., 2008).

The dietary fiber levels in carob pods have been reported to be as low as 3 g/100 g (Iipumbu et al., 2008) to as high as 39.9 g/100 g (USDA, 2006). Besides inter-cultivar differences, such large variations might be attributed to the possibility that methods based on different principles have been applied (Marakis, 1996). Methods based on the Englyst and Cummings procedure (1988) yield lower levels than those utilizing the AOAC enzymatic-gravimetric methods (Iipumbu et al., 2008).

The high dietary fiber content of carob is of high nutritional benefit since carob fiber has been reported to exhibit valuable health-promoting effects including reduced risk of gastro-intestinal cancer, blood cholesterol lowering and anti-oxidative properties (Brandt, 2005; Haber, 2002).

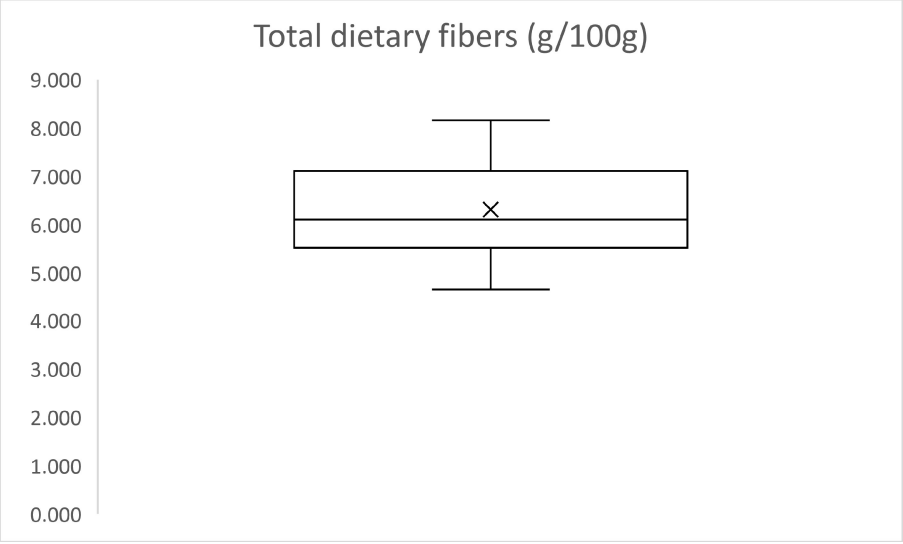

Figure 12: Boxplot of the total fiber content (g/100g) of the carob pod varieties.

Table 9. Mean values of total dietary fiber content of carob powder.

Carob pod variety	Total dietary fiber content (%± SD)
1	5.8 ± 0.19
2	6.9 ± 0.56
3	8.1± 0.2
4	5.8 ± 0.17
5	6.0 ± 0.36
6	7.0 ± 0.37
7	5.8 ± 0.56
8	4.7 ± 0.39
9	5.5 ± 0.36
10	4.7 ± 0.37
11	5.1 ± 0.28
12	6.5 ± 0.17
13	7.1 ± 0.22
14	6.1 ± 0.11
15	5.5 ± 0.11
16	8.2 ± 0.14
17	6.3 ± 0.55
18	5.1 ± 0.5
19	6.4 ± 0.533
20	5.8 ± 0.19
21	7.8 ± 0.18
22	7.7 ± 0.16
23	7.5 ± 0.01

E. Sucrose, glucose and fructose

The sucrose contents of the surveyed carob samples ranged between 4.8 g/kg (V3) and 358.3 g/kg (V19) (Figure 13, Table 10). With the exception of V3, the values of sucrose obtained in this study were within the reported range for sucrose content of carob pods (Biner et al., 2007). The low sucrose content in V3 might be attributed to high levels and/or activity of the enzyme invertase. Invertase is a key metabolic enzyme responsible for the hydrolysis of the disaccharide sucrose to glucose and fructose. Invertase has been reported to exist in higher plants in several isoforms, different subcellular locations and biochemical properties (Fotopoulos *et al.*, 2005). Plant invertase enzyme activity and gene expression are influenced by a number of factors which modulate its activity either by activation and repression. Among factors attributed to increased activity are plant growth regulators (PGRs) (Tymows-ka-Lalanne & Kreis, 1998) and the infection with plant pathogens (Storr & Hall, 1992). The high sucrose content as shown in this study explains carob's sweet taste in general. Furthermore, it is suggested that food products with high carob content would be naturally sweet, and thus the need for additional sweeteners is reduced (Biner *et al.,* 2007).

The glucose contents of the samples were found to range from 21.3 g/kg for V2 to 96.3 g/kg for V14 (Figure 13, Table 10). and were within the reported ranges for carob (Biner *et al.,* 2007).

The fructose contents of the samples ranged between 50.3 g/kg (V2) and 140.9 g/kg (V13) (Figure 13, Table 10) in accord with the reported values for fructose in carobs (Biner *et al.,* 2007).

Table 10. Mean values of total sucrose, glucose and fructose contents of carob powder.

