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Influence of pH, Temperature and Ionic Strength on some Functional Properties of Defatted Flour from *Ricinodendron heudelotii* (Bail.) and *Tetracarpidium conophorum* (Müll. Arg.)

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Abstract: *Ricinodendron heudelotii* and *Tetracarpidium conophorum* are two non conventional oil seed plants belonging to the Euphorbiaceae family. Their kernels constitute one of the main nutritional components of food habit for the western and littoral populations in Cameroon. To contribute to the valorisation of these two non conventional oil crops, some functional properties of the deffated flour of their kernels were studied. Protein solubility of the two oil seeds depends on a couple of factors: pH and temperature, pH and ionic strength. These proteins showed two isoelectric points (pH 4 and pH 8) at which 21 to 48% (*T. conophorum*), 1 to 8% (*R. heudelotii*) of the crude proteins were soluble. Protein solubility of the deffated flour from *T. conophorum* were found to be very high (more than 86%) in the water at 50°C at pH 11, in 0.6N (pH 11) and 0.8N NaCl (pH 2). The maximum value of soluble protein for *R. heudelotii* (more than 20%) were found when solubilised in the water at 50°C (pH 11) and in NaCl 0.8N (pH 11). Foaming and emulsifying properties of the two oilssed flours were protein solubility dependent and were higher in *T. conophorum* compared to *R. heudelotii.*

Key words: *Ricinodendron heudelotii, Tetracarpidium conophorum*, deffated flours, Solubility and functional properties

Introduction

R. heudelotii and *T. conophorum* are two non conventional oil crops belonging to the Euphorbiaceae family that grow in the tropical african forest. Their exploitation is a source of food and income for local populations. Kernels from *R. heudelotii* are used like spices and as soup thickner in the african cook (Mosso *et al.*, 1998), while those from *T. conophorum* are considered as mouth fruits. Recent studies have shown that kernels of the two plants contained more than 50% total oil and more than 20% total proteins (Ige *et al.*, 1984 ; Tchiégang *et al.*, 1997and 2001). Oil from *R. heudelotii* contained high levels of α-elaeostearic acid (Kapseu and Tchiégang, 1995), while those from *T. conophorum* mainly contained conjugate linolenic acid (Tchiégang *et al.*, 2001). Therefore, *R. heudelotii* and *T. conophorum* could be non negligeable alternative sources of oil and protein though the quantities produced are unknown. Protein utilisation in different food systems principaly depend on their functional properties. However, this research have been little explored for the two plants. The general objective of this study was to contribute to the valorisation of deffated flour from *R. heudelotii* and *T. conophorum* seeds. Specifically, studies were carried out to determine the influence of pH, temperature and ionic strength on the protein solubility and functional properties (foam and emulsion) of defatted flours of the two plants.

Materials and Methods

Production of Deffated Flours: Cooked and dried of *R. heudelotii* kernels were purchased from Mbalmayo markets (Cameroon). Dried *T. conophorum* fruits were purchased from the market in Melong (Cameroon), boiled and sun dried for 14 days, dehulled manually to obtain kernels. Kernels from the two samples were separetely ground in a hammer mill (moulinex, France). Meals obtained were defatted for 12 hours with hexane.

Proximate Analysis: Analyses were carried out on defatted flour. Crude protein, real proteic fraction were determined according to the standard methods described by the Association of Official Analytical Chemists (AOAC, 1990) and Okezie and Bello (1988).

Protein Solubility: For this study, a fraction of the defatted flours lower than 400μm were used. The influence of pH, temperature and ionic strength on the protein solubility were measured according to the modified method of Padilla *et al.* (1995). 0.5g of defatted flour, 50ml of water at (25, 30, 40, 50°C) or salt solutions (0.2; 0.4; 0.6; 0.8N NaCl) were added and the pH of the suspension adjusted to the desired value of 2, 4, 6, 8, 10, 11 with 0.2N NaOH or HCl. The suspension was then stirred for 20 min at the desired temperature and the pH adjusted every 5 min. After centrifugation for 25 min at 3500 rpm, the total volume of the aliquots was noted, 10ml were

mineralized and the nitrogen content estimated by the method of Devani *et al.* (1989). Crude protein solubility of each sample was calculated and expressed as percentage solubility in the dry matter basis.

