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Microstructure and *In Vitro* Beta Carotene Bioaccessibility of Heat Processed Orange Fleshed Sweet Potato

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Abstract Orange fleshed sweet potato (OFSP) has been identified as a good source of β-carotene but the β-carotene bioaccessibility is affected by processing. In this study, the effect of traditional heat processing methods on the microstructure and *in vitro* bioaccessibility of β-carotene from OFSP were investigated. Bioaccessibility was determined using simulated *in vitro* digestion model followed by membrane filtration to separate the micellar fraction containing bioaccessible β-carotene. Processing led to decrease in the amount of *all-trans*-β-carotene and increase in 13-*cis*-β-carotene. Processed OFSP had significantly higher ($P<0.05$) bioaccessible β-carotene compared to the raw forms. Bioaccessibility varied with processing treatments in the order; raw < baked < steamed/boiled < deep fried. Light microscopy showed that the microstructure of OFSP was disrupted by the processing methods employed. The cell walls of OFSP were sloughed by the traditional heat processing methods applied. The findings show that heat processing improves bioaccessibility of β-carotene in OFSP and this was probably due to disruption of the tissue microstructure.

Keywords β-carotene · Bioaccessibility · Orange fleshed sweet potatoes · Microstructure · Traditional heat processing methods

Introduction

Orange fleshed sweet potato (OFSP) (*Ipomoea batatas L.)* is a rich source of provitamin A carotenoids [1]. β-carotene content of up to 276.98 μg/g fresh weight has been recorded in OFSP varieties [2]. The β-carotene retention and bioaccessibility determine bioavailability of the provitamin A carotenoids to consumers. It has been shown that only a fraction of provitamin A carotenoids retained after heat processing is bioaccessible [3]. The unaccessible provitamin A remains entangled in the matrix. This shows that bioaccessibility is an important determinant of the bioavailability of provitamin A carotenoids.

The release of carotenoids during digestion is determined by the extent to which the cell wall is degraded during processing [4]. The changes in plant microstructure vary with processing treatments applied [5]. Maceration of plant tissues breaks up the cell wall structure, releasing the cell content thus making nutrients more available for absorption [6]. Thermal treatment increases bioaccessibility by disrupting the cell walls and breaking the protein complexes in which the carotenoids are embedded [7].

Despite the ongoing promotion of OFSP as a rich source of β-carotene in developing countries, there is inadequate information on the effect of common preparation methods on tissue microstructure and the bioaccessibility of β-carotene. The purpose of this study was therefore to determine the effect of traditional processing methods on the OFSP microstructure and *in vitro* bioaccessibility of β-carotene.

Materials and Methods

OFSP Samples

Five OFSP varieties (*Ejumula, SPK004/6/6, SPK004/6, SPK004* and *SPK004/1)* were obtained from a farm in Bombo, Luwero District, Uganda. The roots were harvested at 4.5 months. After harvest the roots were sorted, washed

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with water, kept under ice-packed containers and were transported to the laboratory within 2 h of harvest.

Chemicals and Standards

All chemicals unless stated otherwise, were obtained from BDH (London, UK). The all-*trans*-β-carotene and 9-*cis*-, 13-*cis* and 15-*cis*-β-carotene standards were obtained from CaroteNature GmbH (Lupisingen, Switzerland). Enzymes porcine pancreatin and pepsin as well as porcine bile extract were procured from Sigma Chemicals (St. Louis, MO). The water used for analytical work was double distilled.

Sample Preparation

Previously published procedures [8] for preparing OFSP products in Uganda were used with minor modifications. For each variety, four OFSP roots were randomly selected from the pool, washed and peeled to remove the skin completely. The roots were quartered longitudinally and two opposite quarters were cut using a sharp knife into slices with 15–20 mm thickness.

Sample Processing

OFSP slices (250 g) for boiling were placed in an aluminium saucepan and about 300 ml of water were added. The slices were boiled for 20 min at 92 °C. The OFSP slices (250 g) for steaming were assembled into banana leaves and wrapped properly to ensure that no water entered into the porch. Banana stalks were sliced and first lined on the bottom of the cooking pot to form a bed of about 8 cm onto which the banana leaves porch was placed. Enough water to cover the banana stalk was measured and poured into the cooking pot and then the porch containing slices was placed on top. The samples were steamed at 94 °C for 30 min. The OFSP slices (200 g) for frying were immersed in preheated 300 ml of unfortified sunflower cooking oil (Mukwano Industries, Kampala, Uganda) and were deep-fried at a temperature of 170 °C for 10 min. The OFSP slices (200 g) for baking were placed on a baking pan and placed in a preheated resistance coil electric oven (Infrared Aroma Aeromatic Oven, San Diego, CA) and baked at 180 °C for 15 min.

