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Characterization of Tunisian carob pods (*Ceratonia siliqua* L.) and processed juice

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Abstract: The present manuscript presents a contribution to give value addition to the Tunisian carob pods by the production of natural juice. It illustrates the main nutritional composition of carob pods as the raw material, some properties of carob juice processed with hot water extraction (50 °C for 2 h) and the effects of thermal pasteurization (70 °C for 15 min) as a preservation process. The characterization of carob pods showed a high amount of sugar and low levels of lipids. The characterization of corresponding juice showed high viscosity, high content of soluble sugars and absence of pathogenic flora. The effects of juice pasteurization on color, microflora and vitamin C content were investigated. An important reduction in microflora number was observed. Significant increases were also observed in color and clarity values and significant decrease was detected in vitamin C content in pasteurized juice compared to raw juice. The outcomes of this work suggest the industrial production of natural carob juice.
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Keywords: carob pods; carob juice; hot water extraction; thermal pasteurization

1. Introduction

Carob tree (*Ceratonia siliqua* L.) is an evergreen plant cultivated or naturally grown in Mediterranean countries, including Tunisia where it is naturally grown along the coasts (Tounsi & Kechaou, 2017).

The mature fresh fruit (brown carob pods) is made up of 90% of pulp and 10% of seeds. The nutritive composition of carob pod differs widely according to carob parts, variety and climate (Biner et al., 2007). The carob pulp is characterized by high sugar content (40– 60%), predominantly sucrose, appreciable amounts of protein (3–6%) and low levels of fat (0.2–1%) (Avallone et al., 1997; Biner et al., 2007). The carob seeds contain more protein (50%), more fat (3–6%) and less total sugars (1–5%) compared to the carob pulp (Avallone et al., 1997; Bengoechea et al., 2008).

In recent years, carob has been used in the food industry. Several products are produced from the seeds and pulp of carob. The endosperm is extracted from the seeds to produce galactomannan also called carob gum or Locust Bean Gum (LBG). It is a valuable natural food additive for its strong gel characteristics (Dakia et al., 2008). Other products, such as carob germ flour is used as dietetic human food or as a potential ingredient in cereal derived foods for celiac people (Bengoechea et al., 2008). In Western countries, carob powder is produced from roasted and milled pulp. It is a natural sweetener with flavor and appearance similar to chocolate. Therefore, it is often used as cocoa substitute free from caffeine and theobromine (Yousif & Alghzawi, 2000). In many Arabian countries, the carob pulp is also used for preparing traditional carob molasses as an energetic food because of its high sugar content (Dhaouadi et al., 2014).

Few studies were conducted on carob drinks (El Batal et al., 2016; Elfazazi et al., 2020; Karkacier et al., 1995; Rababah et al., 2013). For that reason, the present study intended to prepare carob juice from Tunisian carob pods, mainly for their nutritive value and relatively low cost. In its natural form, dry carob pods could not be processed with press methods, which are typically used in the fruit juice industry, so that they need to be treated with hot water extraction (Turhan et al., 2010). This method often used to maximize juice yield, color and flavor extraction. The heat breaks down the pulp enough for extraction of juice and flavor (Sin, Yusof, Abdul Hamid, et al., 2006). So that, a simple procedure was developed in this work for the preparation of carob juice using hot water extraction.

Heat treatment is the most common method for preserving fruit juices. The juice is kept at a given temperature for a specified time to inactivate microorganisms and enzymes that could alter the product or render it unfit for human consumption

(Cheftel & Cheftel, 1984). Both batch and continuous methods are used in fruit juice pasteurization and the treatment may be carried out before or after packing the product in the container. In batch pasteurization, individual volumes are treated in jacketed stainless steel vessels for both heating and cooling. Continuous pasteurization may be carried out by passing the juice through plate heat exchangers, which usually comprise the stages of pre-heating, heating, holding and cooling (Aguilar-Rosas et al., 2007). Thermal pasteurization is so efficient in preventing microbial spoilage of fruit juice, but the applied heat may also cause undesirable biochemical and sensory changes which may affect overall quality of the final product (Aguilar-Rosas et al., 2007; Albagnac et al., 2002).

The characterization of Tunisian carob pods and the processed juice (before and after pasteurization) was the major objective of this study.

2. Material and methods

2.1. Origin of fruits

The mature carob pods were purchased from the local market of Sfax (Tunisia). The fruits were kept at room temperature prior to chemical analysis and extraction.

