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Processed *Z. Mauritiana* Lamk in the Formula of High Nutritional Value Cake

Zozio S^{1,2}, Servent A², Hiol A³, Mbeguie-A-Mbeguie D^{1,2}, Cosmidis L², Lucien JM² and Pallet D²

¹CIRAD, UMR QUALISUD, F- 97130 Capesterre-Belle-Eau, Guadeloupe, France

²CIRAD, UMR QUALISUD, F-34398 Montpellier, France

³CIRAD, UMR QUALISUD University of Reunion – ESIROI, Specialty Food, PTU – 97490, France

Abstract

The nutritional value of jujube fruits *Ziziphus mauritiana* Lamk was processed through an optimized traditional cake procedure. The characteristics of jujube fruit polysaccharides from an accession known as P3 were determined for each of the 5 ripening stages. Therefore, the content of the Alcohol Insoluble Materials, Water Soluble Polysaccharide and Galacturonic Acid was determined at each ripening stage. The degree of methylation (DM) of jujube pectins was less than 50% therefore was classified as low methoxylated pectin (LM). Using the 3rd and the 5th ripening stage, the impact of the drying and cooking was evaluated on selected nutritional characteristics, including vitamin C, total phenolics content and antioxidant capacity. Remarkably, using the fruits from the 3rd stage, the drying process decreased the vitamin C content (74.5%, $p < 0.05$) whereas an increase of 20% ($p < 0.05$) was observed for the cake. Interestingly, the antioxidant activity was unchanged during the drying process. In contrast, after the cooking process the phenolics content and the antioxidant capacity had both increased, by 64% and 30% ($p < 0.05$) respectively.

Overall, our results indicated that stage 3 fruits would exhibit higher nutritional qualities than stage 5 fruits. We strongly recommend stage 3 fruits of accession P3 for food applications, including jujube cake processing.

Keywords: Jujube process; Cake nutritional; Polyphenols; Vitamin C; Antioxidant

Introduction

The jujube fruit (*Ziziphus mauritiana* Lamk.), known as “pommesurette” in Guadeloupe, is underutilized despite its high nutritional value and its biological properties, underlined by various triterpenoid acids, flavonoids, phenolic acids, cytokinins and tannins [1]. Furthermore, previous studies have revealed a high antioxidant capacity [2-4]. Nevertheless, a huge range of food products have been established, including compotes, alcoholic beverages, flours, chutneys, pickles and some cakes in India [5]. However, rapid perishability is a problem for postharvest management and further processing [6]. Depending on their ripening stages, the fruit skin color shifts from green to yellow, eventually reaching a reddish-brown color. Then, the harvested fruits can be classified into five ripening stages as showed in the previous work. A recent study on polysaccharides from *Ziziphus mauritiana* indicates that they have rheological properties [7]. High DM pectin (high methoxylated (HM), DM >50%) can form a gel in acidic conditions in the presence of high sugar concentration. Conversely, gelation of low methoxyl pectin (LM, DM <50%) occurs at higher pH in the presence of divalent ions, such as calcium, which acts as a bridge between pairs of carboxyl groups of different pectin chains. The main industrial sources for pectin extraction are apple pomace and citrus peels, which provide HM pectin. LM pectin can be obtained after chemical de-esterification of HM pectins. However, this process often induces pectin depolymerization, thus reducing the gel-forming ability of pectin [8]. Furthermore, polysaccharides extracted from plants and fungi have been identified for their anti-oxidative and hepatoprotective effect [9] and also for their immunobiological, anti-viral, anti-tumor and other biological activities [10]. Hence, the composition of polysaccharides from the species *Ziziphus jujuba* growing in China has been elucidated [11,12]. In Africa, a traditional cake known as “yaabande” is made with harvested mature fruits and dry grains fallen from jujube trees onto the ground [13]. However, the ripening stage has not been clearly defined. Thus, in order to combine

processing and the biochemistry characteristics of jujube during the ripening, we evaluated the nutritional value of an optimized cake. Hence, we first characterized the polysaccharides during ripening in order to assess the rheological properties. Subsequently, we chose 2 ripening stages based on nutritional properties and the traditional processing method, to evaluate the impact of ripening on the quality of jujube cake, previously optimized. In addition, the impact of the process (drying and steam cooking) on the content of vitamin C, total phenolics and antioxidant capacity of the jujube cake was investigated. The optimized process was set as follows: drying parameters (45°C/30 h), size grading of jujube flour (465 µm) and cooking parameters (10 mins/100°C in a steam oven). The present work was carried out to improve the seasonal feature of jujube for a diet application as flour and as cake. Our results strongly suggested that jujube fruits taken at stage 3 may provide high nutritional value and elevated antioxidant activity, in both the flour and the cake.

