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Physico-chemical characterization and evaluation of the antioxidant properties of Tunisian kaki (*Diospyros kaki* L.) fruit jelly

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Abstract
Kaki fruit is a source of bioactive compounds important for maintaining a good health. In view of its short shelf life and perishable nature, we aimed to develop a kaki jelly utilizing the fruit pulp with the characterization of the physico-chemical parameters and the evaluation of the antioxidant properties. The obtained results were compared to those of the fresh pulp.
Based on the results of the physico-chemical parameters of the kaki jelly, all the values were significantly lower than those of the fresh pulp except for the total soluble solids due to the effect of the sugar used in the preparation and the cooking temperature.
Furthermore, our results demonstrated a decrease in total phenolic compounds and antioxidant activity in the kaki jelly. For instance, the kaki jelly showed a 40% decrease in total phenols, a 30% reduction in flavonoids, and a 20% decline in condensed tannins compared to the fresh pulp. Despite this decline, the antioxidant activity, tested by DPPH and ABTS tests, remained considerable when compared to the fresh pulp.
This study highlighted the challenging potential of transforming the kaki fruit into a stable and palatable jelly while preserving its essential bioactive components.

Keywords: *Diospyros kaki* L., pulp, jelly, phenolic compounds, antioxidant activity

Introduction

The fruit of *Diospyros kaki* L., commonly known as kaki, is an indigenous fruit of Asian countries such as China, Korea and Japan, which are the world's leading producers [1]. Asia is the main producer of kaki with a production of 4,079,171 tons over an area of 977,276 hectares, covering thus 90.90% of the world production [2]. Kaki fruits are mainly classified according to their astringency and pollination. As a whole, four varieties are identified: Pollination Constant Non-Astringent (PCNA) (Fuyu, Hana Fuyu, Jiro, O 'Gosho), Pollination Variant Non-Astringent (PVNA) (Amankaki, Tipo, Thiene), Pollination Variant Astringent (PVA) (Aizumishirazu, Giombo, Rojo Brillante, Tone Wase, Triumph, Rama Forte), Pollination Constant Astringent (PCA) (Fuji, Taubate, Hachiya, Pomelo, Rubi) [3, 1].
This fruit stands out a rich source of bioactive compounds. It is rich in vitamins, carotenoids, fibers, and phenolic compounds implicated in improving the overall health and well-being [4]. Kaki pulp is a valuable source of many vitamins like vitamin C and vitamin A, a number of minerals such as calcium and iron, as well as phenolic compounds (ferulic, p-coumaric and gallic) and carotenoids (p-cryptoxanthin, lycopene, carotene, zeaxanthin and lutein) [5]. Peels are also a good source of fibers with an average of 40.35 g. 100 g^{-1} of fruit, ascorbic acid, phenolic components, carotenoids. They are characterized by higher levels of minerals than pulp including manganese, iron, zinc, copper, calcium, and magnesium [3]. Seeds are known to contain high levels of palmitic, oleic and linoleic fatty acids, representing 70.4% to 78.3% of total fatty acids. They constitute an important source of minerals in the range 47.1 - 85.1 mg/kg in addition to significant levels of essential amino acids (50.9 to 54.0 mg/kg), organic acids (1.550 to 2.413 mg/kg) and polyphenol contents (1.228 to 1.308 µg gallic acid equivalent/g) [4, 6].
In the last years, the studies about the bioaccessibility of carotenoids and their relationship with health are increasing continuously [5]. From a standardized protocol, some researchers reported an *in vitro* digestion method adapted for carotenoids to improve their bioaccessibility. Recently, an important benefit of high hydrostatic pressures and thermal treatment are the improvement of the extraction of carotenoids due to induction of changes in plant food structure during processing [5, 7]. Pressurized and pasteurized persimmon samples showed different carotenoid bioaccessibility, which was dependent on the particular structure of the molecule [5].
Kaki is a seasonal fruit, with an inherent shelf life limited to the months of October to December. The lack of knowledge on how to preserve the fruit makes it conservation a hard challenge for the agri food sector. Therefore, it is crucial to develop strategies of processing and transformation to create more value to a final product made from the kaki fruits. Different end products were developed such as jams, jellies, juices, dried persimmon and vinegar [8].
Thus, the objective of the current study was to make the Tunisian *Diospyros kaki* L. fruit available for consumer during the off-season by the development of a shelf-stable product “jelly”. The physico-chemical parameters as well as the antioxidant properties of the obtained jelly were evaluated, with their comparison with the pulp characteristics.

