Evaluation of some physical, chemical, and biological characteristics of the traditional Al-Jabal Al-Akhdar carob Rub "Libya"

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Abstract: The aim of this research was to study of some physical, chemical, biological and functional properties of carob Rub produced by traditional methods in Al Jabal Al Akhdar region, where seven samples of carob rub were purchased from the local market in a random manner. The results were the total solids, dissolved solids, moisture, pH and color are (59.76±6.46%, 57.94±6.73%, 40.23±6.46%, 5.23±0.26, L*21.14±0.18 a*-26.00±0.16 b* 0.53±0.02) respectively. While the average percentage of acidity, total sugars, reducing sugars, sucrose, ash, protein and hydroxymethylfurfural compound were 0.57 ± 0.16%, 41.99 ± 7.50%, 15.42 ± 1.11%, 26.57 ± 6.39%, 1.47 ± 0.50%, 23.15 ± 0.07 mg/kg) respectively. Regarding the biological properties, the mean of total phenols, flavonoids, tannins, and antioxidant activity were DPPH, ABTS (6.082 ± 0.06mEqGA/100g , 2.339 ± 0.05mEq/100 g Catechins, 3.125 ± 0.91% , 35.69 ± 0.14 , 519.38 ± 4.18) respectively, and the studied functional property, which is the ability to emulsify, was (10.3% ±0.15).

Key words: Carob, Pekmez, Chemical and functional properties, Concentrated fruit juices.

تقييم بعض الخواص الفيزيائية والكيميائية والبيولوجية لرب الخروب التقليدي في منطقة الجبل الأخضر "ليبيا" د.عبد الرسول عوض بوسلطان د. مبروكه ميلاد موسى أ. حنان محمود صالح قسم علوم وتقنية الاغذية –كلية الزراعة– جامعة عمر المختار*

الكلمات المفتاحية: الخروب، الخواص الكيميائية والوظيفية، بكميز، المركزات، شراب الفاكهة.

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1. Introduction:

