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Physico chemical properties of defatted flours from *Ricinodendron heudelotii* (Bail.) and *Tetracarpidium conophorum* (müll. arg.) with respect to particle sizes

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Particle fractions from *Ricinodendron heudelotii* and *Tetracarpidium conophorum* defatted flours were obtained after sieving with 160, 400 and 500 μ m sieve mesh. Each particle fraction size was used for the evaluation of proximate and amino acid compositions. Predicted Protein Efficiency Ratio (PER), Biological Value (BV) and the hydrolysis degree of high protein fractions for both plants were performed. The unsieved defatted flours were used as standard. Total proteins (N x 6.25) varied between 2.01 (<160 μ m) to 40.53% (400 – 500 μ m) for *R. heudelotii* fractions and 2.76 (<160 μ m) to 43.91% (400 – 500 μ m) for *T. conophorum* fractions. Total ashes contents were higher in 400 – 500 μ m (3.88 and 8.56% respectively) fractions and lower in fractions < 160 μ m (0.65 and 1.06% respectively). For both defatted flours, fractions > 500 μ m were the highest in crude fibres (5.86 – 6.55%). Phytates, total polyphenols and oxalates were 0.04, 0.31 and 0.61% respectively in different fractions. Essential amino acids from 400 – 500 μ m fractions were close to the Food Agriculture Organization requirements, except lysine for which the chemical score in *R. heudelotii* sample was 69%. The proteins hydrolysis degree indicated high values with sieved defatted flours (6.81-7.79%) compared to the corresponding unsieved flours (4.72-5.29%). Predicted PER and BV of 400 – 500 μ m particles size from both plants were more than 3, and between 69 – 107% respectively. There was a significant effect ($P < 0.05$) of particle size on the different chemical properties. These results indicate desirable fractions for technological purposes as far as defatted flours of these two plants are concerned.

Keywords: *R. heudelotii*, *T. conophorum*, defatted flours, particle sizes, chemical composition, nutritive value.

1. INTRODUCTION

Ricinodendron heudelotii and *Tetracarpidium conophorum*

are two non-conventional oil bearing crops belonging to the family of Euphorbiaceae that grow in equatorial forest of Madagascar and Central Africa (Nigeria, Congo, Gabon, Ivory Coast). In Cameroon those plants grow in the south, west and coastal regions. Their kernels are highly used as

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spice (*R. heudelotii*) and “mouth fruit” (*T. conophorum*) in Central Africa and particularly in Cameroon by the western and coastal habitants (Mosso et al., 1998).

Much information is available in the literature on the physico - chemical characteristics of these oilseeds. Physico – chemical properties of kernels from *R. heudelotii* and *T. conophorum* revealed more than 50% of crude fat, 55 – 62% of crude proteins in corresponding defatted flours (Ige et al., 1984; Tchiégang et al., 1997). Amino acids of proteins from *R. heudelotii* defatted flour are equilibrated on the nutritional view point (Tchiégang et al., 1997). As for *T. conophorum*, its defatted flour contained proteins with good functional properties (Ige et al., 1984; Mezajoug Kenfack et al., 2011). In the human diets, proteins are obtained from animal and plant sources, but the cost of animal protein is increasing making it unavailable to many people in developing countries (Ekpo, 2011). Thus, many families look for alternative sources of protein from plant origin like cereal and legumes (Li, 2013). In Subsaharian Africa, protein malnutrition induces 34.6% of mortality for children less than 5 years of ages (FAO, 2008). In Central Africa two areas that have often been neglected in augmenting available raw food materials, especially oilseeds for food protein production are the use of under exploited local substitutes of proteins and the non development of their production at industrial scale. The under – exploitation of some wild plants in Cameroon has stimulated our interest on *R. heudelotii* and *T. conophorum*.

Studies on grinding showed that particle sizes is one of the chemical and nutritional properties factors of food variation (Guillou and Landeau, 2000). In the aim of valorising defatted flours, it is important to have flours with the same particle size, so as to appreciate their functional, nutritional and technological behaviours as food ingredients. There is a need of looking for raw materials with high protein content with very low antinutritional components to produced infant slurries. The need to produced defatted flours from oilseeds with high proteins and low antinutritional components to prevent protein malnutrition is the purpose of this research. Thus, the aim of this work was to determine the influence of particle sizes on the distribution of some nutritional and antinutritional substances of *R. heudelotii* and *T. conophorum* defatted flours.