Materials and methods

Kaki fruits samples

The experiments were conducted in the Laboratory of Innovation and Valorization for Sustainable Food Industry (LR21AGR04) at the Higher School of Food Industries of Tunis (ESIAT).

The kaki fruits were harvested from the region of Nefza in the Northwest of Tunisia from the astringent variety. Fruits were collected as they reached their physiological ripeness.

In laboratory, damaged and unripe fruits were discarded after a selection, and then we proceeded to the washing using water and peeling. The obtained pulp was conserved in hermetic containers at -18°C before analysis.

The main ingredients for preparing the products were kaki pulp, sugar, citric acid. Other chemicals of analytical grade were used from the laboratory.

Kaki jelly fruit formulation

Kaki jelly was formulated as previously mentioned by Curi *et al*. [9] with a few adjustments. Peeled fruits were mixed using a blender with 20% water for 5 min and filtered to obtain a clarified juice. The proportions of the inputs required to produce the jelly formulation, based on their total weight (sugar and pulp), were as following: 60% clarified juice, 40% sugar, 6% aqueous citric acid solution (2%, w: v). Initially, the clarified juice and sugar were treated in an open stainless-steel pan at medium temperature (+100°C). This process was continued until a level of 65°Brix soluble solids was reached, as measured by a manual refractometer (Euromex Holland). Citric acid is added at the end of the cooking process. The jelly is poured hot into sterilized glass bottles, cooled and stored at +4°C.

Analytical analysis

The physicochemical characterization was performed to the pulp and jelly of kaki. The pH was determined according to NF V 05-108.1970, titratable acidity was estimated by using NF V 52.36 (1982). Total soluble solids were quantified with a refractometer 0-85 (Euromex Holland) [10]. Moisture content was carried as described by Üçer *et al*. [11] Ash content was determined according to NF V05-113 (1972). The color parameters L* (100 (white) 0 (black)), a* (green (negative value) red (positive value)) and b* blue ((negative value) yellow (positive value)) were measured using a Minolta CR300 chromameter.

Phenolic compounds extraction

The phenolic extracts of kaki pulp and jelly were obtained by solubilizing in ethanol 80% (1:10, w: v) and macerating at room temperature for 24 hours with stirring at 250 rpm. The filtrate was centrifuged and concentrated at +40°C using a rotary evaporator (Buchi R-200).

Total Phenols Content

The total phenol content was estimated using the colorimetric method adapted by Snoussi *et al*. [12]. 100 µL of kaki jelly/pulp extract was combined with 500 µL of the Folin-Ciocalteu reagent (10%) and 1mL of distilled water. After stirring for 1 minute, 1.5mL of sodium carbonate (Na_2CO_3, 20%) was added. After 120 min at ambient temperature in darkness, the absorbance measurements were taken at 760 nm and the results were expressed as mg gallic acid equivalent per gram (mg GAE/g) of extract.

Flavonoids Content

The flavonoid content was measured according to that reported by Snoussi *et al*. [13] with slight modifications. One mL of kaki pulp or jelly extract was mixed with the same volume of aluminium chloride solution (2%). After 15 min incubation, absorbance measurements were taken at 430 nm. The total flavonoid content was reported as mg quercetin equivalent per gram (mg QE/g) of extract.