Material and Methods

Materials

Jujube fruit from the cultivar P3 were harvested in January 2012 on a local farm based in the south of the island under wild conditions, following the five ripening stage as described in the previous work. The fruits were washed with 1% chlorinated water and rinsed with

***Corresponding author:** Abel Hiol, 3CIRAD, UMR QUALISUD University of Reunion – ESIROI, Specialty Food, PTU – 97490, France, Tel: +33 2 62 21 16 26; E-mail: abel.hiol@univ-reunion.fr

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water. Then the fruits were stored for four days in air at 20°C in order to homogenize their internal temperature and to reveal any putative injured fruits that might not have been observed during harvesting. Ethylene production was measured in order to check the physiological stage of the fruit samples. The fruit from ripening stage 3 and 5 were kept, the fruits from stage 2 were matured until stage 3, and the stage 4 fruits until stage 5. Then 2 jujube lots were frozen before processing: stage 3 fruits were designated “Fruits 3” and stage 5 fruits, as “Fruits 5”.

Jujube cake processing

The processing of jujube cake comprised three individual steps: drying, grinding and cooking (Figure 1). The drying was optimized with a horizontal air outlet dryer (UTA, Marmande, France) with three parameters: temperature, time and fruit configuration (whole/sliced/grinded). The impact of the flour grading on the consistency of the cake was also evaluated. The cooking parameters (time, quantity of flour and type of mold) were optimized with an Emeraude 3 steam oven (Thirode, Mitri-mory, France).

Sensory evaluation of the cake samples was carried out by 5 semi-trained panelists from CIRAD Montpellier.

Physico-chemical parameters analysis

Determination of jujube flour grading: The particle size was determined by the Mastersizer 3000 laser diffraction particle size analyzer (Malven Instruments, Malvern, Worcestershire, UK) at a grinding speed of 1500 rpm. The mean of 6 measurements was used to estimate the particle size of 3 grading flours.

Determination of jujube cake firmness: Jujube cake firmness was measured by a texture analyzer (Stable Micro Systems TAXT PLUS). Preliminary experiments were conducted to optimize the process conditions with a ball probe adjusted for 70% deformation of the cake, with a speed of 0.7 mm/s. The force recorded in Newtons (N) was given

as firmness. This measurement corresponds to the force needed to give a deformation of 70%. The more flexible the cake, the less it was deformed.

Biochemical analysis

The chemical analysis was performed on the fruits, flours and cakes from the ripening stage 3 and 5 in order to evaluate the effect of processing on nutritional qualities of jujube fruit.

Total soluble solids and titratable acid content: The fruits, flours or cakes were homogenized with a blender and centrifuged for 1 h at 10,000×g and 4°C. The supernatant was collected for analysis of total soluble solids, pH and titratable acidity. The level of total soluble solids was determined using a digital Refracto 30PX/GS refractometer from Mettler Toledo, (Grosseron, Saint-Herblain, France). pH and titratable acidity expressed as citric acid were determined by titration with 0.1N NaOH using a TitroLine easy apparatus from SCHOTT Instrument (Bioblock, Illkirch, France).

Determination of ascorbic acid content: Five hundred milligram of fruits, flours or cakes were stirred in 10 ml of methaphosphoric acid 4% for 10 mins, and then centrifuged for 10 mins at 10,000 rpm. The remaining supernatant was then filtered through a 0.45 µm filter (Millipore) and then analyzed by HPLC using a 1200 series HPLC Agilent System.

