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Compositional and Nutritional Studies of Two Defatted Flours Obtained from *Ricinodendron heudelotii* (Bail.) and *Tetracarpidium conophorum* (Müll. Arg.)

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ABSTRACT

The chemical composition and nutritional value of two edible Euphorbiaceae (*Ricinodendron heudelotii* and *Tetracarpidium conophorum*) from Cameroon were determined. Protein content of defatted flours from these plants was high (44.5 – 47.9% of dry weight). Dietary fiber contents were 9.7 and 10.9%, respectively. Phospholipids were in low quantities and they were close to 9% of dry weight in both defatted flours, with oleic and linoleic acids representing more than 33% of total fatty acid. For all the essential amino acids the amount in *T. conophorum* defatted flour exceeded the value in FAO/WHO (2007) scoring pattern. There were no significant ($P > 0.05$) differences between the values obtained for Protein Efficiency Ratio, Net Protein Ratio, True Digestibility and Net Protein Utilization of diets containing *T. conophorum* and casein. True Digestibility Corrected Amino Acid Scores and Net Protein Utilization Corrected Amino Acid Scores were more than 150% with *T. conophorum* defatted flour, indicating the nutritional superiority of this sample.

Keywords: chemical composition, defatted flours, nutritional value, phospholipids
Abbreviations: AAS, Amino Acid Score; **AAS_1**, Lowest AAS; **DMRT**, Duncan's Multiple Rangle Test; **DW**: Dry Weight; **NPR**, Net Protein Ratio; **NPRCAAS**, Net Protein Ratio Corrected Amino Acid Score; **NPU**, Net Protein Utilization; **NPUCAAS**, Net Protein Utilization Corrected Amino Acid Score; **PER**, Protein Efficiency Ratio; **PERCAAS**, Protein Efficiency Ratio Corrected Amino Acid Score; **TD**, True Digestibility; **TDCAAS**, True Digestibility Corrected Amino Acid Score

INTRODUCTION

Oilseeds are grown and used in many parts of the world. These seeds are used as vegetable sources of lipids (40 – 50%) and proteins (20 – 40%) (Moure *et al.* 2006). Some of them like rapeseed, soya bean and peanuts are known as proteins sources (Moure *et al.* 2006). With the continuing increase of the population in Subsaharan Africa, conventional oilseeds do not meet the nutritional need (FAO 2008).

Ricinodendron heudelotii and *Tetracarpidium conophorum* are two of many non-timber forest products in Cameroon. Both plants belonging to the Euphorbiaceae family are used as oilseeds. *R. heudelotii* is a tree of 30 m in length with 3 m circumference. Kernels known as *djansang* (*duala* and *bassa* dialects in Cameroon) are used as soup thickener. *T. conophorum* is a sarmentous plant of about 30 m in length. Fruits known as *ngak* (*bangangte* dialect in Cameroon) are usually eaten as mouth fruit after cooking (Edem *et al.* 2009).

Kernels of *R. heudelotii* and *T. conophorum* contained more than 50% of total lipids with 70% of conjugated linolenic acid and more than 50% of crude proteins (Tchiégang *et al.* 2006). Nutritional research has been carried out on lipid extracted from kernels for their valorization as nutraceutic and therapeutic oil (Tchankou Leudeu *et al.* 2009). Because of their high protein content, both defatted flours of *R. heudelotii* and *T. conophorum* have a nutritional interest. The electrophoretic pattern of their defatted flours showed that albumins and prolamins were more represented (Mezajoug Kenfack 2010).

Millions of people in Sub-Saharan Africa are depending on vegetable products, mainly cereals and legumes as daily dietary proteins. So that protein malnutrition in this region is increasing. In the other hand, demography is growing up: 2.6/year (FAO 2008). It is therefore a call for a continuous search for cheap plant proteins. *R. heudelotii* and *T. conophorum* which are tropical non conventional oilseeds could be potential candidates in this regard.

Few studies have been worked on the nutritional value of defatted flours from *R. heudelotii* and *T. conophorum* (Tchiégang and Mezajoug Kenfack 2003; Tchiégang *et al.* 2006). In this investigation, we examined the proximate chemical composition, amino acids, *in vitro*, and *in vivo* protein digestibility of defatted flours of these two Euphorbiaceae in order to assess their potential nutritive qualities.