2. MATERIALS AND METHODS

2.1. Production of defatted flours

Kernels of *R. heudelotii* and nuts of *T. conophorum* were purchased respectively from Mbalmayo and Nkongsamba markets (Cameroon). *T. conophorum* nuts were cooked for 30 min in water (1/5: p/v) and dehulled manually by crushing. Kernels from the two samples were separately ground in a

hammer mill (Moulinex, France) for 5min. The charge of grinding container was fixed at 2/3 (w/v). Meals obtained were defatted for 12 h with hexane using Soxhlet apparatus (AOAC 1990).

2.2. Sieving procedure

The efficiency of sieves was determined after sieving using the method described by Melcion (2000). Sieves of 160, 400, 500µm sizes were used.

50g (M_1) of sample were placed on a set of three sieves of 160, 400 and 500µm sizes and submitted to vibration (50Hz) during 15 min in a Merck vibrator. Particle sizes less than the mesh size of sieves used passed (M_2), while those bigger than the mesh sizes (M_3) were retained. All fractions were weighed to determine the particle distributions of samples. The efficiency of sieves was calculated as follows: $[(M_2 \text{ or } M_3) / M_1] \cdot 100$. Figure 1 shows the general flow diagram of the work plan.

2.3. Chemical analyses

Analyses were carried out on unsieved and sieved defatted flours. Unsieved defatted flours were used as standard. Total nitrogen was determined by the microkjeldahl method. Crude protein contents were estimated using a conversion factor of 6.25. Ashes, total sugar, total fibres, total oxalate acids and phytate contents were performed using the standard method describe by AOAC (1990). Polyphenolic compounds were determined according to the Marigo (1973) method.

2.4. Nutritional analyses

2.4.1. Amino acids composition

Samples were hydrolyzed with 6 N HCl at 150°C for 60 min and derivatized with phenyl – isothiocyanate. Amino acids were determined by high performance liquid chromatography at the Protein Sequencing Laboratory of the Faculty of Science, Université Henri Point Carré, Nancy 1, France. The analyses were done in triplicate for each amino acid determination and results were validated when the variation coefficient was less than 0.02.

For tryptophan contents, defatted flours were hydrolysed using 2.5 N $\text{Ba}(\text{OH})_2$, $8\text{H}_2\text{O}$ and measured spectrophotometrically with para dimethylaminobenzene (De Vries et al. 1980). The method described by Tchiégang et al. (2006) was used to calculate amino acid concentrations. Results were expressed as mg of amino acid per 100 g of defatted flour.

2.4.2. Essential Amino Acids Index

First, chemical scores were calculated using amino acids of the reference proteins (FAO/WHO, 2011) and essential