2.2. Preparation of carob juice

The extraction process of carob juice from carob pods is shown in Fig1.

The carob pods were first washed in water, sun dried and then fragmented manually to promote their crushing using a mechanical crusher (Etschmühl, Germany).

Hot water extraction was used to prepare carob juice. When the water temperature reaches 50 °C, crushed carob was added at a ratio of 1 to 5 (w/v). The mixture was kept at the selected temperature using a water bath for 2 h. After extraction, the juice was first strained through a filter cloth and then centrifuged at 5000 g for 15 min to separate the solid particles from the juice.

After bottling, the obtained juice was stored at –20 °C prior to the chemical, physical, microbiological and sensory analysis.

2.4. Thermal pasteurization

Thermal treatment was performed by using the technique of pasteurization after packaging. After bottling, raw carob juice was heated in a water bath to 70 °C for 15 min and then cooled with cold water (Albagnac et al., 2002).

2.5. Chemical analysis

Dry matter, protein, lipids and ash were determined according to the AOAC (2000) methods. Dry matter was determined by oven-drying at 105±1 °C to constant weight. Total nitrogen was determined by the Kjeldahl method and protein content was calculated using a factor of 6.25 (Avallone et al., 1997). Lipids were determined by the Soxhlet mothed, using hexan as a solvent. Ash was determined by total combustion in a muffle furnace at 550 °C. The mineral constituents (Ca, Fe, K, Mg, Na, Cu, Zn and Mn) were analyzed separately using an atomic absorption spectrophotometer (Analytik Jean, ZEEnit, 700, Germany) according to AFNOR (1994).

Sugars content was determined using the phenol-sulfuric method (Dubois et al., 1956) after ethanolic extraction for soluble sugars and acid hydrolysis for insoluble sugars according to the methods described by (Abbès et al., 2011). Total pectin content was determined according to the method described by Englyst, Quigley, et Hudson (1994) and crude cellulose (cellulose, pentosans and lignin) content was determined after acid and alkaline treatments according to AFNOR (1977).

Polyphenols content of carob juice was determined according to Folin-ciocalteau method (Singleton & Rossi, 1965). The polyphenols content of carob pods was determined by the same method, but after a step of extraction according to (Avallone et al., 1997).

Vitamin C content was determined according to the titration method using the 2.6-dichlorophenol indophenol (D.C.P.I.P.) (AOAC, 1984).

The acidity of carob juice was determined by a potentiometric titration with NaOH 0.1N using phenolphthalein as an indicator (AFNOR, 1974).

2.6. Physical analysis

Soluble solids content, expressed as °Brix, was measured using a hand refractometer (OpTech, Germany) and the pH was measured at 20 °C using a MP 220 pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

The water activity (a_w) of carob juice was measured by a NOVASINA a_w Sprint TH-500 apparatus (Novasina, Pfäffikon, Switzerland). The measurement was performed at 25 °C.

The turbidity of the juice was measured using a turbidimeter (WTW TURB 550 IR, Germany) previously calibrated.

The viscosity of the juice was measured with a Brookfield viscosimeter (HA, DV-II+ Pro, Brookfield Eng. Lab., USA) at 20 °C.

Color and clarity of the juice were determined by measuring the absorbance at 420 nm (Ibarz et al., 2005) and at 660 nm against the distilled used as a reference (Liew Abdullah et al., 2007; Sin, Yusof, Sheikh Abdul Hamid, et al., 2006) respectively using an UV-vis spectrophotometer (Shimadzu UV-vis spectrophotometer, Germany).

2.7. Microbiological analysis

Total and fungal floras, total and fecal coliforms and Staphylococcus were assessed using the horizontal method (AOAC, 1984) to check the microbiological aspect of carob juice. The results were expressed as CFU/g.

2.8. Sensory analysis

Hedonic evaluation was done by an untrained panel consisting of 40 subjects from different generations (kids: [5, 15[; youngs: [15, 30[; adults: [30, 50[and elders: ≥ 50).

Carob juice was compared with the most consumed juice in Tunisia (fruit cocktail juice) in order to predict the acceptance of the carob juice by consumers. Fruit cocktail juice was purchased from the local market of Sfax (Tunisia) and was stored under the same conditions.

Juices were evaluated for color, odour, taste and viscosity based on a five point hedonic scale (1: null, 2: mediocre, 3: acceptable, 4: good and 5: excellent). Carob juice was also evaluated for overall acceptability, expressed as percentage (%).

