GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL 15, NO. 3&4, 2009: 305-313

 ISSN 1118-057

HYGIENIC QUALITY AND PECTIN POLYSACCHARIDE CHARACTERISTICS OF DRIED MANGO PULP CHIPS OF A TOGOLESE VARIETY OF MANGO, *MANGIFERA INDICA* L.

YAOVI AMÉYAPOH, JEAN-PIERRE ELE EKOUNA, PHILIPPE PHEULPIN, JEAN-YVES LEVEAU, COMLAN DE SOUZA, BERNARD COURTOIS AND JOSIANE COURTOIS

(Received 29, August 2008; Revision Accepted 1, July 2009)

ABSTRACT

Hygienic quality of dried mango pulp chips and structural characteristics of pectin polysaccharides of a Togolese variety of mango "mangovi" were studied for check pectin production or other final product.
Results of microbiological analysis showed that mesophilic total microbes, yeasts (*Saccharomyces cerevisiae* and *Candida krusei*) and moulds (*Aspergillus niger, Penicillium spp*. and *Geotricum spp)* were involved in the contamination of the products and could cause spoilage during the storage.
The pectin extraction yields obtained with these products were 1.45 to 1.78 per 100g of weight.
The ^{1}H NMR spectrum of the extract revealed two types of pectin. These pectins were constituted of glucose (26.7%), galactose (15.5%), arabinose (27.2%) and rhamnose (26.2%).
Six uronic acids were also determined, of which two were identified, namely glucuronic acid (31.1%) and galacturonic acid (29. 9%).
Pectins of the dried pulp mango chips contained Na (590 mg/kg), Ca (2650 mg/kg), and Mg (1392 mg/kg).

KEYWORDS: Mango; dried pulp chips, Hygienic quality, Pectin; mineral.

1. INTRODUCTION

It is known that mango (*Mangifera indica* L.) is one of the most flavoured fruit growing throughout the tropics (Iagher *et a*l*.,* 2002). There is a high production of mango during the harvest time in Togo (West Africa). Therefore, seasonal post harvest losses of this fruit are high in the tropics due to environmental heat, moisture levels and lack of appropriate systems of preservation and processing. These losses represent up to 25% of mango production per year (M.A.E.P, 1999).

Fresh sliced mango treated with aqueous solutions from 0.5% to 2.0% chitosan was proposed to prolong the shelf life of mango pulp (Chien *et al.,* 2007); but this process needs a storage at 6°C (in refrigerator). It will become more expensive for most of African population.

Alcoholic fermentation by improved strain of *Saccharomyces cerevisiae* of this mango's juice showed in the end of processing an important residual sugar determined by refractometer. This fermentation has been limited by finish of fermentable sugar (Ameyapoh *et al.,* 2006). However, most studies of exploitation of mango peels have been dealing with their use as a source of pectin, which is considered a high quality dietary fibre (Berardini *et al.,* 2005). Various studies (Kratchanova *et al*., 1991 ; Iagher *et al*., 2002 ; Bedouet *et al*., 2006; Chien *et al.*, 2007) performed on structural characterisation of pectin were used following analytical methods/ GC-MS analysis, IR and ^{1}HNMR and ^{13}NMR measurements *and* Size-exclusion chromatography analyses.

The molecular mass of the mango pectin ranged from 72,000 to 83,000 and the gel strength was between 162 and 232° according to Kratchanova *et al.* (1991) Pectin, an acidic polysaccharide, is found in high concentrations in mango pulp (Ollé, *et al.,* 2000). Polysaccharides are of industrial importance in the manufacture, distribution, storage and consumption of food products (Iagher *et al.,* 2002).

It was shown that *Mangifera indica* cv Alphonso

Yaovi Améyapoh, Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées Alimentaires, Ecole Supérieure des Techniques Biologiques et Alimentaires, Université de Lomé, BP1515 Lomé, Togo
Jean-Pierre Ele Ekouna, Laboratoire des Glucides, EPMV CNRS FRE 2779, IUT d'Amiens, Département de Génie Biologique, Avenue des Facultés, Le Bailly F-80025, France.
Philippe Pheulpin, Laboratoire des Glucides, EPMV CNRS FRE 2779, IUT d'Amiens, Département de Génie Biologique, Avenue des Facultés, Le Bailly F-80025, France.
Jean-Yves Leveau, Laboratoire de Microbiologie Industrielle, ENSIA, 1 Av. des Olympiades, 91744-Massy cedex France.
Comlan de Souza, Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées Alimentaires, Ecole Supérieure des Techniques Biologiques et Alimentaires, Université de Lomé, BP1515 Lomé, Togo
Bernard Courtois, Laboratoire des Glucides, EPMV CNRS FRE 2779, IUT d'Amiens, Département de Génie Biologique, Avenue des Facultés, Le Bailly F-80025, France.
Josiane Courtois. Laboratoire des Glucides, EPMV CNRS FRE 2779, IUT d'Amiens, Département de Génie Biologique, Avenue des Facultés, Le Bailly F-80025, France.

