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Influence of pectinase and cellulase extracts on carob juice yield and quality

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Abstract: This work presented the first report producing a juice from carob pods using hot water extraction without and with the addition of cellulase and pectinase extracts. It focused on the physico-chemical characterization of carob pods and raw juice, as well as the effect of enzymatic treatment on juice yield and quality. Carob juice was prepared from whole carob pods with hot water extraction (50 °C for 2 h) and enzymatic treatment was carried out by the addition of fungal enzyme extracts (cellulase extract and pectinase extract) during juice processing at different concentrations. The raw carob juice was characterized by high sugars content, but also, high viscosity and turbidity. The enzymatic treatment induced significant decreases in the physical parameters values (turbidity, viscosity and clarity), and thus significant increase in juice yield. Among enzymatic treatment, the extraction with 2% of pectinase extract gave the highest recovery yield and the lower physical parameters values if compared to the control without enzyme (recovery yield: 85.60 vs 68.80 %; turbidity: 204 vs 681.5 NTU; viscosity 14.4 vs 256 mP.s; clarity: 0.22 vs 0.87). Enzymatic treatment with pectinase extract was proved to be efficient to improve the physical quality of carob juice with the maximum yield. Therefore, this study suggests the production of clarified carob juice using pectinase treatment on industrial scale.
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Keywords: carob pods; carob juice; enzymatic extraction, turbidity, viscosity, clarity

Introduction

Carob tree (*Ceratonia siliqua* L.) is an evergreen tree cultivated or naturally grown mainly in Mediterranean countries, including Tunisia where it is naturally grown mostly along the coasts. Carob fruit, called also carob pods, consists of pulp and seeds (80–90 and 10–20 % of the fruit weight, respectively) (Tounsi and Kechaou, 2017). Many researches focused on the chemical composition of carob pulp and seeds (Avallone et al., 1997; Ayaz et al., 2007; Dakia et al., 2008; Oziyci et al., 2014; Yousif and Alghzawi, 2000). However, few studies have been reported regarding the chemical composition of the whole fruit (Owais and Abdelrahman, 2010; Özcan et al., 2007).

Carob pods have traditionally been used as animal and human food. Currently, the main use is the production of carob bean gum from the seed endosperm which is used as additive (E410) in food and pharmaceutical industry (Dakia et al., 2008). Carob pulp is also used for preparing either powder often used as cocoa substitute for its flavor or health feature (Yousif and Alghzawi, 2000) or juice widely consumed as popular beverage in many Arabian countries especially for its high energetic sugar content (Rababah at al., 2013).

Fruit juices are known to contain colloids, mainly polysaccharides, which could affect their physical quality, for that reason, clarification is sometimes required to improve their appearance characteristics. In this respect, pectinolytic and cellulolytic enzymes have been used during juice processing to degrade the fruits polysaccharides, in order to increase the extraction yield and to reduce turbidity and viscosity (Abbès et al., 2011; Al-Hooti et al., 2002; Liew Abdullah et al., 2007; Lee et al., 2006; Sin et al., 2006). Therefore, the extraction of carob juice using enzymatic treatment could be a valuable addition to juice production.

This work was undertaken to study, on the one hand, the chemical composition of carob pods from Tunisia, as well as the physico-chemical properties of carob juice produced by hot water extraction, on the other hand, the effect of enzymatic extraction on juice yield and physical quality.

Material and Methods

Material

Carob fruit

The mature carob pods were purchased from the local market of Sfax (Tunisia). They were first washed, sun dried for one day and then milled by a hummer mill (Etschmühl, Germany). The ground

carob was kept at 4 °C prior to chemical analysis and extraction processing.

Enzyme extract

Two fungal enzyme extracts were used for enzyme treatment: an enzyme extract rich in cellulases derived from the strain RUTC30 of *Trichoderma reesei* and an enzyme extract rich in pectinases derived from the strain CT1 of *Penicillium occitanis*.

The extracts were provided free by Biomass Valorization and Protein Production in Eukaryotes Laboratory in the Biotechnology Center of Sfax (Tunisia). They were obtained in already available liquid form and stored at 4 °C.

Methods

Juice preparation

Carob fruit, as a hard pod, is different from other pulpy fruits which juices are obtained by pressing (e.g. citrus and grapes). Therefore, hot water extraction was used to make carob juice, as shown in Fig. 1. Ground carob was mixed with water at a ratio of 1:4 (g:ml) and placed in a water bath at 50 °C for 2 h. The produced juice was first strained through a filter cloth (muslin) and then centrifuged at 5000 rpm for 15 min. After bottling, carob juice was stored at 4 °C prior to the physico-chemical analysis.