The results were treated statistically to analyze the responses of subjects and to measure the degree of carob juice appreciation by the consumers.

2.9. Statistical analysis

Analytical values were determined using three independent determinations. Values of different parameters were expressed as the mean ± standard deviation ($\bar{x}$ ± SD).

Student's t-test, at the level of $P < 0.05$, was applied to establish significance of difference between the samples. Statistical analyses were performed by Excel Software.

3. Results and discussion

3.1. Nutritional composition of carob pods

The proximate composition of carob pods (pulp and seeds) from Sfax (Tunisia) is presented in Table 1. Carob pods were characterised by low water content (12.45%). Therefore, hot water extraction was adopted to prepare carob juice instead of extraction by pressure. Results also showed dominance of total sugars (~65 g/100 g dry matter). The sugar fraction of carob pods was essentially formed by insoluble sugars (44 g/100 g dry matter). This could be attributed to the hard shell that surrounds the pod (Tounsi & Kechaou, 2017). Carob pods also contained 10.42 g of crude cellulose/100 g dry matter and 4.9 g of total pectin/100 g dry matter. These contents are higher than that of dates (1.98 g of cellulose/100 g dry matter and 2.27 g of pectin/100 g dry matter) (Masmoudi et al., 2008). Furthermore, carob pods had an appreciable amount of protein (~10 g/100 g dry matter). This amount was more important than that in dates (2.84 g/100 g dry matter) as reported by Masmoudi et al. (2008). The protein fraction of carob pods was composed mainly of seeds proteins that present an interesting biological value. In fact, Dakia, Wathelet, et Paquot (2007) and Bengoechea et al. (2008) detected an interesting amount of essential amino acids in carob germ seeds. The mineral fraction of carob pods (3.35 g/100 g dry matter) was predominated by potassium (332.47 mg/100 g dry matter), followed by calcium (147.91 mg/100 g dry matter), magnesium (101.34 mg/100 g dry matter) and sodium (52.65 mg/100 g dry matter). Ayaz et al. (2007) reported also similar mineral composition of carob fruit from Turkey. Potassium and sodium participate in the ionic balance of vascular membranes by promoting vasodilatation and reduction of blood pressure (Miranda et al., 2009). Calcium and magnesium are essential minerals for the development of strong bones and teeth, and for cardiovascular function (Cotruvo & Bartram, 2009). Therefore, carob pods could be considered as a good source of beneficial minerals for health.

Tunisian carob pods contain low amounts of lipids (0.28 g/100 g dry matter). (Yousif & Alghzawi, 2000) reported similar results for Jordanian carob pulp (0.3 g/100 g dry matter). Therefore, carob fruit could be considered a natural healthy food.

Few studies focused on vitamin C content in carob fruit. So, this work showed that carob pods had relatively low vitamin C content (~7 mg/100 g dry matter) compared to other fruits. Indeed, (Nojavan et al., 2008) reported higher vitamin C content for orange (76 mg/100 g dry matter) and dog rose fruit (417 mg/100 g dry matter).

In addition to that, the studied carob pods contain important polyphenols content (~ 2130 mg gallic acid/100 g dry matter). Many studies were conducted on phenolic composition of carob pulp and seeds (Avallone et al., 1997; Ayaz et al., 2007), but no one was conducted on phenolic composition of whole carob pods. (Avallone et al., 1997) reported much different polyphenols content in Italian carob (19 mg gallic acid/g dry matter for carob pulp and 40.8 mg gallic acid/g dry matter for carob germ). This could be due to various factors such as variety, analyzed part, growing conditions, climatic conditions, maturity, season, geographic origin, soil type, storage conditions (Ayaz et al., 2007).

3.2. Carob juice characterization

3.2.1. Physical characterization

Table 2 shows some physical characteristics of carob juice.

Soluble solids, pH and water activity of prepared juice were 10 °Brix, 5.36 and 0.93 respectively. Knowing these parameters is very useful for product preparation. In fact, the physical conditions of carob juice (°Brix, pH and a_w) could make it vulnerable to bacterial alterations if it was not stored appropriately. Thus, it needs conservation treatments such as thermal treatment and concentration (Cheftel & Cheftel, 1984).

The juice turbidity was 692 NTU. The turbidity in fruit juices is mainly attributed to polysaccharides such as pectins which passe into juice during extraction and make it cloudy. For some fruit juices such as orange and tomato juices, this cloudy effect is desirable and acceptable by the consumers (Liew Abdullah et al., 2007; Sin, Yusof, Sheikh Abdul Hamid, et al., 2006).

