Obtaining a Concentrated Fresh Product of *Capsicum Annuum* by Reverse Osmosis Process and Analysis of Its Bioactive Constituents and Mineral Composition

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Abstract: The aim of this work was to evaluate the quality of fresh extract product of bell pepper *Capsicum annuum* obtained by reverse osmosis in terms of bioactive compounds and mineral composition. Quantitative analysis of this product showed the presence of total alkaloids, total polyphenols and vitamin C at contents of 14.42 ± 0.23 %, 69.65 ± 0.43 g/LGA equivalent and 157.48 mg/100g, respectively, indicating the preservation of these bioactive compounds after the application of this membrane technology. Regarding mineral composition, the contents were estimated to 61.68±0.41, 16.66±2.66, 20.69±5.31 and 11.52±0.7 mg/100g, respectively for potassium, magnesium, calcium and iron, also indicating the preservation of these minerals in the fresh concentrated product.

The bioactive compounds of fresh concentrated product were analyzed using Gas chromatography-mass spectrometry (GC-MS). Thirty four compounds were identified of which 4-Hydroxyphenylethanol. di-TMS; Benzoic acid 4-methoxy-3-(trimethylsilyl) oxy-. methyl ester; Bis(trimethylsilyl)isovanillate. Benzoic acid. 3.4-bis[(trimethylsilyl)oxy]-. trimethylsilyl ester. Vanillylpropionic acid bis(trimethylsilyl) - are phenolic compounds and cis-4-Trimethylsilyloxy-cyclohexyl (trimethylsilyl) carboxylate is one derived fatty acid. The presence of these various bioactive compounds in the fresh concentrated product demonstrated that the application of membrane technology by reverse osmosis could constitute a good alternative for obtaining the viable finished product of Capsicum fruits.

Keywords: Bell pepper, Capsainoids, GC-MS. membrane technology, Phenolic compounds.

INTRODUCTION

Due its high content of vitamin C (ascorbic acid), pro-vitamin A (carotene) and calcium, bell pepper (*Capsicum annuum* L.) is one of the most consumed vegetable worldwide [1, 2]. It constitutes an important tropical crop, not only because of its economic value, but also for the combination of color, taste and nutritional values of its fruit [3, 4]. In addition, it is well-known that the interest in the consumption of pepper fruits is to a large extent related to its content of bioactive compounds and their importance as dietary antioxidants [3, 5]. In West Africa, fruits of Capsicum are essential in the confection some local dishes [6], particularly in Côte d'Ivoire in elaboration of very spicy soups such as "kédjénou" and "biokesseu" [4].

To date, research on pepper fruits has shown that alkaloids, in particular capsaicinoids have a wide variety of biological and physiological activities which provide them functions such as antioxidants [5, 7], anticarcinogenics [8], promotion of energy metabolism and suppression of fat accumulation [9] and anti-inflammatories [9, 10].

Many reports have suggested that there is an association between the consumption of bioactive compounds and the prevention of some diseases [8, 11, 12]. Evidence for such an association has increased interest in the behavior of bioactive compounds during various industrial processes. Thus, in addition to increasing nutritional and pharmacological interest in phenolic compounds, attention to technological aspects of food production has also been growing [13-15].

In despite its rich constitution of course, the pepper is not available on all year because it is perishable and the technologies of transformation and preservation are limited [16, 17]. In Côte d'Ivoire, according to Tano *et al.* [18], post-harvest losses in pepper carriage are a

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major bane for producers and sellers preservation of the pepper is traditionally accomplished by solar drying which can result in loss of volatile aromatic components freshness and vitamins, as well as in a reduction of the antioxidant properties provided by its content of phenolic compounds. Besides, peppers powder sold on the market contains impurities and additives non-conventional.

