Orange-Fleshed Sweetpotato Flour: Characterization of Carotenoids and Conservation

M. Koala,[1] A. Hema,*[1] R. Spina,[2] E. Palé,[1] F. Dupire,[2] C. Françoise,[2] A. Sérémé,[3] M. Nacro,[1] Y. Chapleur,[2] and D. Laurain-Mattar,[2]

[1] Laboratoire de Chimie Organique et de Physique Appliquées, Département de Chimie, UFR-SEA, Université Ouaga I Pr. Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso
[2]UMR 7565 CNRS, Faculté des Sciences et Techniques, BP 70239, 54506 Vandoeuvre-lès-Nancy
[3]CNRST/IRSAT, Département des substances naturelles, 03 BP 7047 Ouagadougou 03, Burkina Faso

Corresponding author: mnacro@hotmail.com

Abstract
Orange-fleshed sweetpotato (OFSP) contains carotenoids that are responsible for its nutritional value and beneficial for consumer health. Orange-fleshed sweetpotato tubers, with their high water content, are very highly perishable in tropical conditions and therefore, have a short time of conservation. Provitamin A including some carotenoids, help in the biofortification of infant food flours in local processing in Burkina Faso. It is a strategy that could stimulate the consumption, marketing and production of OFSP. But the conservation of fresh tubers through different value chains is still problematic. This work aimed to identify the best conditions for conservation of semi-finished products from OFSP tubers (flours). By coupling TLC/MS (Thin Layer Chromatography/Mass Spectrometry) made with CAMAG TLC-MS interface, five molecules of carotenoids such as β-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein, and zeaxanthin and possibly isomers of β-carotene (α-carotene, ε-carotene, γ-carotene and lycopene) have been identified in extracts of OFSP flour. Total carotenoids retention rates went from 84.13% under freezer conservation (SFC) conditions to 27.39% and 26.10% under STC and SADC conservation conditions, respectively, for fourteen weeks. Strongly correlated (R = 0.94) to total carotenoids contents (TCC), total antioxidant contents (TAC) were preserved at 73% (P < 0.05) in freezer conservation conditions, the most suitable to prevent carotenoids from photooxidation phenomena. In these conditions, antioxidant micronutrients which are responsible for its nutritional value could be preserved up to 72% over six months of storage. This type of conservation appears to be an alternative in the conservation of fresh tubers OFSP.

Key words : TLC-MS, Folin-Ciocalteu Reagent, antioxidant, phenolics

Farine de Patate Douce à Chair Orange: Caractérisation des Carotenoïdes et Conservation

Résumé
La patate douce à chair orange (PDCO) contient des caroténoïdes responsables de sa valeur nutritive et de ses effets bénéfiques sur la santé du consommateur. Les tubercules de patate

douce à chair orange avec leur forte teneur en eau, sont très fortement périssables dans les conditions tropicales et ont donc une courte période de conservation. Provitamines A, y compris certains caroténoïdes, aident à la bio fortification des farines alimentaires infantiles dans la transformation locale au Burkina Faso. C'est une stratégie qui pourrait stimuler la consommation, commercialisation et la production de PDCO. Mais la conservation des tubercules frais à travers différentes chaînes de valeurs reste problématique. Ce travail vise à identifier les meilleures conditions de conservation des produits semi-finis à partir de tubercules OFSP (farines). Par couplage CCM/SM (chromatographie sur couche mince / spectrométrie de masse) réalisé avec l'interface CAMAG TLC-MS, cinq molécules de caroténoïdes comme le β-carotène, l'α-cryptoxanthine, la β- cryptoxanthine, la lutéine et la zéaxanthine et éventuellement des isomères de β- du carotène (α-carotène, ε-carotène, γ-carotène et lycopène) ont été identifiées dans des extraits de farine de PDCO. Les taux de rétention des caroténoïdes totaux sont passés de 84,12 % dans les conditions de conservation du congélateur (SFa), 27,39 et 26,10 % dans les conditions de conservation de la SAT et de la SAD respectivement pendant quatorze semaines. Fortement corrélé ($R^2 = 0.94$) aux teneurs totales en caroténoïdes (TCC), les teneurs en antioxydants totaux (TAC) sont conservées à 73 % (P < 0,05) dans les conditions de conservation du congélateur, les plus appropriées pour empêcher les caroténoïdes des phénomènes de photooxydation. Dans ces conditions, les micronutriments antioxydants responsables de leur valeur nutritionnelle pourraient être conservés jusqu'à 72 % pendant six mois de stockage. Ce type de conservation semble être une alternative dans la conservation des tubercules frais.