Regarding viscosity, carob juice could be considered more viscous compared to melon juice. In fact, Table 2 shows a viscosity of 256 mPa.s for the carob juice, while (Vaillant et al., 2005) reported a viscosity of 2.8 mPa.s for melon juice. This result

may be explained by the presence of soluble polysaccharides responsible for a high

viscosity (Liew Abdullah et al., 2007). The carob juice was characterized by a dark color compared to other fruit juices. Indeed, the color value of carob juice was 2.683, while color values of apple, peach and lemon juices were 0.632, 0.626 and 0.726 respectively (Ibarz et al., 2005). The dark color of carob juice is due to the brown pigments characteristics of the carob pods (Tounsi Jammeli, 2012). Clarity is an important index for juice limpidity (Liew Abdullah et al., 2007; Sin, Yusof, Sheikh Abdul Hamid, et al., 2006). As seen in Table 2, the clarity value of carob juice was 0.873. This value was higher than that found by (Tasselli et al., 2007) for kiwi juice (0.71).

3.2.2. Nutritional characterisation

The main nutritional composition of the prepared carob juice is given in Table 3.

Like carob pods, carob juice was rich in sugars (~60 g/100 g dry matter). The sugar fraction was mainly composed of soluble sugars which had the highest content (51.74 g/100 g dry matter). Further, carob juice had low content of insoluble sugars (6.12 g/100 g dry matter) and pectin (2.6 g/100 g dry matter) which pass into juice during separation and make it cloudy (Liew Abdullah et al., 2007).

The mineral content of carob juice was high (6.45 g/100 g dry matter), compared to that of other juices such as date juice (2.68 g/100 g dry matter) (Masmoudi et al., 2008) and apple juice (0.16 g/100 g dry matter) (de Bruijn et al., 2003).

Carob juice showed low protein content (~5 g/100 g dry matter) compared to the corresponding raw materials. This could be explained essentially by the involvement of the proteins in Maillard reaction during heat processing (Cheftel & Cheftel, 1984). However, the protein content of the carob juice was more important than that of date juice obtained by hot water extraction (1.79 g/100 g dry matter) (Masmoudi et al., 2008).

The acidity of carob juice was 27.15 mg total acids/100 ml. This value seems less important than those reported by (Pilando & Wrolstad, 1992) for fig, raisin, prune and pineapple juices (234, 550, 942 and 976 mg total acids/100 ml, respectively).

The vitamin C content was 13.51 mg ascorbic acid/l of carob juice. This value could be considered also low compared to that of other juices such as cactus pear juice (115.15 mg ascorbic acid/l) (Cassano et al., 2010) and apricot juice (215 mg ascorbic acid/l) (Versari et al., 2008).

Moreover, carob juice had a relatively low content of polyphenols (1.35 mg gallic acid/l). Even at low concentration, polyphenols retain their functional properties. Indeed, they neutralize free radicals and thus help to prevent various degenerative diseases such as immune deficiency diseases, cardiovascular and central nervous system (Miranda et al., 2009).

3.2.3. Microbiological characterization

It is so important to check the microbiological aspect of foods to ensure their hygienic quality.

Table 4 shows the microbial flora of carob juice. The count of fungal flora present in prepared juice was conformed to the standards ($9.3 \times 10^2 < 10^4$ CFU / g) according to (Clabots, 2007). Besides, it was noted a total absence of pathogenic microorganisms (Coliforms and Staphylococcus).

3.2.4. Sensory characterization

In order to evaluate its sensory quality, carob juice was compared to a commercial product (fruit cocktail juice) via a hedonic test. This test allows the study of the pair (product, subject) by referring to the pleasant or unpleasant character (Depledt, 2003).

Significant differences ($P < 0.05$) in sensory properties were observed between the studied carob juice and the reference juice (Fig.2). Considering the preference of consumers, carob juice was appreciated for viscosity while the commercial product was appreciated for color, odour and taste. Indeed, all consumers, except elders, preferred the taste and the color of fruit cocktail juice (commercial product) ($P < 0.05$). Moreover, commercial juice was more appreciated for the odour by kids and adults than carob juice ($P < 0.05$). The viscosity of carob juice was appreciated by youngs and adults better than that of reference product.