MATERIALS AND METHODS

Sample preparation

R. heudelotii and *T. conophorum* kernels were collected from Mbalmayo and Melong markets respectively in Cameroon in August 2008. The samples were acheminated to the laboratory and treated one week after. They were ground and then extracted with hexane in a Soxhlet extractor for 8 h to remove triacylglycerols lipid fraction. The defatted flours were ground with a household flourmill (Moulinex, France) and sift by sieve of 500 µm size (AFNOR). *R. heudelotii* and *T. conophorum* defatted flours with particles size lower than 500 µm were used as the starting material.

Received: 27 September, 2010. Accepted: 10 April, 2011.

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Chemicals and reagents

Trichloroacetic acid, glucose and permanganate potassium ($KMnO_4$) solvents of analytical grade were purchased from Fischer Bioblock Scientific (France).

Chemical analyses

Moisture, crude protein and ash contents were determined using AOAC (1990) approved methods. For determination of non-nitrogen protein, 1 g defatted flours was extracted by stirring in 50 ml of aqueous trichloroacetic acid 24% (w/v) for 1 h. The slurry was centrifuged at 4500 rpm and the supernatant recovered to determine nitrogen content (AOAC 1990). Total fiber was estimated (Lee *et al.* 1992), soluble sugars were measured using a standard curve of glucose (Tood Deal *et al.* 2002). Phytate and polyphenols were determined according to AOAC (AOAC 1999). The oxalate contain was determined by titrating an aliquot of extracts from the homogenized samples with 0.09 N $KMnO_4$ (AOAC 1990).

Residual lipids associated with defatted flours were extracted following the modified method of Folch *et al.* (1957). Lipids classes of the crude oils were separated by the thin layer chromatography Iatroscan MK-5 (Iatron Laboratories Inc., Tokyo, Japan) coupled with a flame ionization detector. Fatty acid methyl esters were prepared and analyzed by gas chromatography (Périchrom, Saulx-les-Chatreux, France) (AOAC 1990; Grodji *et al.* 2006).

Nutritional analyses

1. Amino acid composition

The amino acids of two defatted flours were performed after hydrolysis of samples with 6 N HCl for 60 min at 150°C. The amino acid compositions were determined after chromatography on HPLC Applied Biosystems model 172A (Applera Corp., Foster City, CA, USA). The injections were done in triplicate for each amino acids determination and results were validated when coefficient of variation was less than 0.02. For tryptophan analysis, samples were hydrolyzed with 2.5 N $Ba(OH)_2 \cdot 8H_2O$ while the colorimetric analyses were carried out with para-dimethylaminobenzene after basic hydrolysis of proteins from defatted flours (De Vries *et al*. 1980).

The method described by Tchiégang *et al.* (2006) was used to determined amino acid concentration. Results were expressed as mg of amino acid per 100 g of defatted flour.

2. In vitro protein digestibility

In vitro protein digestibility was determined using a multienzyme digestion system (Fasasi *et al.* 2005). Enzymes used were trypsin from porcine pancreas (Sigma, USA, activity: 15900 units/mg of proteins), chymotrypsin from bovine pancreas (Sigma, activity: 62.6 units/mg of powder) and peptidase from porcine intestine (Sigma, activity: 1024/mg of powder).

3. In vivo protein digestibility

Biological assays: Weanling male rats of the Wistar strain weighting 60-70 g were used in this experiment. They were obtained from the animal holding unit of the Department of Biochemistry, Laboratory of Pharmacology and Toxicology, University of Yaoundé I, Yaoundé, Cameroon. Animals were divided into 4 groups of six animals, so, the weight difference between the 4 groups will not exceed 5 g. Rats were housed individually in plastic metabolic cages at room temperature (22 ± 3°C) and 12 h light–dark cycle. After 5 days acclimatization, animals were fed with diets and given distilled water *ad libitum* for 14 days (modified method of Giami 2005). Feed intake and body weight were recorded everyday and then every two days. During the last five days, urine and fecal were daily collected made from each animal, pooled and frozen. One milliliter of 4.8M HCl was added in 10 ml measuring flasks before urine collection to prevent nitrogen lost in form of ammonia.

The protocol and rat use were approved by the experimental research committee of the faculty of medicine of Nancy (France).