Carob pod variety	Sucrose content (g/kg of carob powder ± SD)	Glucose content (g/kg of carob powder ± SD)	Fructose content (g/kg of carob powder ± SD)
1	297.8 ± 1.76	35.8 ± 2.02	69.9 ± 1.64
2	304.3 ± 13.63	21.3 ± 1.39	50.3 ± 2.63
3	4.8 ± 3.54	84.3 ± 4.95	138.6 ± 4.41
4	236.3 ± 6.57	51.8 ± 0.66	95.2 ± 3.05
5	268.8 ± 1.76	50.8 ± 1.44	89.2 ± 1.44
6	240.0 ± 3.53	35.8 ± 2.88	82.5 ± 0.00
7	269.17 ± 3.82	36.6 ± 2.26	69.6 ± 1.66
8	355.0 ± 3.53	40.0 ± 2.50	85.8 ± 1.44
9	318.8 ± 5.83	30.8 ± 2.88	71.3 ± 1.76
10	336.3 ± 5.30	32.5 ± 2.50	81.7 ± 1.44
11	240.5 ± 3.18	57.8 ± 1.46	113.6 ± 3.22
12	200.9 ± 0.88	56.5 ± 0.50	116.5 ± 3.96
13	109.9 ± 0.17	95.9 ± 3.92	140.9 ± 3.00
14	163.1 ± 5.89	96.3 ± 0.70	126.3 ± 4.95
15	285.4 ± 0.17	45.4 ± 2.89	75.4 ± 2.26
16	151.9 ± 0.53	49.6 ± 2.15	91.8 ± 5.03
17	327.1 ± 5.26	26.1 ± 2.56	64.3 ± 3.88
18	349.3 ± 5.23	39.5 ± 1.63	83.1 ± 3.00
19	358.3 ± 5.04	23.7 ± 0.41	50.7 ± 1.46
20	279.1 ± 5.62	26.2 ± 1.52	59.6 ± 3.00
21	137.5 ± 2.50	65.0 ± 2.50	97.5 ± 0.00
22	170.1 ± 3.39	63.3 ± 1.06	120.0 ± 0.00
23	159.6 ± 5.056	61.8 ± 2.03	95.9 ± 1.94

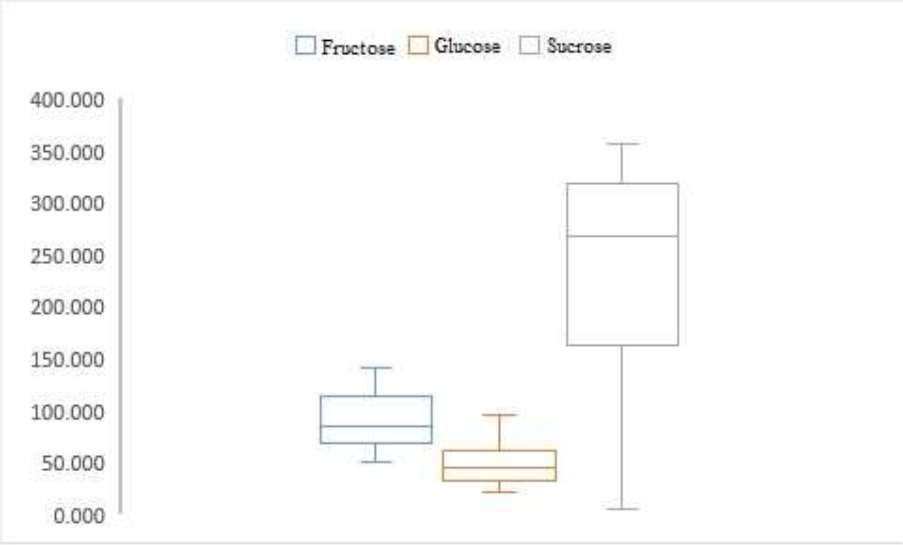

Figure 13: Boxplot of the total fructose, glucose and sucrose contents (g/Kg) of the carob pod varieties.

F. Phenols and antioxidant activity

1. Phenolic content

The phenolic content of the samples ranged between 1 g GAE/ 100g for V23 and 3.3 g GAE/ 100g for V16 (Figure 14, Table 11) in agreement with values reported by other workers (Iipumbu *et al.,* 2008; Makris & Kefalas, 2004; Roseiro *et al.,* 2013). It should be noted that the Folin-Ciocalteu method used in the present work does not measure the absolute contents of specific phenols since the values are expressed as gallic acid equivalents (Li *et al.,* 2007).