Foaming Properties and Stability in NaCl 0; 0.05; 0.5; 0.75N: These properties were analyzed according to the method described by Srinivas & Rao Narasinga (1986) with a slight modification. 3 grams of the defatted flour were dispersed in 100ml of distilled water or NaCl 0.05; 0.5; 0.75N and whipped for 5 min with a food mixer (model Supermix 150, France) at high speed and poured immediately into a 250ml graduated cylinder. Foam volume formed was then recorded as the foam capacity (FC). Foam stability (FS) expressed as percentage volume was determined by measuring the volume of foam after 5, 30, 60 and 90 minutes compared to the initial volume.

Emulsifying Properties and Stability in NaCl 0; 0.05; 0.5; 0.75N: These properties were determined by the method of Yatsumatsu *et al.* (1972) with a slight modification. One gram of the defatted cake was dispersed in a graduated tube containing 3ml of distilled water or NaCl 0.05; 0.5; 0.75N followed by the addition of 3 ml of soybean oil. The mixture was then vigorously mixed for 10 min using a magnetic agitator. The resulting emulsion was centrifuged at 6000g for 30 min. The height of the emulsified layer divided by that of the whole slurry expressed as a percentage was taken as the emulsifying capacity (EC) of the defatted flour. Emulsifying stability (ES) was determined after heating the homogenized mixture at 80°C for 30 min before centrifugation. The Emulsifying stability was then calculated as the height of the emulsifying layer divided by that of the heated slurry height multiplied by 100.

Statistical Analysis: All measurements were carried out in triplicate and data obtained were subjected to an analysis of variance and then to the Duncan's muliple range test where there was a significative difference at 5%. Statgraphics Plus 3 (Statgraphics, 1998) logiciel was used.

Results and Discussion

Proximate analyses showed that the crude protein content were 50.69 and 54.19g / 100g of dry matter respectively for *R. heudelotii* and *T. conophorum*. Their real proteic fraction represented respectively 80.5 and 93.20% of the total crude proteins.

Protein Solubility: The influence of pH and temperature on protein solubility in water are shown in Figs. 1 and 2 for *R. heudelotii* and *T. conophorum* respectively. Both deffated flours are less soluble at pH 4 and pH 8 but more soluble at pH 11. pH 4 and pH 8 might represent the isoelectric pH (pI) region. This suggests the existence of two families of proteins: one acid and the other basic.

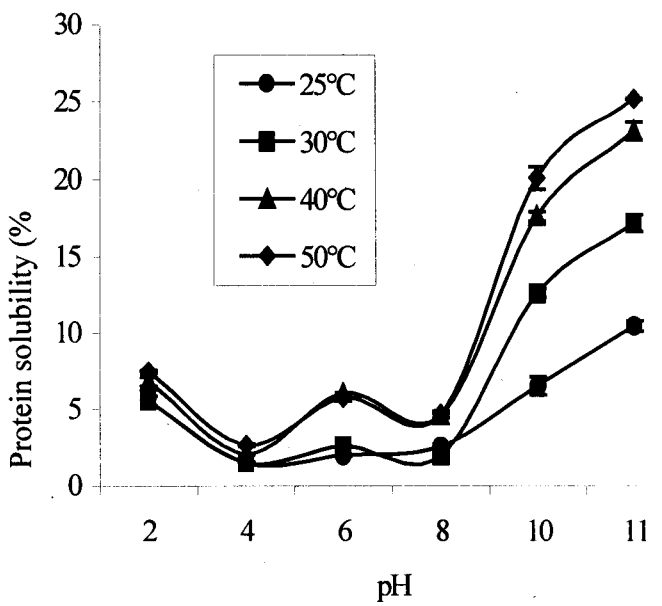

Fig. 1: Protein solubility of *R. heudelotii* in water as a function of pH and temperature