Fig.3 shows the overall acceptability of carob juice according to generations. Carob juice had an overall acceptability of 25% for all consumers. It was observed that the elders were the most consumers who preferred the carob juice (80 %). However, kids did not like it, while youngs and adults accepted it at list of 27.77% and 8.33% respectively.

3.3. Influence of the thermal pasteurization on carob juice properties

Thermal treatment is often used for preserving fruit juices. The thermal pasteurization of carob juice was performed at 70 °C for 15 min. the effects of this treatment on color and clarity, microbial quality and vitamin C content were studied.

3.3.1. Color and clarity

It was observed from Fig.4 that the thermal pasteurization significantly (P < 0.05) increased the absorbance values of carob juice color and clarity to 3.01 and 1.04 respectively compared to raw juice (2.87 and 0.87 respectively). This result is probably due to the production of brown pigments during the non-enzymatic browning (Maillard reactions and caramelizetion) under the effect of high temperature during the pasteurization process (Chen et al., 2009). Similar observation was also reported by (Lee & Coates, 2003) for thermal processing of orange juice at 90 °C for 30 s.

3.3.2. Microbial quality

Table 5 shows an important reduction of microbiological counts after thermal pasteurization of carob juice. The total flora count was reduced from 1900 to 270 CFU/g, while the fungal flora was reduced from 930 to 400 CFU/g. (Parish, 1998) noted also a reduction of orange juice microflora to below detectable limits under pasteurization treatment (98 °C for 10 s).

3.3.3. Vitamin C content

Vitamin C (ascorbic acid) is a highly sensitive biomolecule to various thermal processing conditions. So that, it is used as reference in different industrial processes since its presence ensures a high nutritional quality of the final product due to its easy degradation (Igual et al., 2010). Figure 5 shows that the thermal pasteurization (70 °C,15 min) decreased significantly (P<0.05) the vitamin C level of carob juice from 13.51 to 8.92 mg ascorbic acid/l. (Tiwari et al., 2009) reported also a significant decrease in ascorbic acid content of orange juice due to the high temperature of thermal treatment (98 °C, 21 s).