galactan/galacturonan backbone, which is occasionally involved in side chain branches consisting of single residues of galactose and arabinose or oligomeric 1,5-linked arabinofuranose residues linked through 1,3-linkages. Xyloglycan type having 1,4 linked glucan backbone with branching by non-reducing terminal arabinose and xylose residues were also identified on the hemicellulosic fraction of unripe mango (Yashoda *et al.,* 2005).

The 1,4- linked α-D- galacturonic acid residues in either free acid (salt) or methyl-ester form were determined in the mango pulp pectin (Iagher *et al.,* 2002). The ranges of the galacturonic acid (40-70%), arabinose (2-4%), rhamnose (1-2%), xylose (1-7%), mannose (1-3%), galactose (14-22%) and glucose (8-22%) were estimated for two Guinean mango varieties, Ceni and Springfield (Kratchanova *et al.,* 1991).

The aim of this study was to appreciate the hygienic quality of dried mango pulp chips and to determine the structural characteristics of pectin polysaccharides of a Togolese variety of mango "mangovi" for it valorisation by pectin production or other final product.

2. MATERIALS AND METHODS

2.1. Plant material

Mango (*Mangifera indica* L.) selected for this study was a local variety (mangovi) cultivated in Togo, Republic of Benin and Ghana in West Africa. Mature green mango fruits were freshly harvest from backyards and markets in Lomé (Togo) and selected for their absence of defects, firmness, and uniformity of size, symmetry and yellow green color. The middleweights of the fruits were 205.70 ± 22.50g.

The production flow charts for dried mango pulp chips is shown in figure 1, with the following nine steps: 1) mango ripening, 2) washing, 3) peeling in slices with a stainless steel knife, 4) cutting, 5) pounding, 6) bleaching, 7) drying in oven at 70°C for 240 min, 8) putting in plastic packet, 9) storage. Water chlorinated (1 mg / liter) during 5 min was used in the second step.

From 60 kg of fresh mango pulp, 13.5 kg of dried mango pulp chips were collected, the equivalent of 270 samples of 50g (yield = 23%). The mango pulp chips have 6cm length, 4 to 5cm width and 0.3 to 0.4cm thickness. One hundred thirty five samples of mango pulp chips were produced with electric oven in the laboratory (University of Lomé in Togo, Laboratory of Biochemistry and Food Technology).

Seventy five samples of dried mango pulp chips were collected for analysis for microbiological and physicochemical parameters, out of which 25 samples were used for pectin extraction.

2.2. Microbiological analysis

Dried mango pulp chips samples setting in packet were collected. Samples were aseptically collected with a sterile spoon in sterile bags and stored at 3°C +/- 1°C in a cooler and analyzed 2 hours later. Mango fruit samples were also collected and stored similarly.

Gram-positive and gram-negative bacteria, including *Bacillus*, coliform, thermotolerant coliform, *Escherichia coli*, anaerobic sulfite-reducing bacteria, thermophilic sporeforming bacteria, and yeasts and moulds were isolated and enumerated from the raw material and the dried mango pulp chips produced. Samples were ground and suspended in tryptone-salt water (0.85% w/v). The resulting suspensions were diluted from 10^0 to 10^4 in the same tryptone-salt water. The previously mentioned microbial genera, species, groups were enumerated in duplicate on decimal dilutions using respectively the following media: Plate count agar (Diagnostics-Pasteur, France), Tryptone Sulfite Neomycin (Diagnostics-Pasteur, France), Yeast Glucose Chloramphenicol (Bio-Rad France), The enumeration results of these microorganisms are given in colony forming units per gram of wet weight product (cfu/g) (Ameyapoh *et al.,* 2007).

Coliform and thermotolerant coliform (formerly referred to as fecal coliform) were determined by the Most Probable Number (MPN) using the Mackenzie test according to Guiraud and Galzy (1980). Broths used in this case were bromocresol purple lactose (Diagnostics-Pasteur, France), Brilliant green bile (2%) (Bio-Rad France) and Peptone water (Diagnostics-Pasteur, France). The cultures were incubated at 30°C to isolate coliform and yeasts and moulds; at 37°C for Bacillus and 44°C for thermotolerant coliform and anaerobic sulfite reducing bacteria.

The values of the enumeration range and the frequency distribution were chosen according to microbiological limits of French Association of Normalization (AFNOR) (BOCC du 21/12/1978) (Leyral and Vierling, 2001).