Condensed Tannins Content

Total condensed tannins were assessed following a procedure of Ma *et al*. [14]. For this, 25 µL of kaki pulp or jelly extract was mixed with 150µL of methanolic vanillin solution (4% (w:v)). Then, 25 µL of sulphuric acid methanolic solution (32% (v:v)) was added to the mixture that was incubated in the dark at room temperature for 15 min. The absorbance measurements were taken at 500 nm and the total condensed tannin content was given as mg catechin equivalent per gram of extract (mg CE/g).

Antioxidant assays

DPPH Radical scavenging assay

The test was carried out in accordance with the previously procedure of Snoussi *et al*. [12]. 2 ml of a serial kaki pulp or jelly extract was added to the same volume of a solution of DPPH in ethanol (2.10^{-4} M) and incubated for 30 min in the darkness at ambient temperature. A control was prepared with 2 ml of ethanol. Absorbance measurements were performed at 517 nm. The antioxidant activity was reported as IC_{50} (mg/ml), i.e., the dose at which 50% of the DPPH radicals were inhibited. The percentage of DPPH radical inhibition was assessed by means of the formula follows:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where $A_{control}$ and A_{sample} represent the absorbance of DPPH radicals in methanol and sample extracts, respectively.

ABTS radical scavenging assay

The ABTS radical scavenging test was evaluated using the modified assay of Sicari *et al*. [15]. 2 mL of kaki pulp or jelly extract were mixed with 2 mL of ABTS solution, containing 7 mM of ABTS, 140 mM of potassium persulfate $K_2S_2O_8$ (1:1, v: v) and diluted in PBS buffer. After 6 min, the absorbance was measured at 734 nm. The free radical scavenging capability was calculated by the equation below and expressed as the percentage of inhibition of the ABTS radical compared with the blank

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where $A_{control}$ and A_{sample} represent the absorbance of ABTS radicals in PBS buffer and sample extracts, respectively.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using SPSS Statistics version 20.0 (IBM Corp, Armonk, NY, USA). Duncan's test was used to detect significant differences among kaki pulp and jelly samples.

Results and discussion

Physico-chemical characterization

The average values for the different physico-chemical parameters of kaki pulp and jelly are shown in the table 1.

Table 1: Physico-chemical parameters of the kaki pulp and jelly

Parameters	Kaki		Sing.
	Pulp	Jelly	
pH	6.44 ± 0.01	3.86 ± 0.00	***
Acidity (%)	0.97 ± 0.21	0.45 ± 0.02	*
TSS (°Brix)	16.50 ± 0.01	65.00 ± 0,00	***
Moisture (%)	82.32 ± 0.58	23.24 ± 0.5	***
Ash (%)	1.10 ± 0.54	1.07 ± 0.07	Ns
Color			
L*	38.04 ± 0.40	33.26 ± 0.54	**
a*	4.58 ± 0.40	2.98 ± 0.40	***
b*	24.66 ± 0.03	11.83 ± 0.03	***

Data are mean values ± standard deviation; ns: not significant; *p<0.05; **p<0.01; ***p<0.001.

Based on the results (Table 1), kaki jelly showed significantly (p<0.001) lower value of pH of 3.86 and acidity (p<0.05) about 0.45%. These findings agreed well with those found in persimmon jelly prepared with different cultivars with pH and acidity values ranging from 3.67 to 4.60 and 0.29% to 0.43%, respectively [9]. Furthermore, the acidity result obtained within the present study, is very close to that previously obtained by Islam *et al.* [16] (0.44-0.46%) for dragon fruit jelly. These differences in acidity could be therefore due to the conversion of organic acids into sugars during the preparation of the jellies [9].

The evaluation in terms of total soluble solids (TSS), moisture and ash content of the jelly made with kaki, obtained levels of around 65°Brix, 23.24% and 1.07%, respectively. Compared to the fresh pulp, the obtained jelly presents higher total soluble solids (TSS) (p<0.001) and lower moisture content (p<0.001) which may be caused by the heating effect and other ingredients added to the formulation such as sugar and citric acid.