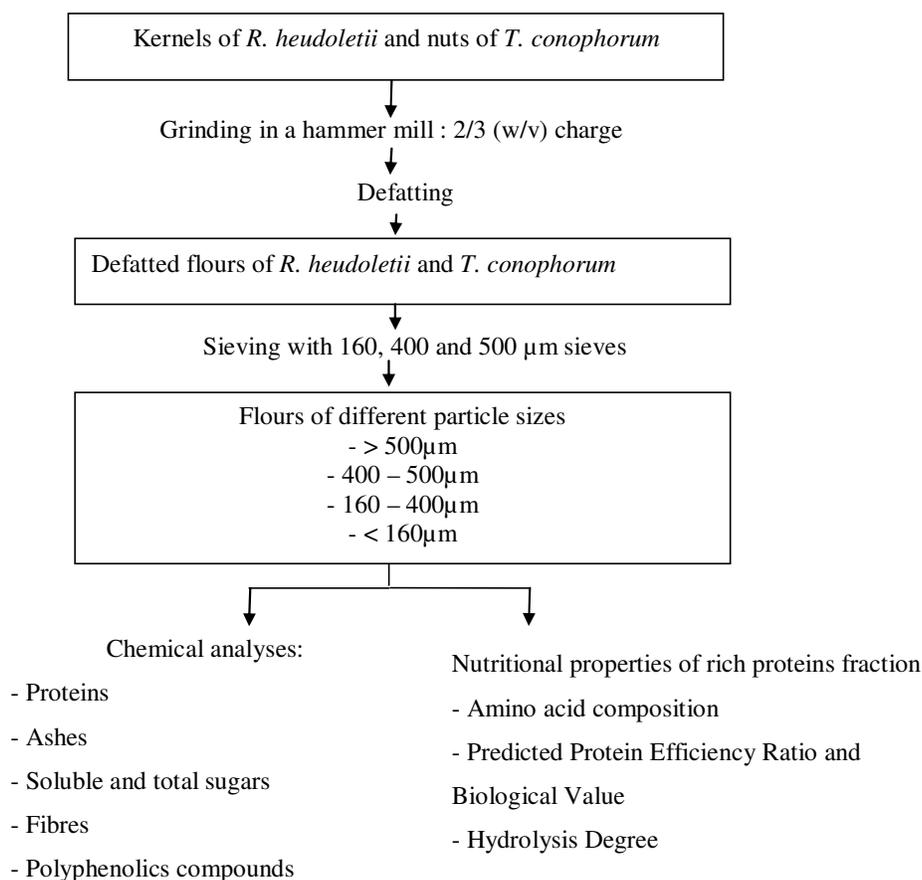


Figure 1: Flow diagram of work plan

amino acid contents of samples. Then for a given essential amino acid, Essential Amino Acid Index (EAAI) was obtained by dividing the content of this amino acid in the sample by the content of the same amino acid in the reference.

2.4.3. Protein Efficiency Ratio (PER)

The Protein Efficiency Ratio (PER) of defatted flours were estimated according to the following regression proposed by Alsmeyer et al. (1974): $PER = 0.0630[X_9] - 0.1539$.

Where $[X_9] = Thr + Val + (Met + Cys) + Ile + Leu + (Phe + Tyr) + Lys + His + Tyr$.

2.4.3. Biological Value (BV)

The Biological Values (BV) were calculated using the equation of Oser (1959). $BV = 1.09 (EAAI) - 11.7$.

