Impact of temperature on the content and functionality of miraculin in Beninese Sisrè berries (*Synsepalum dulcificum* Shumach. & Thonn.)

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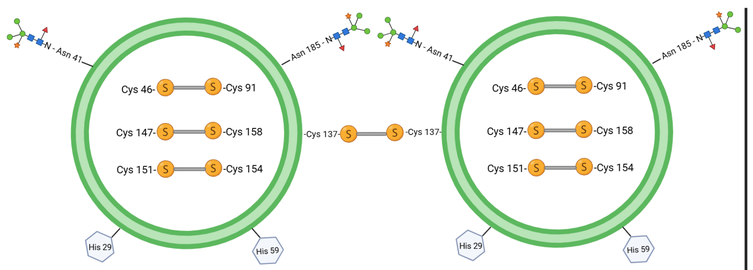
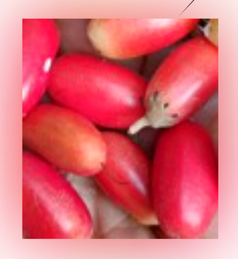
Introduction



Miraculin active form (dimer 50 kDa)

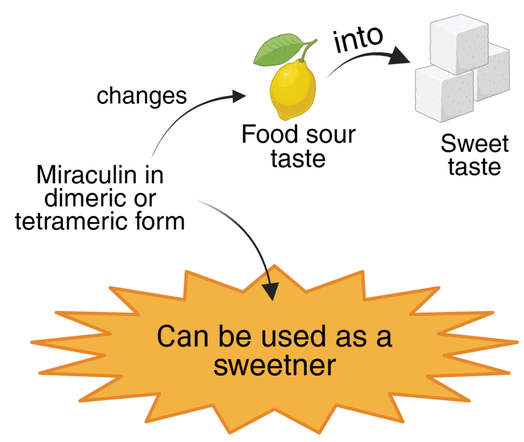


Sisrè berries

Miraculin's characteristics

- Glycoprotein not sweet & not caloric.
- Monomer weighted 25 kDa with 220 amino acids chain (Kurihara *et al.,* 1992).
- Sweetening power 400,000 times greater than that of saccharose on a molar basis.

Functionality



Miraculin's weakness

- Inactive at pH > 6 (Paladino *et al.*, 2008).
- Monomer form is not active (Kurihara *et al.*, 1992).
- Heat sensibility but not precisely known (Choi & Garza, 2020).

Objectives

Assess the effect of temperature on this glycoprotein, to define the optimum conditions for the stabilization of the pulp, preserving its functional properties.

Methods

- Sisrè collected in the locality of Sèhouè (Benin).
- Frozen fruits pitted, pulp crushed and frozen (-20°C) until heat treatments.
- Heat treatments on pulp batches in water bath at 40, 45, 50, 60 and 70°C during 5 min.

- Freezing (-20°C) heated pulps labeled PT40, PT45, PT50, PT60 and PT70, until sensory and laboratory analyses.

Sensory analysis

1. Consumption of 2.5 g of frozen heated pulp
2. Consumption of citric acid solution pH 3
3. Evaluation of sweet and sour tastes of the previous sour solution on a 0-10 scale
4. Sweetness persistence evaluation each 30 min until 2h 30min

Panel: 9 Beninese people

Laboratory analyses

- Miraculin extraction: Washing with water + extraction with NaCl 0.5 M buffer.
- Miraculin quantification was carried out in the extract by RP-HPLC according to the method used by Demesyeux *et al.* (2020) with some modifications (Béhanzin *et al.*, 2025).
- SDS-PAGE under reducing conditions carried out on miraculin extract.

Fundings

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CIRAD, Montpellier (France).

References

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- Paladino A. *et al.* (2008). *Biochemical and Biophysical Research Communications,* 367 (1), 26–32.
- Choi S. E. and Garza J. (2020). *Journal of Food Science*, 85 (1), 36–49.
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Results

Pulp heated at temperature ≤ 50°C induced sweetness but the sweet taste intensity was reduced by half comparing PT50 to PT40 (**Fig.1**).