Sample Handling After Processing

Baked, boiled, deep fried and steamed samples were placed on absorbent paper, cooled for about 15 min at room temperature, packaged in polyethylene bags (125 micron) and frozen at −50 °C. Cooked and reference (raw) samples were freeze dried using a Virtis Genesis (American Lyophiliser, Inc., USA) freeze drier before carotenoid analysis. The freeze dried samples were packaged under nitrogen in polyethylene bags (125 micron) before storage at −50 °C. The samples were analyzed for carotenoids within 2 months of being cooked. Before analysis, the freeze dried OFSP samples were milled in a coffee grinder (Wagtech, UK) and passed through a 0.2 mm mesh. Determination of fat content of deep fried samples was determined using Folch extraction method [9].

OFSP Carotenoid Extraction

OFSP carotenoid extraction was done according to a method described by Bengtsson et al. [8]. The OFSP flour samples (~0.2 g) were weighed in triplicate into a test tube and reconstituted with 1 ml of deionized water for 20 min followed by addition of 2 ml of acetone containing 0.1% (w/v) butylated hydroxy toluene (BHT). The test tube was vortexed for 3 min and sonicated for 15 min and then centrifuged in a MicroR centrifuge (Fisher Scientific, UK for 3 min at 4750×*g*. The resulting supernatant was saved into a new test tube. The residue was extracted with 2 ml of acetone and centrifuged again. This was repeated up to four times until the residue was colourless. To the resulting acetone extract 3 ml petroleum ether (40–60 °C) was added together with 5 ml deionised water to aid in the separation of the phases. The organic and water phases were separated by centrifugation at 4750×*g* for 4 min and the organic phase was pippeted into a new test tube. This step was repeated once. The pooled organic phases were dried in a rotary evaporator at 35 °C under a stream of nitrogen. The residue was dissolved in 5 ml mobile phase (methanol: methyl-tert-butyl-ether (1:1, v/v). All the steps described above were performed under dim light and care was taken to make sure there was no contact with acids and the solvents used were all of high purity. Carotenoids in raw and processed OFSP were calculated based on dry weights of the samples and corrected for changes in dry matter due to loss or uptake of water and uptake of fat in the deep fried samples.

HPLC Analysis of Carotenoids

Carotenoids were analyzed by reversed phase High Performance Liquid Chromatography (HPLC) using a Gilson HPLC system (Gilson, USA) equipped with a pump, a degasser and a UV6000LP photo diode array detector operating at 450 nm. The data were stored and processed by means of PC1000 Version 3.5 Software. Absorption spectra were recorded between 250 nm and 500 nm. Separations were carried on ProntoSil C_{30} carotenoid column (5 μm, 250 mm×4.6 mm i.d). The mobile phase used for isocratic elution consisted of methanol: methyl tert-butyl ether: water (55:41:4, v/v/v). The flow rate was 1.3 mL/min and the injection volume was 20 μl. Carotenoid identification was done by use of authentic standards and comparing the

spectral data with reported values. All-*trans*-β-carotene, 9-*cis*-, 13-*cis* and 15-*cis*-β-carotene were quantified using the individual carotenoids as external standards.

Determination of *In Vitro* Bioaccessibility of β-Carotene in OFSP

The *in vitro* digestion was done according to the procedure described by Hedren et al. [10] with modifications. Since OFSP contains high starch content, the mouth digestion step was included [11]. At the end of *in vitro* digestion, the micellar fraction was separated by centrifugation followed by filtration [12]. Briefly, 0.5 g of freeze dried OFSP powder was reconstituted with 10 ml distilled water containing 1% ascorbic acid (w/v) and was subjected to simulated gastric digestion at pH 2 and 37 °C in the presence of pepsin (5 mL of 0.5% porcine pepsin solution in 0.1 mol L^{-1} HCl). This was followed by simulated intestinal digestion in the presence of porcine pancreatin-bile extract mixture (4 g L^{-1} and bile salt extract 25 g L^{-1}) at pH 7.5 for 2 h. After *in vitro* digestion, the digesta was filtered through a Millipore membrane (0.65 μm pore size) following centrifugation in a MicroR centrifuge (Fisher Scientific, UK) at 5,000×*g* for 20 min. The micellar fraction was analyzed for bioaccessible β-carotene.