Preparation of the diets

Rats were fed with diets containing 10% of crude proteins. The composition of the experimental diets is shown in **Table 1**. Diets 1 and 2 were prepared with defatted flour of *R. heudelotii* and *T. conophorum* as protein sources, respectively. Diet 3 was the standard (casein) and diet 4 was the protein-free diet.

Nitrogen content of the feed and feces were performed by the semi-micro- Kjeldahl method using 6.38 as conversion factors for casein and 6.25 for *R. heudelotii* and *T. conophorum* defatted flour (AOAC 1984).

Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), True Digestibility (TD) and Net Protein Utilization (NPU) were the criteria used to evaluate protein digestibility and were calculated by the following formulae (Giami 2005):

$$\text{PER} = \frac{\text{Weight gain}}{\text{Protein intake}}$$

$$\text{NPR} = \frac{(\text{weight gain} + \text{weight loss})}{\text{Protein intake}}$$

$$\text{TD} = \frac{(\text{NI} - \text{F})}{\text{NI}} * 100$$

where NI = Nitrogen intake; F = Fecal nitrogen

$$\text{NPU} = \frac{\text{NI} - (\text{F} - \text{Fm}) - (\text{U} - \text{Um})}{\text{NI}} * 100$$

where Fm = metabolic fecal nitrogen; U = urinary nitrogen; Um = urinary metabolic nitrogen.

Chemical scores were calculated using amino acids of the reference proteins (FAO/WHO 2007) and essential amino acid contents of samples. For a given essential amino acid, amino acid score (AAS) was calculated by dividing the content of this amino acid in sample by the content of the same amino acid in the reference. The lowest AAS for each sample was noted AAS_1. PER, NPR, TD and NPU Corrected Amino Acid Score were the product of the lowest AAS (AAS_1) in a sample by PER (PERCAAS), NPR (NPRCAAS), TD (TDCAAS) and NPU (NPUCAAS) of the food.

Statistical analysis

Analyses were done in triplicate. One way analysis of variance was performed using Statgraphics for windows software. Significant differences were defined at $P < 0.05$. Duncan's multiple range test was used to compare means.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of *R. heudelotii* and *T. conophorum* defatted flours are shown in **Table 2**.

In both samples, the main nutrients were proteins. The true protein of defatted flours from *T. conophorum* was higher (47.9%) than that of *R. heudelotii* (44.5%). The true protein was calculated from protein nitrogen (total nitrogen minus non-protein nitrogen) × 6.25. Non-protein nitrogen for both samples was 5.8 and 4.9%, respectively and could be from ammonia, urea and free amino acids. We obtained the same results (47.36 g/100 g DW) as those obtained with *T. conophorum* from Nigeria (Edem *et al.* 2009). Protein contents of *R. heudelotii* and *T. conophorum* defatted flours are comparable with that of some edible defatted conventional oilseeds as soya bean: 40% (Vollman *et al.* 2000) peanut: 30% (Ng Chin *et al.* 2006) cowpea seeds: 20.31% (Kabas *et al.* 2007).

The total ash content of *R. heudelotii* defatted flour was higher (16.3%) compared to 6.0% found in the *T. conophorum* flour. The higher value of ash was also reported (Kapseu and Tchiégang 1995). 2.03% of ash was indicated in Nigerian *T. conophorum* nuts (Edem *et al.* 2009).

The soluble sugars contents were similar in both species and close to 5.4%. The crude fiber content varied from 9.7 to 10.9%. The fairly high level of fiber in these oilseeds is

Table 1 Diets composition of rats used in the experiments (g/1000 g of the mixture).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
R. heudelotii defatted flour	22	–	–	–
T. conophorum defatted flour	–	21	–	–
Casein	–	–	10	–
Mineral mixture*	40	40	40	40
Vitamin mixture**	10	10	10	10
Corn oil	80	80	80	80
Cellulose	30	30	30	30
Sucrose	374	374	374	374
DL-Methionine	3	3	3	3
Corn starch	443	442	453	463

*Salt mixture (composition/100 g): phosphate (36.5 g); calcium carbonate (22.77 g); potassium chloride (7.75 g); sodium chloride (7.75 g); anhydrous magnesium (4 g); ferrous sulfate (1.13 g); zinc sulfate (0.9 g); manganese sulfate (0.4 g); copper sulfate (0.1 g); potassium iodine (1 mg).
**Vitamin mixture (composition/100 g): Vit B_1 (1.5 mg); Vit B_2 (2.5 mg); Vit B_3 (15 mg); Vit B_5 (5 mg); Vit B_6 (1.5 mg); Vit B_9 (0.5 mg); Vit B_8 (150 µg); Vit B_{12} (10 µg); Vit A (1 mg); Vit D (37 µg); Vit E (40 mg); Vit K (3 mg).