Moulds have been identified by macroscopic observation of the isolated colonies followed by microscopic observation of the fungal organs after coloration to lactophenol cotton blue (Ameyapoh *et al.,* 2007).

2.3. Chemical analyses

2.3.1. Ascorbic acid, water activity and pH determination

The following parameters were evaluated: pH, moisture content on a dry weight basis water activity (aw) by the cryometric method (Ameyapoh *et al*, 2007), and ascorbic acid (Vitamin C) by the 2-6-dichlorophenolindophenol method (AOAC, 1984).

2.3.2. Characterization of pectin polysaccharides of « mangovi »

2.3.2.1. Extraction of the pectins

Dried mango pulp chips (5g) of each sample were powdered and ground to powder in 50mM sodium acetate buffer, pH 4.5 (200 ml) for 1 min at 4°C using a liquidiser Janke & Kunkel (France), model Ultra-Turrax T25. After filtration, addition of 50mM HCl, washing in chloroform/methanol solution (v/v), and centrifugation (10 min, 12,000g, 15°C), the supernatant obtained was mixed with 3 volumes of ethanol and incubated overnight at 4°C. The pectin collected after centrifugation were solubilised in water and the solution was kept at -20°C until use (Bédouet *et al.,* 2006).

2.3.2.2. Analytical methods

Neutral carbohydrates and uronic acids were determined by resorcinol and meta-hydroxydiphenylphenol methods respectively (Iagher *et al.,* 2002). L-arabinose (Sigma USA) and D-galactose

ASigma USA) were used as standards. The uronic acids concentration was determined with m-HBP test. This assay performance determined neutral carbohydrates and uronic acids content with 5 and 8% errors which were considered in the calculation (Bédouet *et al.,* 2006).

Size fractions of pectins was performed with low pressure size-exclusion chromatography by adaptation of the method used by Iagher *et al.* (2002). Glass column (100x1 cm) containing Sephacryl S-400 gel (Pharmacia France) was used at flow rate of 6 ml/h in 50 mM sodium acetate buffer, pH 5,5% ethanol. The exclusion and inclusion volumes were estimated using amylopectin and galactose A, respectively. The yields of recovery of pectins following the chromatography separations were around 75%.

Total proteins content was estimated by Bradford's method, using bovine serum albumin as standard (Zor and Selinger, 1996; Prasanna et al., 2006).

Determination of sugars in pectins was performed by gas chromatography (GC) according to the method adapted from (Quemener and Thibault, 1990**;** Levigne *et al.,* 2004).

Pectins were digested with pectinase (0.1 U/mg of pectin) for 24 h in 50 mM pyridine acetate, pH 5 at 37°C. The product was lyophilised, and methanolyzed in 0.5 M HCl (Methanolic instant kit, Alltech France). Afterwards the assay was performed onto the hydrolysates for 10 h at 80°C with mesoinositol (Sigma France) as internal standard. The methanol/HCl solution was evaporated under argon and O-methyl-glycosides were derived at 4°C in pyridine with BSTFA/1% TMCS solution (Alltech). Resulting solutions were injected in splitless mode on a HP1 capillary column (25 m x 320µm) of a HP 6890 gas chromatograph (Hewlett Packard France) at 250 °C injector and detector temperature with oven temperature, 120 °C. Speed of nitrogen, carrier gas, was 0.9 ml/ mn.

The purification of pectin components was performed by HPLC P680 Hplc pump1 ASI-100 automated sample injector (Chrompack, France) with analytical column TSK gel G 2000 SW (7.5 mm x 30 cm, 10µm). Spectral detection was performed by UVD 340 U using UV 166 Beckman system Gold.

^{1}H NMR spectroscopy was performed with a Bruker AC-300 spectrometer at frequency of 300 MHz, with complete proton decoupling at 80 °C, using D_2O (Sigma France) as solvent of dry pectins at 10 mg/ml. The spectral window was 3000 Hz for 8 k data point with a pulse of 7 µs, an acquisition time of 1.36s and a relaxation delay of 1.0s. A number of 256 scans were recorded.

2.3.3. Minerals detection:

Minerals detection was achieved by atomic absorption method (Larrauri *et al.,* 1996). A dry sample was incinerated in a furnace at 525°C for 8 h. The residue was dissolved in HNO_3 with 50 g/L of $LaCL_3$ to determine Ca, Mg and Na in a Perkin Elmer model 5100 PC atomic spectrophotometer.

All chemical analyses were performed in triplicate and results were given on a dry weight basis.

3. RESULTS AND DISCUSSION

3.1. Microbiological analysis

Table 1 displays the results of the microbiological analysis of the fruit of the local mango "mangovi" and the dried mango pulp chips.