The result of TSS was slightly higher to those showed in a previous study which ranged between 45.44 and 54.67°Brix [9] and similar than those of the jellies prepared from blackberries with 65°Brix [17]. The moisture content obtained was slightly lower than reported previously in Dragon fruit jellies (28.96-30.12%) [16], however, the ash content was slightly higher to that found in the same study (0.59-0.62%).

With regard to the color parameters of persimmon jelly, we found similar results to those previously reported for persimmon jelly from different cultivars (L* 32.38-40.27, b* 5.55-15.07, a* 0.58-1.44) [9] and we found a decrease in the L* (p<0.01), a* and b* (p<0.001) values in comparison to fresh pulp with L* (33.26), a* (2.98), b* (11.83) and L* (38.04), a* (4.58), b* (24.66), respectively. The jelly obtained had less lightness intense, this indicates the significant effect of temperature on the change in fruit color during cooking. Achour *et al.* [18] show that the loss of the initial color of the fruit is due to the Maillard reaction produced after temperature increasing.

Phenolic compounds contents

According to data shown in Figure 1, the kaki jelly prepared in this study has considerable levels of total phenols, flavonoids and condensed tannins with amounts of about 4.41 mg AGE/g, 2.77 mg QE/g and 1.63 mg CE/g, respectively. However, these values are significantly (p<0.001) lower than those of fresh pulp.

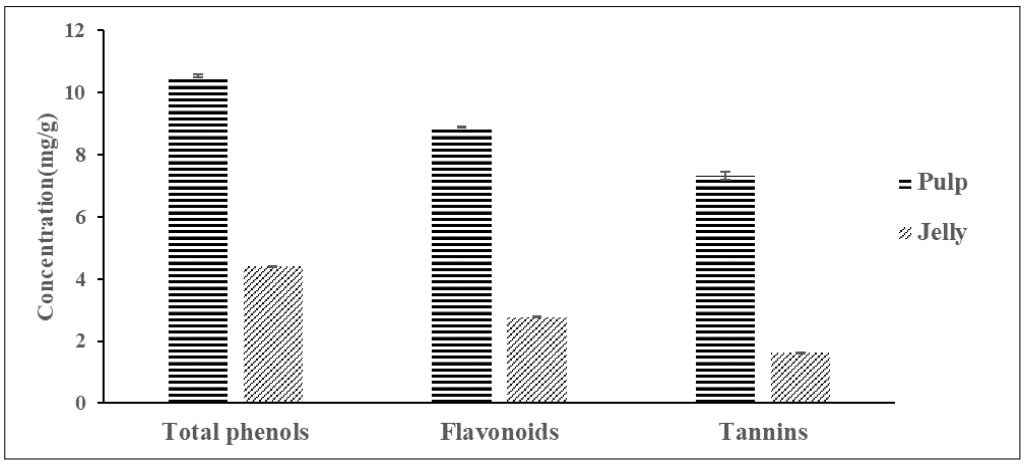

Fig 1: Total phenol (mg GAE/g extract), flavonoid (mg QE/g extract) and condensed tannin (mg CE/g extract) contents of kaki pulp and jelly Data are mean values ± standard deviation.

As reported by de Sousa *et al.* [17] our jelly may be classified with a medium phenol content and consequently can be considered good source of phenols. Although, the degradation of phenolic compounds is important during manufacturing, which may be related to the effect of the high temperature employed during processing of jelly.

The total phenol content of our jelly is between the range of total phenols found by de Souza *et al*. [17], on the formulation of blackberry jelly from different varieties (357.68 – 467.88 mg GAE/100g). The total flavonoids level obtained in the current study was higher than that reported for jelly palm which was only 63.11 µg QE/g. While that recorded for total tannins condensed was almost twice than that of our study [14]. It's crucial to preserve bioactive compounds concentration in fruit jam/ jelly by using high sugar and pectin in addition to well-controlled storage conditions [19].