Reverse osmosis is a membrane separation process in which a hydraulic pressure that is greater than the osmotic pressure of the solution is applied so that water permeates from a high to low solute concentration [19]. It has been pointed out as an alternative technique to thermal concentration. It can be used to concentrate fruit juices at room temperature without any changes in the physical structure of water, hence minimizing the damages caused by the utilization of heat. In addition, the costs of processing and energy are lower, and high quality products are obtained due to the maintenance of the aroma and flavor compounds and those responsible for nutritional characteristics [20]. The use of reverse osmosis in the concentration of many fruit juices has been carried out and the results are very promising. Thus, concentrated fruit juices of good quality with soluble solids content were obtained [21-23]. This process can be applied to concentrate pepper fruits reducing the damage caused by solar drying and resulting in superior maintenance of their nutritional and sensory characteristics.

The aim of this work was to achieve a fresh concentrated product of pepper Capsicum by the reverse osmosis process and to evaluate its quality in terms of bioactive constituents and mineral composition.

MATERIAL AND METHODS

Plant Material

Fresh *Capsicum annuum* fruits were collected at the beginning of the rainy season (May-June 2013), in the central part of Côte d'Ivoire. The collected peppers were sent in LAPISEN laboratory (INPHB Yamoussoukro, Côte d'Ivoire), where they were carefully washed before being use.

Extraction Procedures

The maceration and bioprocesses membrane separation was carried out according Adjé *et al*. [24]. Fresh fruits were carefully washed and crushed. Weighted aliquots of fresh plant material were macerated into volumes to obtain plant/liter ratio (5kg/200L). The maceration was slightly stirred to wet completely the crushed material and let for 3 h maceration time, at room temperature. The crude extract was successively pre-filtered through a fine sieve (pore diameter of 1 mm) and a nylon fabric lower porosity (pore diameter = 25µm). Crude extract (CE) is obtained.

Clarification of Crude Extract

The clarification of crude extract was conducted in the microfiltration (MF) unit featured a ceramic multichannel membrane (P19-40, France) that had a total effective filtration area of 0.24 m^2.and an average pore diameter of 0.2 µm. Transmembrane pressure for feeding is set at 1.2 bar, and the CE was clarified against a transmembrane pressure of 0.6 bar. All trials were carried out with continuous crude product feed and permeate collection at flow. The feed-and-bleed procedure was also followed in the long term trials by implementing continuous extraction of retentate at flow. Thus, the clarified extract is obtained.

Concentration Procedure

Reverse osmosis (RO) was performed with a composite polymer membrane of type SW 30-2540 (Filmtec) from Polymem (France). The characteristics of which were effective membrane area of 2.5 m^2. The operation conditions were 40 bar of transmembrane pressure and temperature of 30°C. The extract circulated inside the fibers. The loop was continuously fed with cold clarified extract (20°C). When the total soluble solid content set up (TSS_{final}) was reached. Concentrate was extracted at rate VCF_t (VCF_t; the volumetric concentration factor is defined as the ratio between the initial volume and the concentrate volume at any time) which was calculated according to equation (1)**:**

$$VCF = V_i / V_C = V_i / (V_i - V_w) \quad (1)$$

where V_i is the initial volume permeated during a determined time t. V_C is concentrate volume and V_w is permeate volume.

Analytical Methods

Samples of crude extract (CE) and concentrate (C) were analyzed with respect to the following physical and chemical parameters. pH and total soluble solids content [25] were measured respectively with pH-meter

Testo 230 type 4 (Testo, France) and Abbe refractometer Leica infra-red (Barloworld, USA).

The method of AOAC [26] was used to determine minerals. Samples of freeze-dried extracts previously were calcite at 600 °C for 5 hours and were wet digested with concentrated nitric and percholoric acids. The minerals calcium (Ca), Potassium (K), iron, Phosphorus (P) and magnesium (Mg) were determined by atomic absorption spectrophotometer (VARIAN AA20 Techtron Pty. Ltd, Australia)).