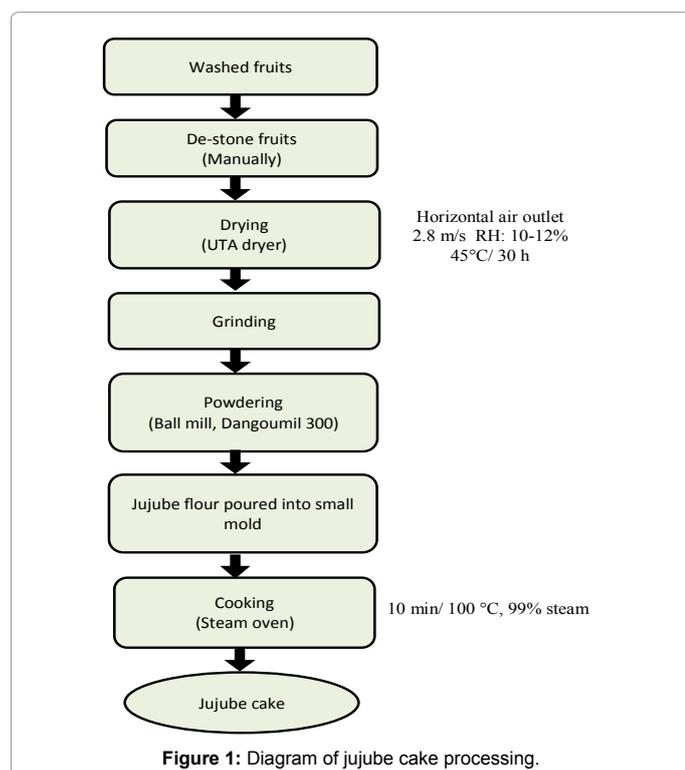
Total phenolics (TP) content: Total phenolics content was evaluated spectrometrically method using the Folin-Ciocalteu reagent as per the method of [14] modified for a TECAN Infinite 200 96-well plate reader. Catechin was used as a standard to quantifying the TP content in fruits, flours and cakes. The results were expressed in mg catechin equivalent (CE)/100 g.

Antioxidant capacity determination: The FRAP assay was carried out on a TECAN Infinite 200 96-well plate reader (TECAN Austria GMBH) as per [15] Trolox was used as a standard to quantify the TP content in fruits, flours and cakes. The results were expressed in mmol Trolox equivalent (TE)/100 g.

Polysaccharide analysis

Extraction method: Polysaccharide extraction from jujube fruits was carried out as per the modified method based on [16] Lyophilized jujube fruits were refluxed with 96% ethanol at 70°C for 1 h, and this step was repeated 3 times. Subsequently, the dried ethanol-extracted residue was extracted with distilled water at 80°C for 3 h. After one night of decantation at 4°C, the aqueous part was recovered by centrifugation (4°C/20 mins/10000 g) and concentrated. The polysaccharide was isolated by mixing 3 volumes of cold 96% ethanol. The precipitate was recovered by centrifugation (4°C/20 mins/10000 g), and finally lyophilized. Brown water-soluble polysaccharide (WSP) was obtained.

Galacturonic acid content determination: Galacturonic acid content was determinate as previously [17] reported with slightly modifications. Five milligrams of polysaccharide from each ripening stage was poured into a screw-capped tube, then 1 ml of sulfuric acid was added for hydrolysis for 3 h at 20°C. After dilution and filtration thought gauze, 500 µl was mixed thoroughly with 2.5 ml of 0.125M sodium tetraborate in sulfuric acid and immediately cooled in an ice-bath. Then, all the tubes were heated to 80°C for 6 mins, cooled, added to 50 µl of 0.15% m-hydroxybiphenyl in 0.125M sodium hydroxide, and vortex agitated; the absorbance at 520 nm was then measured every 2 mins for 20 mins. The maximum absorbance was used to determine the galacturonic content based on the standard curve, which was



prepared using 7 concentrations (5, 10, 20, 40, 60, 80 and 100 µg/mL) of galacturonic acid standard. The straight-line equation obtained for the standard curve was $y=0.0112x-0.0147$ with an R^2 value of 0.9902.