Sanitation of these products was evaluated in samples collected from five lots of the same product five times. Bacteria, yeasts and moulds were isolated, enumerated and identified. Fresh mango was contaminated by aerobic mesophilic bacteria, coliforms, yeasts and moulds with respective middle values of 63.0x 10^3 ufc/g, 23.5x 10^2 ufc/g, 31.0x 10^3 ufc/g and 7.5 ufc/g. Neither gram-negative bacteria, anaerobic sulfite reducing bacteria nor thermophilic sporeforming bacteria**,** *Staphylococcus aureus* and *Salmonella sp*. were detected in these products.

The dried mango pulp chips produced only contained total germs (5.6x 10^{+1} ufc/). The percentage of aerobic mesophilic bacteria concentration reduction was 97.6% from the raw material to dried mango pulp chips. These dried mango pulp chips contained spores of three moulds species: *Aspergilus niger* (78%)*, Penicillium spp* (13%)*, Geotricum spp* (9%).

Two types of microorganisms (moulds and bacteria) could be involved in spoilage during the storage.

The presence of mould spore in the final product indicated that the pasteurization condition was insufficient or the couple temperature - time (70°C / 12 h) could be used for a better hygienic production. However the environmental sanitation of the production as putting in plastic packet step was not appropriate and should be controlled to avoid an ulterior contamination and deterioration of the product during its preservation. The processing using ultraviolet (UV-C) light could be also added to thermal treatment of the product to reduce the number of microorganisms and to limit toxin risks (Keyser *et al.,* 2007).

Table 1: Hygienic quality of the traditional production line of mango pulp chips

Products studied	Microorganism concentration (CFU/g wet weight).						
	Total mesophilic microbes	Total coliform	Thermo-tolerant coliform(44°C)	Anaerobic sulfite reducing bacteria	Salmonella	*Yeasts	**Moulds
Fresh ripening mango	$63 \times 10^{+3} \pm 2.05 \times 10^{+2}$	$23.5 \times 10^{+2} \pm 9.2 \times 10^{+1}$	MDL	MDL	MDL	$31 \times 10^{+3} \pm 5 \times 10^{+1}$	7.5 ± 5.2
Dried mango pulp chips	$5.6 \times 10^{+1} \pm 1.8 \times 10^{+1}$	MDL	MDL	MDL	MDL	7.75 ± 2.1	6.3 ± 1.5

* Yeasts identified were: *Saccharomyces cerevisiae* and *Candida krusei*.
** Moulds identified were: *Aspergillus niger, Penicillium spp*. and *Geotricum spp*
Mean value of 5 samples (X) ± standard error.
MDL = Minimum detection limit. $\varepsilon = 2.0 \times 10^{+1}$ cfu/g

3.2. Ascorbic acid, water activity and pH determination

Results of the determination of physicochemical characteristics of “mangovi” pulp ships showed that its middle pH was 4.5, the water content value was 15% with 0.47 for water activity (aw). Total glucides of the dried pulp ships were 35% and ascorbic acid content was 46% (w/w).

3.3. Characteristics of « mangovi » pectins

Figures 2, 3 and 4 shows pectin component values of “mangovi” mango. The pectin extraction yields obtained from “mangovi” mango were 1.45 to 1.78 per 100g of weights. These results were similar at those obtained by El Zoghbi (1994) for mango variety “zebda” (0.66 to 1.50g per 100g) and for mango variety “baladi” (0.47 to 1.33g per 100g) in Egypt.

The ^{1}H NMR spectrum of the extract reveals that two types of pectin occurs mango variety: the first one represents 95.27% and the second 2.74%. The pulp of that mango is also characteristic of the low content in polyphenols (0.3). These pectins comprise following four (04) monosaccharides: glucose (26.7%), galactose (15.5%), arabinose (27.2%) and rhamnose (26.2%).

Six (06) uronic acids were also determined, of which two have been identified. These are glucuronic acid (31.1%) and galacturonic acid (29.9%). The glucuronic acid and the galacturonic acid represent 66% of the total uronic acids of these pectins. The four others have contents within 3% and 15%. The glucose ratio on glucuronic acid and the galactose ratio on galacturonic acid were respectively 0.74 and 0.52. These ratios showed that there are more uronic acids than their corresponding neutral carbohydrates.

“Mangovi” mango contained more arabinose than rhamnose while these two monosaccharides had low amount in the two varieties of the Guinea mangoes called “Ceni” and “Springfield” with contents in arabinose of (2 – 4%) and in rhamnose of (1 – 2%) (Kratchanova et *al*., 1991).