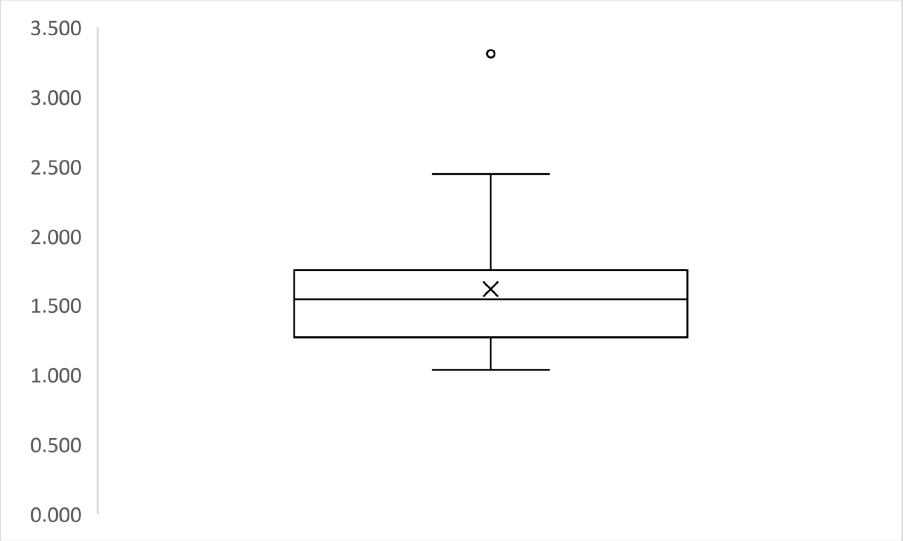

Figure 14: Boxplot of the total phenolic content (g GAE/ 100g) of the carob pod varieties.

Table 11. Mean values of total phenolic content of carob powder.

Carob pod variety	Total phenolic content (g GAE/ 100g carob kibbles ± SD)
1	1.39 ± 0.01
2	1.26 ± 0.01
3	1.82 ± 0.02
4	2.44 ± 0.04
5	1.75 ± 0.02
6	2.04 ± 0.03
7	1.64 ± 0.01
8	1.12 ± 0.01
9	1.72 ± 0.03
10	1.19 ± 0.03
11	1.59 ± 0.00
12	1.53 ± 0.04
13	1.49 ± 0.02

14	1.46 ± 0.02
15	1.60 ± 0.01
16	3.31 ± 0.00
17	1.29 ± 0.03
18	1.70 ± 0.04
19	1.27 ± 0.03
20	1.11 ± 0.03
21	1.22 ± 0.02
22	2.22 ± 0.02
23	1.03 ± 0.04

2. Antioxidant activity

The antioxidant activity of the carob samples was determined using the FRAP, DPPH and ABTS antioxidant assays.

The FRAP procedure measures the antioxidants' ability to reduce the TPTZ-Fe^{3+} complex into the blue TPTZ-Fe^{2+}. The range of antioxidant capacity determined by this procedure varied between 1.00 µM Fe (II)/100g of carob (V21) and 3.38 µM Fe (II)/100g of carob (V16) (Figure 15, Table 12).

The DPPH assay measures the degree of decolorization after exposure to the radical scavengers present in the carob. The antioxidant capacity was expressed as the carob extract concentration providing 50% inhibition (IC_{50}) in mg/L which ranged between. 0.03 mg/L (V16) to 0.55 (V23) mg/L (Figure 15, Table 12).

The ABTS assay is based on the decolorization of the ABTS•+cation in the presence of antioxidants and is normally expressed as the extract concentration providing 50% inhibition (IC_{50}) in mg/L. The IC50 values obtained in the present work ranged between 0.32mg/L and 1.03 mg/L with V16 exhibiting again the highest antioxidant capacity (Figure 15, Table 12).

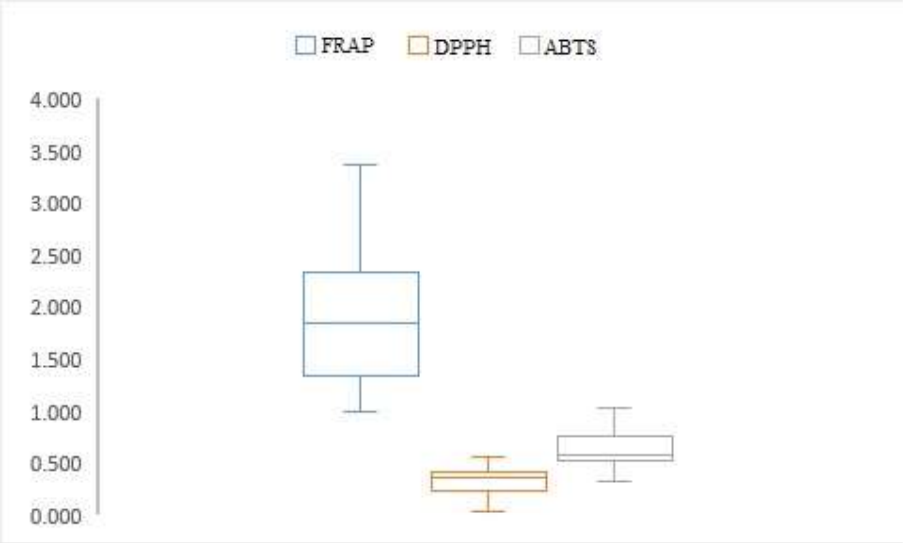

Figure 15: Boxplot of the ferric-reducing antioxidant capacity (FRAP) (µM Fe (II)/100g), DPPH• inhibition by the carob extracts (IC_{50} mg/L) and ABTS•+ inhibition by the carob extracts (IC_{50} mg/L) of the carob pod varieties.