Degree of Methylation (DM) estimation: The degree of methylation of jujube pectins from the five ripening stages was determined using a modified method based on Huisman et al. [18]. The pectin DM is expressed as the percentage of the total number of galacturonic acid residues esterified with a methoxyl group. SPME/CG by standard addition was used to quantify methanol released from pectic material by saponification. Five milligrams of WSP was weighed into a headspace vial (in quadruplicate) and 1 ml of 2N NaOH was added. 1 ml of deionized water was added to the samples (duplicate), and 1 ml of methanol to the spikes (duplicate). The vials were sealed and kept at 4°C for 1 h, and then 20 mins at room temperature, and subsequently analyzed. The vials were heated to 85°C for 15 mins in a the head-space sampler, then an SPME fiber PDMS/DVB (85 µm stableflex, Chromoptique, Courtaboeuf, France) was exposed to the headspace vials while the extract was continuously stirred for 15 mins. Methanol was desorbed by inserting the SPME fiber into a GC injector (injector temperature 250°C) for 30 s in splitless mode connected with DB-WAX column (30 m, 0.25 mm ID, 0.25 µm film thicknesses) for 60 mins. The integration was achieved using MSD ChemStation software. The degree of methylation was estimated using Equation 1 below:

$$DM = \frac{m \text{ Methanol}}{MM \text{ Methanol}} \times \frac{MM \text{ Uronic acid}}{m \text{ Uronic acid}} \times 100 \quad (1)$$

Where: m=mass (g), MM Methanol=32 g/mol, MM Uronic acid=176 g/mol

Statistical analysis

The data were subjected to Analysis of Variance (ANOVA) using STATISTICA software (Statsoft, version 7).Duncan's Multiple Range test ($p<0.05$) was applied to calculate the significant difference between the different ripening stages and treatments. Unless otherwise stated analyses were performed on three or more biological replicates and the results were expressed as the mean ± standard deviation.

Results

The jujube cake processing

Impact of temperature and fruit configuration on quality of drying: The temperature and fruit configuration have a high impact on quality of drying, as shown in Table 1. Whole and scalped fruits were dried only on the skin, and were finally burned, whereas the pulp was candied. However fruits sliced before drying exhibited acceptable drying. Conversely, the ground fruits formed a kind of mesh during drying, and became very hard.

Step of experiment	Configuration of fruits	Temperature applied °C	Quality of drying	Observations
1	Whole	60	+	Burnt skin, Pulp not dried
2	Whole	45	+	Pulp not dried
3	Scalped	50	+	Skin burned
4	Sliced	35	+	Correctly dried
5	Ground	35	++	Pulp not dried
6	Sliced	45	+++	Formation of a hard mesh

Table 1: Impact of fruit configuration and temperature on quality of drying. The quality of drying was defined by the number of (+) symbols: slightly dry (+), moderately dry (++) and correctly dry (+++).

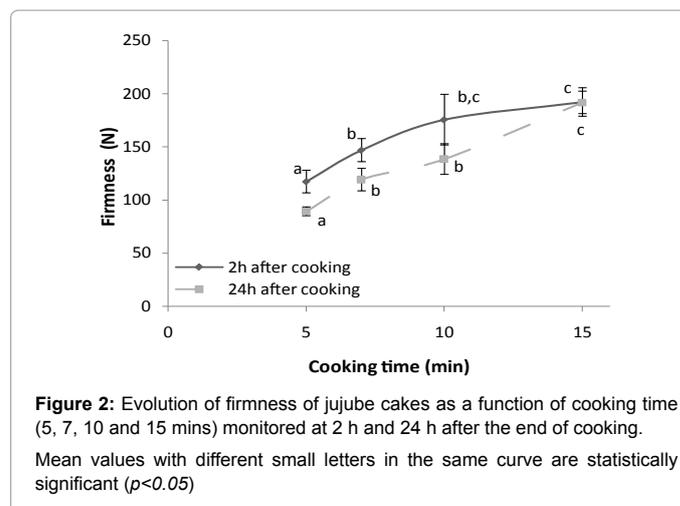


Figure 2: Evolution of firmness of jujube cakes as a function of cooking time (5, 7, 10 and 15 mins) monitored at 2 h and 24 h after the end of cooking. Mean values with different small letters in the same curve are statistically significant ($p<0.05$)

Grading nomenclature	Grading (µm)	Consistency of cake
Fine	75 ^a (1.70)	Pasty and sticky
Intermediate	465 ^b (24.70)	Soft and melting
Coarse	812 ^c (61.78)	Grainy

Values are means with standard deviations of triplicate determinations. Mean values of grading with different small letters are statistically significant ($p<0.05$)

Table 2: Impact of the jujube flour grading on cake consistency.