Antioxidant activity

The antioxidant activity results, estimated as IC_{50}, which is the concentration required to reduce the initial forms of the DPPH and ABTS radicals by 50%, are shown in Figure 2.

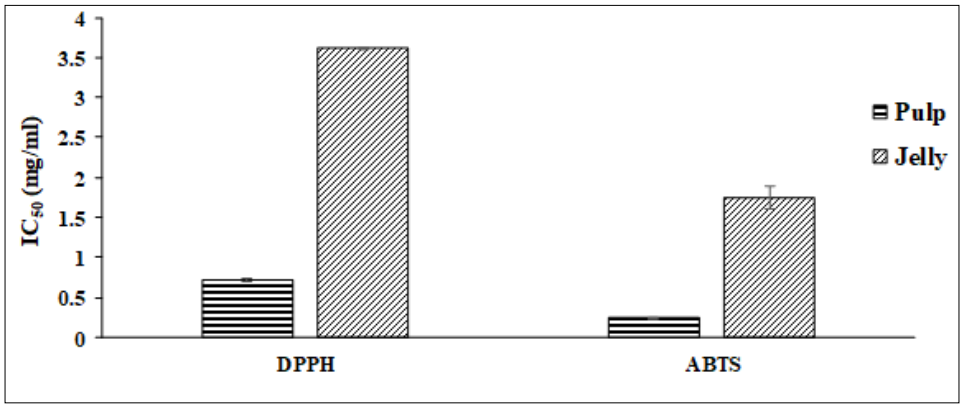

Fig 2: IC_{50} values obtained by DPPH and ABTS tests of pulp and jelly of kaki fruit. Data are mean values ± standard deviation.

As shown in figure.2, the IC_{50} values of the kaki jelly by means DPPH and ABTS assays were 3.61 and 1.75 mg/mL, respectively. They are significantly ($p<0.001$) lower than those estimated in the fresh pulp. Therefore, kaki jelly showed five times less antioxidant activity than pulp in the case of DPPH and seven times less in the case of ABTS.

Many studies have shown that when processing fruits into jellies, antioxidant activity decreases [17, 20, 21, 22]. This loss may be due to the effect of heat treatment and storage time on phenolic compounds. As the jelly is complex product, the change in bioactive compounds during processing vary with various factors like type and variety of fruit, sugar and pectin concentration, and other ingredients as well. Also, the interaction among the components of food might be the reason [22].

However, it is important to point out that the processing of vegetables can have a beneficial effect on the bioavailability of some molecules of nutritional interest such as carotenoids. Indeed, on the basis of the work carried out by Dhuique-Mayer *et al*. [23], the bioavailability of carotenoids in formulations based on beta-carotene fortified potatoes is improved by industrial processes such as sterilization despite losses in concentrations reaching the 30%. The authors suggest a structural modification at the origin of this difference. The reduction of particle sizes is essential to ensure significant bioavailability. Likewise, a mechanical processing such as grinding has a beneficial effect on the availability of carotenoids.

Interestingly, Shinwari *et al*. [19] have suggested new strategies for improving the stability of bioactive compounds in fruit jams and jellies by encapsulating them and implementing advanced food manufacturing technologies.

Conclusion

In this study, the results demonstrated That the physico-chemical characteristics of kaki jelly were significantly lower than those of the fresh pulp except for the total soluble solids. Phenolics compounds amounts were also lower than those of the fresh pulp. However, the antioxidant activity remained considerable with value of IC50 of about 3.61 and 1.75 mg/mL when tested by the DPPH and ABTS assays respectively.

This study highlighted the challenging potential of transforming the kaki fruit into a stable and palatable jelly while preserving its essential bioactive components. This jelly can be, also, incorporated into food products to enhance their nutritional and organoleptic properties.

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