Mots clés: CCM-MS, Réactif de Folin-Ciocalteu, antioxydant, composés phénoliques

Introduction

Orange-fleshed sweetpotato (OFSP) contains many chemical substances which guarantee its nutritional value. It is a potential source of vitamin A (Mark et al,.2009; Haskell *et al.* , 2004), minerals (Fe, Zn, Mn), and other micronutrients such as polyphenols and carotenoids (Haskell *et al.* 2004). These micronutrients have biological properties of interest for humans, and pharmacological or nutritional properties. However, these phytomicronutrients are not always stable during processes of transformation of the fresh tubers.

Phtytomicronutreals undergo various degradations due to the conditions of processing and preservation, thus, altering the nutritional value of the food which contains them. This work identifies molecules of carotenoids (by coupling TLC/MS with TLC - MS interface CAMAG) extracted from Jewel variety flour and a determination of the levels of carotenoids, phenolics and total antioxidants. Then, losses of these phytomicronutrients were evaluated by spectrophotometry in relation to the time when this flour was kept at ambient (transparent and dark) either in the refrigerator or in the freezer.

Materials and Methods

Identification of the carotenoids found in the extract: The extracted solutions were sprayed using the semi-automatic Linomat 5. HPTLC-ESI-MS spectra were directly recorded using the TLC-MS interface (CAMAG). The mass spectrometer used was a Bruker microTOF-Qde in mode ESI positive.

Storage conditions: 200 g of flour of OFSP

was stored at ambient (laboratory) in transparent containers (SATC), Ambient (laboratory) in dark containers (SADC); stored in refrigerator (SR) and Freezer (SF). 10 mg of this flour were collected, extracted and dosed every two weeks.

Extraction of plant material: extractions were performed by maceration at 4°C with each of the following solvents systems: acetone-water-acetic acid (70: 29.5: 0.5 v/v) (Koala *et al.* ,2013a) for assays of total antioxidants (TAC) and phenolics (TPC) contents and acetone-hexane (50: 50 v/v) (Kowalski *et al.* 2000, Koala *et al.* 2013b) for the determination of total carotenoids contents (TCC).

Determination of total antioxidants, phenolics and carotenoids content: these contents were determined spectrophotometrically using a microplate in quartz 96 wells (MP96, SAFAS spectrophotometer) and choosing standards. Thus, total carotenoids were assessed following the slightly modified method described by McMurry *et al.* 2008, Jun *et al.* 2011, and Koala *et al.* 2013.β-carotene, which absorbs at 450 nm was taken as standard. Total carotenoids content were obtained by reporting absorbance of extracts on a standard curve ($y = 25.56x + 0.016$; $R^2 = 0.999$) established using β-carotene. Total carotenoids content were expressed in mg of β-carotene Equivalents (BCE) per gram of sample. All measurements were carried out in triplicate.

Using Ferric Reducing Antioxidant Power (FRAP) (Benzie *et al.* ,1996;Pulido *et al.* , 2000; Koala *et al.*, 2013), total antioxidants content which reacted with FRAP reagent to give intense blue color (λ =593 nm) were determined. Results obtained from the calibration curve equation ($y = 28.67x + 0.066$; $R^2 = 0.999$), were expressed in mg of Trolox Equivalent (TE) per gram of sample.

Total phenolics content were determined using the Folin-Ciocalteu reagent (Nihal *et al.* 2007; Koala *et al* 2013a). Using Gallic acid as standard, a calibration curve was established. Results determined from the calibration curve equation ($y = 46.41x + 0.063$, $R^2 = 0.998$), were expressed in mg of Gallic Acid Equivalents (GAE) per gram of sample. All measurements were carried out in triplicate.

Data analysis: the experiments were repeated three times and the results are expressed as mean ± SD calculated on the threshold of probability less than or equal to 95%. Analysis of variance (ANOVA) to determine the differences between formulations for their