The xylose and mannose found again by the same authors have not been identified in the pulp of “mangovi”. The arabinose ratio on galactose (1.75) t we found was higher than that found by Yashoda *et al.* (2005) which was of 1.38 for the “Alphonso” mango at the mature green state.

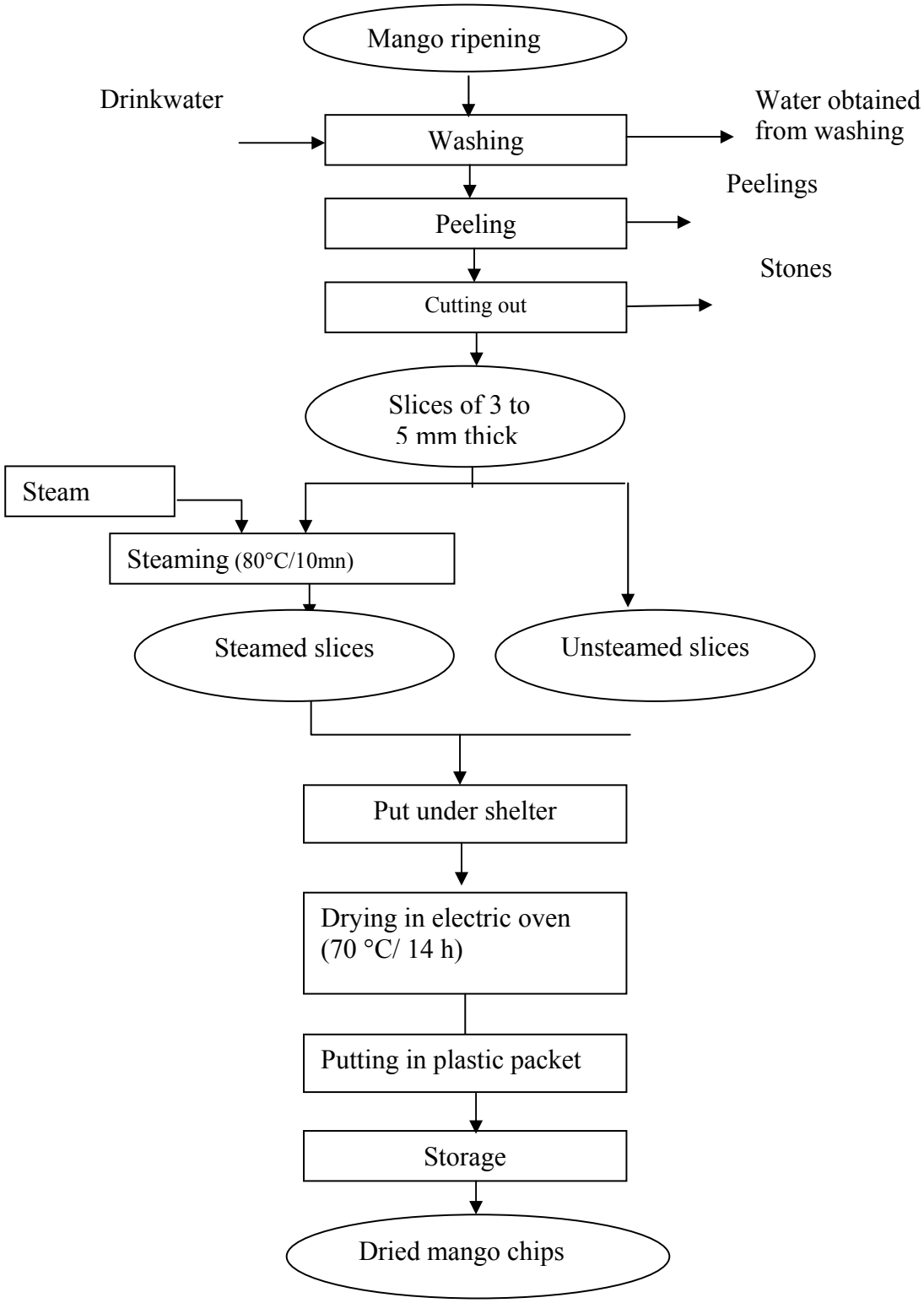
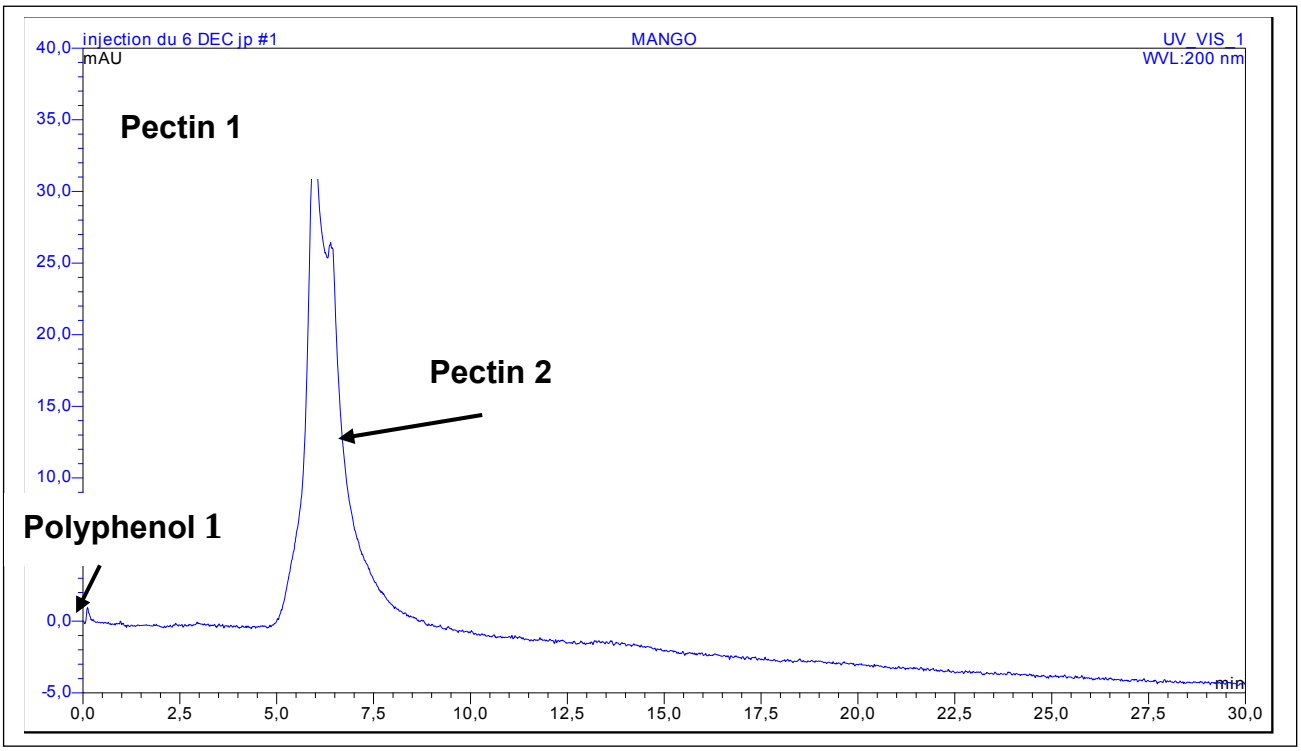