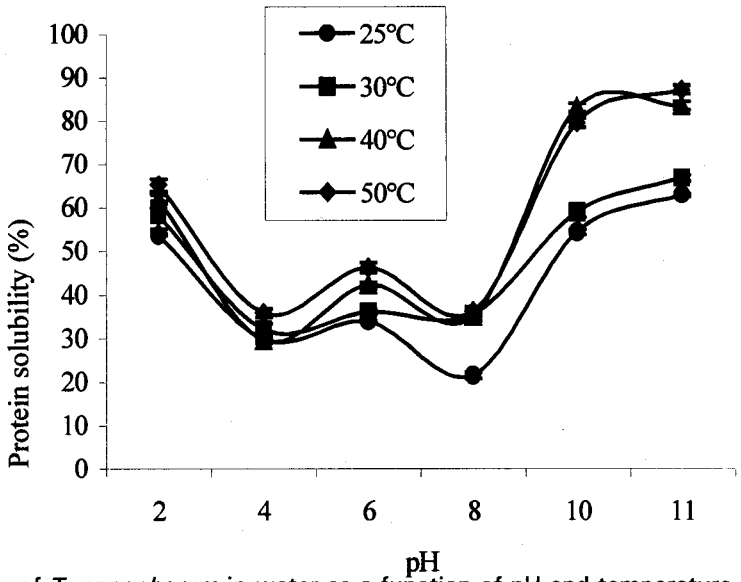

Fig. 2: Protein solubility of *T. conophorum* in water as a function of pH and temperature

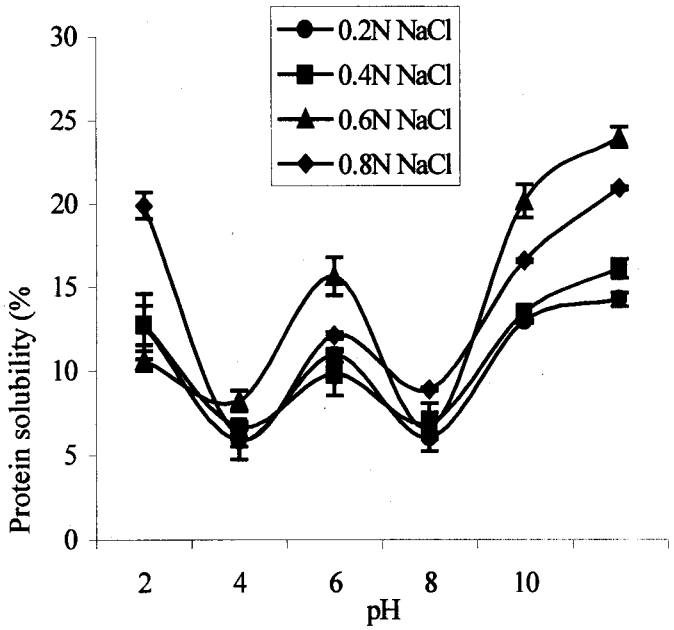

Fig. 3 : Protein solubility of *R. heudelotii* as a function of pH and ionic strength

Protein solubility of the two samples increased both above and below the isoelectric point. This behaviour is similar to many other seeds flours, as reported by Cheftel *et al.* (1985). At acid pH, more than 50% of crude protein of deffated flour from *T. conophorum* were soluble whatever the temperature. This result can be explain by the water – protein interactions and the overall positive charge of protein molecules (Lorient and Mesnier, 1990). Deffated flour from *T. conophorum* can be used to enrich food systems like beverages as reported by Ige *et al.* (1984). Whatever the temperature, the proteins of the two samples are more soluble at pH 11. This suggest that at alkaline pH there is greater extraction of the soluble proteins as has been indicated by Ma and Harwalker (1984). Fig. 3 and 4 shows the effect of pH and NaCl concentration on the proteins solublity of *R. heudelotii* and *T. conophorum* respectively. With respect to pH and NaCl