Table 12. Mean values of ferric-reducing antioxidant capacity (FRAP), DPPH• inhibition by the carob extracts and ABTS•+ inhibition by the carob extracts.

Carob pod variety	FRAP (µM Fe (II) / 100g of carob ± SD)	DPPH (IC50 mg/L ± SD)	ABTS (IC50 mg/L ± SD)
1	1.69± 0.01	0.38 ± 0.01	0.53 ± 0.01
2	1.23 ± 0.03	0.24 ± 0.009	0.75 ± 0.01
3	1.53 ± 0.00	0.39 ± 0.04	1.03 ± 0.00
4	2.41 ± 0.03	0.08 ± 0.02	0.44 ± 0.02

5	1.04 ± 0.02	0.29 ± 0.05	0.53 ± 0.02
6	3.04 ± 0.04	0.32 ± 0.01	0.67 ± 0.04
7	1.51 ± 0.02	0.39 ± 0.01	0.69 ± 0.01
8	1.34 ± 0.02	0.41 ± 0.01	0.78 ± 0.01
9	2.63 ± 0.01	0.302 ± 0.01	0.43 ± 0.00
10	1.30 ± 0.01	0.45 ± 0.01	0.58 ± 0.01
11	1.13 ± 0.01	0.38 ± 0.01	0.77 ± 0.00
12	1.86 ± 0.00	0.41 ± 0.02	0.53 ± 0.00
13	2.42 ± 0.02	0.40 ± 0.02	0.53 ± 0.06
14	2.27 ± 0.05	0.31 ± 0.05	0.48 ± 0.02
15	1.85 ± 0.04	0.15 ± 0.03	0.59 ± 0.05
16	3.38 ± 0.04	0.03 ± 0.01	0.32 ± 0.01
17	1.78 ± 0.03	0.51 ± 0.02	0.73 ± 0.02
18	2.34 ± 0.08	0.09 ± 0.06	0.53 ± 0.05
19	1.60 ± 0.01	0.40 ± 0.01	0.57 ± 0.02
20	1.92 ± 0.03	0.15 ± 0.03	0.81 ± 0.01
21	1.00 ± 0.02	0.36 ± 0.02	0.81 ± 0.02
22	2.16 ± 0.01	0.24 ± 0.02	0.76 ± 0.03
23	1.33 ± 0.02	0.55 ± 0.04	0.93 ± 0.04

The ABTS•+ procedure registered a stronger scavenging activity than that observed with the DPPH• assay in all the carob samples consistent with the lower rate of scavenging of antioxidative compounds by the DPPH radical as compared to the ABTS radical (Almanasrah et al., 2013). However, a high correlation exists between the DPPH• and ABTS•+ assays (Jiménez-Escrig *et al.*, 2001) as observed in the present

work (Table 13). Furthermore, the correlation coefficients between the antioxidant assays and total phenolic content were very high, thereby indicating the pivotal role of the phenolics in modulating the antioxidant effects of carob (Table 12).

Table 13. Correlation coefficients between the total phenolic content and the antioxidant capacities.

	TP	**FRAP**	**DPPH**	**ABTS**
TP	1	0.744**	0.794**	0.586**
FRAP	-	1	0.610**	0.731**
DPPH	-	-	1	0.734**
ABTS	-	-	-	1

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

The FRAP, ABTS and DPPH assays are based on different mechanisms and are often expressed in different ways. Accordingly, the total antioxidant capacity of the carob samples was expressed as relative antioxidant capacity index (RACI) (Sun et al., 2007).

A clear difference in the antioxidant capacity between the carob samples is evident (Figure 16) indicative of the presence of different types and levels of antioxidants in the surveyed carobs. The carobs contain different classes of phenolics (e.g. flavonoids, proanthocyanidins) and these classes have different antioxidant capacities due to variations in the # and positions of the hydroxyl groups on the aromatic rings.

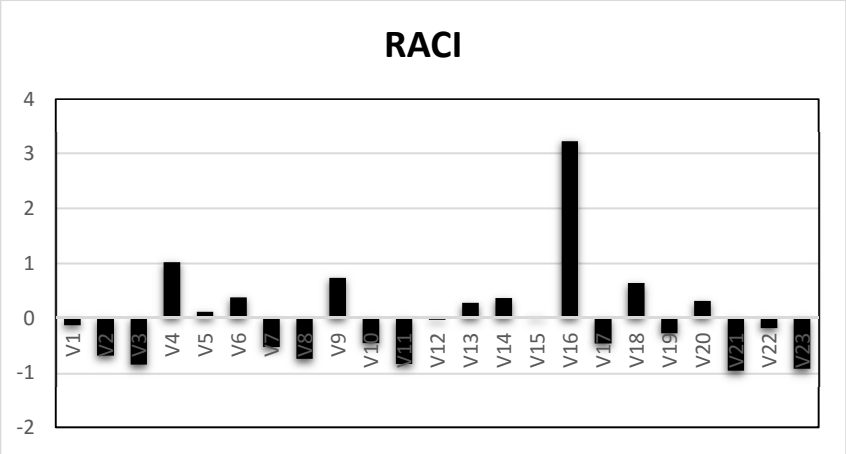

Figure 16: Relative Antioxidant Capacity Index of the 23 carob samples.