Enzymatic extraction

Four lab flasks containing the same water volume were placed in a water bath. When the water temperature reached 50 °C, ground carob was added at a ratio of 1:4 (g:ml). Enzyme extracts were also added at different concentrations (% = ml/100 g), including 0 % (without enzymes), 1 % C (cellulases extract), 2 % P (pectinases extract) and 1 % C + 2 % P (cellulases extract + pectinases extract). The four mixtures were kept 2 h at 50 °C for juice extraction and then 5 min at 90 °C for enzyme inactivation. Next, they were filtered through a filter cloth (muslin) and centrifuged at 5000 rpm for 15 min. The produced juices were then bottled and stored at 4 °C for analysis. The recovery yield of carob juice (RYJ) was determined as follows:

$$\text{RYJ (\%)} = \frac{\text{volume of obtained juice}}{\text{volume of introduced water}} \times 100$$

Chemical analysis

Moisture, protein, fat, crude fiber and ash were determined according to AOAC (1995). Moisture was determined by oven-drying samples at 105 °C to constant weight. Total nitrogen was determined by the Kjeldahl method and protein concentration was calculated using the conversion factor of 6.25 (Avallone et al., 1997). Fat was determined by the Soxhlet method using hexane as a solvent. Crude fiber of carob pods was determined by the Weende method using a Fibertec system (Tecator, model 1020 M6, Suede). Ash was determined by sample combustion in a muffle furnace at 550 °C for 8 h. The residue was dissolved in nitric acid (65 %) and the mineral elements (Ca, K, Mg, Na, Cu, Zn and Fe) were analyzed separately using an atomic absorption spectrophotometer (Analytik Jean, ZEEnit, 700, Germany), while phosphorus was analyzed colorimetrically by the molybdo-vanadate method (AFNOR, 1994).

Soluble sugars extraction was determined using the method described by Perez et al. (1997) with slight modifications. 5 g of samples were homogenized twice with 10 ml of aqueous ethanol (95 %) and then centrifuged at 5000 rpm for 20 min. The two supernatants were collected and evaporated under vacuum to a minimal volume (~1 ml). The extract was dissolved in 10 ml of distilled water and analyzed for determination of soluble sugars content by the phenol-sulfuric method using glucose solution (100 mg/l) as a standard (Dubois et al., 1956).

Polysaccharides were determined according to Nerd and Nobel (1991). After soluble sugars extraction, the pellet of the ethanolic extract was evaporated overnight at room temperature. The polysaccharides were hydrolyzed with 10 ml of aqueous HCl (35 %) at 60 °C for 2 h in a water bath. The hydrolyzate, filtered through Whatman no. 1 filter paper, was analyzed for determination of sugar concentration by the phenol-sulfuric method using glucose solution (100 mg/l) as a standard (Dubois et al., 1956).

Total pectin was determined by hydrolysis of sample (500 mg) with 5 ml of H_2SO_4 (12 mol/l) at 30°C for 1 h. Distilled water (12.5 ml) was then added to the hydrolyzate and kept at 100 °C for 1 h. The hydrolyzate obtained after filtration through Whatman no. 1 filter paper was analyzed for the determination of pectin concentration as described by Englyst et al. (1994), using galacturonic acid aqueous solution (500 mg/l) as a standard.

Total polyphenols of carob fruit were determined according to Avallone et al. (1997). Crushed carob (1 g) was extracted twice with 10 ml of acetone (70 %) using a homogenizer at room temperature. After centrifugation (10 min, 5000 rpm), the supernatants were collected and evaporated under vacuum to a minimal volume (~1 ml). The extract was dissolved in 10 ml of distilled water and then filtered through Whatman no. 1 filter paper. Carob extract and carob juice were assayed for the determination of polyphenols content according to Folin-Ciocalteu method using gallic acid aqueous solution (500 mg/l) as a standard (Singleton and Rossi, 1965).

Vitamin C (ascorbic acid) was extracted twice from crushed carob (20 g) with 20 ml of metaphosphoric acid solution (10 %). After centrifugation (5000 rpm, 15 min), the supernatants were collected and then adjusted with distilled water to 50 ml. 50 mg of oxalic acid were added to 5 ml of the samples (carob extract and carob juice). Vitamin

C content was determined by the titration method with the 2,6-dichlorophenol indophenol solution (0.3 g/l) using ascorbic acid solution (0.2 g/l) containing 4 g of oxalic acid as a standard (AOAC, 1980).