The evergreen carob tree (Ceratonia siliqua L.) is found all over the world, One of its domestication sites, in particular, has been the Mediterranean region. Carob has been grown for millennia not only as a forage crop or as food for humans, but also to treat a variety of ailments (Sidina et al., 2009). Because of its multiple applications, this species has recently gained a lot of attention and has become socioeconomically important. In the food, pharmaceutical and cosmetic industries, pods and seeds are utilized as row material; particularly for gum, extraction (Barracosa, et al., 2007). It has been introduced and grown in many dry areas of the world. However, its cultivation and production are centered in Spain, Italy and Portugal (Batlle and Tous, 1990). In reality, it is utilized in reforestation programs in dry locations that serve both environmental and economic goals, as well as to valorize marginal lands or as a drought-tolerant species substitution, when compared to other Mediterranean species, its ecophysiological behavior has been described as more resistant to water stress (Sakcali and Ozturk, 2004). The carob tree matures slowly and bares pods in the autumn but only in July every second year, and partially renews leaves in spring. The world carob production from the year 2012, Spain with 40000 tons was ranked the first, Italy with 30841 ton ranked the second, Portugal with 23000 tons ranked the third, and Turkey with 14218 tons ranked the sixth. In 5119 hectares in Turkey in 2013 produced 14261 tons of carobs (Anonymous, 2014). Components of the carob pod have been used as a sweetener, as a food ingredient in the production of confectionary, beverages, bread and paste. The nutritive value of carob pods was attributed to its high level of carbohydrate67.48% appreciable amounts of protein 6.64% and low level of fat 2.24% considering the minerals content of fruit (calcium, potassium, magnesium, sodium, and iron)are abundant, but it also contains a large amount of condensed tannins 16-20 % (Can et al., 2007 ; Goulaset al., 2016). Molasses is produced from sugar rich fruit juices by boiling until achieving 70-80% soluble dry matter content (Akbulut and Özcan, 2008). Fruit is cut into different sizes (small, medium, large and mixed) the mixed equally and soaked for several hours, keeping in mind that no significant difference is seen between brix values of one hour and three hours or between two hours and six hours or between three hours and nine hours (Dimassi et al., 2019). Molasses is a traditional confectionery widely prepared in many Mediterranean countries, known as "Rub" in Tunisia Pekmez" in Turke and "Débès" in Lebanon. It is generally produced by the concentration of fruit juice up to 65-80 °Brix without the addition of sugar or other food additives (Maskan, 2006). Molasses contains mainly high amount 50-80% of natural sugars, some minerals (iron: phosphorous, calcium and potassium), as well as organic acids. Therefore, such product could be considered as natural, energetic and healthy food, which is important for human nutrition. Furthermore, molasses can easily pass into the blood without digestion because it contains simple sugars (glucose and fructose). So, it is an important food. especially for babies and children who need urgent energy requirement (Toker et al. 2013).Molasses concentration is traditionally carried out through the use of thermal evaporation, which could greatly affect the sensorial and nutritional quality of fruit juices (Maskan 2006). Indeed, juice concentration as a heat treatment promotes non-enzymatic browning reactions occurring in molasses during processing and storage. It is contribute to food quality and acceptability. Those reactions include mainly caramelization and Millard reaction. Caramelization occurs by the sugar decomposition, while Millard reaction takes place between amino acids and reducing sugars at high temperatures during juice concentration (Benjakul et al., 2005). Therefore, the molasses production represents an interesting fruit processing in food industry as it determines its quality features (flavour, colour, aroma, appearance and mouth feel) so that, molasses can be used as ingredient in many food formulations such as ice creams, fruit syrups, jellies and fruit juices beverages (Maskan 2006). At the local level, carob Rub is produced by traditional methods and is available in the local market and due to the lack of previous studies of this local product, the aim of this study was to evaluate some of the physical, biological and chemical properties of traditional carob rub.

2. Material and methods:

Seven samples of locally produced carob rub were purchased from various places in locale market, and kept chilled until all laboratory testing were done. On each sample, three analyses were carried out.

1.2. Chemical and Physical Analyses:

Total and soluble dry matter, protein, ash, pH and titrable acidity were determined according to the standard methods(AOAC1997).pH was measured at 25°C using a pH meter (professional meter pp-15 Sartorius, Germany).Total sugars (T.S), reducing sugars and sucrose were determined by the Lane -Eynon volumetric method. Sucrose concentration was calculated by subtracting the reducing sugar concentration from the total invert sugar and multiplying the result by 0.95. Then the reducing sugar and non-reducing sugar (sucrose) concentrations were taken together as TS concentration. Hydroxymethylfurfural (HMF) was determined as described in (AOAC, 1997), Hunter Lab was used to determine the color of carob rub with a Hunter Lab parameters, L refers for (Brightness; 100: white, 0: black), a (+: red, - green), and b (+: yellow, - blue).

Functional properties:

Emulsifying capacity of carob rub was investigated using the method previously described by (Tounsi *et al.*, 2017).

3.2. Estimation of Total phenolic compounds and antioxidant activity:

Total phenolic content (TPC) was quantified following the method previously described by (Singleton & Rossi, 1965) with some modifications . The carob rub extract (100 μ L) was mixed with100 μ L of Folin-Cioalteau reagent. After 5 min of incubation at room temperature, 800 with some modifications, where 100 μ L of the solution were taken with 200 μ L of Folin-Cioalteau reagent and then incubated for 5 min at a temperature800 μ L of 7.5% sodium bicarbonateNa₂CO₃was added to the mixture, followed by the addition of 1ml of distilled water. The mixture was kept in the dark for 2 h, and the absorbance was then measured at 750nm using a device aqua mate plus UV-Vis Spectrophotometer (Thermo scientific, England) and the results were obtained after performing the standard curve for Gallic acid, which was prepared using concentrations of 20-120 mg / ml, and the results were expressed as the equivalent of mg Gallic acid / 100 g Rub.