Figure 1: Flow charts showing the production of dried mango pulp chips

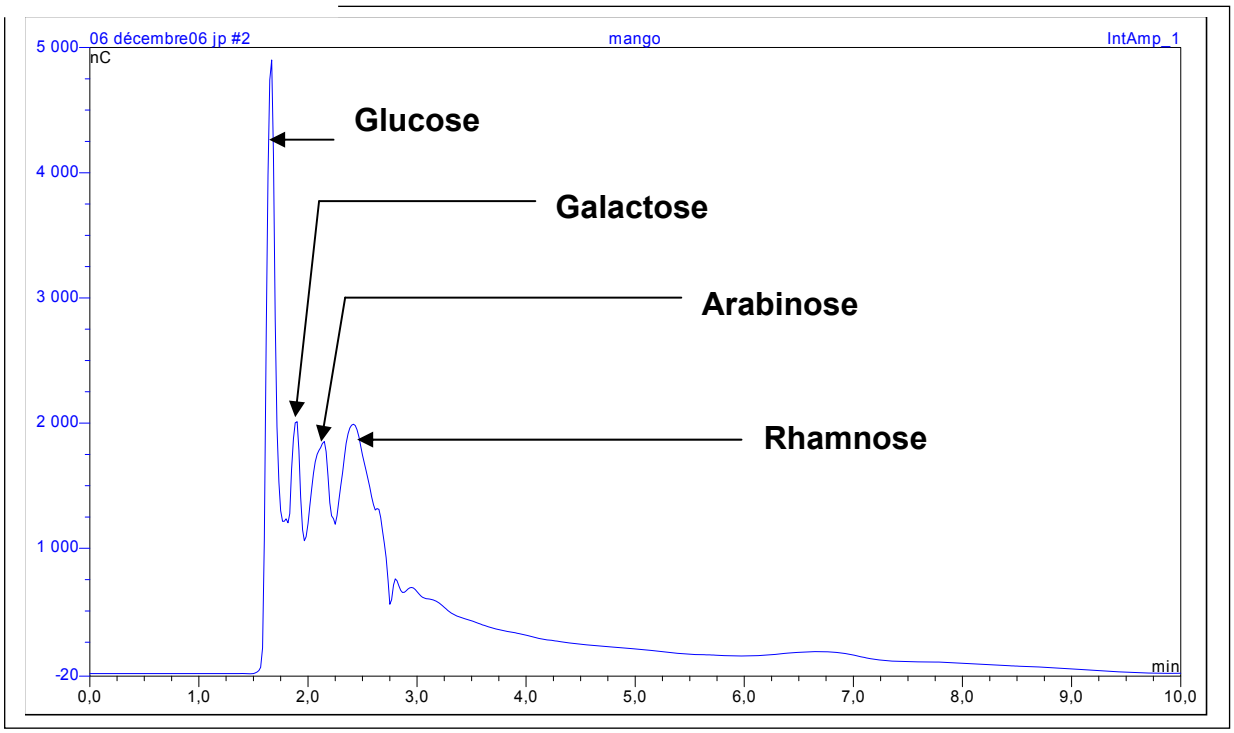
Relative area (%)