The acidity of carob juice was determined by the titration method with NaOH solution (0.1N) using phenolphthalein as an indicator (AFNOR, 1997).

Physical analysis

Physical analyses were carried out only on carob juice. Soluble solids content (°Brix) was measured using a refractometer (OpTech, Germany). pH was measured using a pH meter (METTLER TOLEDO MP 220, Switzerland).Water activity (a_w) was determined by a_w meter (NOVASINA, Sprint TH-500, Switzerland). Color was measured by a colorimeter (CR-300 Konica Minolta Chroma Meter, CR 400-410, Japan) and the results were expressed as color coordinates: L* (0=black, 100=white), a* (-a*=greenness, +a*=redness), and b* (-b*=blueness, +b*=yellowness). Turbidity was conducted with a turbidimeter (WTW TURB 550 IR, Germany) and the results were reported as Nephelometric Turbidity Units (NTU). Viscosity was measured with a viscosimeter (HA, Brookfield, USA) at 25 °C. Clarity was determined by measuring the absorbance at 660 nm against distilled water as a reference (Liew Abdullah et al., 2007).

Statistical analysis

All physico-chemical measurements made on carob samples were determined in triplicate. Values of different parameters were expressed as the mean ± standard deviation ($\bar{x} \pm SD$).

The ANOVA test (Duncan's multiple range) was used to evaluate the effect of enzymatic treatment, while Student's t-test was used for to evaluate the consumers preference of the carob juice compared to the commercial juice. Statistical analyses were conducted by SPSS software (Statistical Package for Social Science) version 13.0 and differences were considered to be significant at $p < 0.05$.

Results and Discussion

Chemical composition of carob fruit

Table 1 shows the main chemical composition of carob pods from Tunisia used in this study compared to those from Turkey cited in literature.

Moisture content of the studied carob pods was in the range of 12.45 %. This value seems to be higher than that reported by Özcan et al. (2007) for Turkish carob pods (6 %). Many factors may affect the moisture content of the fruit, including dryness and irrigation. In fact, carob tree is well adapted to dry climates and could grow with annual average rainfall between 250 and 500 mm per year (Battle and Tous, 1997).

The studied carob pods were also characterized by high content of total sugars in dry mater (DM) (~65 g/100 g DM), constituted essentially by polysaccharides (44 g/100 g DM) and soluble sugars (~21 g/100 g DM). A previous study by Özcan et al. (2007) reported also that sugar was found to be the major nutrient in Turkish carob fruit (48.35 g/100 g DM). According to Battle and Tous (1997), the carob pulp is the most part rich in soluble sugars (sucrose, glucose and fructose), while the polysaccharide molecules come mainly from either the hard husk surrounding the pod or the endosperm of carob seeds.

Beside sugars, Tunisian carob pods were found to contain crude fibers (10.42 g/100 g DM), including total pectins (~5 g/100 g DM). The fiber content mentioned in this study was closed to that reported by Özcan et al. (2007) for Turkish carob fruit (9.69 g/100 g DM). Similar finding was observed for fiber content in carob pulp (~11 g/100 g DM) according to Yousif and Alghazawi (2000). Carob fibers (lignin, cellulose, hemicellulose and pectins) are mainly present in the pulp (Battle and Tous, 1997).

Tunisian carob fruit was also found to contain a high amount of proteins (10.42 g/100 g DM) when compared to the Turkish carob fruit (4.71 g/100 g DM; Özcan et al., 2007) and the carob pulp (3-7 g/100 g; Tounsi and Kechaou, 2017). In this respect, the protein fraction of carob pods is mainly composed of seeds proteins containing essential amino acids according to Dakia et al. (2008).

The mineral content of both Tunisian and Turkish carob pods was observed to be in the range of 3.3 g/100 g DM. As can be seen in Table 1, the mineral fraction of carob fruit was predominated by potassium. Özcan et al. (2007) reported also similar mineral composition for Turkish carob fruit, but with different concentrations. Many factors could affect the mineral content of the fruit, for example, temperature, salinity, dryness, irrigation and fertilization (Oziyci et al., 2014).