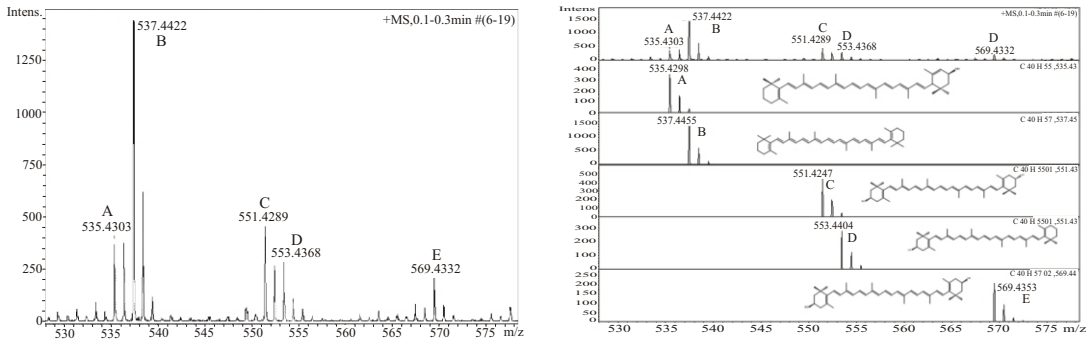


Figure 1: mass spectra of carotenoids

Table 1 : Structures of identified carotenoids

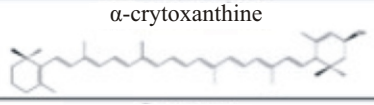
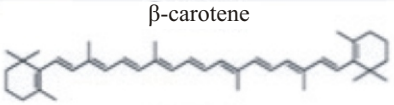
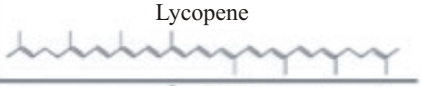
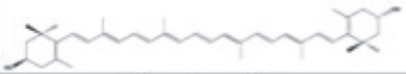
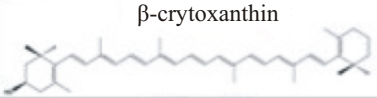
Molecular ion peaks	m/z [M+H]*	Formula	Pseudo molecular Ions	Structures of carotenoid suggested
A	553.4	$C_{40}H_{56}O$	535.4 $[M-H-18]^{+*}$	α-crytoxanthine 
B	537.4	$C_{40}H_{56}O$	-	β-carotene  α-carotene  γ-carotene  ε-carotene  Lycopene 
C	569.4	$C_{40}H_{56}O_2$	551.4 $[M-H-18]^{+*}$	Luteine 
D	553.4	$C_{40}H_{56}O$	-	β-crytoxanthin 
E	569.4	$C_{40}H_{56}O_2$	-	Zeaxanthine 

Table 2 : Total antioxidants, phenolics and carotenoids contents remaining after 14 weeks of conservation

Conditions of Storage	SATC	SADC	SR	SF
TCC (%)	27.39	26.10	55.71	84.13
TAC (%)	28.74	45.16	54.56	73.08
TPC (%)	71.42	73.04	75.03	75.48

total antioxidants, phenolics and carotenoids content were done using Genstat, edition 14.

Results and Discussions

Identification of carotenoid compounds from OFSP flour extract Jewel extract carotenoids isolated by the TLC, were identified on the basis of the combined information obtained from Liquid Chromatography and the mass spectrum. The analysis of the Spectra TLC-

MS/MS of the spot 1 (Rf = 0.48) with the solvents system hexane/ethyl acetate 96/4 v/v) eluent demonstrates several compounds of carotenoids. On the spectrum of Electrospray mass positive mode (+ ESI), we observed five quasi-molecular ions (A, B, C, D and E) were found at m/z 535,4 [M + H]+, m/z 537,4 [M + H] +, m/z 551.4 [M + H]+, m/z 553,4 [M + H]+ and m/z 569,4 [M + H]+, suggesting Atomic masses of 534 u 536 u, u 551, 552 u and 568 u, respectively (Figure 1). These Atomic masses are characteristic of the molecular weight of some carotenoids known in the literature. By analyzing mass spectra (Figure 1 and Table 1), it was clear that in addition to ordinary molecular ion peaks, there were the so-called very intense peaks pseudo-molecular ions. Their high intensity reflected their greater stability. These pseudo-molecular ions peak come from fragmentation of molecular ions whose structures are appropriate (De Rosso *et al.* , 2007a ;De Rosso *et al.* 2007b; AF de Faria *et al.* 2009).

Conclusion

This study has revealed increase in five molecules of carotenoids when the existent infant food flour is enriched with OFSP flour. Flour formulation containing 30% of OFSP lead to an optimum of total antioxidant content (0.469 mg TE / g), and total carotenoid contents (0.8968 mg BCE/g). This formulation with the best energy value (802.526 ± 5.14 kcal / 200g), can be recommended in combating vitamin A deficiency and protein-energy malnutrition in Burkina Faso.

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