4.2. Estimation of Total Flavonoids:

The Total flavonoids (TP) were estimated using the aluminum chloride colorimetric assay in the method used by (Yoo *et al.*, 2008), where 250 μ L of the solution was taken with the addition of 1.25 ml of deionized water and 75 μ L of 5% sodium nitrate, then after 6 minutes was added 150 μ L of 10% aluminum chloride and then incubate at room temperature for 5 minutes. After incubation, 0.5 ml 1 M NaOH and 2.5 ml deionized water are added, then the solution was shaken, and the color formed was measured at a wavelength of 510 nm using the Aqua mate plus device. UV-Vis Spectrophotometer (Thermo scientific, England) and the standard curve was prepared using Catéchine, which was used at concentrations ranging from 20-60 μ g/ml. The results were expressed as the equivalent of mg catechins/100g of rub.

5.2. DPPH assay:

The DPPH free radical-scavenging activity of each sample was determined according to the method described by (Sudha *et al.*,2012).

6.2. ABTS assay:

The scavenging activity against radical cation 2,2 '-azinobis (3-ethylbenzothiazoline-6sulfonic acid) (ABTS⁺⁺) was estimated according to (Re et al., 1999).

Statistical analysis:

Data were expressed as an averages and standard deviations. The results were analyzed using Microsoft Excel 2007.

3. Results and discussion:

3.1. Chemo physical characteristics of carob rub .

Table 3.1. The chemical and physical characteristics of Carob Rub	
Parameters	Mean value ±SD
Total dry matter %	59.76 ±6.46
Soluble dry matter%	57.94±6.73
Moisture%	40.23±6.46
рН	5.23±0.26
Color	
L*	21.14 ±0.18
a*	-26 ±0.16
b *	0.53 ±0.02
Titratable acidity (citric acid) %	0.57±0.16
Total sugar%	41.99±7.50
Reduced sugar%	15.42±1.11
Sucrose%	26.57±6.39
Ash%	1.47±0.50
Protein%	1.59±0.23
Hydroxymethylfurfural (HMF mg/kg)	23.15±0.07
Emulsifying capacity%	10.3±0.15

3.1.1 Total dry matter and Soluble dry matter:

Through Table (3.1)it was found that the total dry matter content of the samples of traditional carob rub was % 59.76 ±6.46. This result was less than (Karhan et al., 2005) which found $72.18 \pm 0.61\%$ in samples pekmez Turkish carob. Also from Table (3.1) that the mean of the total dissolved solid was 57.94%. This result is considered less than (Sengul et al., 2007) the results found, where it was found that the value of the total dissolved solid is 72% the degree of Brix, and it was less than the results found (Tounsi et al., 2020). In his study of the chemical properties of a group of locally made carob samples and commercial samples in Tunisia, it was found that the content of dissolved solids was within 75% of the degree of (Brix). According to previous studies, the total dissolved solids (TDS) content did not exceed 75% and ranged from 66.6 to 73.7% for Turkish carob, the total soluble solids values for carob bean is mainly due to its contents of soluble sugars, especially glucose, fructose and sucrose (Tetik et al., 2010). The water-soluble dry matter in fruits is primarily formed by sugars, including fructose, glucose and sucrose, and by acids, such as citric acid and malic acid (Türkben et al .,2016).

3.1.2 Moisture content:

The moisture content of carob Rub was 40.23% ±6.46, as in table (3.1). This result was higher than the results obtained (Sengul et al., 2007), Whose found the moisture content of Turkish carob molasses was 24.10%, and also it was similar to(Tounsi et al., 2020) whose found in his

study of the physical, chemical and functional properties of carob molasses was the percentage of moisture in the processed carob molasses 41.95%, and compared to the date rub, The differences in the ratios may be due to the degree of concentration during the heat treatment. The moisture decrease is explained essentially by water evaporation during juice boiling (Maskan,2006).