Carob pods, whatever from Tunisia or Turkey, were almost devoid of fat (~0.2 g/100 g DM). This finding was in agreement with those reported in the literature for carob pulp (0.1-0.75 g/100 g) (Tounsi and Kechaou, 2017). Despite the low fat content, carob pulp and seeds have an interesting fatty acid composition due to the presence of essential fatty acids, such as linoleic and alpha-linolenic acids (Dakia et al., 2008; Gubbuk et al., 2010).

Regarding polyphenols, results presented in Table 1 showed an appreciable amount for Tunisian fruit in the average of 2130 mg GAE/100 g DM. Several studies have been conducted on the phenolic composition of carob pods (pulp and seeds) from different geographic origin (Avallone et al., 1997; Ayaz et al., 2007; Papagiannopoulos et al., 2004). They reported different phenolic contents, and these differences are probably attributed to the cultivar, cultural conditions, analyzed parts and even methods used for polyphenolic extraction and estimation. The

identification of phenolic compounds detected gallic acid as the most abundant phenolic acid present in the extractable polyphenols comprising also flavanols, hydrolyzable and condensed tannins.

The vitamin C content of Tunisian carob pods (~7 mg/100 g DM) seems to be in close proximity to that of the Turkish carob pulp (~8-10 mg/100 g) as reported by Gubbuk et al. (2010). A previous study conducted on organic acids of carob pulp (Ayaz et al., 2007) did not detect ascorbic acid, but it detected malic acid (2.4 mg/g dry weight). Carob fruit had generally low content of vitamin C on comparison with other fruits, such as orange and dog rose fruits (76 and 417 mg/100 g DM, respectively) according to Nojavan et al. (2008).

This study showed that Tunisian carob pods had an interesting chemical composition mainly sugars, fibers, proteins and minerals. Therefore, they could be considered as a raw material in different applications such as fiber extraction, biofuel production and protein isolation.

Physico-chemical characteristics of carob juice

The main physico-chemical characteristics of the prepared carob juice are given in Table 2. Only one scientific work is available in the literature regarding the phytochemical and sensory properties of carob juice (Rababah at al., 2013). Therefore, our results were compared to those reported for other fruit juices.

Knowing the quality parameters of fruit juice (°Brix, pH and a_w) is very useful for its conservation. In fact, according to the high water activity (0.93), the carob juice could be susceptible to bacterial alterations if it was not appropriately stored. Thus, it needs conservation treatments such as thermal treatment and concentration.

Turbidity and viscosity are the main quality attributes of fruit juices. Their values were recorded as 692 NTU and 256 mPa.s, respectively, for the studied carob juice. Vaillant et al. (2005) reported higher turbidity value (3000 NTU) and lower viscosity value (2.8 mPa.s) for melon juice, while Bruijn et al. (2003) reported lower turbidity and viscosity values (23.6 NTU and 1.37 mPa.s, respectively) for apple juice. This difference between values is mainly attributed to the content of soluble polysaccharides in fruit juices, mainly pectins which are responsible for high viscosity and cloudy aspect (Liew Abdullah et al., 2007).

According to the colorimetric coordinates (L*, a* and b*) presented in Table 2, carob juice had reddish coloration due to positive a* values, and brown coloration defined as a yellow color with low positive b* values. Consequently, the studied carob juice was characterized by a reddish brown color. The brown color is considered as the natural color of carob pods (Battle and Tous, 1997), while the reddish color is probably due to the formation of colored compounds as products of Maillard reaction occurring during thermal extraction of carob juice (Ibarz et al., 2005).

Regarding the chemical composition of the studied carob juice (Table 2), soluble sugars were found to be the major component with high content in the range of 62.54 g/100 g DM. Accordingly, it could be considered as a natural sweet juice which did not require sugar addition during fruit processing. Several studies reported also high levels of natural sugars (mainly glucose, fructose and sucrose) in other fruit juices, such as grape juice (Bozkurt et al., 1999), melon juice (Vaillant et al., 2005), peach juice (Versari et al., 2002), cherry and apricot juices (Aider and Halleux, 2008). The high sugar content of fruit juices should justify either their consumption as a good source of rapid energy, or their use as a source of liquid sugar suitable to many food products such as ice cream, beverages, pastry and confectionery products.

In addition to soluble sugars, the prepared carob juice was found also to contain polysaccharides (4.12 g/100 g DM) which might be passed into juice during separation according to Liew Abdullah et al. (2007). Among polysaccharides, pectins were present in carob juice with low content (2.61 g/100 g DM) if compared to that of carob fruit (~5 g/100 g DM). Aider and Halleux (2008) reported also the presence of pectin in apricot and cherry juices with higher levels in the range of 3.16-5.51 g/100 g DM, respectively.