Retention time (min)

Figure 2 : Characteristics of « mangovi » pectins by Nuclear Magnetic Resonance

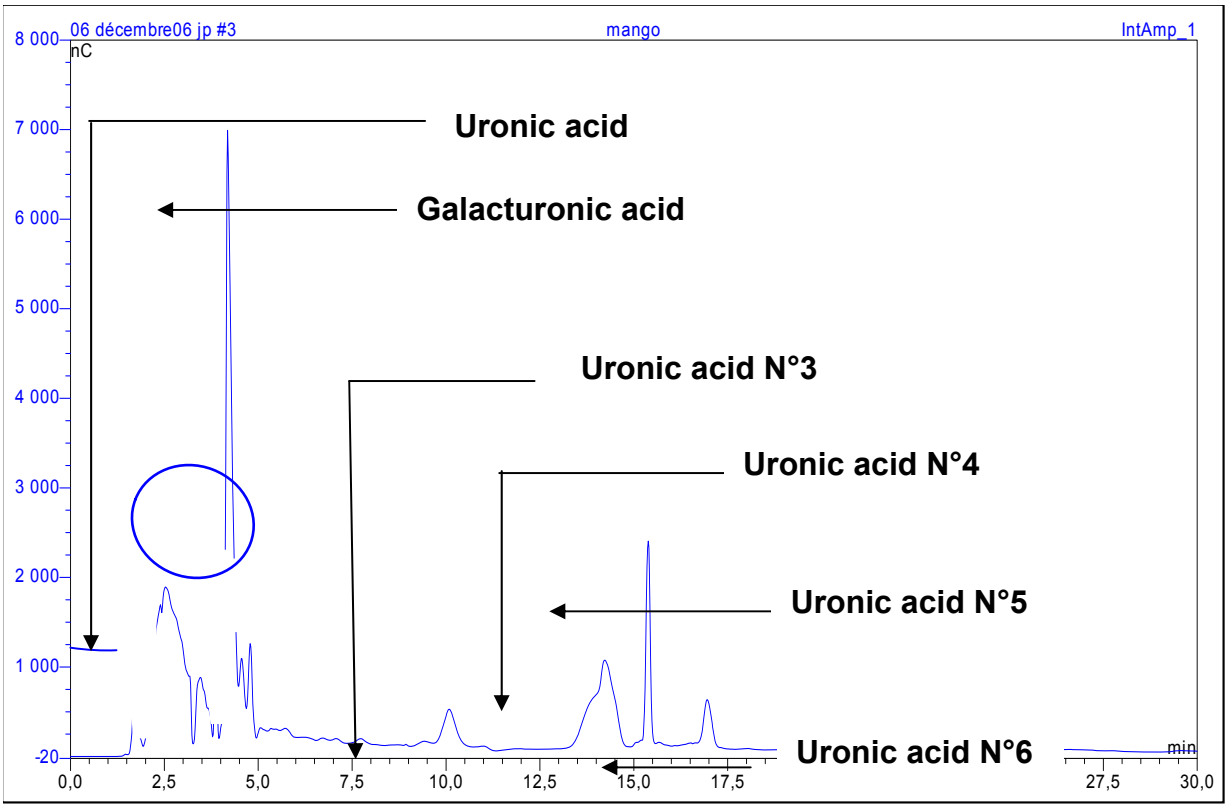
Relative area (%)



Retention time (min)

Figure 3: Neutral carbohydrates composition of «mangovi» pectins

Relative area (%)



Retention time (min)

Figure 4: Uronic acids composition of «mangovi» pectins

3.4. Minerals detected

Pectins of the dried pulp mango chips contained Na, Ca and Mg (table 2). These contents of Na, Ca and Mg are comparable to values reported by Larrauri *et al.* (1996) for mango pellets. However, results of dried pulp mango chips mineral components showed that its Mg^{2+} content was raised in relation to that of the mango peel.