Vitamin C content was determined according Durust *et al*. method [27]. Samples were ground with oxalic acid at 0.4% in a ratio of 1:10 w/v and put in a dark room for 20 min before its centrifugation at 660 rpm. Later, 1 ml of the supernatant was mixed with sodium acetate buffer solution and a 2, 6-dichlorophenol indophenol solution. Then, absorbance of the solution was measured by spectrophotometer at a wavelength of 520 nm, and the vitamin C was calculated on the basis of an adjusted calibration curve of L-ascorbic acid standard (99% purity; Reg. 84272 Sigma, St. Louis, Missouri, USA). The estimated concentration of vitamin C was reported as ascorbic acid mg 100 g^{-1} fresh weight.

Total extracted polyphenol content was determined according to the Folin-Ciocalteu method reported by Singleton and Rossi [28] and modified by Wood *et al*. [29]. To 30 µl sample extract. 2.5 ml of diluted Folin-Ciocalteu's phenol reagent (1/10) were added. The mixture was kept for 2 min in the dark at room temperature and 2 ml of calcium carbonate solution (75 $g.L^{-1}$) were added. The mixture was heated at 50°C for 15 min then cooled down. The absorbance was measured at 760 nm against water as blank. Analyses were performed in triplicate. Total polyphenols content was quantified as gallic acid equivalent per liter of extract equivalent Gallic acid (g/L Gallic acid Equivalent).

Total extracted Alkaloids content was determined according to method AOAC [30]. A weight of 5g of freeze-dried extracts previously seed were ground in an ethanol solution containing acetic acid diluted to 10% (1:10 v/v).The mixture was shaken all the 30 min during 4 hours and filtered on paper Whatman. The filtrate obtained was evaporating in a water bath at 100°C until a quarter of the initial volume. Ammonia concentrated drop was added to filtrate to precipice alkaloids. The extracts were filtered using Whatman filter paper previously weighed. This filter paper was dried (60°C), cooled in the desiccate and weighed. Total alkaloid content expressed as percentages (%) was calculated according to equation (2):

$$\% \text{ Alkaloids} = (\text{mass of alkaloids}) / (\text{mass of samples}) \quad (2)$$

GC-MS analytical conditions of the concentrate were as follows:

Hydrolysis

Within a three necked round bottom flask containing aqueous methanol (25ml, 95%) and 25ml of hydrochloric acid (3M). was added to 0.5g of freeze-dried sample. The mixture was refluxed in a water bath at 90°C for 2 h and cooled. In separator funnel containing the mixture, ethyl acetate (3x50ml) was then added to recover the aglycones of O-glycosyl compounds. Anhydrous ($MgSO_4$) was added to remove moisture, filtered and evaporated of the solvent.

Silylation

In general, the hydrolysis extract contain groups (phenols, alcohols and carboxylic acids) which can be derivatised to improve the chromatographic properties and separation on the GC-column. The most common derivatisation procedure of compounds containing –OH and – COOH groups is silylation. Among the many possibilities of silylating agents. derivatisation experiments were performed by considering BSTFA. For the silylation procedure, a mixture of N.O-bis (trimethylsilyl) trifluoroacetamide BSTFA (0.5 ml) and dichloromethane (0.5 ml) were added to 10 mg of residue (extract with ethyl acetate after hydrolysis). The mixture was placed in a water bath at 70°C for 1hour.

Injection

The silylated samples were injected into a GC-MS system (Shimadzu- QP2010SE) consisted of a gas chromatograph coupled with a mass spectrometer (quadrupole) in the EI (Electron Impact) brand fitted with a split / split less injector. A capillary column low-bleed Zebron ZB-WAX (20m x 0.18µm) was used. The flow rate of carrier gas (helium) was maintained at 0.9 $ml.min^{-1}$. The injector temperature was 280°C. The own temperature increased from 70 to 270 °C with 4 °C/min and then held to 270°C for 20 min. The detector temperature was 290°C. Identification of components was based on comparison of their mass spectra with those of Wiley and NIST libraries and those described

by Adams [31] as well as on comparison of their retention indices with literature [32].