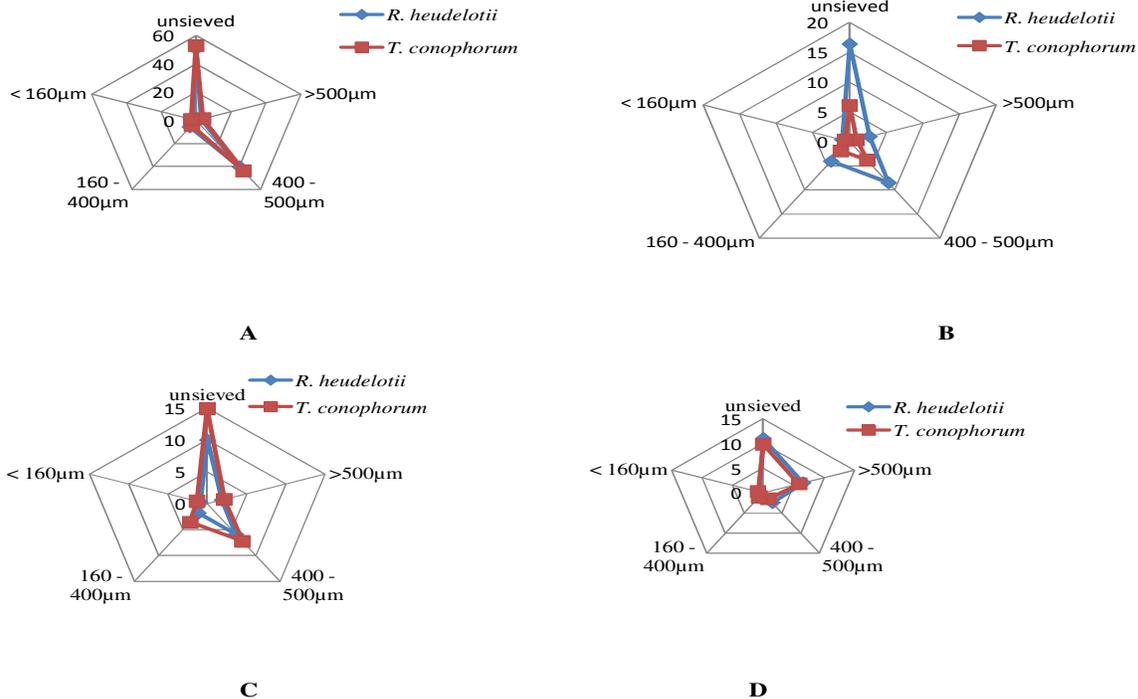
3. Statistical analyses

The statistical analyses (ANOVA) were carried out in order to compare the averages. The software Statgraphic version 15 was used for these purposes. Duncan's test was performed at 5% probability in order to compare the means. Radar plot was used to represent nutritional compositions.

4. RESULTS AND DISCUSSION

4.1. Efficiency of sieving

Efficiency of sieving is the percentage of flour that could pass through the considered sizes. For the two samples, the highest fraction obtained was with 400 - 500μm particle sizes with corresponding values of 86.53 and 80.77% for *R. heudelotii* and *T. conophorum* respectively. *R. heudelotii* had a high sieving efficiency compared to that of *T. conophorum* but the difference was not significantly ($P > 0.05$). This could be due to the difference of a consequence of different textures and the dry matter



Figures 2: Total proteins (A), ashes (B), sugars (C) and fibres (D) of defatted flours from *R. heudelotii* and *T. conophorum* with respect of particle sizes

(97.30% for *R. heudelotii* and 95.10% for *T. conophorum*) of the defatted samples as suggested by Melcion (2000).

4.2. Chemical composition

4.2.1. Total proteins

Figure 2A showed the total protein (N x 6.25) contents with respect to different particle fractions. The unsieved defatted flours of *R. heudelotii* and *T. conophorum* had a total proteins contents of 50.30 and 52.81% respectively. There was a significant difference ($P < 0.05$) between the mean values of the total protein contents of the different fractions for both oilseeds. Protein content ranged from 2.01 (*R. heudelotii* particle size less than 160µm) to 43.91% (400 - 500µm *T. conophorum* particle size). For both species, particles between 400 - 500µm showed the highest protein contents with 40.53 and 43.91% respectively. Compared to the unsieved flours, the recovery of proteins was 80.57 and 83.31% respectively.

The highest content of protein contents in unsieved and sieved (400 – 500µm fractions) defatted flours is of interest to be used for infant weaning foods and at technological purposes. These defatted flours are good sources of proteins as soybean (40%) (Vollman et al., 2000) and peanuts flours (30%) (Ng et al., 2006), which are oil seeds commonly used for the preparation of infant slurries. The

use of these fractions would depend on antinutrient factors and the bioavailability of amino acids.

4.2.2. Total ashes

Total ashes represent the total mineral content. The unsieved defatted flour of *R. heudelotii* contained more ashes (16.35%) than that of *T. conophorum* (6.05%) and values were significantly ($P < 0.05$) higher than those in different particles size (figure 2B). Total ashes of unsieved defatted flours corroborate with values reported by Enujiugha (2003), Tchiégang et al. (2006) who indicated 16 and 9% on *R. heudelotii* and *T. conophorum* defatted flours respectively. For *R. heudelotii* and *T. conophorum*, the fraction with the highest ashes content was 400 - 500µm (8.56 and 3.88%), while the lowest was fraction < 160µm (1.06 and 0.65g% DW respectively) (figure 2B). Compared to unsieved flours, the recovery of ashes in 400 – 500µm particle fractions were 52.35 and 64.41 % for *R. heudelotii* and *T. conophorum* respectively.