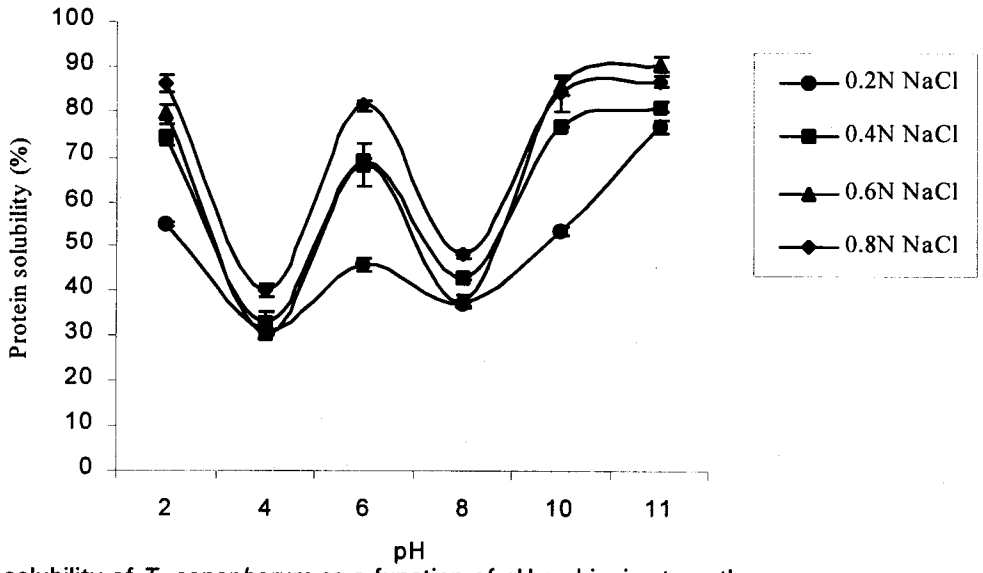

Fig. 4: Protein solubility of *T. conophorum* as a function of pH and ionic strength

Table 1: Foaming capacity (%) and stability of *R. heudelotii* as a function of NaCl concentration

Time (min)/ [NaCl]	0.5	5	30	60	90
0.00	5.24 ± 0.20^{ah}	0^{bf}	0^{bg}	0^{bf}	0^{be}
0.05	10.42 ± 1.80^{afg}	0^{bf}	0^{bg}	0^{bf}	0^{be}
0.5	19.62 ± 1.20^{agf}	5.24 ± 1.00^{be}	4.73 ± 0.80^{cf}	0^{df}	0^{de}
0.75	23.07 ± 0.30^{ahe}	22.05 ± 0.70^{ad}	15.47 ± 1.60^{be}	6.25 ± 0.00^{ce}	0^{de}

a,b,c,d: Means on the same line with different superscripts are significantly different at 5%.
e,f,g,h: Means on the same column within time with different suprescripts are significantly different at 5%.

Table 2: Foaming capacity and stability of *T. conophorum* as a function of NaCl concentration

Temps (min)/ [NaCl]	0.5	5	30	60	90
0.00	84.12 ± 0.08^{ec}	84.12 ± 0.08^{ae}	83.81 ± 0.05^{ae}	81.17 ± 0.13^{be}	79.9 ± 0.08^{be}
0.05	84.23 ± 0.03^{ae}	83.95 ± 0.08^{af}	83.33 ± 0.00^{ae}	81.21 ± 0.07^{be}	76.8 ± 0.20^{cf}
0.5	84.63 ± 0.40^{ae}	82.45 ± 0.14^{bg}	82.29 ± 0.07^{bf}	79.86 ± 0.19^{cf}	75 ± 0.01^{dfg}
0.75	84.91 ± 0.61^{ae}	84.21 ± 0.19^{ae}	83.75 ± 0.05^{be}	78.47 ± 0.05^{cf}	74.92 ± 0.12^{dh}

a,b,c,d: Means on the same line with different superscripts are significantly different at 5%
e,f,g,h: Means on the same column within time with different suprescripts are significantly different at 5%

concentration, proteins of the two samples also presented two pI (pH 4 and pH 8). At these pH, the solubility is higher than that obtained when used distillled water (Fig. 1 and 2). This difference in solubility at the isoelectric point can be explained by the fact that at these points, although global protein charge is null, the increase of ionic strength by monovalent ions improved solubility by reducing electrostatics attractions between protein molecules. The solubility increases with NaCl concentration up to 0.8N for the two Euphorbiaceae. This behaviour is due to the low ionic strength of the NaCl ($\mu < 1$) which allows its dissociation and consequent interaction with the proteins, thus increasing their solubility (*salting in* effect) (Lorient and Mesnier, 1990). Proteins of the two samples are more soluble in the NaCl medium than in distilled water. These results corroborate with the remarks of Ekpenyong and Bochers (1981) according to whom neutral salt ions react with protein molecules thereby reducing the electrostatic attraction between opposite charges of adjacent protein molecules.