G. Statistical analysis

Principal Component Analysis was performed to objectively interpret and evaluate the most important variables in order to compare the morphological parameters and chemical characteristics of the carob pods. Furthermore, to investigate similarities between varieties and confirm PCA, analysis was completed by a hierarchical cluster analysis.

1. Morphological parameters

1.1. Principal component analysis

Principal component analysis of the morphological data identified 3 principal components that explained 91.4% of the variance (Figures 17 & 18, Table 14). The first component A1 accounted for 46.6% of the total variance and was obtained by the combination of the weight, length, width and number of seeds. These variables are coherent with the description of pod size. Thus, A1 describes “big carob pods”. The

second principal component A2 accounted for 30.6% of the variance and correlated negatively with the weight and the thickness. A3 accounted for 14.5% of the variance and correlated positively with the number of seeds and the thickness.

The plot of the two principal components showed a high dispersion of populations (Figure 19).

However, a group containing V5, V11, V12, V13, V14, V15, V16, V17, V19 and V20 was obtained and positioned at the center of the PCA biplot. The varieties (V11, V12 and V13) situated in the upper right quadrant correlated positively with both PC1 and PC2, those situated in the lower left quadrant correlated negatively with both PC1 and PC2 (V19, V20, V22), those situated in the lower right quadrant correlated positively with PC1 and negatively with PC2 (V14, V15 and V16), V5 correlated negatively with A1 and positively with A2, and V17 correlated negatively with A2. The varieties belonging to this group can be described as average sized varieties.

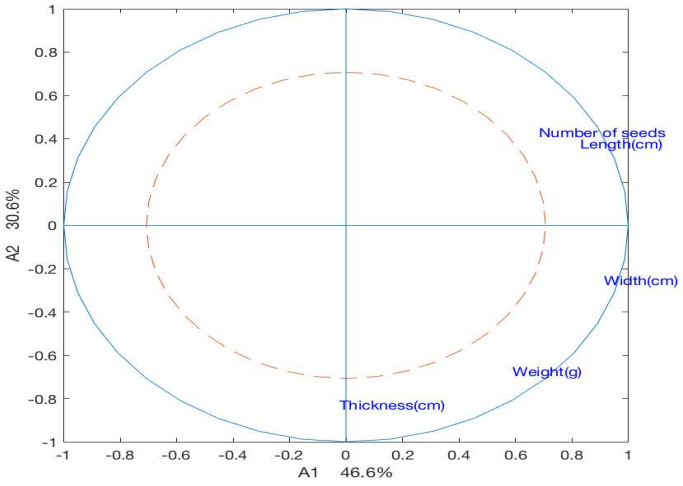

Figure 17. Variables factor map (A1 & A2).

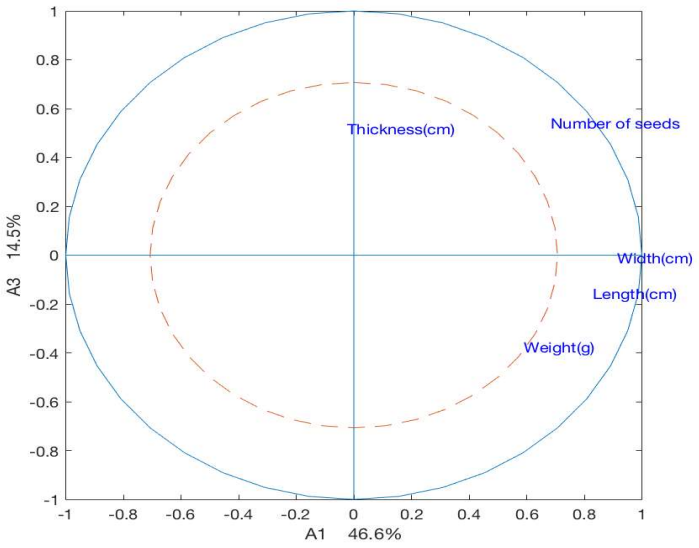

Figure 18. Variables factor map (A1&A3).