Microstructure Analysis

Tissues with dimensions of 6×3.4×3.4 mm were sectioned from the outer parts of OFSP roots using a dissection blade. Tissues were first fixed in 10% formol saline solution [13] and were then processed using an automatic Leica TP 1020 Histokinette tissue processor (Leica Microsystems, Germany). OFSP samples were dehydrated using alcohol in ascending order of concentration starting with 70%, 80%, 90%, 96%, 100%, 100%, 100% for 1½ h per concentration. Samples were later cleared in two changes of xylene for 1 h and 1½ h, respectively. Lastly samples were impregnated using two changes of molten paraffin wax at 50 °C for 2 h per change.

After processing, the samples were embedded in paraffin wax, blocked and sectioned using a Leica RM 2235 rotary microtome (Leica Microsystems, Germany). Sections of 5–7 μm were cut and floated on a Leica H1120 water bath (Leica Microsystems, Germany). The wrinkle-free sections were picked on grease-free slides and then dried in the oven at 53 °C. The cut sections were de-waxed using two changes of xylene for 1–2 min per change. They were then dehydrated using alcohols starting with two changes of 95% and 80% alcohol for 3–5 min per change. The breakdown of cell wall material was studied using Periodic Acid Schiffs (PAS)-reaction for visualization of totally insoluble carbohydrate. The sections were stained with PAS for 15 min and passed through several changes of ethanol. The sections were then dehydrated using ethanol in ascending order of concentration starting with 95% and then two changes of absolute ethanol for 3–5 min per change. The sections were cleared in two changes of xylene for 1–2 min and were mounted using depex. After mounting, the slides were allowed to air dry and were examined using a light microscope in Objective 40 (Carl Zeiss, Germany) connected to a PC and mounted with a Canon Power Shot A640 digital camera. The micrographs were captured using Arcsoft Photostudio 5.5 software (Carl Zeiss, Germany).

Data Analysis

All experiments were performed in triplicate. Results were subjected to analysis of variance (ANOVA) using Stata statistical software (Stata Corporation, TX, USA). Multiple comparisons of means were done using the Bonferroni method. P values$\leq$0.05 were considered significant. Image analysis of micrographs was done using AxioVision Release 4.7 software (Carl Zeiss, Germany).

Results and Discussion

β-Carotene Content of Cooked OFSP

Mean *all-trans*-β-carotene content differed with variety from 67.33 μg/g dm in SPK004/1 to 314.48 μg/g dm in *Ejumula* (Table 1). The traditional processing methods generally reduced β-carotene content. The boiled, deep fried and steamed OFSP samples retained more *all-trans*-β-carotene compared to baked samples for all varieties studied. All- *trans*-β-carotene contributes twice to the amount of retinol activity equivalents compared to the *cis*-isomers [14]. Studies show that all-*trans*-β-carotene is the preferred substrate during uptake, transportation and tissue accumulation [15]. All the varieties studied showed no significant difference (P>0.05) in the provitamin A content between boiled and steamed OFSP samples. There was a higher level of 13-*cis*-β-carotene in processed OFSP than in the raw forms for all varieties. Heat processing is known to induce formation of 13-*cis*-β-carotene. The extent of isomerisation and oxidation is associated with the duration and severity of heat processing [16]. The proportion of 13-*cis*-β-carotene formed in relation to the total β-carotene in cooked samples was the highest in steamed SPK004 (10.29%) and was the lowest in boiled *Ejumula* (5.72%). The amount of 13-*cis*-β-carotene formed during heat processing appeared to increase with total β-carotene content of the OFSP sample. The major provitamin A carotenoid found in the raw and cooked samples was all-*trans*-β-carotene. All samples contained 13-*cis*-β-carotene but no 15-*cis* and 9-*cis*-β-carotene were detected.