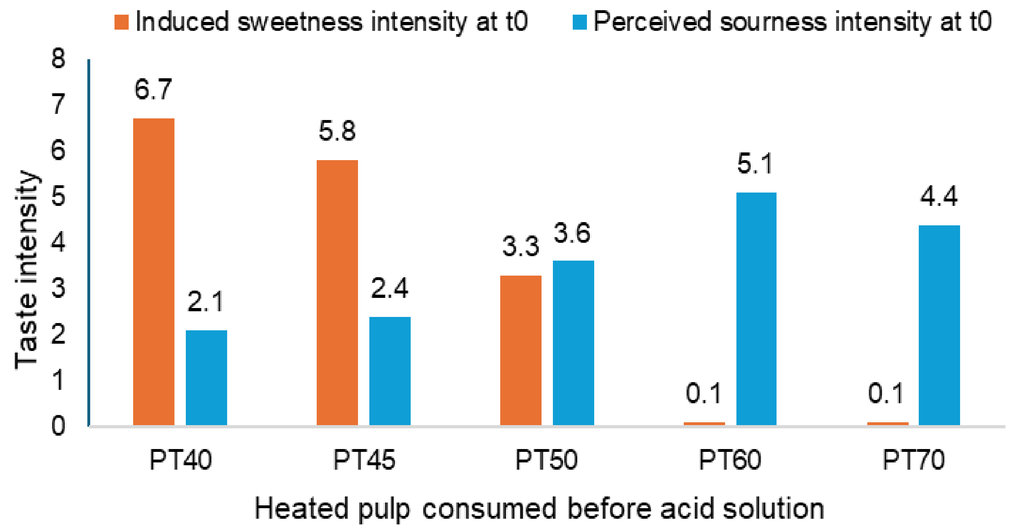

Fig. 1: Taste intensities after citric acid solution consumption

The induced sweet taste lasted at least 150 min for pulps heated at 40 and 45°C but 30 min for the one heated at 50°C (**Table 1**).

Table 1: Persistence time of the induced sweetness and miraculin content in the extracts

Pulp samples	PT40	PT45	PT50	PT60	PT70
Total persistence time of effect (in min)	**150**	**150**	**30**	**0**	**0**
Miraculin concentration in the extract (mg/L)	**615**	**642**	**608**	**499**	**434**

- The higher the temperature, the more miraculin is lost. Heating resulted in denaturation of miraculin, which led to aggregation or loss of solubility, thus reducing its extractability by the buffer.
- Despite the loss of functionality, residual miraculin is still present in samples heated above 50°C. It could represent the monomeric form initially present in the fruit pulp.

Only one band was observed on the polyacrylamide gel at 25 kDa (**Fig. 2**), representing the molecular weight of miraculin's monomer (**Fig. 3**). The intensity of this band decreased for the PT60 extract and was almost invisible for the PT70 extract compared to the extracts from the three lower temperatures.

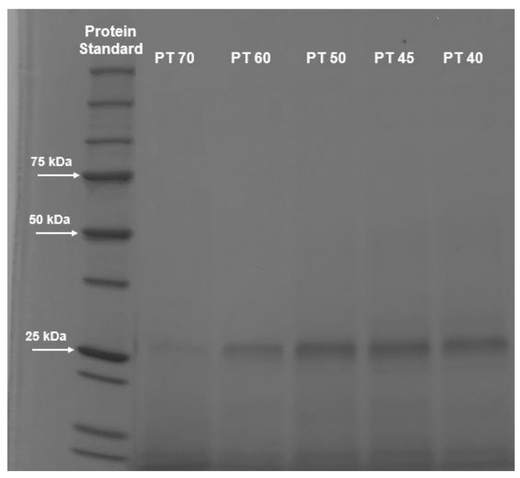

Fig. 2: SDS-PAGE on miraculin extracts

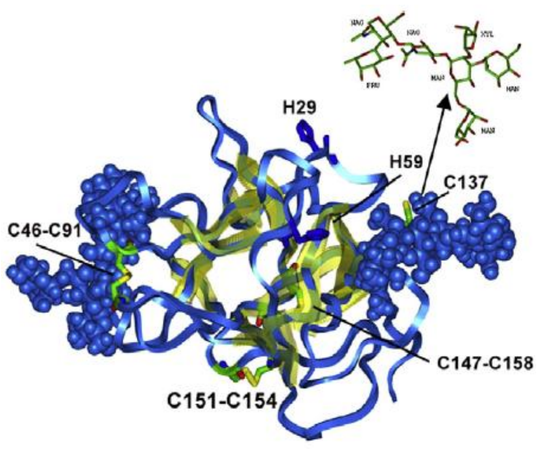

Fig. 3: Miraculin's monomer (Paladino *et al.,* 2008)

Conclusions

- ✓ Above 50°C, miraculin is denatured and loses its functionality.
- ✓ Pasteurization stabilization techniques are therefore not feasible.
- ✓ It would be interesting to explore stabilization techniques such as low-temperature drying or membrane sterilization of miraculin extracts.