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Solubility and emulsifying properties of aqueous extracts and protein concentrate from African palm weevil larvae

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Introduction

Today, edible insects are evaluated for their potential to be used as food ingredients (1,2,3). The larvae of Rhynchophorus phoenicis (Rp), edible insect eaten in Central and Western Africa, are a good source for proteins and unsaturated lipids (4). Now, they can be farmed which could be the opportunity for sufficient production at small-scale to medium level to provide a new source of protein ingredient for small-size formulation industries. In the present work, the influence of pH on the emulsifying properties of aqueous extracts (AE) and protein concentrate (PC) of Rhynchophorus phoenicis larvae was studied.

Results & Discussion (2)

Material & Methods 1. Preparation of aqueous extracts and protein concentrate of *Rp* larvae Lipid layer **Rp Larvae Ground Rp** Homogenization Aqueous larvae /buffer (20 min) extracts (AE) (ratio 1:10) and centrifugation pH 3-10 (10,000 x g; 20 min) Insoluble components pH adjusted to 9.0 **Dried insect** Centrifugation pH of supernatant with 2N NaOH adjusted to 4,5 with 2N HCI powder/water at 10,000 x g Stirring for (ratio 1:10) (30 min, 4 ° C) Stirring for 45 min à 45 °C 45 min at 55°C (5) Protein **Dispersion at pH 7 Precipitate washed** Centrifugation concentrate and centrifuged at 2,000 at 2,000 x g and (PC) x g (10 min, 4°C) (X2) freeze drying (72 h) (15 min, 4° C) ≈ 70 % protein 3. Preparation and characterization 2. Analysis of AE & PC of Rp larvae of emulsions **Protein content** Protein solubility Droplet (BCA Method) (6) (g/100 g total protein) AE (protein] = 1 mg/mL) or flocculation **PC (protein] = 7,1 mg/mL)** (optical in pH 3 - 10 buffers microscopy)

Molecular weight

profile (SDS PAGE)

3. Lipid composition of Aqueous Extracts of Rp larvae at different pHs 1.8 (1.6 (1.4 (1.4) (1.2) ■ TAG ■ FFA ■ Total FA

For a given lipid fraction, values with different letter are significantly different. (p<0.05; a> b)



- Lipids present in Rp aqueous extracts were mainly composed of triacylglycerols (TAG) and free fatty acids (FFA).
- Free fatty acids were majorly extracted at pH 9.0 and 10.0. They can result from lipid hydrolysis before (grinding of the larvae) or during preparation of aqueous extracts

Figure 3: Triacylglycerols, free and total fatty acid contents of Aes of *Rhynchophorus phoenicis* larvae prepared at different pHs

4. Droplet size and aggregation of emulsions stabilized by Aqueous Extracts of *Rp larvae*







AE or PC solution (4,95 g)

Results & Discussion (1)

AE prepared at

pH 3 to 10

PC solubilised

at these pHs

1. Effect of pH on solubility of proteins of Rp and Rp protein concentrate



The protein solubilites of Rp (AE) and Rp PC increased with pH. They were minimum at acid pHs corresponding to the pI of most PC proteins as confirmed by isoelectrofocalisation (not shown).

sonication

Emulsion

- Protein solubility was maximum at pH 10. This is why protein concentrate was prepared in alkaline conditions (pH 9)
- At a given pH, proteins of Rp PC were more soluble than total proteins of Rp larvae (AEs) showing that a fraction of the larvae proteins remained insoluble even at high pHs.

2. Molecular weight distribution of proteins according to pH

Values of histogram with different lower case letter are significantly different (P< 0.05; a>b>c

Figure 4: d3,2 of the droplets of the emulsions

according to extraction pH (n = 3)



Figure 5: Microscopy of the emulsions prepared at pH 5 to 10

■At pH 5.0 to 8.0, presence of flocs and very large oil droplets (d3,2) > 9 µm), which led to unstable emulsions that creamed in less than 30 min after emulsification.

•At pH 9.0 and 10.0, the droplets were small : d3,2 < 1,5 μ m, and no flocculation was observed. Emulsions had been stable for more than a week.

At alkaline pHs, FFA (ionized) form alkali metal salts (soap), which have important properties as association colloids and are surface active agents (8)

Proteins and free fatty acids extracted at alkaline pH contribute to provide emulsifying properties to AE.

5. Droplet size distribution of emulsions with Protein Concentrate of Rp **larvae (with and without SDS 1%)**



Figure 5: Distribution of emulsion size of protein concentrates of *Rhynchophorus phoenicis* larvae at different pH levels in the presence and absence of SDS

- Whatever the pH, with and without 1 wt % SDS, the droplet size distributions of the emulsions were monodisperse
- Fine droplet distributions and stability against flocculation and coalescence were observed at pH 3.0, 7.0



For many proteins were AEs, different profiles with extracted, according to pH. Less proteins were visible at pH 4 because most of Rp proteins are not soluble at this pH.

• For Rp PC, apart for one protein band not present at pH 4.0 and 5.0, the profiles were similar whatever the pH.

Figure 2: SDS PAGE of proteins of aqueous extracts of Rp larvae (A) and Rp larvae protein concentrate (B).

and 9.0, while the pH 5 emulsion was coarser and unstable

Conclusions Aqueous Extracts of *Rhynchophorus phoenicis* larvae prepared at pH 9.0 and 10.0 have, contrary to acid and neutral AEs, good emulsifying properties probably due to the presence of both free fatty acids and proteins. They could be used to emulsify and stabilize complex formulations. **Protein Concentrate of** *Rhynchophorus phoenicis* larvae was able to form emulsions with small droplet size at studied pHs. Rp protein concentrate can be used to prepare emulsions for food applications.

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