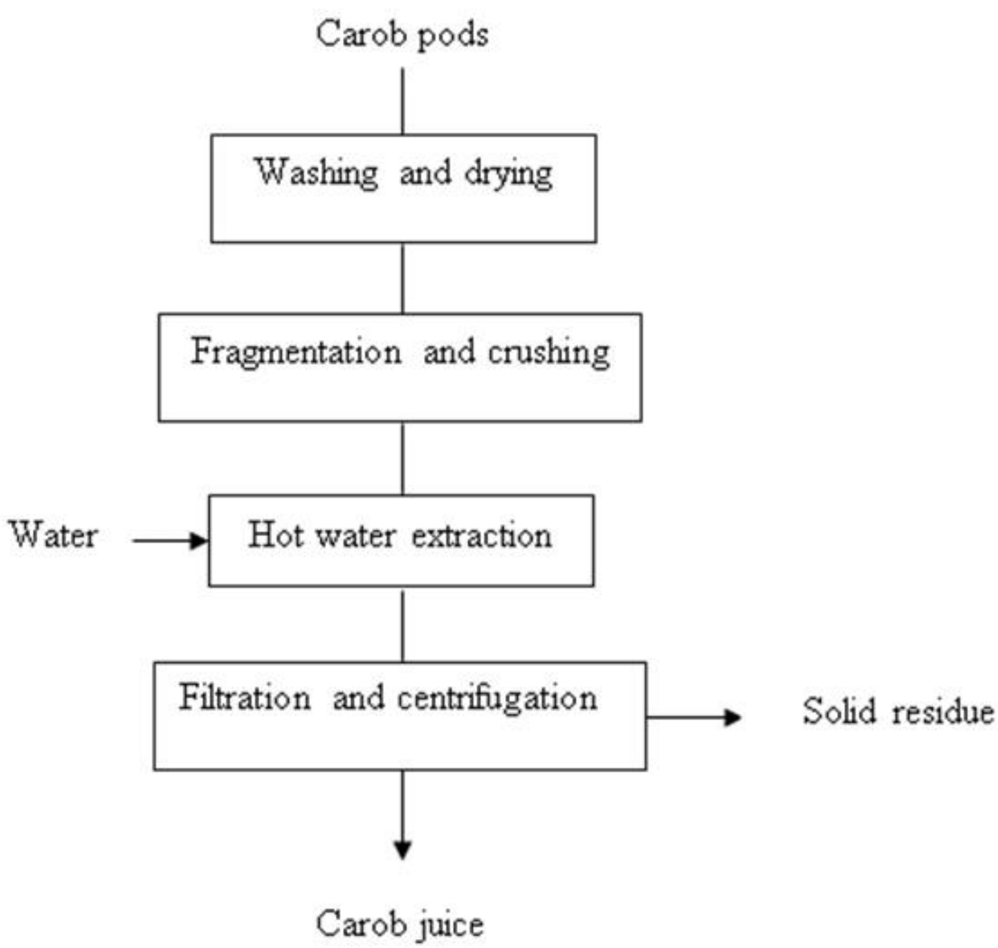

Fig.1. General processing diagram of carob juice preparation.

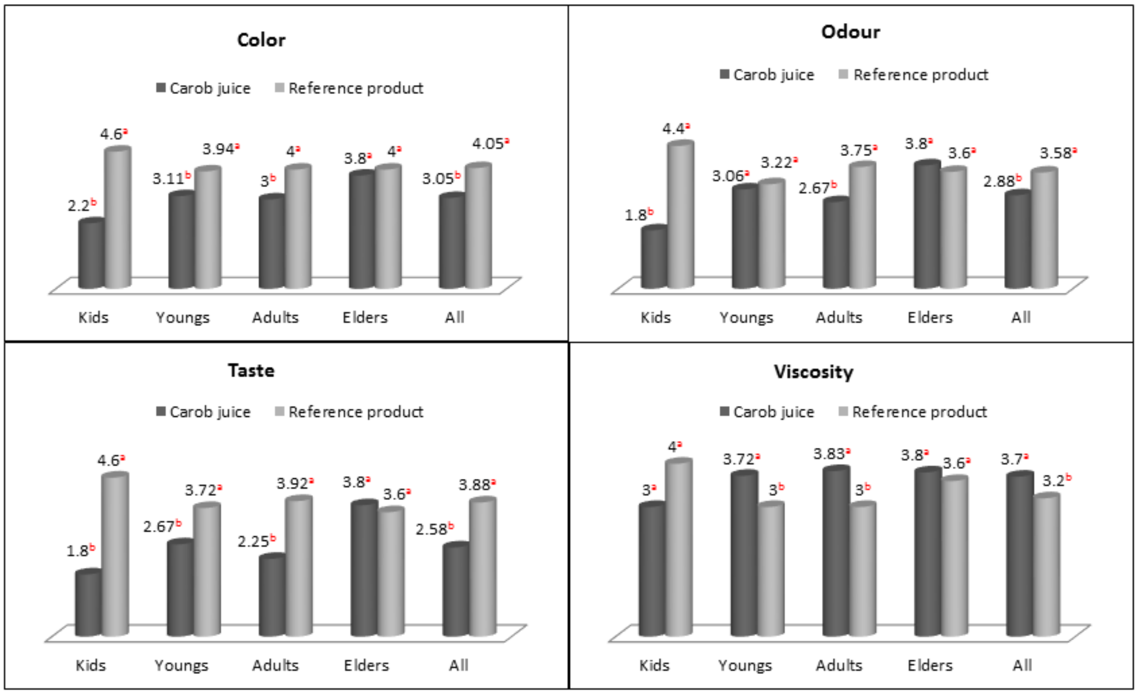

Fig.2. Sensory properties of carob juice, compared to fruit cocktail juice as reference. For each generation, means marked by different letters are statistically different (P <0.05).