Table 1 The content of total β-carotene, all-*trans*-β-carotene and 13-*cis*-β-carotene in OFSP varieties processed by different traditional methods (μg/g dm)

Variety/processing method	Total β-carotene (μg/g dm)	*All-trans*-β-carotene (μg/g dm)	13-*cis*-β-carotene	
			(μg/g dm)	% of total β-carotene
Ejumula				
Raw	315.71±11.64^{a}	314.48±11.43^{a}	1.22±0.22^{b}	0.39
Boiled	263.65±6.06^{bc}	248.54±4.99^{bc}	15.1±1.49^{a}	5.72
Baked	246.57±11.79^{c}	229.71±8.12^{c}	16.85±3.67^{a}	6.8
Steamed	266.08±11.07^{bc}	246.87±8.50^{bc}	19.2±2.68^{a}	7.2
Deep fried	279.11±11.39^{b}	259.85±11.19^{b}	19.25±2.95^{a}	6.9
SPK004/6/6				
Raw	213.11±11.73^{a}	212.35±16.06^{a}	0.75±0.52^{c}	0.37
Boiled	164.47±7.87^{bc}	153.45±7.34^{bc}	11.02±0.53^{a}	6.7
Baked	136.53±5.46^{c}	126.98±5.08^{c}	9.56±0.38^{b}	7.0
Steamed	166.80±10.96^{bc}	156.96±10.31^{bc}	9.84±0.64^{b}	5.9
Deep fried	181.25±9.97^{b}	169.82±9.34^{b}	11.42±0.63^{a}	6.3
SPK004/6				
Raw	189.29±16.45^{a}	188.41±16.65^{a}	0.88±0.53^{b}	0.48
Boiled	135.98±7.77^{b}	126.87±7.25^{b}	9.11±2.52^{a}	6.7
Baked	104.96±5.68^{c}	96.64±5.33^{c}	8.39±0.45^{a}	8.0
Steamed	130.02±3.86^{bc}	116.22±8.03^{bc}	11.79±4.88^{a}	9.07
Deep fried	133.78±7.93^{b}	123.07±7.28^{b}	10.7±0.63^{a}	8.0
SPK004				
Raw	101.38±3.74^{a}	100.61±3.39^{a}	0.76±0.53^{a}	0.74
Boiled	69.94±7.82^{b}	64.76±6.75^{b}	5.21±1.22^{b}	7.41
Baked	55.12±4.21^{b}	50.71±3.86^{b}	4.41±0.34^{b}	8.1
Steamed	66.49±5.06^{b}	59.60±4.28^{b}	6.86±0.87^{b}	10.29
Deep fried	68.44±14.9^{b}	64.02±13.94^{b}	4.42±1.01^{b}	6.45
SPK004/1				
Raw	67.33±2.31^{a}	66.79±2.47^{a}	0.83±0.2^{a}	0.8
Boiled	52.99±2.89^{b}	49.52±2.57^{b}	3.46±0.33^{b}	6.53
Baked	48.31±2.68^{b}	44.45±2.47^{bc}	3.86±0.22^{b}	8.0
Steamed	50.89±2.32^{b}	47.58±2.19^{b}	3.31±0.31^{b}	6.5
Deep fried	42.08±3.97^{b}	38.18±4.15^{c}	3.87±0.88^{b}	9.26

The values are mean±standard deviation (n=3). For each variety, means in the same column with different superscripts are significantly different at $P \leq 0.05$

In Vitro β-Carotene Bioaccessibility in Cooked OFSP

Heat processing reduces β-carotene content but increases bioaccessibility [17]. In all the varieties studied, the bioaccessibility values for all-*trans*-β-carotene of the raw OFSP was significantly lower ($P<0.05$) than heated samples (Table 2). The bioaccessibility of the all-*trans*-β-carotene for different processing treatments varied in the following order; raw < baking < steaming/boiling < deep frying (Fig. 1). The low β-carotene bioaccessibility of baked OFSP could be attributed to hardening of the surface which limited the extent of matrix disruption at the centre of the OFSP. Some studies have shown that despite reduction in carotene content due to cooking, heat processing has a potential to increase the bioavailability of carotenoids in vegetables [18, 19]. Mild heat processing could increase the bioavailability of carotenoids by disrupting or softening plant cells and carotenoid-protein complexes [20].