4.2.3. Total sugars

Total sugars in the unsieved defatted flours of *R. heudelotii* and *T. conophorum* were 9.98 and 14.91 g% DW respectively (figure 2C). For both defatted flours, fractions < 160µm had the lowest value of total sugars, while the

Table 1: Total polyphenolics, phytates, total oxalate contents (mg%) of defatted flours of *R. heudelotii* and *T. conophorum*

		<i>R. heudelotii</i>		
		Total polyphenolic compounds	Phytates	Total oxalates
Unsieved		940.00 ± 5.02 ^a	17.57 ± 0.54 ^a	2690.87 ± 0.05 ^a
	>500µm	170.01 ± 3.10 ^d	3.03 ± 0.23 ^d	620.01 ± 2.02 ^d
	400 - 500µm	210.02 ± 5.40 ^c	6.54 ± 0.18 ^b	1060.01 ± 7.72 ^b
Sieved	160 - 400µm	490.05 ± 6.57 ^b	5.03 ± 0.32 ^c	790.00 ± 6.61 ^c
	< 160µm	80.05 ± 4.63 ^e	3.07 ± 0.36 ^d	150.03 ± 5.51 ^d
		<i>T. conophorum</i>		
Unsieved		1590.04 ± 8.26 ^a	18.46 ± 0.36 ^a	2200.02 ± 0.14 ^a
	> 500 µm	250.07 ± 6.55 ^d	4.37 ± 0.07 ^b	104.24 ± 4.04 ^e
	400 - 500µm	480.12 ± 4.13 ^c	8.76 ± 0.12 ^c	780.03 ± 3.80 ^c
Sieved	160 - 400µm	730.01 ± 7.43 ^b	4.14 ± 0.46 ^d	1140.04 ± 8.80 ^b
	< 160µm	83.08 ± 3.09 ^e	1.02 ± 0.27 ^e	287.07 ± 2.79 ^d

Means within the same line followed by the same superscripted letter are not statistically different ($P < 0.05$)

highest value was recorded in 400 – 500 µm particle sizes. These values represented 59.41 and 48.55% of unsieved flours from *R. heudelotii* and *T. conophorum* respectively. Total sugars in unsieved flours and fractions 400 – 500µm were lower than those indicated by Felberg et al. (2009) (32.43%) in soybean.

4.2.4. Total fibres

The total fibres contents of the unsieved defatted flours and sieved fractions of *R. heudelotii* and *T. conophorum* are found in figure 2D. There was a significant difference ($P < 0.05$) between the mean values of the crude fibre of different particle fractions of the two oil seeds. Fibre contents of unsieved defatted flours were significantly higher when compared to the corresponding sieved defatted flours (0.68 to 5.86g%). *R. heudelotii* contained the highest value of fibre (10.98g%), compared to *T. conophorum* (9.76g%).

The defatted flours with particle size > 500µm for *R. heudelotii* and *T. conophorum*, contained the highest total fibre (6.55 and 5.86 respectively). These values represent 50.54 and 49.79% of unsieved flours respectively. Fractions with the lowest crude fibres content being the fractions < 160µm (0.68 – 0.82g%). Physiological roles of crude fibres in the body is to maintain an internal distention for proper peristaltic movement of the gastro - internal tract and thereby prevent constipation. Diets with high fibre content have been used for diabetic and atherosclerosis patients (Groff et al. 1985; Oduor et al. 2008). *R. heudelotii* and *T. conophorum* defatted flours with 400 – 500µm particles sizes could reduce constipation when added in

the food of diabetic and atherosclerosis patients as indicated by Liu et al. (2000).

4.2.5. Antinutritional substances

Polyphenolic compounds

The total polyphenolic contents in defatted flours and the different particles fractions are given in table 1. Polyphenolic compounds have been associated with colour, sensory qualities, nutritional and antioxidant properties of food (Wang et al., 2013). For the two plants species, particle sizes between 160 – 400µm had the highest polyphenolic compound contents (490 and 730mg%). These values represented 52.13 and 45.91% of the respective unsieved flours (940 and 1590mg%). The lowest values were noted for fractions < 160µm for both *R. heudelotii* and *T. conophorum*.

Phytates

Table 1 shows that in both defatted flours, phytic acid contents were less than 1g%. From one fraction to other, the level of phytate was different ($P < 0.05$) for each sample. For both flours, particle size between 400 – 500µm had the highest phytates contents. Results indicate that phytates contained in both Euphorbiaceae could not affected bioavailability of proteins through the formation of protein – phytate – mineral (cations) complexes during food processing (Anhwange et al. 2005).