Table 2: Mineral content of dried pulp mango chips

Mineral	Concentration (mg/kg)[a]	
	$\overline{X}$	S_X
Na	590	40
Ca	2650	48
Mg	1392	31

[a] Mean value ($\overline{X}$ ıd standard deviation (S_X) of three determinations

CONCLUSION:

Dried pulp mango chips of the Togolese local variety “mangovi” may be used as a food for the recovery of functional compounds. It may be considered as a source of food ingredients and fruit dietary product needed for health-promoting. Two types of microorganisms (moulds and bacteria) could be involved in the spoilage of product in storage. Treatment with ultraviolet (UV-C) light could be added to thermal treatment of the product to reduce the number of microorganisms and their toxin products.

REFERENCES

Ameyapoh Y.,Wokpor K.,de Souza C., 2006. I dentification et sélection des souches de levures performantes pour la production d'alcool. Journal des Sciences, 1, 30-40.

Ameyapoh Y., de Souza C., and Traore A. S., 2007. Hygienic quality of traditional processing and stability of tomato (*Lycopersicon esculentum*) puree in Togo. In: Bioresource Technology, 99, 5798-5803.

AOAC, 1984. Official Methods of Analysis of the Association of Analystical Chemists. 14è edition, Washington, DC., USA.

Berardini N., Knödel M., Schieber A., and Carle R., 2005. Utilization of mango peels as a source of pectin and polyphenolics. Innovative Food Science and Emerging Technologies, 6, 442-452.

Bedouet L, Courtois B, and Courtois J, 2006. Rapid quantification of *O*-acetyl and *O*-methyl residues in pectin extracts: Carbohydrate Research, 338, 379–383.

Chien P., Sheu F., and Yang F., 2007. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. Journal of Food Engineering 78, 225-229.

El Zoghbi, 1993. Biochemical changes in some tropical fruits during ripening, Food Chemistry, 49, 33-37.

Guiraud and Galzy, 1980. L'analyse microbiologique dans les industries agroalimentaires. (Collection génie-Alimentaire). Les éditions de l'Usine Nouvelle, 349.

Iagher F., Reicher F., and Ganter, J. L. M. S., 2002. Structural and rheological properties of polysaccharides from mango (*Mangifera indica* L.) pulp. International Journal of Biological Macromolecules, 31, 9-17.

Keyser M., Muller I. A., Cilliers F. P., Nel W., and Gouws P. A., 2007. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innovative Food Science and Emerging Technologies, in press.

Kratchanova, M., Bénémou, C., and Kratchanow, C., 1991. On the pectic substances of mango fruits. Carbohydrate Polymers, 34, 597-282.

Larrauri J. A., Rupez P.,Borroto B. and Saura-Calixto F., 1996. Mango peels as a new tropical fibre: preparation and caracterization. Lebensmittel-Wissenschaft und-Technologie, 29, 729-733.

Levigne, S., Ralet, M.-C., Quemener, B., and Thibault, J.-F., 2004. Isolation of diferulic bridges ester-linked to arabinan in sugar beet cell walls. Carbohydrate Research 339, 2315–2319.

Leyral and Vierling, 2001. Microbiologie et toxicologie des aliments : Hygiene et sécurité alimentaires. Biosciences et Techniques, Serie Sciences des aliments,3^{e} éd.CRDP Bordeaux France.

M.A.E.P, 1999. Stratégie de croissance du secteur rural. Analyse économique des options stratégiques et des sources de croissance. Ministère de l'Agriculture, de l'Elevage et de la Pêche. Togo.

Ollé D., Baron A., Lozano Y. F., and Brillouet J. M., 2000. Enzymatic degradation of cell wall polysaccharides from mango (*Mangifera indica L.*) puree. Agric Food Chem, 48, 2713.

Prasanna V., Prabha T. N., and Tharanathan R. N., 2006. Multiple forms of polygalacturonase from mango (*Mangifera indica* L. cv Alphonso) fruit. Food Chemistry 95, 30-36.

Quemener, B and Thibault, J.-F, 1990. Assessment of methanolysis for the determination of sugars in pectins. Carbohydrate Research 206, 277–287.

Yashoda H. M., Prabha T. N., and Tharanathan R. N.J., 2005. Mango ripening-chemical and structural characterization of pectic and hemicellulosic polysaccharides In Sci. Food Agric., communicated, 340, 1335-1342.

Zor T., Selinger Z., 1996. Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. Analytical Biochemistry, 236, 302-308.