Table 2 Proximate composition of defatted flours from *R. heudelotii* and *T. conophorum* (g/100 g of dry matter)

Constituents	*R. heudelotii*	*T. conophorum*
Crude protein (N*6.25)	50.3 ± 1.4 b	52.8 ± 0.5 a
True protein (N*6.25)	44.5 ± 0.0 b	47.9 ± 0.5 a
Ash	16.3 ± 0.9 a	6.05 ± 0.7 b
Residuals lipids	8.7 ± 1.1 a	9.1 ± 0.5 a
Soluble sugars	5.3 ± 1.6 a	5.5 ± 0.0 a
Crude fiber	10.9 ± 0.0 a	9.7 ± 0.3 b
Polyphenols	0.94 ± 0.04 b	1.60 ± 0.11 a
phytate	0.01 ± 0.0 a	0.01 ± 0.0 a
Oxalic acid	2.70 ± 0.05 a	2.20 ± 0.14 a

Values in a row with different letters indicate significant differences ($P < 0.05$) according to DMRT

desirable characteristic, since their positive effects on diabetic and arteriosclerose diseases have been proved (Liu *et al.* 2000).

Polyphenols contents in defatted flours were 0.94 and 1.60% for *R. heudelotii* and *T. conophorum*, respectively. Oxalate contents were around 2% in both defatted flours. These antinutritional substances can complex proteins and reduce their nutritional value. Phytate was below 0.1% in both defatted flours.

Although, the kernels were extracted with hexane, lipids were not completely removed and part of them remained in the flours (**Table 3**). Lipids tend to associated with proteins which affect the flavor and nutritional value of defatted flours (Kikugawa *et al.* 1981). *R. heudelotii* and *T. conophorum* defatted flours contained 8.7 ± 0.7 and 9.1 ± 0.2% lipids, respectively, that could be extracted with a mixture of chloroform/methanol (Folch *et al.* 1957). The lipids classes of these oils separated by thin layer chromatography (Iatroscan MK-5) showed that polar lipids are more represented (90 and 92% from *R. heudelotii* and *T. conophorum* defatted flours, respectively) than non polar

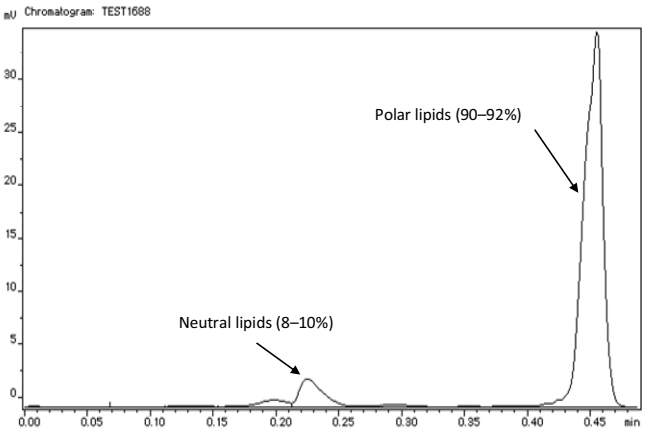

Fig. 1 Lipids classes of *R. heudelotii* and *T. conophorum* defatted flours obtained from kernels oils extracted with hexane.

lipids (**Fig. 1**). These lipids, mainly polar, play an important role in the emulsifying activity and stability of emulsions. Emulsifier agents as phospholipids decrease the interfacial tension and facilitate formation of stable oil–water interfaces, increase the stability of lipids oil against oxidation (Belhaj *et al*. 2010). Phospholipids prevent food behaviour disorders (Marsollier 2009).