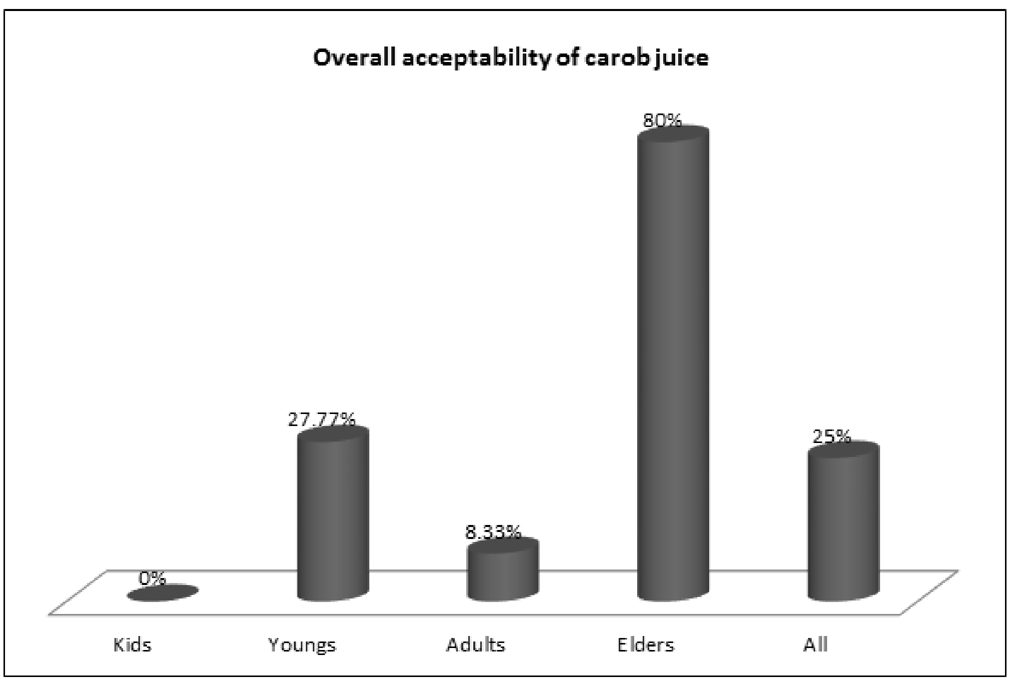

Fig.3. Evaluation of overall acceptability of carob juice according to the generations

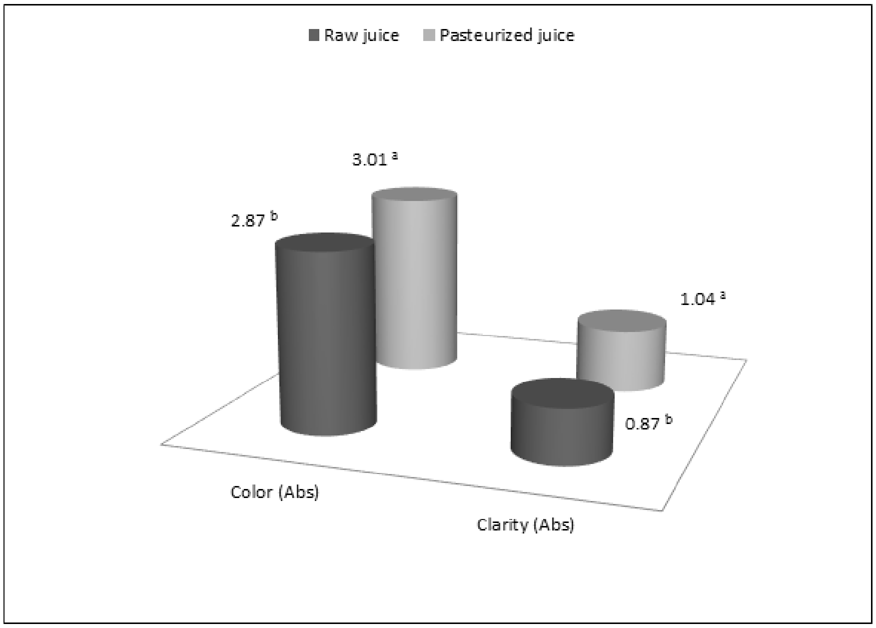

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Fig. 4. Effects of thermal pasteurization on color and clarity of carob juice

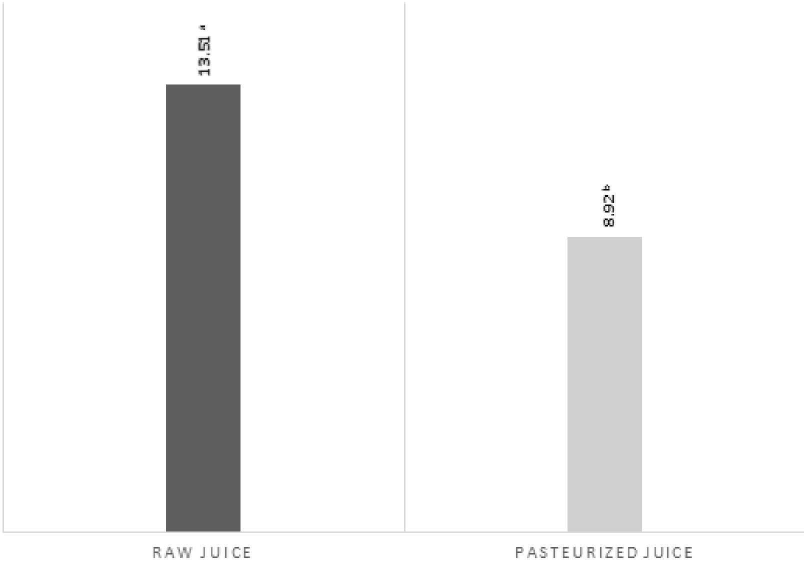

Fig. 5. Effects of thermal pasteurization on vitamin C content (mg ascorbic acid/l) of carob juice