There was no significant difference between the all-*trans*-β-carotene bioaccessibility values of boiled and steamed OFSP ($P>0.05$). The processing conditions employed in steaming and boiling may not have affected the sweet potato matrix differently. Similar retention levels of all-*trans*-β-carotene have been reported for boiled and steamed OFSP [8]. OFSP processed by deep frying had

Table 2 The amount of bioaccessible *all-trans*-β-carotene (μg/g dm) in raw and processed OFSP varieties

Variety	Raw	Boiled	Baked	Steamed	Deep fried
Ejumula	34.77±10.17[a]	106.53±5.02[a]	78.32±8.14[a]	105.79±4.4[a]	150.79±9.67[a]
SPK004/6/6	41.53±3.51[a]	70.74±3.98[b]	48.03±5.22[b]	64.64±4.87[b]	101.14±9.04[b]
SPK004/6	33.78±4.38[a]	69.25±1.24[b]	44.35±2.64[b]	49.43±2.67[c]	58.80±3.94[c]
SPK004	18.18±2.07[b]	37.18±3.07[c]	18.54±2.51[c]	25.34±1.16[d]	40.54±3.73[d]
SPK004/1	7.63±1.08[b]	18.36±1.26[d]	11.46±1.33[c]	13.48±0.94[e]	19.30±1.05[e]

The amount of bioaccessible *all-trans*-β-carotene in μg/g dm in micellar fraction was determined by High Performance Liquid Chromatography (HPLC) technique. The values are means ± standard deviation (n=3). Means in the same column with different superscripts are significantly different at $P \leq 0.05$

the highest percentage of bioaccessible all-*trans*-β-carotene compared to other processing treatments (Fig. 1).The mean fat content of deep fried OFSP was 4.9±0.4%. The presence of fat in the diet is known to improve the bioaccessibility of β-carotene [21]. A study by Veda et al. [12] reported bioaccessibility of 63% in carrots and 53% in pumpkin stir-fried in 9% w/w oil. However, Huang et al. [22] reported when men were fed with sweet potato balls, bioavailability was 37%. The apparently lower bioavailability could have resulted from the fact that the sweet potato balls used were prepared by mixing sweet potato with other food materials such as cassava, sugar and wheat which may have substantially modified the matrix structure.

The highest bioaccessible amount of β-carotene was found in *Ejumula* (150.79±9.67 μg/d dm) and *SPK004/6/6* (101.14±9.04 μg/g dm) cooked by deep frying (Table 2). Total β-carotene content of OFSP varieties and the oil medium in which the OFSP was prepared seemed to influence bioaccessibility. The amount of provitamin A carotenoids in the meal and the fat content are some of the factors that determine their bioaccessibility [23].

Effect of Heat Processing on the Microstructure of OFSP

The storage parenchyma of the raw OFSP was found to be composed of polyhedral cells with a diameter of approximately 98 μm. The raw OFSP root cells contained starch granules ranging from globular (Fig. 2a, b, c, and d) to ellipsoid (Fig. 2b) and of varying sizes. Several studies have showed that in plants, carotenoids occur as membrane-bound semi-crystalline structures within the chromoplasts or chloroplast, and may be found dissolved in oil droplets [4]. The cell wall of the raw OFSP was smooth and intact. The parenchyma of the sweet potato contained several intercellular spaces which were approximately 6.8 μm in size. There were no differences between micrographs of different varieties processed by the same method (micrographs not shown).

After heat processing, the starch granules hydrated and swelled forming a reticulum of amylose and amylopectin thereby filling the cell lumen (Fig. 3). After gelatinization the starch also formed dense clusters. There was less clustering of the gelatinized starch in baked OFSP (Fig. 3a) as compared to OFSP processed by other methods (3b, c, and d).