The mineral content of the studied carob juice was found to be in the range of 4.71 g/100 g DM. Regarding the mineral composition, it was observed that the mineral elements (K, P, Ca, Na, Mg and Fe) detected in the prepared carob juice (Table 2) were also detected in carob pods used as raw material for juice manufacturing (Table 1) with potassium dominance. Bozkurt et al. (1999) reported also similar mineral profile for grape juice, but with different concentrations. This is may be explained by the mineral composition of fruits used as raw material or the process used for juice production.

The prepared carob juice showed low protein content (3.32 g/100 g DM) when compared to the corresponding raw material (~10 g/100 g DM). This could be explained essentially by the involvement of the proteins in Maillard reaction during thermal juice processing (Bozkurt et al., 1999). Although, the protein content of carob juice was in agreement with those of other fruit juices such as apple juice (2.79 g/100 g DM) as reported by Bruijn et al. (2003), cherry juice (3.75 g/100 g DM) and apricot juice (4.33 g/100 g dry mater) as reported by Aider and Halleux (2008).

The carob juice prepared from carob pods was found also to contain phenolic compounds with an average of 1184.48 mg GAE/ 100 g DM. Rababah at al. (2013) reported higher total phenolics content in the carob juice prepared from carob pulp (1980-2030

mg GAE/ 100 g DM). Compared to other fruit juices, carob juice had more levels of total phenolics than grape juice (620 mg GAE/ 100 g DM, Rababah at al., 2013) and apple juice (150.21 mg/100 g DM, Bruijn et al., 2003). The differences in phenolic contents of juice samples may arise from the differences between phenolic composition of fruits, juice processing, or methods used for phenolic extraction and estimation. In general, natural polyphenols, including those present in fruit juices, are responsible for antioxidant activities. Indeed, they are able to neutralize free radicals and thus to prevent various degenerative diseases such as immune deficiency diseases, cardiovascular and central nervous system (Rababah et al., 2013).

The acidity of the studied carob juice (178 mg malic acid/100 g DM) was found to be more important than those reported by Aider and Halleux (2008) for cherry and apricot juices (10.80 and 12.56 mg malic acid/100 g DM, respectively). This high acidity could explain the acidic pH of the studied carob juice (5.36).

Considering carob juice characterization, the present study describes for the first time the physico-chemical quality of carob juice. It could serve as a good source of sugars, proteins, minerals and polyphenols, whereas, it is characterized by dark color and high viscosity and turbidity. In order to improve the physical quality of carob juice, it was necessary to test the effect of enzyme treatment.

Influence of enzymatic treatment on carob juice

This study focuses on the effects of different enzymatic treatments (with cellulases extract, pectinases extract and cellulases+ pectinases mixture) mainly on recovery yield and physical quality of carob juice.

Influence on the recovery yield

Enzymes have been exploited in fruit juice industry to break down all polymeric carbohydrates such as pectin and celluloses for maximum juice yield (Abbès et al., 2011).

It was clear from Fig. 2 that the extraction of carob juice with the addition of enzyme extracts increased significantly ($p < 0.05$) the recovery yield. Previous studies reported also that the addition of pectinase/cellulase enzymes during extraction increased the recovery yield of date juice. Abbès et al. (2011) proved that the treatment with 50 U of pectinase and 5 U of cellulase during 120 min at 50 °C increased the recovery of total soluble solids from 66.34 to 72.37 g of total soluble solids/100 g fresh basis. Al-Hooti et al. (2002) indicated that the use of 1 mg/ 100 g of pectinase and cellulase enzyme preparation increased the extraction yield from 35 to 68.22 %. To understand these results, it is highly important to elucidate the rheological properties of the major polysaccharides of plants. Indeed, cellulose and pectin are hydrocolloid molecules able to form gels in water. So, the increase of juice yield is probably due to hydrolytic enzymes (pectinase and cellulase) which degrade gel networks allowing liberation of water molecules and facilitating thus the juice filtration (Liew Abdullah et al., 2007).

Fig. 2 showed also that the carob juice treated with 2 % of pectinase extract during extraction gave the highest recovery yield (85.60 %) compared with the control juice (68.80 %). This could be explained by loss in cellulose accessibility to cellulases as a limiting factor for enzymatic hydrolysis (Abbès et al., 2011).