Table 2: Amino acids contents (g /100g of protein) of sieved (400 – 500µm particles size) and unsieved defatted flours of *R. heudelotii* and *T. conophorum*

Amino acids	Sieved <i>R. heudelotii</i>	Unsieved <i>R. heudelotii</i>	Sieved <i>T. conophorum</i>	Unsieved <i>T. conophorum</i>	FAO/WHO requirements (2011)
Ile	4.90	5.90	5.30	6.46	3.2
Leu	9.70	12.89	8.80	12.22	6.6
Lys	<u>4.26</u>	4.89	6.22	7.12	5.7
Met + Cys	4.16	5.39	5.32	6.20	2.7
Phe + Tyr	9.20	10.21	5.91	7.95	5.2
Thr	5.70	6.80	6.52	7.15	3.1
Val	12.10	13.39	7.62	9.65	4.3
Trp	1.58	2.69	0.92	1.19	0.8
His	<u>2.15</u>	<u>3.28</u>	<u>4.91</u>	<u>3.76</u>	<u>2.0</u>
Non essentials					
Asp	1.97	27.04	28.10	32.40	
Glu	24.41	36.92	21.11	23.75	
Ser	0.79	9.56	9.30	11.10	
Gly	1.87	20.30	31.71	35.22	
Arg	1.62	18.20	10.71	12.72	
Ala	1.36	15.73	11.91	13.86	
Pro	0.89	9.74	8.11	9.25	
ΣEAA	53.73	60.05	51.52	61.7	
ΣTAA	32.91	139.49	120.95	138.30	

*Mezajoug Kenfack et al. (2011). Value underlined is limiting amino acids.

Total oxalates

Sieving induced the variation ($P < 0.05$) of total oxalate in *R. heudelotii* and *T. conophorum* defatted flours (table 1). Oxalic acid contents in unsieved flours were 2.69 and 2.20g%DW respectively. For *R. heudelotii* defatted flour,

fraction between 400 - 500µm had the highest value of total oxalate content (1.06%). As shown on figures 2A and 2B, the same fraction was also rich in total proteins and total ashes. This result suggested that the greatest part of oxalic acid could react with proteins and minerals in the *R. heudelotii* defatted flour. As far as *T. conophorum* defatted flour is concerned, the

highest value of total oxalate (1.14%) was obtained with fraction between 160 - 400µm. In the same sample, the highest level of total proteins was in fraction between 400 - 500µm. Grinding and sieving of *T. conophorum* sample could induce the dissociation of protein – oxalate – mineral cations complexes formed during cooking.

Table 3: Hydrolysis degree (%), volume of NaOH (0,1 N) of unsieved and 400 – 500 particle sizes from *R. heudelotii* and *T. conophorum* defatted flours

	Hydrolysis degree (%)	Volume of NaOH 0.1N (ml)
Casein	11.07±0.02	2.84±0.00
<i>R. heudelotii</i>		
Sieved defatted flour	6.81±0.04 ^b	2.59±0.00
Unsieved defatted flour	4.72±0.02 ^a	2.52±0.01
<i>T. conophorum</i>		
Sieved defatted flour	7.79±0.01 ^b	2.72±0.01
Unsieved defatted flour	5.29±0.02 ^a	2.82±0.00

Means within the same column followed by the same superscripted letter are not statistically different ($P < 0.05$)

4.3. Protein quality

The physico – chemical composition of sieved defatted flours of *R. heudelotii* and *T. conophorum* indicated that the sieved defatted flours withing 400 – 500µm particle sizes were those with high efficiency and high protein contents. These fractions for both defatted flours were used in this work to evaluate the nutritional quality of proteins.

4.3.1. Amino acids composition

Table 2 shows the amino acids profiles of the fractions between 400 – 500µm of both defatted flours, compared to the amino acids scoring pattern of the Food and Agricultural Organisation of the United Nations (FAO/WHO, 2011). Amino acids of sieved defatted flours were also compared with those of unsieved defatted flours of *R. heudelotii* and *T. conophorum* (Mezajoug Kenfack et al. 2011).