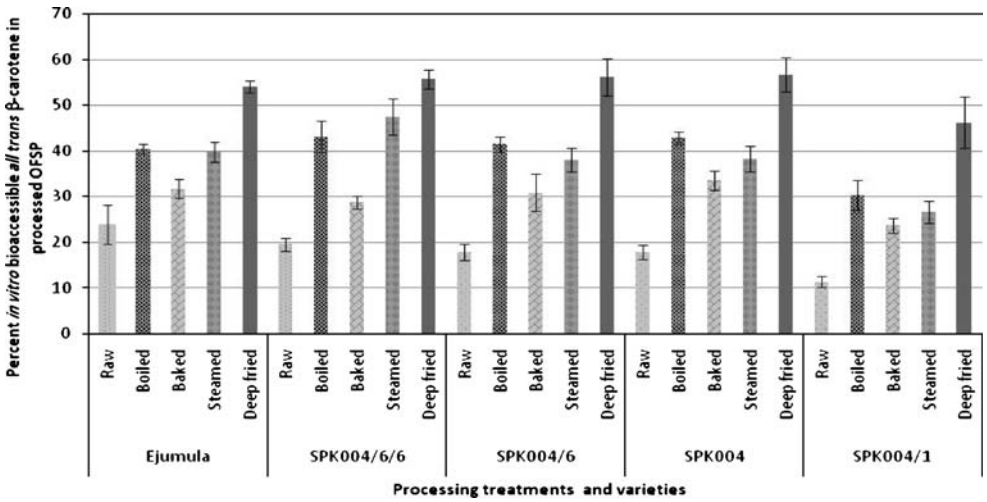

Fig. 1 Effect of traditional cooking methods on the *in vitro* β-carotene bioaccessibility of OFSP. Values (percent bioaccessibility) are given as mean ± SD (n=3). Percent bioaccessibility of *all-trans*-β-carotene was calculated from the amount in the micellar fraction and total β-carotene content in the OFSP

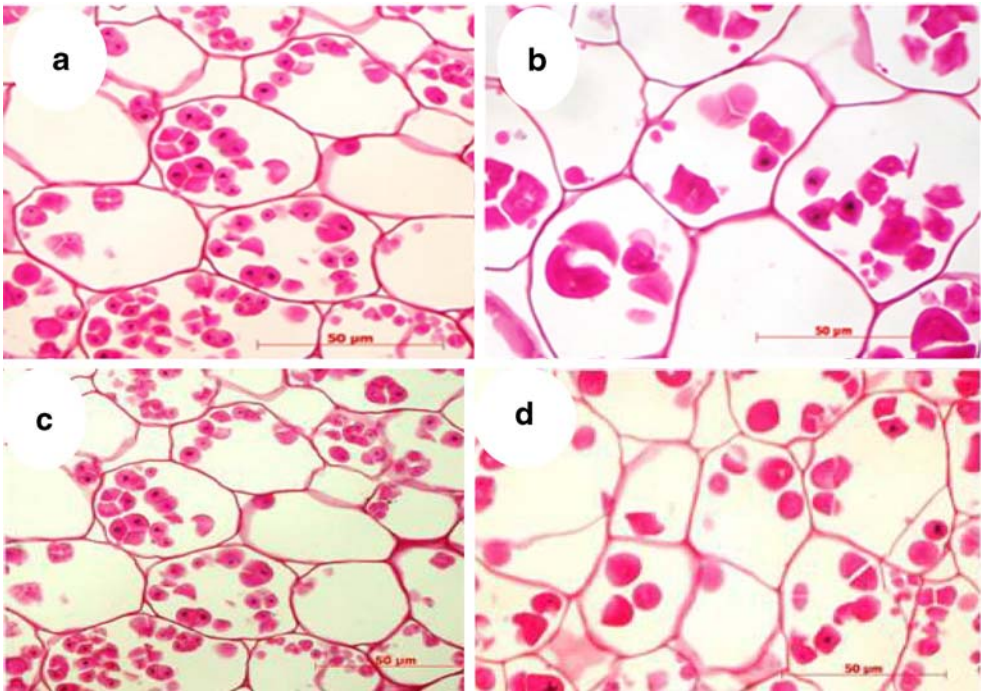

Fig. 2 Micrographs (**a**–**d**) of storage parenchyma tissue of raw OFSP for varieties; Ejumula, SPK004/6/6, SPK004 and SPK004/6, respectively, stained with PAS, Bar=50 µm

This could have resulted from lower heat penetration of the baked OFSP slices than in boiled, steamed and deep fried slices. The micrographs of processed OFSP showed that in boiled, steamed and deep fried samples, the cell walls were thin as compared to the cell walls of the raw roots. This could be a result of the depletion of the cell wall due to the breakdown of the cell wall constituents such as pectin materials (Fig. 3b, c, and d). There was cell separation which led to the increase in intercellular space. Heat processing is known to soften plant tissues by swelling of the cell wall and