Influence on the physical properties

Several studies regarding juice clarification using enzymatic treatment have reported the effect of enzymes on the main quality attributes of fruit juices, namely turbidity, viscosity and clarity (Lee et al., 2006; Liew Abdullah et al., 2007; Sin et al., 2006). Therefore, the influence of the enzyme extraction on the physical quality of carob juice was investigated and the results are illustrated in Fig. 3.

As shown in Fig. 3a, the addition of enzyme extracts during extraction reduced significantly ($p < 0.05$) the turbidity of carob juice. The turbidity reduction is probably due to the hydrolytic action of enzymes on the cellulolytic and pectinolytic particles present in the juice (Abbès et al., 2011). Among all enzymatic treatments, the carob juice treated with 2% of pectinase extract had the lowest turbidity (204 NTU) if compared to the control juice (681.5 NTU). This funding agreed with those of other studies reporting that the use of pectinases was an efficient alternative to reduce turbidity in fruit juices (Abbès et al., 2011; Lee et al., 2006; Liew Abdullah et al., 2007; Sin et al., 2006). In fact, pectinase enzymes degrade pectin which causes mainly the turbidity in fruit juices when it passes into juice during extraction and makes it cloudy (Liew Abdullah et al., 2007).

As observed in Fig. 3b, the viscosity of carob juice had significantly ($p < 0.05$) decreased after enzymatic extraction, mainly with pectinases extract (14.4 mPa.s) if compared to the control juice which had the highest viscosity (256 mPa.s). Several researches (Lee et al., 2006; Liew Abdullah et al., 2007; Sin et al., 2006) reported also that enzymatic treatment with pectinolytic enzymes has been investigated with the objective to break down the pectin molecules responsible for high viscosity in fruit juices. Indeed, pectin degradation leads to a reduction of water holding capacity, and consequently, free water release, reducing thus the juice viscosity and facilitating filtration.

As seen in Fig. 3c, the control carob juice (0 %) had the highest clarity value (0.87), while the carob juice treated with pectinases extract (2 %) had the lowest clarity value (0.22). However, the addition of

cellulases extract (1 %) did not significantly decrease the absorbance value of carob juice clarity (0.8). Previous studies have showed that enzymatic treatment with pectinase was efficient to improve the juice clarity (Lee et al., 2006; Liew Abdullah et al., 2007; Sin et al., 2006). Upon enzyme treatment, pectolytic enzymes break down the pectin molecules, which facilitate the formation of pectin–protein flocs, leaving a clear supernatant and significantly removing the colloidal aspect of fruit juices (Sin et al., 2006).

To our best knowledge, this is the first report detailing the use of enzymatic treatments to improve the yield and the quality of carob juice. Indeed, fungal enzyme extracts, namely pectinase extract and cellulase extract, could be effectively used to degrade polysaccharides components (pectins and celluloses) transferred to juice during processing. Nevertheless, commercial cellulases and pectinases are endowed with potential biotechnological applications in fruit juice manufacturing industry according to Abbès et al. (2011).