3.1.3 Total acidity and pH:

The total acidity value was 0.57%, which is lower than the 0.60% results obtained by Sengul *et al.* (2007), where the total acidity value was 0.60%. The pH of the carob was listed in the table (3.1) as being 5.23. This result is comparable with (Tunisi *et al.*,2020), who observed that the pH range for carob rub was between 5.1 and 4.20. Titratable acidity is inversely proportional to pH a acidity may vary depending on the herbal sources and producing regions (TÜRKBEN *et al.*, 2016).

3.1.4 Total Sugars content:

In carob rub as shown from table (3.1), concentrations of sucrose (26.57 ±6.39),invert sugar(15.42 ± 1.11) and total sugar (41.99 ± 7.50)were found. These results differed with those mentioned by (Tetiket al., 2010) whose found Total sugar 62.80 g/100g, invert sugar17.05 g/100g, and sucrose 45 g/100g were all present in carob pekmez or carob rub. The sugars content of fruit can vary considerably according to species, variety, climatic, storage conditions, harvest season and physiological maturity (Ayaz et al., 2007). Sugars represent the main components of all the pekmez and invert sugars (or reducing sugar), mainly, glucose and fructose have been found to be the major component of pekmez. In addition, simple carbohydrates, known as sugars, have other roles, such as acting as sweeteners to make food more palatable, increasing the viscosity, enhancing flavor, adding texture and color, inhibiting protein coagulation, and assisting in food preservation. The use of invert sugars is very common in the elaboration of beverages, baked goods, and syrups, among others. This equimolar mixture of two simple sugars (glucose and fructose) is commonly used to retard the crystallization of sugar and to retain moisture, and when freshness in the final product is desired. Furthermore, this is one of the factors, along with pH and temperature that greatly influences the quality and storage capabilities of syrups. Thus, in this carob product, invert sugars were found at high concentrations. These compounds encompass monosaccharide's; hexoses (such as glucose, fructose and galactose) and pentoses (such as xylose and arabinose); and disaccharides, maltose and lactose. Which are important for the flavor of heattreated carob products, due to react with amino acids via the Maillard reaction to provide many important flavor compounds, including furanics.

3.1.5 Ash content:

As showed in table (1) that the ash content of carob molasses was 1.47%, and this result was less compared to the results of the total ash of Turkish carob molasses, where it was 2.48%, These differences in ash content are attributed to the different chemical composition of the carob, different varieties and environmental factors (Sengul *et al.*, 2007).

3.1.6 Protein content:

The results revealed that the protein value was 1.59%, which was in agreement with (Tetik*et al.*, 2010) who reported that the protein content in Pekmaz was 1.18 g/100 g. It was also higher than the values reported in some previous studies, where it was discovered that CarobRub contains a very small amount of proteins (0.3 g/100 g) (Sengul*et al.*, 2007).From other hand this result was similar to that of (Abd Allah et al., 2020), who examined the chemical composition of samples of date rub made using traditional and modern procedures and discovered that the date Rub contained a protein 1.98%.

3.1.7 Color properties:

With regard to the characteristics of the color estimation values, the color values were found to be (L *= 21.14, a *=-26 ,b * =0.53) respectively. This result agreed with previous studies, where it was noted that the values of homemade and commercial Tunisian carob molasses match the color black (L*=29.03, a*=-0.69, b*=-2.03) (Tounsi*et al.*, 2020).The color of carob rub tends to dark color. Although the color of the fruits of the carob tends to brown, however, the dark color of carob molasses is generally attributed to the formation of colored polymeric compounds as products of non-enzymatic browning reactions (caramelisation and Maillard reaction) during juice concentration(Turkmen *et al.*, 2006).