RESULTS AND DISCUSSION

pH, Soluble Solids, Contents of Some Bioactive Constituents in Capsicum Fruits Crude Extract and Fresh Concentrated Product

Processing of fresh *Capsicum annuum* fruit on a pilot plant scale provided two co-products: crude extract (CE) and concentrate (C). The most relevant physicochemical properties of CE, and C reported in Table **1.** The concentration factor (CF) values (38 for alkaloids; 7 for polyphenols) indicated that both families were concentrated. Total soluble solids content was higher in the concentrate (5.2^{o}Brix) than in crude extract (0.8^{o}Brix). As already mentioned, this observation is probably related to the presence of high suspended solids content in the finished products C that can interfere with the measurement of the refractive index [33]. The clarification process completely removed the suspended pulp in the permeated extract, as it was already observed by Matta *et al*. [34]. pH of the CE and the C were 6.2 and 5.6, respectively. Generally, in the literature, it indicated that pH of pepper crude extracts increased with levels of ripening and ranged from 4.25 to 5.73 [35]. As expected, the concentration of pepper extract by reverse osmosis increased pH value due to the removal of water that increased concentration of organic acids. All the processes were finished when the concentration factor was equal to 71 (Vi = 199 liters. VC = 2.8 liters).

The phytochemical quantitative analysis of the crude extract (CE) and concentrated extract (C) of Capsicum annuum fruit showed that both extracts contain alkaloids and polyphenols (Table **1**). This is in agreement with the findings of several authors [36; 37; 38]. Indeed, findings of these authors revealed the presence of alkaloids which are responsible for the pungency of the Capsicum species. Total alkaloids content was 14.42 ± 0.23g/100 and 0.38 ± 0.14g/100 gram by liter of fresh material in concentrate and in crude extract respectively. From these results, it was noted that membrane process has significantly increased concentration of alkaloids in the extract. Accordingly, membrane process by reverse osmosis allowed preserving the alkaloids in pepper extract whilst it well established that method of preservation of dry treatment causes a considerable degradation of certain alkaloids such as capsaicinoids [39]. In addition, comparison of the value of alkaloids content (14.42 ± 0.23g/100 gram by liter of fresh material) in concentrate with that obtained (2.36 ± 0.18g/100 gram by dry weight) by Bouchelta et al [40], confirm that dry treatment causes alkaloids losses.

As indicated in Table **1**, vitamin C content was 168.40±0.53 and 157.48±0.18 mg/100g in crude extract fresh concentrated product, respectively. Reverse osmosis process thereby has caused a loss of about 11% of vitamin C. According to Odriozola-Serrano et al. [41], vitamin C losses during processing occur due to the fact that it is a bioactive substance very sensitive to environmental conditions such as exposure to oxygen, temperature and light. So, this reduction of content may be attributed to the possible occurrence of oxidative reactions, since the process was carried out for a long time, due to the small membrane surface. However, it can be concluded that this process of membrane technology has enabled the preservation of the vitamin C in the concentrate.

Total polyphenols contents were estimated to10.06±0.06 and 69.65±0.43 g.L^{-1}Equivalent AG in the crude extract and the concentrate respectively (Table **1**). As alkaloids, the membrane process increased significantly the concentration of phenolic compounds in the pepper extract, indicating preservation of these compounds during this treatment. These results were in agreement with findings of reports focused on

Table 1: pH, Soluble Solids, Total Polyphenols Content, Total Alkaloids Content, and Vitamin C Content in Capsicum Fruits Crude Extract and Fresh Concentrated Product

	Crude Extract (CE)	Concentrate (C)
pH	6.2	5.6
Soluble solids (g kg^{-1})	0.8	5.2
Total polyphenols (g.L^{-1}EqAG)	10.06 ± 0.06	69.65 ± 0.43
Vitamin C (mg/100g)	168.40±0.53	157.48±0.18
Total alkaloids (%)	0.38 ± 0.14	14.42 ± 0.23

concentration of fruits juices by membrane process which indicated also preservation of phenolic compounds [42-43]. Due the essential role of the phenolic compounds as main responsible of antioxidant properties of majority of fruits [43]. This result could be qualified as very promising in capsicum technology transformation.

From these results, it can support that the fouling and flux restoration on reverse osmosis of the membrane separation was efficient for preservation of bioactive compounds such as polyphenols, alkaloids and vitamin C in pepper (*capsicum*) fruits extract.

Mineral Composition of Capsicum Fruits Crude Extract and Fresh Concentrated Product

The data on mineral analysis of Capsicum crude extract and fresh concentrated product are shown in Table **2**. The mineral profile of the fresh concentrated product seems interesting, since calcium, potassium, iron, magnesium, phosphorus whose beneficial roles in the human body are well-known, were found to higher levels than in the crude extract. Thus, iron with concentration of 11.52±0.7 mg/100g is the main metal micronutrient of the bell pepper fresh concentrated product. Calcium (20.69±5.31 mg/100g), potassium (61.68±0.41 mg/100g), magnesium (16.66±2.66 mg/100g) and phosphorus (61.68±0.41 mg/100g) constitute the main metal macronutrients. These results indicated clearly that application of reverse osmosis process for preparation of pepper fresh concentrated product ensures the preservation of minerals. Our findings were in accordance with those obtained during concentration of the pineapple juice by reverse osmosis which has indicated also the preservation of minerals [20].

Consequently, this fresh concentrated product obtained by reverse osmosis could be good a source of calcium, magnesium, potassium, phosphorus.

Identification of Chemical Constituents of Capsicum Concentrated Extract

Using Gas chromatography-mass spectrometry (GC-MS), we identified some main chemical compounds in concentrated extract of *Capsicum annuum* obtained by membrane technology. Figure **1** indicates the chromatographic profile which showed thirty four compounds in Capsicum concentrated product whose the 4-Hydroxyphenylethanol di-TMS at t_R (retention time) 8.72 min ; Benzoic acid. 4-methoxy-3-(trimethylsilyl)oxy-methyl ester at t_R 9.17min; Bis

Table 2: Minerals Composition in Capsicum Fruits Crude Extract and Fresh Concentrated Product (mg/100g)

Co-products	Potassium (K)	Phosphorus (P)	Magnesium (Mg)	Calcium (Ca)	Iron (Fe)
Crude extract	53.03± 1.12	28.11±0.04	14.01±1.30	16,24±4.2	4.21±0.33
Concentrate	61.68±0.41	34.24±0.12	16.66±2.66	20.69±5.31	11.52±0.7

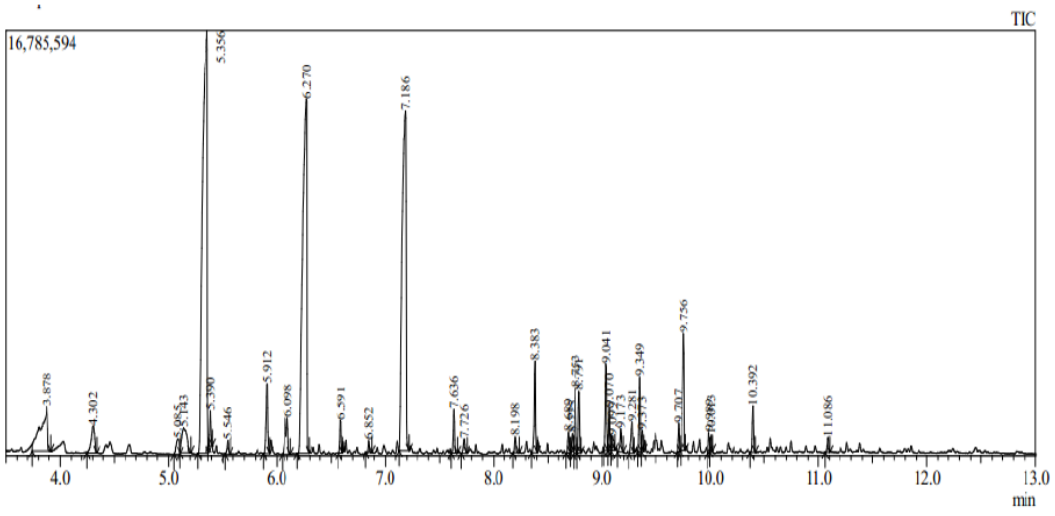


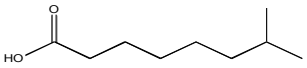
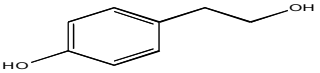
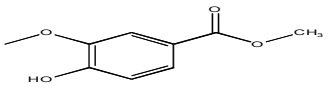
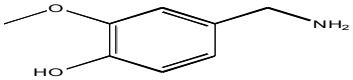
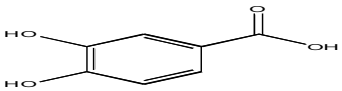
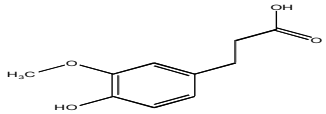
Figure 1: GC-MS chromatogram related to compounds of *C. annuum* fresh concentrated extract.

(trimethylsilyl) isovanillate at t_R 9.75min. Benzoic acid 3.4-bis[(trimethylsilyl)oxy]-. trimethylsilyl ester at t_R 10.01min. Vanillylpropionic acid. bis(trimethylsilyl)- at t_R 10.39min, cis-4-Trimethylsilyloxy-cyclohexyl (trimethylsilyl) carboxylate at t_R 7.63min.

The structures of corresponding phytochemical compounds are presented in Table **3**.

We noted that apart from 7-methyl octanoic acid, these compounds were phenolic compounds. This is in accordance with the high values of content for total phenolic compounds in the concentrated extract previously indicated. Several reports indicated presence of phenolic compounds in pepper extract by using chromatographic methods [44-46]. Thus GC-MS analysis confirmed that concentrated extract obtained contained high level of phenolic constituents and could demonstrate high antioxidant capacity. More over interestingly, vanillylamine and fatty acids were present in the fresh concentrated extract. Indeed, it is well known that capsaicin is the parent compound of a group of vanillyl fatty acid amides isolated from *Capsicum* spp. [47]. The more than twenty known capsaicinoids are all amides formed from condensation of vanillylamine and fatty acids of different chain lengths. This means that of the alkaloids of concentrated product obtained by membrane technology belong to capsaicinoids group, as reported in the literature. In fact, it is widely reported in the literature that the capsaicinoids, particularly capsaicin and dihydocapsaicin are the main alkaloids of pepper and are responsible for 90 % of the pungency [48]. In this same context, Hin *et al*. [49] suggested that GC-MS was efficient for analysis and identification of capsaicinoids. However, findings of Reilly *et al*. [36] showed that some peppers contain up to 22% of nordihydrocapsaicin, another capsaicinoid from *Capsicum* spp. The type of capsaicinoid depends on the number of lateral chain carbons and the existence of unsaturations. Taking account the identification by GC-MS analysis of vanillylamine and 7-methyl octanoic acid in the concentrated product, it could postulate that

Table 3: Nature and Chemical Structures of the Most Prevailing Compounds Identified in the Fresh Concentrated Product of Capsicum Fruits

t_R (min)	Fragment	Name of the Compound Sililated Identified	Chemical Structure Identified
7.63	230/215/199/174	cis-4-Trimethylsilyloxy-cyclohexyl (trimethylsilyl) carboxylate	 7-methyl octanoic acid
8.72	282/267/193	4-Hydroxyphenylethanol. di-TMS	 Tyrosol
9.17	254/239/193	Benzoic acid. 4-methoxy-3-(trimethylsilyl)oxy-. methyl ester	 Isovanilic acid
9.75	297/282/267/193	Bis(trimethylsilyl)isovanillate	 Vanillylamine
10.01	370/355/193	Benzoic acid. 3.4-bis[(trimethylsilyl)oxy]-. trimethylsilyl ester	 Protocatechic acid
10.39	340/325/310	Vanillylpropionic acid. bis(trimethylsilyl)-	 Vanillylpropionic acid

t_R : retention time (minute).

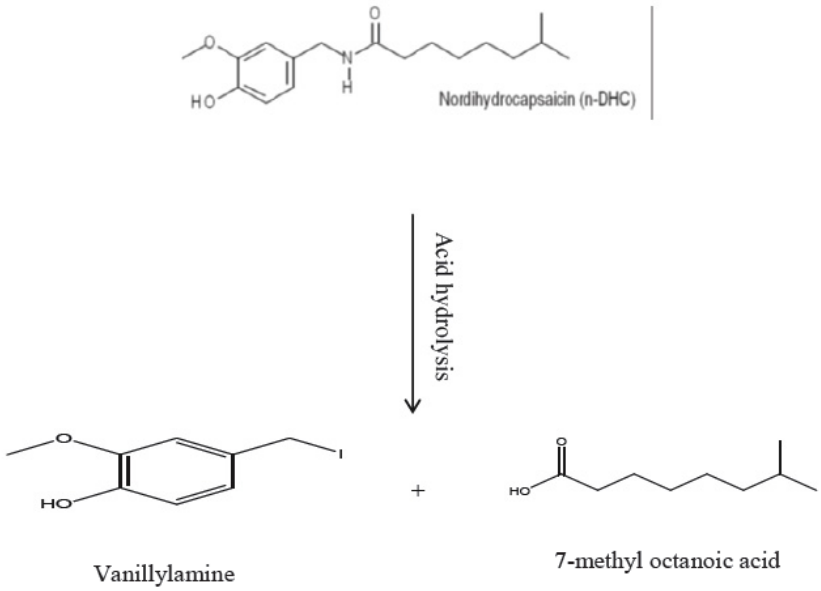


Figure 2: Acid hydrolysis of nordihydrocapsaicin.

these two compounds originated from the acid hydrolysis of the amide bond of nordihydrocapsaicin according the scheme presented in Figure **2**.

Consequently, the concentrated product of pepper obtained contains likely a significant amount of nordihydrocapsaicin. This data constitute a value added for this product of Capsicum fruit since it well established that in addition to the pungency whose contributes nordihydrocapsaicin, it is endowed with pharmacological properties [36] and anti-obese activity [50].

Due the high contents of bioactive compounds (phenolic compounds, alkaloids and vitamin C) and minerals in fresh concentrated product obtained by reverse osmosis process, it can be established that this product displayed a good quality for cookery.

CONCLUSION

This work concluded that the use of membrane technologies involving reverse osmosis could constitute an efficient method for transformation of fruits of pepper Capsicum. Indeed, the concentrated fresh product obtained by using this technology displays a good quality in terms of bioactive compounds such as phenolic compounds (antioxidant properties) and alkaloids (pungency property) and of mineral composition. Thus, an efficient and simple method was successfully developed for concentration pepper Capsicum extract in order to improve the preservation duration and to avoid preservation by solar drying which causes damage in terms of bioactive compounds losses and health safety in Côte d'Ivoire and other countries. However, further studies are needed on this Capsicum product in order to verify preservation of other bioactive constituents such as, carotene (pro-vitamin A) and for stability of these compounds.

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