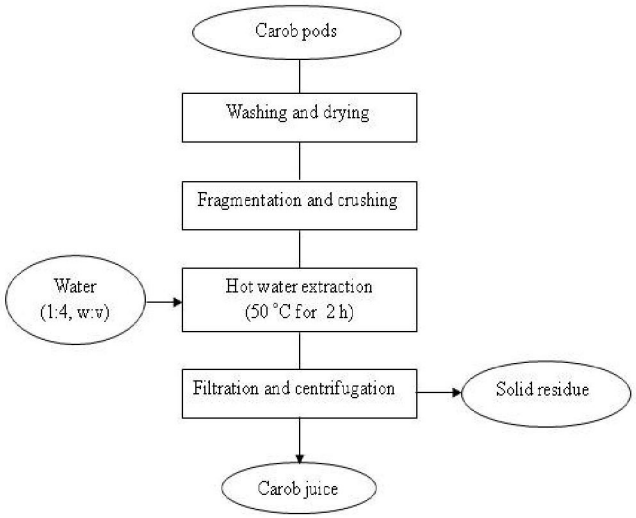

Fig. 1 General processing diagram of carob juice preparation

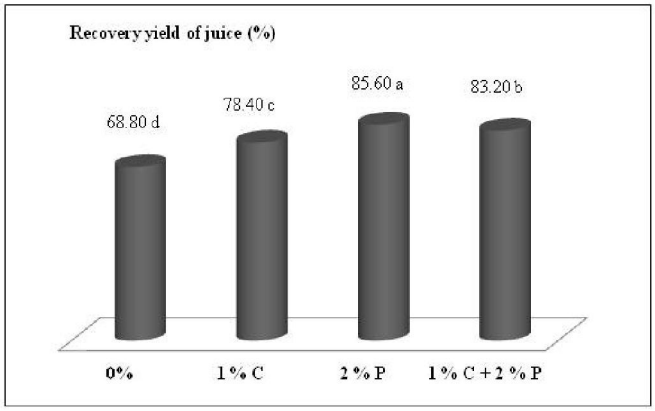

Fig. 2 Influence of enzymatic treatments on carob juice yield. 0 %: without enzymes; 1 % C: 1 ml of cellulase extract per 100 g of ground carob pods; 2 % P: 2 ml of pectinase extract per 100 g of ground carob pods. Means followed by different letters are statistically different ($p < 0.05$)

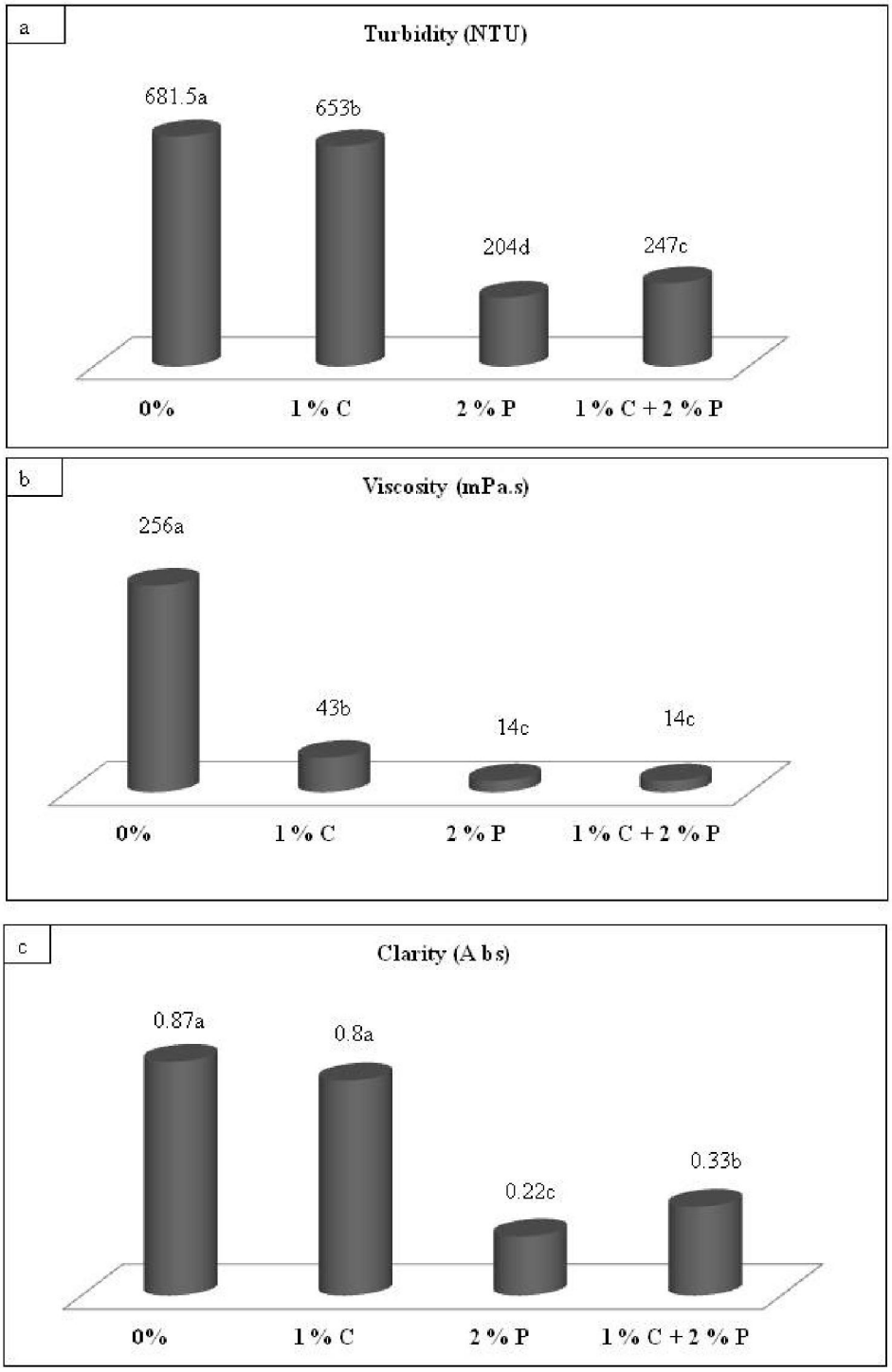

Fig. 3 Influence of enzymatic treatments on the physical quality of carob juice: turbidity (a), viscosity (b) and clarity (c). 0 %: without enzymes; 1 % C: 1 ml of cellulase extract per 100 g of ground carob pods; 2 % P: 2 ml of pectinase extract per 100 g of ground carob pods. Means followed by different letters are statistically different ($p<0.05$)