Table 3 shows the fatty acid composition of polar lipids extracted from two defatted flours analyzed. Results were compared with triacylglycerol oils extracted from kernels of *R. heudelotii* and *T. conophorum* (Tchankou Leudeu *et al.* 2009), from some common used oilseeds in Cameroon as *Coula edulis* (Tchiégang *et al.* 1998), *Arachis hypogea* (Musa Özcan 2009), *Belschmiedia anacardiodes* (Tchiégang and Parmentier 2008). In *R. heudelotii* and *T. conophorum* defatted flours, oleic acid ($C_{18:1(\omega-9)}$) was the main fatty acid (65.2 and 33.9% of total fatty acids, respectively). Linoleic ($C_{18:2(\omega-6)}$) and linolenic ($C_{18:3(\omega-3)}$) acid were also major in *R. heudelotii* and *T. conophorum* with 12.8 and 22.3% of total fatty acids, respectively. The presence of considerable amounts of linoleic acids suggests that oils obtained from both defatted flours are nutritious due to their ability to reduce serum cholesterol (Tarek *et al.* 2001). Oils samples analyzed were rich in oleic and inoleic acids as triacylglycerols from *Arachis hypogea*, one conventional oilseed, and from *B. anacardiodes*, one soup thickeners in Cameroon (Tchiégang and Parmentier 2008). Lipids extracted from *R. heudelotii* and *T. conophorum* defatted flours were rich in oleic acid than those extracted from their kernels. It is also important to point out that, lipids extracted with hexane in *R. heudelotii* kernels were rich in α–elaeostearic (32.5%) (Tchankou Leudeu *et al.* 2009). Residuals lipids extracted from *R. heudelotii* and *T. conophorum* defatted flours could be used as edible cooking, salad oils or for margarine manufacture (Tarek *et al.* 2001). Polyunsaturated fatty acids have received considerable interest,

Table 3 Fatty acid composition of lipids (expressed as percentage of total fatty acid) extracted from defatted flours (*10*) and from kernels of *R. heudelotii*, *T. conophorum*, *Dacryodes edulis*, *Elaeis guineensis*.

Fatty acids	Triacylglycerols*				Polar lipids**	
	R. heudelotii	*T. conophorum*	*Dacryodes edulis*	*Elaeis guineensis*	*R. heudelotii*	*T. conophorum*
$C_{14:0}$	0.02 ± 0.0	0.4 ± 0.0	0.09 ± 0.0	0.8 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
$C_{15:0}$	–	–	–		–	4.9 ± 0.0
$C_{16:0}$	6.6 ± 0.1	1.7 ± 0.0	43.88 ± 0.0	41.6 ± 0.0	18.4 ± 0.2	6.7 ± 0.1
$C_{17:0}$	–	–	–		–	0.3 ± 0.0
$C_{18:0}$	6.5 ± 0.8	–	0.1 ± 0.0	4.9 ± 0.0	–	8.1 ± 0.0
$C_{18:1(\omega-9)(oleic\ acid)}$	6.0 ± 0.0	10.8 ± 0.1	30.4 ± 0.0	41.0 ± 00	65.2 ± 0.3	33.9 ± 0.1
$C_{18:2(\omega-6)\ (linoleic\ acid)}$	27.9 ± 1.4	14.83 ± 0.0	24.55 ± 0.0	10.41 ± 0.1	12.8 ± 00	10.7 ± 0.0
$C_{18:3(\omega-3)\ (linolénic\ acid)}$	–	72.2 ± 0.3	0.8 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	22.3 ± 0.1
$C_{18:3\ (\alpha-elaeostearic\ acid)}$	52.6 ± 0.2	–	–	–	–	–
$C_{20:0}$	–	–	–	0.5 ± 0.0	0.5 ± 0.0	2.9 ± 0.2

* Fatty acid composition of lipids extracted with hexane from kernels (*26*)
** Fatty acid composition of lipids extracted from defatted flours

Table 4 Amino acid contents (mg/g of protein) and chemical scores (%) of defatted flours from *R. heudelotii* and *T. conophorum*.

Amino acids	*R. heudelotii*		*T. conophorum*		FAO / WHO (2007) pattern
	Amino acid contents	Chemical scores	Amino acid contents	Chemical scores	Amino acid contents
Essential amino acids					
Ile	59.0	200.0	64.6	215.4	30
Leu	128.9	118.4	122.2	207.2	59
Lys	48.9	108.7*	71.2	158.7	45
Met + Cys	53.9	245.4	62.0	281.9	22
Phe + Tyr	102.1	268.7	79.5	209.4	38
Thr	68.0	295.6	71.5	311.2	23
Val	133.9	372.2	96.5	268.1	36
Trp	26.9	250.0	11.9	198.5*	6
His	32.8	219.2	37.6	251.3	15
Non-essential amino acids					
Asp	270.40		324.09		
Glu	369.26		237.54		
Ser	95.1		111.09		
Gly	203.00		352.21		
Arg	182.09		127.24		
Ala	157.32		138.61		
Pro	97.44		92.54		

* Underlined values correspond to the lowest Amino Acid Score (AAS_1) for each sample.