Impact of cooking time on consistency of cakes: The cooking time (5, 7, 10 and 15 mins) was monitored. The consistency changed and became more compact as time increased, exhibiting loss of the flavor and aroma characteristics. Firmness was determined 2 h and 24 h after baking, to evaluate the possible modification of the consistency due to water absorption.

The cake firmness increased with cooking time (5 to 15 mins, $p<0.05$), both 2 h and 24 h after the end of cooking. However an insignificant increase ($p>0.05$) was observed between 7 mins to 10 mins of cooking, regardless of the measurement (2 h or 24 h). In addition, no significant difference ($p>0.05$) between 2h and 24h was observed after 7 mins of cooking (Figure 2).

Optimization of flour grading on cake consistency: The flour grading was shown to have an impact on the cake consistency. The fine and coarse grading gave a sticky consistency irrespective of the cooking time. However, the intermediate grading (465 µm) was chosen for baking jujube cakes, because of the soft and melting texture (Table 2).

Nutritional quality improvement of the reengineered jujube cake

Nutritional quality of Fruit 3 compared to Fruit 5: In order to evaluate the impact of ripening stage on the nutritional quality of jujube cake, Fruits 3 and Fruits 5 were used in processing to make jujube cake. Chemical analyses were carried on fruits, flours and cakes. Fruits 3 exhibited high ascorbic acid content (267.78 mg/ 100 g DW), with a large decrease in Fruits 5 (98%) (Table 3). This last ripening stage (Fruit 5) also showed a lower total phenolics content (61%) and antioxidant capacity (87%) than Fruit 3 (Figures 3 and 4). Previous works have highlighted the decrease in nutritional quality during ripening.

Combined effects of ripening stages and processing on nutritional quality of jujube cake: Flour from the ripening stage 3 was designated "Flour3", and flour from stage 5 "Flour 5". Likewise, cakes

Characteristics	Fruits		Flours		Cakes	
	Stage 3	Stage 5	Stage 3	Stage 5	Stage 3	Stage 5
Pulp (%)	88.89 (0.28)	84.25 (1.48)	-	-	-	-
pH	3.33 (0.05)	3.52 (0.03)	3.43 (0.01)	3.43 (0.02)	3.43 (0.04)	3.43 (0.06)
TSS*(% DW)	60.2 (3.67)	81.27 (0.40)	82.63 (1.19)	74.47 (1.2)	80.12 (2.32)	75.21 (1.21)
Titrateable acidity (%)	1.23 (0.01)	1.41 (0.049)	7.29 (0.05)	7.38 (0.02)	6.54 (0.06)	6.66 (0.04)
TSS/Titrateable acid	9.75	10.71	11.11	9.65	12.25	10.60
Total sugar (%)	-	-	24.00 (0.67)	24.42 (0.72)	23.65 (0.86)	23.84 (0.97)
Dry matter (%)	19.92 (0.09)	18.58 (0.38)	98.05 (0.02)	95.63 (0.05)	96.77 (0.75)	93.89 (0.37)
Ascorbic acid (mg/100g DW)	267.78 (7.54)	5.64 (0.32)	68.28 (4.15)	1.37 (0.04)	82.21 (6.23)	6.60 (0.09)

*TSS: Total Soluble Solid

Values are means with standard deviations of triplicate determinations

Table 3: Physico-chemical characteristics of jujube fruits, flours after drying and cakes after cooking from ripening stages 3 and 5.

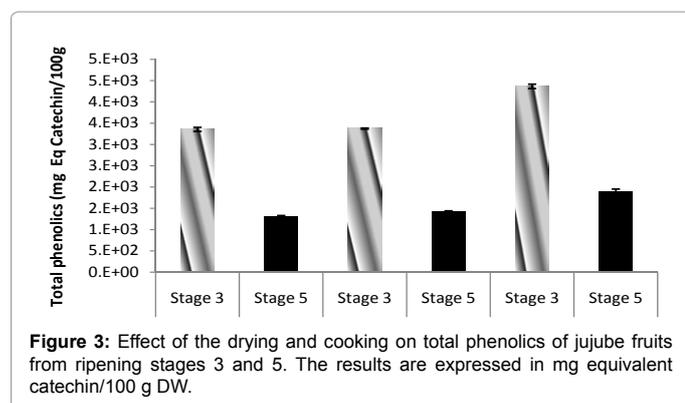


Figure 3: Effect of the drying and cooking on total phenolics of jujube fruits from ripening stages 3 and 5. The results are expressed in mg equivalent catechin/100 g DW.