Table 1 Main chemical composition of Tunisian carob fruit used in this study compared to Turkish one reported by Özcan et al. (2007)

Component	Tunisian carob pods	Turkish carob pods
Moisture (a)	12.45 ± 0.22	6.01±0.11
Total sugar (b)	64.95 ± 2.62	48.35±0.52
Soluble sugar (b)	20.95 ± 0.19	-
Polysaccharide (b)	44.00 ± 2.81	-
Crude fiber (b)	10.42 ± 1.01	9.69±1.2
Total pectin (b)	4.90 ± 0.86	-
Protein (b)	9.77 ± 0.57	4.71±0.66
Ash (b)	3.35 ± 0.03	3.33±0.2
Potassium (c)	2332.47 ± 7.07	2466.56±28.65
Calcium (c)	547.91 ± 5.96	420.67±10.02
Magnesium (c)	201.34 ± 2.49	143.56±19.14
Sodium (c)	52.65 ± 6.07	126.16±21.85
Phosphorus (c)	200.80 ± 3.21	542.7±43.92
Iron (c)	4.21 ± 0.78	4.26±0.34
Zinc (c)	2.18 ± 0.16	0.03±0.00
Copper (c)	1.24 ± 0.72	0.24±0 .03
Manganese (c)	0	0
Fat (b)	0.28 ± 0.07	0.23±0.02
Polyphenols (d)	2127.47 ± 87.23	-
Vitamin C (e)	7.03 ± 0.97	-

Values are presented as means ± standard deviation; -: not determined;
a: g /100 g FM; b: g /100 g DM; c: mg /100 g DM; d: mg GAE /100 g DM;
e: mg ascorbic acid /100 g DM

Table 2 Physico-chemical characteristics of carob juice prepared from carob pods with hot water extraction (50°C, 2 h)

Component	Value
Soluble solids (°Brix)	12.00 ± 0.00
Water activity (a_w)	0.93 ± 0.00
pH	5.36 ± 0.04
Turbidity (NTU)	691.67 ± 6.66
Viscosity (mPa.s)	256.00 ± 0.00
L*	57.22±0.41
a*	8.28±0.04
b*	15.68 ± 0.01
Soluble sugar (a)	62.54 ± 4.24
Polysaccharide (a)	4.16 ± 0.44
Total pectin (a)	2.61 ± 0.10
Ash (a)	4.71 ± 0.13
Potassium (b)	1488.79±146.81
Calcium (b)	816.74 ± 100.94
Sodium (b)	525.87 ± 27.15
Magnesium (b)	266.67 ± 22.60
Phosphorus (b)	110.98±4.45
Iron (b)	3.99 ± 0.58
Protein (a)	3.32 ± 0.09
Polyphenols (c)	1184.48 ± 28.89
Acidity (d)	17.80 ± 0.00

Values are presented as means ± standard deviation.
L* (100: white, 0: black), a* (+: red, -: green), b* (+: yellow, -: blue)
a: g/100 g DM; b: mg/100 g dry DM;
c: mg GAE/100 g DM; d: mg malic acid/100 g

Conclusions

This study is the first document regarding the production of carob juice from pods using hot water extraction (50 °C for 2 h) without and with enzyme addition. The carob juice prepared without enzyme treatment was characterized by natural compounds (sugars, proteins, minerals, and phenolic compounds), reddish brown color and high viscosity and turbidity. The use of fungal enzyme extracts for enzymatic extraction of carob juice was found to be beneficial to increase significantly the juice yield and to decrease significantly its physical parameters values (turbidity, viscosity and clarity). Among the different treatments used for the production of carob juice, the addition of 2 % of pectinase extract seems to be the most efficient treatment to have the best physical quality with the maximum yield. In future works, the effect of enzymatic extraction on the chemical composition of carob juice will be investigated.

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