3.1.8. Hydroxymethylfurfural (HMF) content:

According to table (3.1), the results showed that the carob rub value for the HMFcompound was 23.15 mg/100 g. This result was slightly higher than the results reported by Sengul *et al.* (2007), who found that the carob molasses value for the HMF compound was 21.23 mg/100 kg. HMF is not naturally found in fruits; rather, it is formed from monosaccharide's by the action of heat and acid and is a limited compound for preventing the application of excess heat in many products. It is an important quality factor that reflects the severity of heat treatment (temperature and time) that were applied to the foods concentrated with the application of a heat treatment (Tosun & Üstün 2003).The most significant factor contributing to the increase in the value of HMF for traditional carob rub is the high temperature used for heating and storing. Another factor is the high pH, which when combined with a high concentration of sugars and amino acid promotes the Maillard process.

3.2 Functional properties

3.2.1 Emulsifying capacity:

Table (3.1) shows that the carob rub's emulsion-forming capacity is 10% lower than that of egg yolk lecithin, which reaches an emulsion ratio of 80%. This outcome fell short of what (Tounsi *et al.*, 2020) reported Compared to soybean lecithin, which has a 100% emulsifying capability, carob molasses has an emulsifying capacity of about 80%. The different ingredients in carob paste could be the cause of this variation. According to (Alpaslan *et al* .,2002), the sugars and Millard reaction products that increase the stability of the emulsions it's what give carob rub its ability to emulsify.

Parameters	Mean ± SD
Total phenols (mEq/100g)	6.082± 0.06
Total flavonoids (mEq/100 g Catechins)	2.339± 0.05
Tannins (%)	3.125± 0.91
DPPH (µg/ml)	35.69±0.14
ABTS (µg/ml)	519.38±4.18

Table (3.2) Values of total phenols, flavonoids,	, tannins, and antioxidant acti	i <mark>vity of</mark>
traditional carob rub:		

3.2 Total phenolic compounds, total flavonoids and tannis

The total phenolic compounds, total flavonoids and tannins of traditional carob rub samples were determined and the values are given in table (3.2). According to table (3.2), the average amounts of phenolic components were 6.082 ± 0.06 mgEq Gallic acid/100g ww. And flavonoids were 2.339 ± 0.05 mgEq catechins/100DW, whereas tannins were $3.15\% \pm 0.91$. Our study's findings on the total phenolic content were much more considerable than those of (Fidan *et al.*, 2019), which were2.14 ± 0.07mg GAE/g DW. which were also less than the results of (Ben Ayache *etal.*, 2020), which was 13 ± 0.8 EAG/100 g DW, while with regard to

flavonoids and tannins, our results were different with(Ben Ayache *etal.*, 2020)which were 2.4 ± 0.1 g EC/100 g DW and 1.2 ± 0.1 g EC/100 g DW respectively. On the other hand, our results regarding tannins were in agreement with the results of (Ayaz *et al.*, 2007) that were3-5%.These differences may be due to genetic, environmental and extraction methods according to(Makris *etal.*, 2007).

3.4. Antioxidant activity:

As shown in table (3.2) the results of evaluating the antioxidant activity of carob Rub using the two radical scavenging tests, DPPH, ABTS, expressed in the form of EC₅₀ (the concentration that inhibits 50% of free radicals), was 35.69 µg/ml and 519.38 µg/ml, respectively. Carob has a significant activity in the ability to scavenge free radicals. A previous study carried out in different molasses samples (including carob molasses) reported strong and positive correlations between the free radical inhibition activity and the polyphone levels due to their hydrogen donating ability. In addition, it suggested the antioxidant properties of Maillard reaction products (MRPs), mainly melanoidins obtained from a heated glucose-glycine reaction (Wang B. *etal.*,2011) (Turkmen *et al.*, 2006).Accordingly, the studied carob molasses with high antioxidant activity could be either directly consumed to avail their potential health benefits, or used in food industry to prevent lipid oxidation in food systems, thereby improving their oxidative stability and prolonging their shelf life.

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