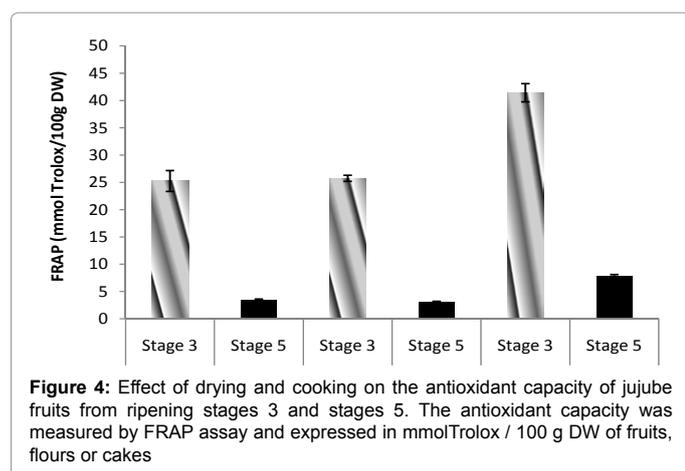


Figure 4: Effect of drying and cooking on the antioxidant capacity of jujube fruits from ripening stages 3 and stages 5. The antioxidant capacity was measured by FRAP assay and expressed in mmol Trolox / 100 g DW of fruits, flours or cakes

from Flour 3 were designed “Cakes 3” and cakes from flour 5, “Cakes 5”.

Unexpectedly, the drying process did not negatively affect the total phenolics content or the antioxidant capacity, irrespective of the ripening stage (Figures 3 and 4). Conversely, ascorbic content was reduced dramatically ($p < 0.05$): 74.5% in Flour 3 and 76% for Flour 5 (Table 3). A previous study on *Z. jujuba* showed a decrease of vitamin C (65%), phenolic content (32%) and antioxidant activity (40%) during drying at 65°C [19]. Surprisingly, the Cakes 3 and Cakes 5 exhibited an increase of total phenolics content (30%, $p < 0.05$), and also for the

antioxidant capacities (60%, $p < 0.05$) compared to the corresponding flour (Figures 3 and 4). A similar antioxidant improvement during steam cooking was found in jujube cake. This enhancement may be due to naturally occurring compounds or formation of new compounds, such as Maillard reaction products with antioxidant activity [20]. Furthermore, cooking was found to increase total phenolics in green beans, pepper and broccoli [21]. It was reported that heat treatment increased the level of free flavonols in tomatoes by releasing conjugated quercetin as rutin [22]. A study on phenolic acids of citrus peel showed that the free compounds increased after heat treatment; as opposed to ester, glycoside and ester-bound compounds which declined, as did flavanone glycosides [23]. Phenolic compounds are present in different bound states in plants [24,25] and may be cleaved and rearranged into more soluble forms by thermal processing, which leads to an increase in antioxidant activity [26]. In a previous study, an increase of p-coumaric acid (1.8 to 4.3 mg/kg DW) and ferulic acid (48%) were found after sun-drying of *Z. jujuba* [27] and after microwave, vacuum and roasting treatment for p-coumaric acid [28]. Likewise, ascorbic acid content showed a significant increase ($p < 0.05$) from the Flour 3 to Cakes 3 (20%). Surprisingly, the ascorbic acid content increased 5 fold with Cakes 5 compared to Flour 5 (Table 3).

Characterization of the polysaccharide extract from jujube

WSPs from jujube fruits were obtained by precipitation with alcohol from the aqueous extract of the alcohol-insoluble material (AIM). The AIM increased slowly from the 1st to the 4th ripening stage (38.47% to 47.40% DW, $p < 0.05$), before a decrease at the end of the ripening (5th stage: 39.13% DW, $p < 0.05$). The resulting WSPs exhibited a constantly high value ($\approx 6\%$) during ripening. Previous studies by Kannan et al. [29] pointed to very low pectin content in unripe and ripe *Z. mauritiana* (0.39% and 0.18% respectively). However, an increase was observed during ripening in unripe ($\approx 0.7\%$ DW) and ripe ($\approx 3\%$ DW) fruits of the *Z. jujube* Huanghua cultivar, whereas a constant value ($\approx 3\%$ DW) was obtained for the Zhanhua cultivar [30]. The content of uronic acid extracted from the WSP showed a global increase from the 1st to the 5th ripening stage (41%, $p < 0.05$). For the DM, a global decrease was observed from the 1st to 5th ripening stage (19%, $p < 0.05$) (Table 4).