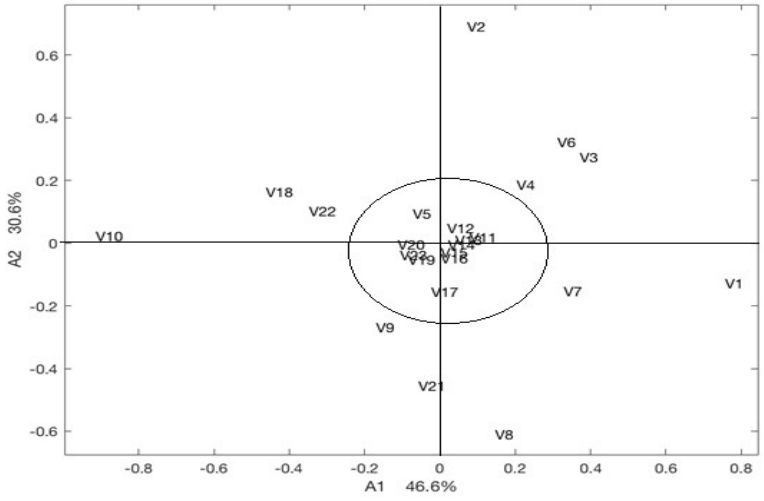

Figure 19. Individual factor map of the morphological parameters according to A1 and A2.

Table 14. Principal components of the morphological parameters.

PC	Factors loading	% of the total variance
A1	Weight, length, width and number of seeds.	46.6
A2	Weight, thickness.	30.6%
A3	Number of seeds, thickness.	14.5%

1.2. Hierarchical cluster analysis

Cluster analysis revealed the presence of 3 clusters (Figure 20). Cluster 1 contained V5, V8, V9, V11, V12, V13, V14, V15, V16, V17, V19, V20, V21 and V23. It primarily comprised the heavy and wide pods. A highly similar cluster was obtained with PCA and contained V5, V11, V12, V13, V14, V15, V16, V17, V19, V20 and V22. Cluster 2 contained V1, V3, V4, V6 and V7 whose pods were, in general, heavy, long, wide and contained a high number of seeds. Varieties in cluster 3 were V2, V10, V18 and V22 with mostly light, short, thin and narrow pods.

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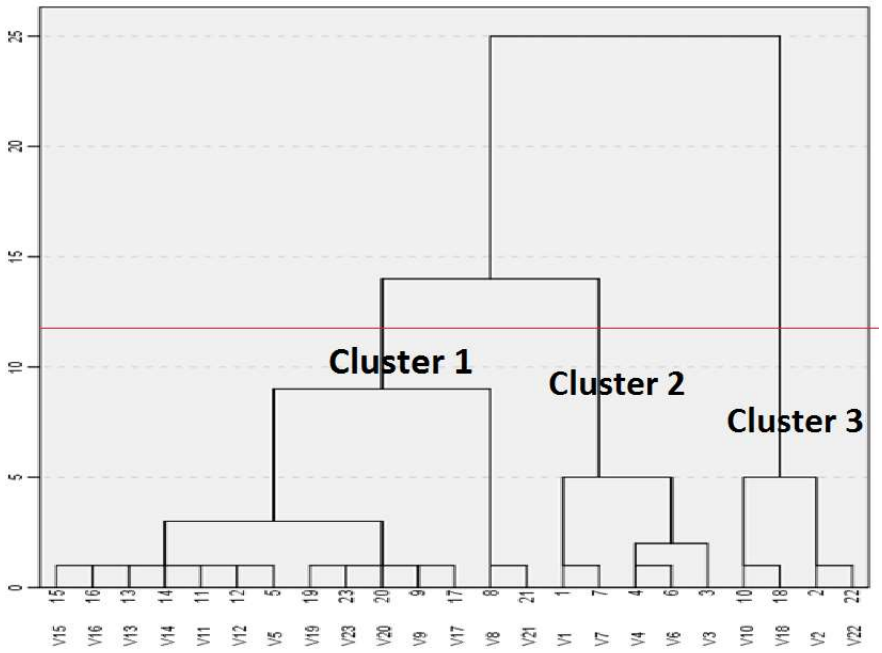

Figure 20. Hierarchical Ascendant Classification on morphological characters.

2. Chemical parameters

2.1. PCA

In order to reduce the number of variables for PCA, the values of FRAP, DPPH and ABTS were combined in RACI; Na, Ca, Mg, P and K contents were added to indicate the macrominerals content, and the Cu, Mn, Zn and Fe contents were likewise combined to present the microminerals content. The other variables for analysis included the total phenols, dietary fiber, sucrose, and the ratio of fructose to glucose (F/G).

PCA reduced the data to 3 principal components: A1 (37.6%), A2 (25.6%) and A3 (17.5%) which accounted for 80.7% of the total variance (Table 15). A1 correlated positively with the total phenols, RACI, the total dietary fibers and the microminerals content, and negatively with the sucrose content and F/G. A2 correlated positively with

the macrominerals, microminerals and total dietary fiber contents and negatively with sucrose content, F/G, total phenolics and RACI. A3 correlated negatively with total phenols, RACI and macrominerals content and positively with sucrose content, F/G, total dietary fibers and microminerals contents (Figures 21 & 22).