Foam Capacity and Stability: Tables 1 and 2 show the effect of ionic strength on the formation and stabilily of foam from *R. heudelotii* and *T. conophorum*. The foam capacity (FC) of *R. heudelotii* varied with time and

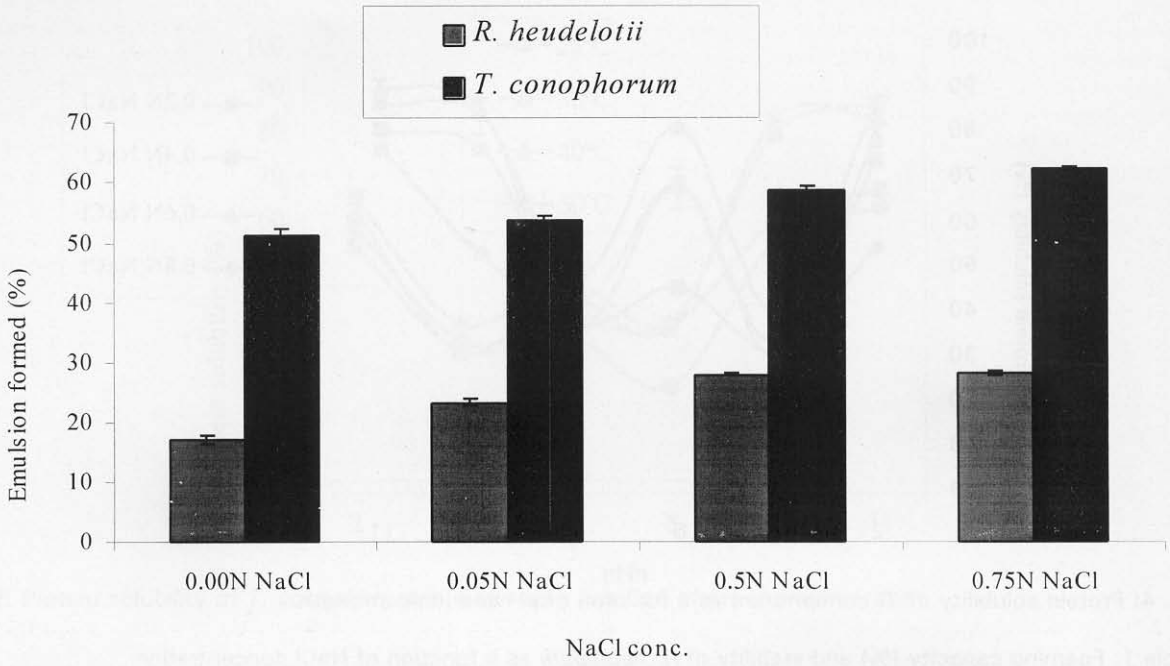

Fig. 5 : Emulsion capacity of *R. heudelotii* and *T. conophorum* as a function of NaCl concentration

