Advanced imaging methods to optimize extraction of natural red colorant from dye sorghum

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Sorghum in the 21st Century-
“Food, Feed and Fuel in a Rapidly Changing World”
Genetic diversity of sorghum is a valuable resource.
Sorghum is a multi-use crop

Sorghum has a huge genetic diversity which is at the origin of a wide range of uses in Africa since a long time:

• Nutritional value: Food and feed ==> Many traditional processes allow different consumption modes: flour, semolina, " tô", malt and local beer “Dolo”

• Health value (traditional medicine) and other uses associated to a high biochemical diversity of poly-phenols (phenolic acids, tannins and anthocyanins) ==> strong variations in concentration depending on the variety
Tinctorial sorghum

Dye sorghum) which red pigment is used traditionally in Africa: textiles, painting masks and pottery, but also to color food products (cheese in Benin: waragashi).

- The leaf sheaths of dye sorghum are very rich in red pigments which are composed of anthocyanins (mainly apigeninidine): up to 49 mg / g of dry matter (Balole, & Legwaila, GM, 2006).

- High content of anthocyanins and phenolic compounds provide antifungal and antibacterial qualities associated with these pigments (Sessou P et al., 2012) and antioxidant capacity (Kayod et al., 2011)
  
  => Multifunctional extracts with big potential in cosmetics and nutraceuticals (Dykes and Rooney, 2006)
Sorghum pigment: new market opportunities

Market for natural pigments is growing <= tightening of health regulations ➔ 5 to 10% of the global dye market; average annual growth rates: 10% -15%.

The anthocyanin market: has been valued at $300 million in 2015 ➔ Annual growth of 4.4% between 2015 and 2021 (transparency market research, TMR)

**Assets:**

- “red“ pigment: high market share; substitutes are sought for cochineal red
- high concentration as compared with other sources of anthocyanins marketed as pigments=> price advantage (savings on the size of equipment and the volumes of consumables needed for extraction)
- high natural stability
Obstacles to overcome for an industrial boom

- Lack of knowledge of the complex composition of the sorghum dye extract (co-pigment ?) : very important to define the conditions of its stability

- Low extraction rate as reported by the bibliography and the patents
  
  ➞ from sheaths 2 to 3% ; from grains about 8%

An important margin of progress to be exploited
How can imaging be used to optimize extraction processes?

- Makes possible to **visualize the effect** of the various conditions of extraction on the structure of the matrix and on the extractables distributed in the different tissues.
- “to extract” is “to draw out structures” and few biochemists are interested in the **residual matrix after extraction**
- imaging should help to no longer do "blind" extraction
Cirad experience in imaging and pigment extraction processes: Pastel (*Isiatis tinctoria*)

*Chiffoleau et al., 2013*

Blue pigment (indigotine)

Isiatis leaf anatomy

Leaf cells
- Before extraction
- During extraction

Pigment movement outside the cells
Cirad Dye Sorghum research consortium

Image Platform / AGAP PHIV
- Histological organization of the tinctorial biomass
- Biomass observation after extraction
  (position of non extracted compounds)

Market study

Extraction / BIOWOOEB
- Biomass processing
- Influence of extraction parameters
  (grinding, solvents, temperature...)

Optimization

Biomass production (Montpellier)

Extracts

Purified compounds

Biochemical analyses / QUALISUD
- Dye quantification
- Biochemical characterization of the extract
  Composition des extraits

Polyphénols Platform UMR SPO/INRA

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In what type of tissues/cells is the dye accumulated?

- Sorghum red dye is localized in the lacunar parenchyma,
- Highly concentrated at the periphery of the lacunar parenchyma
- In the cell layer around the vascular bundles

**IMAGING AND PIGMENT EXTRACTION PROCESSES IN DYE SORGHUM**

- Crystallized Dye
- Lacunar parenchyma
- Vascular bundle
- Autofluorescence

**Thickness of leaf sheaths**: 1010-1250 µm
Imaging at the different steps of the pigment extraction process

Dried raw biomass (Oven)

grinding

Powder

Extraction

Extract + Bagasse

Accelerated Solvent Extraction (ASE® 300) Dionex with conventional solvents at high temperatures and pressures.

Histological analyses
Imaging at the different extraction steps: the grinding

- **Waring Blender**
- **Crushed Biomass**
- **Crushed tissues**

- **Genotype: Chantor**

- **Histology** can provide qualitative and quantitative description of the effect of grinding on tissue fragmentation.

- **Use of imaging methods** to assist biomass grinding before extraction.

- After grinding, many cells containing color remain unopened.
Histology of remaining biomass (bagasse) after extraction by solvents:

- After extraction, many tissue fragments still contain the pigment.
- The pigment remains in unopened cells, particularly in the form of small peripheral vesicles.
- Less remaining dye for EtOH compared with (Aceton and Water), in agreement with the extraction yields (respectively 21%, 15% and 7.5%).
- Even with EtOH, imaging reveals an important margin of progress to be exploited.
Localization of the two major dye sorghum anthocyanidins: apigeninidin and luteolinidin

We could identify cell compartments containing apigeninidin and/or luteolinidin

- Apigeninidin is mainly found inside the vacuole of parenchymatous cells
- Apigeninidin can be detected in the form of vacuolar globules or crystals
- Luteolinidin is mainly localized in small globules (mainly in the cell layer around the vascular bundles)
- Luteolinidin can also be found inside the vacuole (small amount compared with apigeninidin)

Multiphoton microscopy combined with spectral analysis
Follow-up of apigeninidin and luteolinidin in cell compartmentalization during solvent extraction

- High level of apigenidin taken out of the cell structure by solvent
- Low level of Luteolinidin taken out of the cell compartments (globules remain rich in luteolinidin)

Imaging evidence of differential extraction of apigeninidin and luteolinidin
Imaging molecular diversity in sorghum leaf sheath

The spectral analysis reveals a multicolored sorghum reflecting the richness of the leaf sheaths in fluorescent compounds (at least 15 still need to be characterized by biochemical analysis)
New perspectives

• Imaging through the visualization of interactions between molecules of interest / Structure / extraction parameter offers new opportunities for targeted extraction optimization

• Advanced imaging technologies offer new opportunities to assist the biorefinery concept and diversify the uses of dye sorghum extracts
THANK YOU
FOR YOUR KIND ATTENTION

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