Furthermore, the individual factor maps (Fig. 23) allowed separating some varieties according to A1 and A2. The grouped varieties (V3, V13, V14, V21, V22 and V23) situated on the upper right quadrant correlated positively with A1, indicating their high contents of total dietary fibers and microminerals. The other grouped varieties (V1, V2, V11, V15, V17, V19 and V20) situated in the upper left quadrant, correlated negatively with A1, indicating their low contents of total dietary fibers and microminerals. Additionally, and according to the individual factor map, the different carob varieties can be grouped in terms of their antioxidant capacities and sugar contents to further investigate their potential uses in the food industry. The varieties that were situated in the lower right quadrant (V4, V5, V6 and V16), correlated positively with A1 and negatively with A2 and thus indicating their high antioxidant capacities and low sugar contents. Whereas those situated in the upper left quadrant (V2, V11, V15, V17 and V20), had high sucrose contents and low antioxidant capacities as they correlated positively with A2 and negatively with A1.

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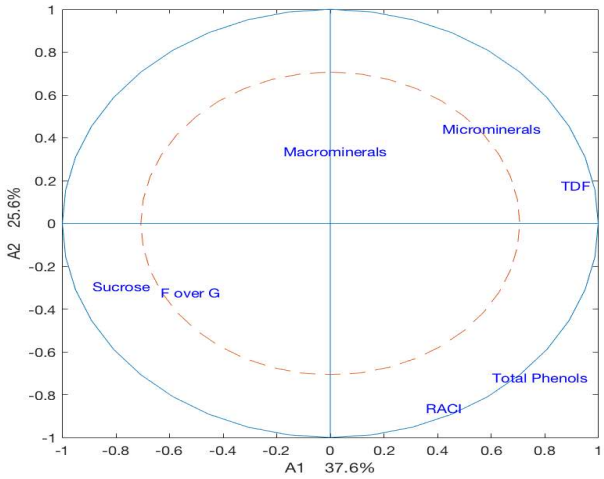

Figure 21. Variables factor map for the chemical parameters of carob (A1 & A2).

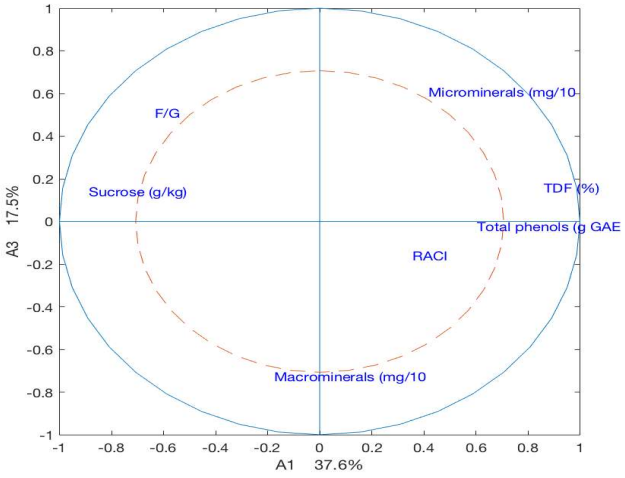

Figure 22. Variables factor map (A1 & A3).

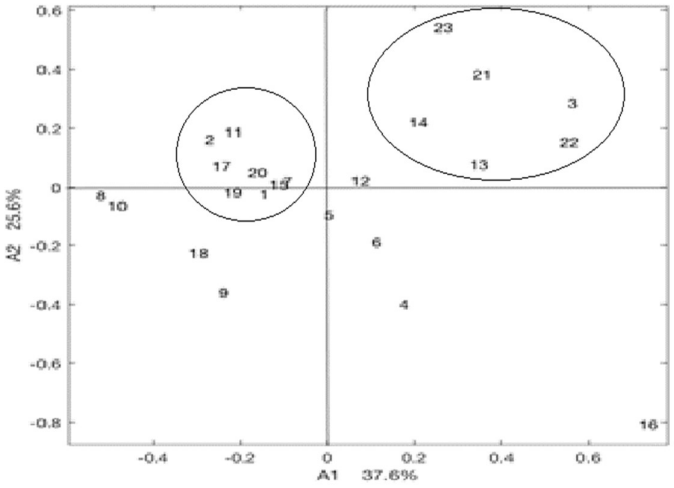

Figure 23. Individual factor map for the chemical parameters of carob (A1 & A2).

Table 15. Principal components of the chemical parameters of carob.

PC	Factors loading	% of the total variance
A1	Total phenols, RACI, the total dietary fibers and the microminerals content.	37.6
A2	Macrominerals, microminerals, total dietary fiber, sucrose content, F/G, total phenolics and RACI.	25.6
A3	Total phenols, RACI, macrominerals content, sucrose content, F/G, total dietary fibers and microminerals contents.	17.5

b.2. Hierarchical cluster analysis

Four clusters were identified from hierarchical cluster analysis on the chemical characteristics of the carob varieties (Figure 24). Cluster 1 contained V4, V9, V10, V19, V21 and V22 with pods having low microminerals contents. Cluster 2 contained V1,

V3, V5, V6, V7, V8, V12, V13, V16, V17 and V20 which had high total phenols and microminerals contents, high relative antioxidant capacity index, low sucrose content and low F/G ratio, which is in accordance with the results obtained with PCA. Cluster 3 contained V2, V11 and V14 grouping pods with low microminerals and macrominerals contents while Cluster 4 comprised V15, V18 and V23 with low phenolic content and high macrominerals content.