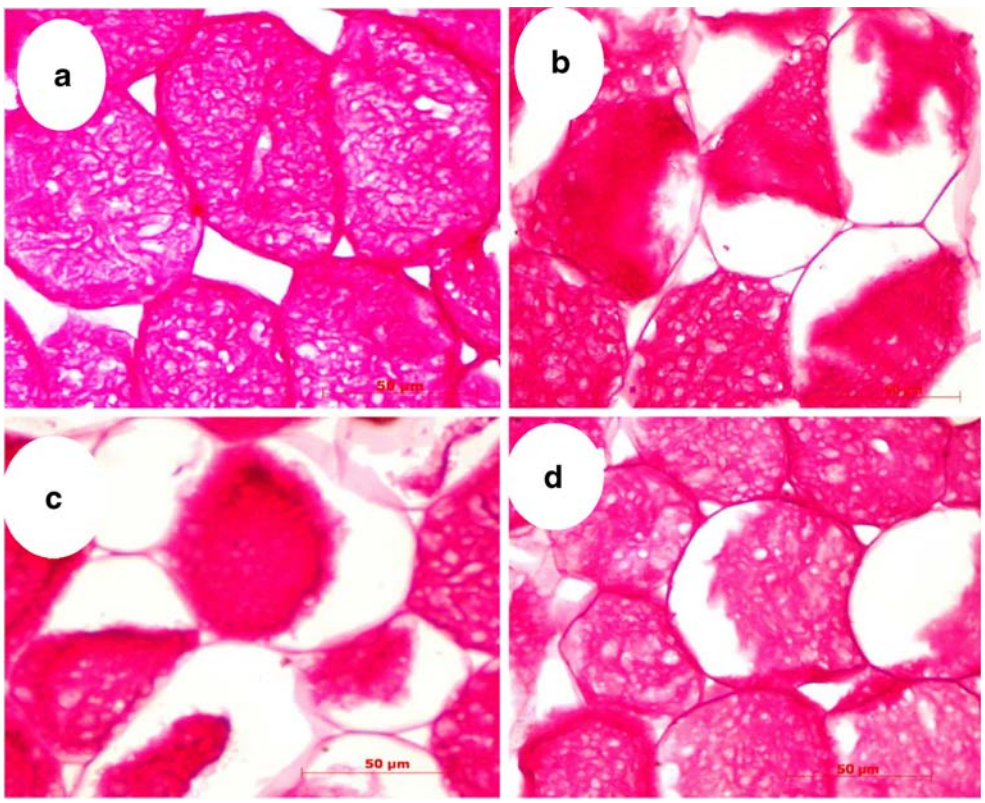

Fig. 3 Light microscope micrographs stained with PAS of processed *Ejumula* variety: **a** (baked), **b** (boiled), **c** (steamed), **d** (deep fried) Bar=50 µm

cell separation [5]. The expansion of the cells results from a combined effect of starch swelling pressure and middle lamella degradation [24].

The micrograph of OFSP roots processed by baking showed thick cell walls and no clustering of gelatinized starch (Fig. 3a) compared to the micrographs from boiled, steamed and deep fried OFSP samples. This could partly account for the low bioaccessibility of β-carotene in OFSP processed by baking. There were no apparent differences in the microstructure of OFSP roots prepared by boiling and deep frying (Fig. 3b and d). The amount of bioaccessible β-carotene was also not significantly different ($P>0.05$) in steamed and boiled OFSP (Table 2). The results show that there is a link between processing treatments and the extent of microstructure disruption in OFSP.

Conclusion

In this study, OFSP processed using traditional processing methods had significantly higher bioaccessible β-carotene than raw OFSP. The change in microstructure corroborates the increase in bioaccessibility but does not show it. Bioaccessibility of β-carotene was less in baked OFSP compared to that prepared by other traditional processing procedures. Deep fried OFSP had more bioaccessible β-carotene than OFSP prepared by other processing procedures suggesting that fat leads to increased bioaccessibility. In general, bioaccessibility of β-carotene among processing procedures varied thus; raw < baking < steaming/boiling < deep frying. Although heat processing is known to reduce β-carotene retention, this study shows that loss in retention is more than compensated for by the improved bioaccessibility. This information could be used in designing interventions aimed at alleviating VAD in developing countries.

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