Table 5 PER, NPR, TD and NPU for casein and defatted flours from *R. heudelotii* and *T. conophorum* defatted flours.

Diet	PER	NPR	TD	NPU
Casein	3.3 ± 0.4 a	5.1 ± 0.7 a	97.6 ± 4.1 a	98.0 ± 3.2 a
R. heudelotii defatted flour	2.0 ± 0.3 b	3.3 ± 0.1 b	58.9 ± 8.9 b	51.4 ± 1.4 b
T. conophorum defatted flour	2.9 ± 0.5 a	4.4 ± 0.4 a	96.3 ± 3.8 a	96.5 ± 1.4 a

Values in a row with different letters indicate significant differences ($P < 0.05$) according to DMRT

Table 6 PERCAAS, NPRCAAS, TDCAAS and NPUCAAS (%) for *R. heudelotii* and *T. conophorum* defatted flours.

	PERCAAS	NPRCAAS	TDCAAS	NPUCAAS
R. heudelotii defatted flour	2.1	3.5	63.6	55.5
T. conophorum defatted flour	4.5	7.0	152.1	152.4

because, their consumption has been associated with beneficial health effects as the reduction of cholesterol and triglycerides levels in rats (Tchankou Leudeu *et al.* 2009).

Protein quality

1. Amino acids composition

The amino acids composition compared to amino acids scoring pattern of the Food and Agricultural Organization of the United Nations (FAO/ WHO 2007) are given in **Table 4**.

Sixteen amino acids including tryptophan were determined. Isoleucine, leucine, valine and sulphur amino acids (methionine + cysteine) were the major essential amino acids. They represented an average of 54 and 134 mg/g of proteins. Essential amino acid accounted for 33.4 (*R. heudelotii*) and 33.0% (*T. conophorum*) of total amino acid contents, indicating a good equilibrium between the amino acids (Tchiégang *et al.* 2006). The amounts of essential amino acids in both species exceeded the value in FAO/ WHO (2007) scoring pattern. Lysine is usually limiting in many vegetable foods, but was present in amounts exceeding the reference protein requirements (FAO/WHO 2007) in *R. heudelotii* and *T. conophorum* defatted flours with a concentration of 49.0 and 71.0 mg/g of protein, respectively. Results obtained by Tchiégang *et al.* (2006) indicated the presence of tryptophan with chemical score of 33.6% and methionine with chemical score of 47.6% as limiting amino acid in *R. heudelotii* and *T. conophorum* defatted flours, respectively. This difference could be due to the fact that protein quality in plants widely varies with the environments (Vollman *et al.* 2000). It is known that basic amino acids and glutamic acid contribute to the flavour properties of mushroom, a wild edible fungus (Maga 1981). The high level of arginin (182.09 369.2 mg/g of protein) and glutamic acid (369.2 mg/g of protein) in *R. heudelotii* defatted flour could contribute to their characteristic flavour since their kernels are used as soup thickener.

2. In vitro and *in vivo* protein digestibility

***In vitro* protein digestibility:** *R. heudelotii* defatted flour had the highest *in vitro* protein digestibility (81.4 ± 1.0%) compared to *T. conophorum* defatted flour (69.2 ± 0.9%). Aromatic amino acid (phenylalanine and tyrosine) contents of the two plants were 102.1 and 79.5 mg/g of protein, respectively. This difference may explain the high *in vitro* protein digestibility of *R. heudelotii*, since pepsin acts preferentially near the basic side of aromatic amino acids (Roufik *et al.* 2006). *In vitro* methods showed the capacity of proteins to be hydrolyse by proteic enzymes, results *in vivo* may give the availability of these amino acids.

***In vivo* protein digestibility:** The values of PER, NPR, TD, and NPU are summarized in **Table 5**. Corresponding corrected values are given in **Table 6**.