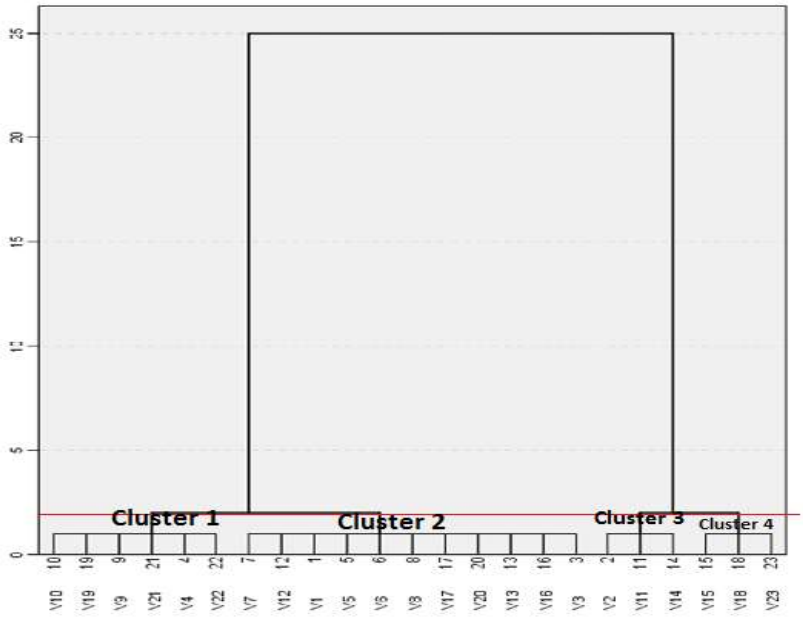

Figure 24. Hierarchical Ascendant Classification on chemical characters.

CONCLUSION AND RECOMMENDATIONS

The carob samples showed wide variations in their morphological and chemical properties. As expected, the unique geography of Lebanon gave rise to a huge diversity in the carob varieties. The morphological characteristics and their ranges were: weight (11.8 - 26.5 g/pod), length (8.2-18.5 cm/pod), width (1.5-3.0 cm/pod), thickness (0.7-1.0 cm/pod) and number of seeds (7-13 seeds/pod. The moisture content of carob pods ranged from 11.0 to 13.2%, ash content 2.4-4.7%, fat content 0.1-1.4%, protein content 3.4 - 6.5%, and total dietary fibers 4.7-8.2%. The average mineral ranges of carob pods were (in mg/100g carob powder): 0.13 – 0.58 copper; 0.18 – 0.73 manganese; 0.23 – 0.56 zinc; 1.77 – 15.34 iron; 5.46 – 21.26 sodium; 20.17 - 103.10 phosphorus; 25.92 - 100.10 magnesium; 110.45 - 442.43 calcium; and 1046.75 - 3992.50 for potassium. Sugar analysis showed that sucrose content of the carob varieties ranged between 109.9 and 358.3 g/Kg, fructose 50.3-140.9 g/Kg and glucose 21.3-96.3 g/Kg. The total phenolic content ranged between 1.03 and 3.30 (g GAE/ 100g carob). The antioxidant capacity of the varieties as determined the FRAP, DPPH and ABTS assays were in the range 1.00 and 3.38 μM Fe (II)/100g of carob, 0.03 to 0.55 mg/L providing an inhibition of 50% (IC_{50}) and 0.32 to 1.03 mg/L providing an inhibition of IC_{50}, respectively.

All antioxidant tests correlated highly with TP and with each other thereby suggesting that the phenolic compounds are responsible for the antioxidant activity of carob. The present work provided further evidence that carob pods are rich sources of compounds with high antioxidant activities.

The variety V16 had the highest dietary fiber total phenolic contents and displayed the highest antioxidant capacity in the FRAP, DPPH and ABTS assays but had relatively-low sucrose content. In contrast, V19 had the highest sucrose content but exhibited a low antioxidant capacity, and low dietary fiber, microminerals and macrominerals contents.

The information obtained in this study is potentially useful to the food industry, farmers, plant breeders and seedlings producers and provides a guide on the carob varieties for use in product formulation. Overall, it was proven that carob can be used as an ingredient in various processed foods as carob pods were found to be rich in sugars, polyphenols, fiber and minerals. Moreover, and depending on the end product, varieties V4, V5, V6 and V16 are recommended for formulation into antioxidant-rich products with high nutritional values, whereas V2, V11, V15, V17, V19 and V20 would find applications in providing sweet taste to food products.

Further studies are needed to identify the components that are responsible for the antioxidant properties of carob pods. To this end, HPLC analysis of the phenolic compounds would particularly be effective in constructing the phenolic profiles and relating these to the observed variations in the antioxidant activity of the different carob varieties. Further, investigating the *in vivo* antioxidant activities of carob pods would provide useful insights into the antioxidant activity of carob in humans. Moreover, studies addressing the antibacterial, anti-inflammatory and anti-proliferative activities of Lebanese carob varieties would further enhance the potential of the crop for varied applications.

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