Table 1: Nutritional composition of carob pods

Component	Content
Dry matter (g/100 g fresh matter)	87.55 ± 0.22
Insoluble sugars (g/100 g dry matter)	44 ± 2.81
Soluble sugars (g/100 g dry matter)	20.95 ± 0.19
Crude cellulose (g/100 g dry matter)	10.42 ± 1.01
Total pectin (g/100 g dry matter)	4.9 ± 0.86
Protein (g/100 g dry matter)	9.77 ± 0.57
Ash (g/100 g dry matter)	3.35 ± 0.03
K (mg/100 g dry matter)	332.47 ± 7.07
Ca (mg/100 g dry matter)	147.91 ± 5.96
Mg (mg/100 g dry matter)	101.34 ± 2.49
Na (mg/100 g dry matter)	52.65 ± 6.07
Fe (mg/100 g dry matter)	4.21 ± 0.78
Zn (mg/100 g dry matter)	2.18 ± 0.16
Cu (mg/100 g dry matter)	1.24 ± 0.72
Mn (mg/100 g dry matter)	Trace
Lipids (g/100 g dry matter)	0.28 ± 0.07
Ascorbic acid (mg/100 g dry matter)	7.03 ± 0.97
Total phenolics (mg gallic acid /100 g dry matter)	2127.50 ± 87.23

All the given values are means of three determinations ± standard deviation.

Table 2: Main physical characteristics of carob juice

Characteristic	Value
Soluble solids (°Brix)	10 ± 0.00
pH	5.36 ± 0.04
a_w	0.93 ± 0.00
Turbidity (NTU)	691. 67 ± 6.66
Viscosity (mPa.s)	256 ± 0.00
Color (Abs)	2.683 ± 0.01
Clarity (Abs)	0.873 ± 0.00

Table 3: Main nutritional composition of carob juice

Component	Content
Dry matter (g/100 g fresh matter)	9.07 ± 0.06
Soluble sugars (g/100 g dry matter)	51.74 ± 4.89
Insoluble sugars (g/100 g dry matter)	6.12 ± 0.21
Total pectin (g/100 g dry matter)	2.61 ± 0.1
Ash (g/100 g dry matter)	6.45 ± 0.18
Protein (g/100 g dry matter)	4.9 ± 0.09
Acidity (mg total acids/100 ml)	27.15 ± 0.00
Vitamin C (mg ascorbic acid/l)	13.51 ± 1.7
Polyphenols (mg gallic acid/l)	1.35 ± 0.04

All the given values are means of three determinations ± standard deviation.

Table 4: Microbiological characteristics of carob juice

Microbial flora	Microbial counts (CFU/g)
Total flora	1.9×10^3
Fungal flora	9.3×10^2
Total coliforms	0
Fecal coliforms	0
Staphylococcus	0

Table 5: Effects of thermal pasteurization on carob juice microflora

Microbial flora	Microbial counts (UFC/g)	
	Raw juice	**Pasteurized juice**
Total flora	1.9×10^3	2.7×10^2
Fungal flora	9.3×10^2	4×10^2

4. Conclusions

Nutritional composition of Tunisian carob pods showed low water content, high sugar content and appreciable protein content, but also mineral compounds which have many health benefits.

Hot water extraction (50 °C for 2 h) was useful to prepare carob juice at lab scale. Nutritional characterization of carob juice showed a high soluble sugars content and mineral content. The carob juice was characterized by high viscosity and dark color. The study of microbial and sensory properties of carob juice showed a total absence of pathogenic microorganisms and 80% of overall acceptability by elders.

On the other hand, thermal pasteurization (70 °C, 15 min) of raw carob juice resulted an important reduction of microflora number, significant increase in color and clarity values and significant decrease in vitamin C content.

Therefore, carob pods and juice may be considered as potential sources of sugar that could be used as additives in many foods. Further studies are necessary to improve the valorization of Tunisian carob pods into others products.

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