Discussion

Jujube cake preparation was optimized in a steam oven using various temperatures and cooking times, different flour grading and

Ripening stage	AIM (%DW)	WSP (% DW)	GuAc (% WSP DW)	DM (%)
1	38.47 ^a (4.39)	6.06 ^a (1.08)	40.58 ^a (2.46)	46.52 ^a (3.75)
2	42.30 ^a (6.75)	5.65 ^a (1.21)	40.99 ^a (2.32)	47.31 ^a (2.61)
3	45.11 ^{a,b} (6.84)	5.44 ^a (1.40)	44.45 ^{a,b} (2.42)	45.77 ^a (4.73)
4	47.40 ^b (4.93)	5.14 ^a (1.12)	46.81 ^b (2.41)	36.07 ^b (3.93)
5	39.13 ^c (6.23)	4.67 ^a (0.83)	57.25 ^c (2.68)	37.61 ^b (2.53)

AIM: Alcohol Insoluble Material

WSP: Water Soluble Polysaccharide

GuAc: Galacturonic acid

DM: Degree of Methylation

Data are presented as means with standard deviations of triplicate determinations. Mean values with different small letters in the same column for a given analysis are statistically significant ($p < 0.05$)

Table 4: Characteristics of polysaccharides extracted from jujube cultivar P3 during ripening.

tins. The main goal was to achieve the flavor, aroma and consistency characteristics ascribed to the traditional jujube cake found in Africa. The optimized drying parameters were 45°C/24 h/sliced fruits, and the cooking parameters were 13 g of jujube flour cooked for 10 mins with an intermediate grading of flour (465 µm). After 7 mins of cooking, jujube flour was compacted, but the cake was better after 10 mins, when the specific jujube flavor was released. This property of compaction may be attributed to the gelling ability of pectin polysaccharides [31]. Indeed, our results showed a high WSP content during ripening (6%), with a high content of galacturonic acid (50%). Furthermore, with regard to its DM (less than 50%), jujube polysaccharides were classified as "low methylated" polysaccharide. Therefore, the gel was created with bivalent ions such as calcium. *Z. mauritiana* Lamk cv Gola from Senegal revealed a high calcium content (488 mg/100 g DW) [32]. Traditionally, the jujube cake was baked using the last ripening stage 5. However, the nutritional quality was very low in fresh fruit picked at this stage, as described in previous work. Fruits 3 exhibited a higher total phenolic content, ascorbic acid content and antioxidant capacities than Fruits 5. Whereas the drying process decreased the ascorbic acid in Flour 3 and Flour 5, it did not affect the total phenolic content or antioxidant capacity. Surprisingly, cooking had a big impact on the flour, producing cakes with higher nutritional quality. Then, since Fruits 3 have a similar WSP content as Fruits 5 (Table 4), it will be interesting to bake the jujube cake with an earlier ripening stage which have more nutritional value. Furthermore, the change in rheological properties of polysaccharides during baking may involve the synergy of other phytochemicals [33], leading to an increase of the antioxidant activity. Moreover, it should be noted that pectins with high degree of esterification (49%) from *Z. jujuba* have greater immunological activity [34]. For the increase of ascorbic acid in cake, it should be explained by the reduction of dehydroascorbate formed during the drying process, leading to ascorbate during cooking [35]. It should be noted that the high quality attributes of some jujube-based products such as beverages, compotes, jam, dried candy, syrup and cakes have been reported [36]. This enhanced nutritional quality has also been highlighted with conventional cakes enriched with 20% dried jujube. This study found an interesting nutritional advantage of processing the cake from a defined ripening stage of jujube fruits.

Conclusion

Overall, our results showed that reengineering traditional process leads to a jujube cake with high nutritional qualities and appreciated flavor. The elevated nutritional value of jujube from ripening stage

3 was found preserved and enhanced after the processing. Thus, the limited post-harvest life of jujube fruits may be overcome by processing including cake cookery. Further investigation would focus on the physiological relevance of the of the cake antioxidant capacity and may extend acceptability studies.

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