Sieved defatted flours with 400 – 500µm particle sizes of *R. heudelotii* and *T. conophorum* showed higher concentrations of Met+Cys, threonine and valine as compared to the FAO/WHO requirements for the essential amino acids (FAO/WHO, 2011). All the essential amino acids of analyzed fractions exceeded the FAO/WHO (2011) requirements, except lysine in *R. heudelotii*, with a chemical score of 69%. As indicated by Mezajoug Kenfack et al. (2011), there were no limiting amino acids in both unsieved defatted flours. The amount of amino acids in unsieved defatted flours was higher than that of the corresponding sieved flours. These results show that sieving influence the amino acids composition of *R. heudelotii* and *T. conophorum*.

The most abundant amino acids were glutamic acid (24.41g/100g of protein) and glycine (31.71g/100g of protein) in *R. heudelotii* and *T. conophorum* sieve defatted flours.

4.3.2. Hydrolysis degree

Results in **table 3** show that hydrolysis degree of proteins after 10min of digestion with enzyme mixture (trypsin, chymotrypsin and peptidase) was maximum with casein (11.07 %) and minimal (4.72 - 5.29 %) with unsieved defatted flours from both plants. This means that the volume of 0.1N NaOH solution used to maintain pH at 8 after 10min was significantly high with casein and sieved fractions of defatted flours. These results could be related to the composition of the defatted flours. In fact, in casein, there was no substance which can reduce the accessibility of enzymes on active sites of proteins (Rubanza et al., 2005). Values were high with sieved defatted flours compared to the corresponding unsieved samples because sieving reduce the level of fibres. Results of chemical composition showed that fraction between 400 – 500µm for both samples presented low quantities of fibres (1.45 – 2.43%), compared to the unsieved flours (9.76 – 10.98%). The use of enzymes mixture (trypsin, chymotrypsin and peptidase) induced an increase of hydrolysis degree of sieved *R. heudelotii* and *T. conophorum*.

4.3.3. Predicted nutritional value

The predicted nutritional value of sieved defatted flours with particle sizes between 400 – 500µm is presented in **table 4**. Essential amino acids accounted for 29.33 and 29.87% of the total amino acids contents for *R. heudelotii* and *T. conophorum* respectively. The predicted Protein Efficiency Ratio (PER) for sieved *R. heudelotii* (3.23) was comparable to that of sieved defatted flour from *T. conophorum* (3.09). Values of the corresponding unsieved defatted flours were 3.79 and 3.65 respectively. A protein – based food material is said to be of good nutritional quality when its protein efficiency ratio is > 2.

Table 4: Predicted nutritional values of 400 – 500µm fractions of *R. heudelotii* and *T. conophorum* particle sizes

	<i>R. heudelotii</i>	<i>T. conophorum</i>
$\frac{\sum \text{EAA}}{\sum \text{TAA}} * 100$	32.10%	34.22%
Protein Efficiency Ratio	3.23	3.09
Biological Value	62.95	107.24
EAAI	68.4	113.65
Limiting amino acid	lysine	none
Highest amino acid	Glutamic acid	glycine

ΣEAA: sum of essential amino acids; ΣTAA: sum of total amino acids; EAAI: Essential Amino Acids Index

(Friedman, 1996). Essential Amino Acids Index (EAAI) were 68.4 and 109.12% for *R. heudelotii* and *T. conophorum* sieved defatted flours respectively. The predicted Biological Values (BV) were 62.95 and 107.24 respectively. According to Oser (1959), proteins with biological value more than 70% are to be of good quality. Results obtained from both defatted flours indicated that sieving reduce the PER and BV predicted values. The parameters analyzed showed that sieved defatted flours with 400 – 500µm particle sizes contained protein of intermediary (*R. heudelotii*) and good quality (*T. conophorum*).

CONCLUSION

Results of the present research establish that the 400 – 500µm fractions of *R. heudelotii* and *T. conophorum* defatted flours were more representative in terms of quantity, contained high level of ashes and protein. Total fibres were more represented in fractions > 500µm. Hydrolysis degree of proteins were higher in sieved samples. The nutritional outcome of sieved defatted flours with 400 – 500µm particles size in terms of essential amino acids index, predicted protein efficiency ratio and biological value showed that the 400 – 500µm particle sizes contained protein of acceptable (*R. heudelotii*) and good quality (*T. conophorum*). These results provide useful indications of the effect of sieving on chemical and nutritional properties of *R. heudelotii* and *T. conophorum* defatted flours.

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