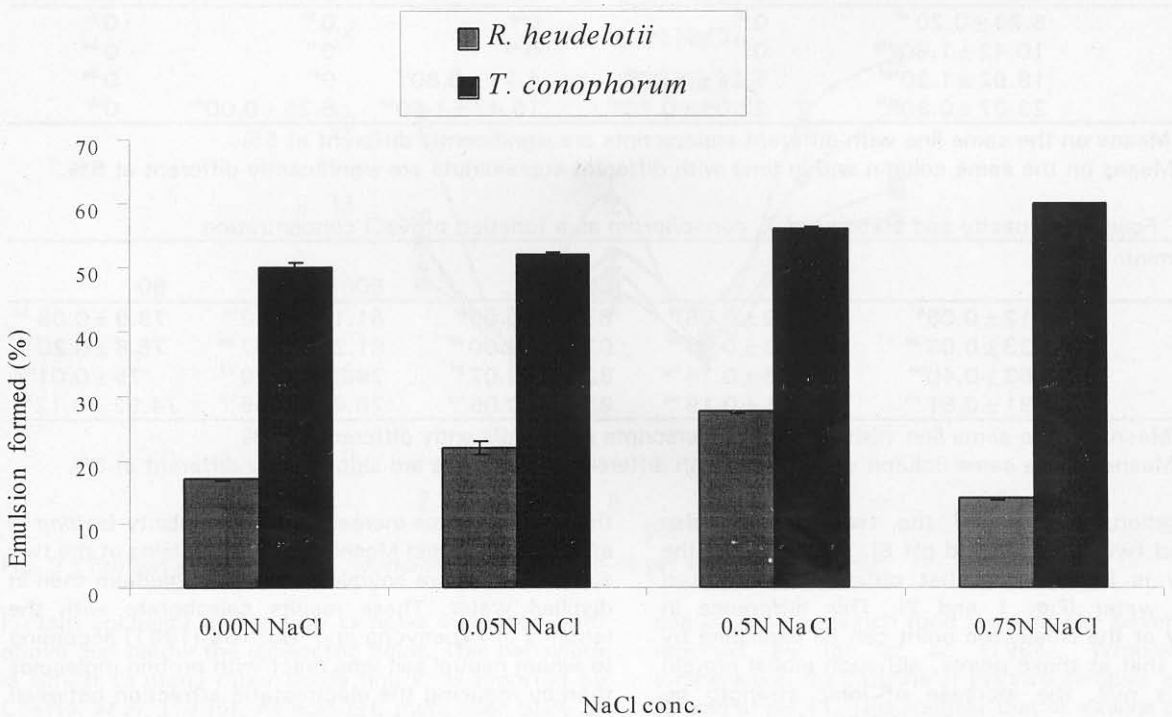

Fig. 6: Emulsion stability *of R. heudelotii* and *T. conophorum* as a function of NaCl concentration

ionic strength, with the optimum value of 23.07% obtained with 0.75N NaCl after 0.5 min. It is the only concentration where there is 6.25% of foam after 60 minutes (table 1). In the case of *T. conophorum*, FC is higher than 80% whatever the NaCl concentration at 60 sec (Table 2). Foam stability (FS) of the two

oilseeds decreased as time increased; so for *T. conophorum*, 74.2% of the foam remained after 90 min (table 2). The FC increases with ionic strength for the two samples analysed. These results may be due to the increase in protein solubility produced by the NaCl effects as reported by Cheftel *et al.* (1985), Shanmugasundaran and Venkatamaran (1989). Nevertheless, *R. heudelotii* deffated cake showed a lower FC than *T. conophorum*, probably due to its low true proteic fraction (40.81%) and higher mineral content (16.5%).

Emulsifying Capacity and Stability According to Ionic Strength: Results obtained for the two defatted flours are presented on the Figs. 5 and 6. For the two oilseeds, emulsifying capacity (EC) increases with ionic strength up to 0.75N NaCl (Fig. 5). However, EC of *R. heudelotii* is lower compared of *T. conophorum* whatever the NaCl concentration. EC of *R. heudelotii* varied from 18 to 28% and that of *T. conophorum*, from 51 to 62% when the NaCl concentration varied from 0 to 0.75N.

Emulsions formed by the two samples are relatively stable. The stability in NaCl concentrations varied from 15 to 29% for *R. heudelotii* and 51 to 60% for *T. conophorum* (Fig. 6). The highest emulsion stability presented by *T. conophorum* can be due to its real proteic fraction (93% of the total crude protein). As has been reported by Duterte (1976) and Cheftel *et al.* (1985), protein in water – oil interface improves emulsion stability. Results obtained with *R. heudelotii* could be explained by the fact that when insoluble proteins are finely ground, they can be together at the water – oil interface (Yatsumatsu *et al.*, 1972 and Duterte, 1976).

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Conclusions

The lowest protein solubility was recorded for *R. heudelotii*. Maximum solubility for the two samples was obtained with NaCl solution concentration of 0.6 and 0.8N at alcaline pH (pH 11). Foam and emulsion properties of the two samples depend on their respective protein solubility. The present results indicated that of *R. heudelotii* and *T. conophorum* deffated cake could be used in the preparation of enriched protein foods.

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