PER and NPR indicated the gain in weight/g of protein eaten. However, NPR was calculated when a group of animals fed protein free diet was included in the experiment (Giami 2005). PER and NPR for *R. heudelotii* were significantly lower ($P < 0.05$) compare to that of *T. conophorum* and casein (**Table 5**). According to these values, animals fed with casein and *T. conophorum* defatted flour give the highest growth, whereas, those fed with *R. heudelotii* defatted flour show the lowest growth. NPR values from *R. heudelotii* (3.3) and *T. conophorum* (4.4) were comparable to those obtained with soya bean (2.74) and rapeseed meal (4.59), respectively (Rozan *et al.* 1997).

PERCAAS and NPRCAAS of both samples increase (**Table 6**) because there is not limiting amino acids and the chemical scores were 108.7 and 158.7% for *R. heudelotii* and *T. conophorum* defatted flours, respectively. PERCAAS of both Euphorbiaceae (2.1 and 4.5 respectively) were higher than those from soybean (1.6) and rapeseed meal (0.9) (Rozan *et al.* 1997). In this regards, proteins from *R. heudelotii* and *T. conophorum* defatted flours could be of good interest as those from conventional oilseeds.

TD is the ratio of nitrogen absorbed versus nitrogen ingested when fecal metabolic nitrogen is considered. Comparing the TD obtained for the test samples with that of casein (**Table 5**), it was observed that casein digestibility (97.6%) was not significantly different ($P < 0.05$) from *T. conophorum* defatted flour (96.3%), but it was significantly higher than that of *R. heudelotii* (58.9%). The lowest value of *R. heudelotii* defatted flour could be attributed to high level of crude fiber (10.9% of DW), because crude fiber has high hydration capacity and could reduced intestinal absorption and increase fecal nitrogen lost (Leterme *et al.* 1997). Nitrogen fecal losses were higher in rats fed *R. heudelotii* defatted flour diet with 0.14% in compared to those fed *T. conophorum* defatted flour diet with 0.09%.

NPU is the ratio of nitrogen retained versus nitrogen ingested when feacal metabolic nitrogen is considered. The NPU of *T. conophorum* (96.5%) was compared to that of casein (98.0%) and was higher than that of *R. heudelotii* (51.4%). The highest level of ash (16.3%) in *R. heudelotii* defatted flour could induced the lowest NPU value because it is known that phytate–mineral–protein complexes may also adversely influence protein digestion and bioavailability (Urbano *et al.* 2000).

TDCAAS and NPUCAAS were calculated by multiplying TD and NPU respectively with values of their amino acid scores (AAS_1). Corrected values of *R. heudelotii* and *T. conophorum* increased due to the high value of AAS_1 (158.7 and 108.7, respectively). Both defatted flours presented important protein digestibility corrected values and therefore, they had a better nutritional protein quality, but, *T. conophorum* had the highest value (152%) in compared to protein from egg and cow's milk with 118 and 121%, respectively (FAO/WHO 1990). The two species analyzed are from Euphorbiaceae family. This difference showed that the quality of protein varies between them. PERCAAS and NPRCAAS presented by rats fed *R. heudelotii* and *T. conophorum* defatted flours diets were more than 2, means that proteins were of good nutritional quality (Friedman 1996). TDCAAS and NPUCAAS values of both Euphorbiaceae were between 55 and 152, means that availability of both protein from samples analyzed were more than 55%.

CONCLUSION

R. heudelotii and *T. conophorum* defatted flours could be used as protein and fiber sources. Phospholipids in both defatted flours represented more than 90% of polar lipids. PER and NPR were two growing parameters measured and corrected values with amino acids score showed that rats fed with *T. conophorum* diet presented the highest growth. TD and NPU represent the percentage of the absorbed or fed protein retained in the body. TDCAAS and NPUCAAS of both defatted flours were between 51-97%. *T. conophorum* defatted flour showed the highest protein quality compared to that of casein, based on its *in vivo* protein digestibility. Results obtained *in vitro* with *R. heudelotii* were different from those *in vivo*, because of the highest level of dietary fiber and ash.

ACKNOWLEDGEMENTS

This work was supported by IFS (International Foundation for Science), France – Cameroon Corporation and Laboratory of Biomolecular Engineering of ENSAIA (INPL – Nancy, France).

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