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Domestication of *Irvingia gabonensis*: 4. Tree-to-tree variation in food-thickening properties and in fat and protein contents of dika nut

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Abstract

Dika nut kernels were extracted from the nuts of 24 *Irvingia gabonensis* fruits collected from 151 trees in three villages in Cameroon and Nigeria. Methods were developed for the milling, de-fatting, hot-water pasting and rapid visco-analysis of the samples, to simulate the cooking of dika nut meal as a food-thickening agent. Two parameters (viscosity and drawability), thought to relate to the soup-thickening quality of dika nut meal, were derived from the traces. The samples exhibited significant tree-to-tree variation in viscosity, drawability and fat content, and differences between their villages of origin were significant. Thickening was found not to be directly associated with protein content. Similarly, the fat content did not contribute to the thickening properties at temperatures above fat melting point. Fat determination and fatty acid profiling indicated that the fat content ranged from 37.5% to 75.5% and identified myristic and lauric acids as the major fatty acid components. This study is part of a wider tree domestication project characterising tree-to-tree variation in fruit, nut and kernel traits of *I. gabonensis* with the aim of improving the livelihoods of subsistence farmers.

Keywords: Dika nut; Bush mango; Rheology; Food-thickening; Fat and protein content

1. Introduction

Many trees throughout the tropics produce edible fruits and nuts with potential as new crop plants. To maximize the opportunity to develop novel foods from these new tree crops, there is an urgent need for the food industry to work with agroforesters domesticating these trees, so that the process of genetic selection includes the traits of importance to the food industry (Leakey, 1999).

Irvingia gabonensis (Aubry Lecomte ex O'Rorke) is a commercially and socially important indigenous fruit tree of West and Central Africa, which has been identified as the most important tree for domestication in the region (Franzel, Jaenicke, & Janssen, 1996). Consequently, it is the prime focus in West and Central Africa of the agroforestry tree domestication programme of the World Agroforestry Centre (formerly the International

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Centre for Research in Agroforestry, ICRAF) and its partners (Leakey & Simons, 1998; Leakey, Schreckenberg, & Tchoundjeu, 2003). This programme has identified very considerable phenotypic variation in the morphological traits of *I. gabonensis* fruits, nuts and kernels, suggesting that there is considerable opportunity for the development of cultivars (Anegbeh et al., 2003; Atangana et al., 2001, 2002). However, regression analysis has found that there are only weak relationships between fruit mass, nut mass, shell mass and kernel mass. This relative independence of fruit and kernel mass has led to the recommendation that separate cultivars would be required to meet the needs of fruit and kernel markets (Leakey, Fondoun, Atangana, & Tchoundjeu, 2000). To select cultivars for kernel production, data is needed on the tree-to-tree variation in the physical and chemical properties of the kernels. Variation between individual tree samples in these kernel properties has not been previously studied. Such variation could be due to variation in the quantity or molecular structure of the polysaccharide itself, or to variation in other components of the de-fatted meal that might modulate the behaviour of the polysaccharide. In the same way, more work is required to examine the nutritional aspects of cultivars for both fruit and kernel production, although this is not planned at present. The nutritional potential of this and other agroforestry tree products have been reviewed by Leakey (1999).

The kernels of *I. gabonensis* are widely marketed domestically, nationally and between countries in West Africa (Ndoye, Ruiz Pérez, & Eyebe, 1998) for their food-thickening properties (Joseph, 1995; Ndjouenkeu, Goycoolea, Morris, & Akingbala, 1996; Onyeike, Olungwe, & Uwakwe, 1995), which are the basis of local 'ogbono' soups and stews (Okolo, 2000). This use of dika nut kernels has led to the initiation of the domestication of the species by local farmers (Leakey et al., 2004). The term "draw-soup" for ogbono, reflects the ability of the mucilage to be drawn out in strings or tendrils, and is a trait of good quality. