WORKSHOP: VALORISATION OF TRADITIONAL PROCESSING OF INDIGENOUS AND UNDERUTILISED FRUITS
Institute of Technology of Cambodia, Phnomenh, Cambodia
January 14-16, 2013.
The workshop is funded under the project "International network on preserving safety and n utrition of indigenous fruits and their derivatives", by the Leverhulme Trust, UK

PURIFICATION OF ANTHOCYANIN BY POLYMERIC ADSORBENT RESINS

Suzie ZOZIO, Dominique PALLET, Max REYNES
Integrated Food Processing Research
UnitCIRAD - Département PERSYST34398.
Montpellier cedex 5

Justine Y.Phuong P. H. BOFFO

Faculty of Food Science and Technology
Nong Lam University, Vietnam

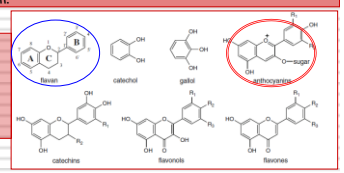
Introduction

- There is a general tendency nowadays toward using **natural coloring** in foods as a substitute to chemical food coloring that has harmful physiological effects.
- Studies are being done accordingly to develop food coloring products from natural sources such as barberry, blackberry…the pigment of which contain various **phenolic compounds/anthocyanins**.
- Prices for anthocyanins or other polyphenol extracts cover a wide range depending on the **concentration of active components**, …..costs into the hundreds of dollars/ kilogram.

Major families of phenolic compounds

The typical structural characteristic shared by most polyphenols: the three-membered flavan ring system.

Their role in fruits/ vegetables:
- responsible for structural and protective functions.
- contributing to flavour, color, astringency and bitterness.



Their role in human health: based on their antioxidant activity against reactive species involved in aging and in chronic, autoimmune, inflammatory, coronary, cancer and degenerative diseases.

Nathan, 2009

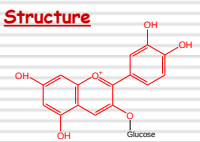
Anthocyanin molecules

Characteristics

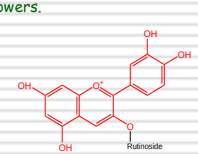
-Belong to the **flavonoid group of polyphenol**

-Water soluble pigments, responsible for the **red**, **purple**, and **blue** colors of fruits, vegetables, and flowers.

Structure



Cyanidin-3-glucoside



Cyanidin-3-rutinoside

Antioxidant interest: problematic research

ORAC value *apply to water-soluble antioxidants*

The higher the number, the stronger the antioxidant properties.

	Açai	Blackberry	Strawberry	Raspberry	Red dragon	White dragon
ORAC value	48,6 - 61,5	13,7 - 25,1	18,3 - 22,9	19,2 - 22,6	7.59-10.76	2.96-5.23

ORAC = (Oxygen Radical Absorbance Capacity), (µmol eq trolox /g)
(Del Pozo Insfran,D.,2007, USDA, 2004)

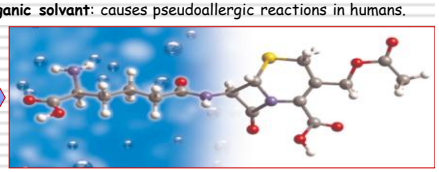
The USDA recommends the consuming of 3,000 to 5,000 ORAC units/day.

Separation process

Conventional process

High T°: leads to color loss, Maillard reactions since extracts may contain high amounts of sugar.
Organic solvant: causes pseudoallergic reactions in humans.

Absorption-desorption process



- **minimizes degradation, simplicity of design, operation and scale up,**
- **ease of regeneration and**
- **low cost.**

Major types of adsorbents

- Activated carbon;
- Mineral adsorbents include siliceous materias, clay and natural zeolites; and
- **polymeric adsorbents (resin).**

RESIN

- Approved by for food contact use, by the European code of regulation.
- Most of the impurities are removed.

7

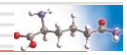
Resin purification process

➢ **2 steps process**

1. Adsorption of the ACNs on resins
2. Desorption

8

Step 1: Which type of Resin could be used for anthocyanin purification?

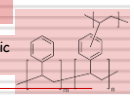


- **Nature of the solvent**: polar, non-polar
- **Functionality of solute**: aromatic, high electron density, saturated C-C bond
- **Polarisation**: the solute has groups that can be easily polarised or not.
  - Easily polarised: $-CO_2H$, $-NH_2$
  - Can be polarised under extreme conditions: >C=O or ethers,
- **Size of solute**:
  - Small molecule: molecular < 1000 D,
  - large molecule: molecular > 10,000 D

Available Resins:

- Polystyrene-divinylbenzene copolymers,
- Polymethacrylate,
- Divinylbenzene-ethylvinylbenzene coopolymers, and
- Vinylpyridine.

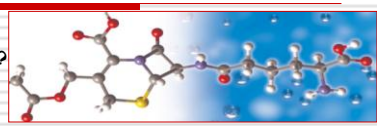
It's a **Polystyrene-divinylbenzene copolymers**, with a specific surface area, porosity, and pore radius ($C_{24}H_{24}$).



9

Step 2: Which solvant for desorption of anthocyanins?

- Alcohols:
  - Methanol ?
  - Ethanol ?
  - Propanol ?

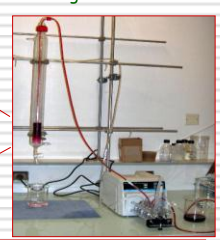


- **Methanol** : **more efficient** *(96% to 100% of anthocyanins could be recovered)* in desorbing anthocyanins from the polystyrene-divinylbenzene copolymerisate than ethanol and 2-propanol.

10

Adsorption of anthocyanins on resins- Step 1.

Hydrophobic interaction, hydrogen bonding



11

Desorption of anthocyanins from resins- Step 2.

With an organic solvent which can weaken the attractive forces between the solute and the polymer for removing ACNs

At the beginning

At the end

12

Result of ACN's purification with resin

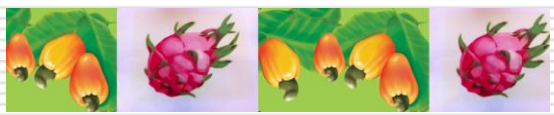
		Juice	Final Extract	Yield ACN % (extract/juice)	Yield DM % (extract/juice)	Purity of final extract (ACN/DM)
BBerry	ACN contents (mg/100mL)	40,8	130,8	96	6,05	19,46%
	$E^{1\%}_{1cm}$	4	36			
	VI/BI	24.6	34.1			
Açai	ACN contents (mg/100mL)	31,2	54,3	87	31,32	8%
	$E^{1\%}_{1cm}$	17,8	48			
	VI/BI	54.4	51			

DM = Dry matter ACN = Anthocyanins VI = Violet Index ; BI = Brown Index
$E^{1\%}_{1cm}$=Strength colour : correspond to the Abs of (x g) pigment in 100mL buffer pH3

- Simplicity , - Great % of ACN's recovery
- Increase of the colour strength, - No colour degradation

Research Proposal

Extraction and purification of Polyphenols from cashew apples waste and of betalains from pitaya by-products



Cashew apple phenolics

- **1978**: Satyanarayana,extracted, purified and characterised three flavonols from an Indian variety, i.e. quercetin 3-O-galactoside, myricetin, and quercetin.
- **2001**, Moura & **2007** de Abreu, colorimetrically measured yellow flavonoids and anthocyanins from Brazilian variety… without further characterisation.
- **2009**, Laetitia extracted Monomeric phenols by acetone/water (60:40) from the *skin and flesh* of four cashew apple from Brazil and Bénin (West Africa).
- **2012**, ADOU, phenolic profile of the two varieties of cashew (anacardium occidentale L.) cultivated in Côte d'Ivoire.

Cashew apple phenolics

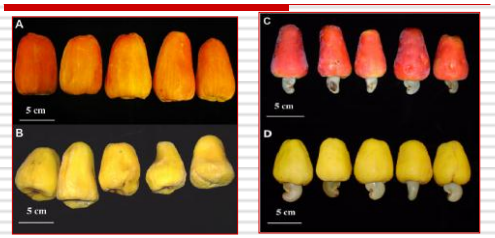


Figure 1. Cashew apple from Brazil (A-B), cashew apple from Benin (C-D). (Laetitia, 2009).

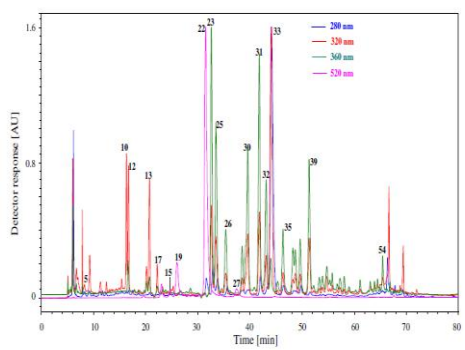


Fig. 2. HPLC chromatograms of skin extract of cashew apple (Parakou Rouge variety) (5) gallic acid; (12, 13) *p*-coumaric acid conjugates; (15) cyanidin hexoside; (19) petunidin hexoside; (22) peonidin hexoside; (23) myricetin hexoside; (25) myricetin hexoside; (26) myricetin pentoside; (27) unknown anthocyanidin hexoside; (30) myricetin 3-O-rhamnoside; (31) quercetin 3-O-galactoside; (32) quercetin 3-O-glucoside; (35) quercetin pentoside; (39) quercetin 3-O-rhamnoside; (54) quercetin. Some unknown peaks are also numbered (10, 17, and 33). Peak heights were normalised at 1.6 AU at λ = 280 nm.

Cashew apple phenolics

- Skins from red Benin variety and organe Brazil: anthocyanidin glycosides.
- Skins from the apples were 1520 times richer in total phenolic compounds than their fleshes, reaching *30→110 and 2→5 mg/100 g* fresh weight, respectively.
- African apples: be richer in skin flavonols and flavonol glycosides than Brazilian ones, while the reverse situation was found in the flesh.

Dragon fruit phenolics

- *Hylocereus undatus* :white dragon fruit and *Hylocereus polyrhizus* : red dragon fruit.
- Total phenolic content (TPC) assay demonstrated that peels of both *Hylocereus* species **contained higher phenolic** content than the pulps.
  - The phenolic content in **peels** of *white dragon* was higher than *red dragon*,
  - but the phenolic content in **pulps** of *white dragon* was much lower than *red dragon*.

Nurliyana, 2010

Dragon fruit phenolics

in order to gain better views on the antioxidant level and activities in *Hylocereus* species,…

→ further studies on purification, identification and quantification of *each phenolic compound and other non-phenolic compounds such as carotenoids and betalains*

Extraction technology and Purification Process

- Extraction techniques, which have been replacing conventional ones, include:
  - supercritical fluid extraction (SFE),
  - pressurized liquid extraction (PLE),
  - microwave-assisted extraction (MAE) and
  - ultrasound-assisted extraction (UAE).
  - Membrance extraction

These alternative techniques reduce considerably the use of solvents and accelerate the extraction process.

- Purification polymeric adsorber resin: polystyrene-divinylbenzene copolymerisate polymer (PSDVB).

WORKSHOP: VALORISATION OF TRADITIONAL PROCESSING OF INDIGENOUS AND UNDERUTILISED FRUITS
Institute of Technology of Cambodia, Phnomenh, Cambodia
January 14-16, 2013.
The workshop is funded under the project "International network on preserving safety and n utrition of indigenous fruits and their derivatives", by the Leverhulme Trust, UK

Thank you for your welcome and for your listening

Justine Y.Phuong P. H. BOFFO

Faculty of Food Science and Technology
Nong Lam University, Vietnam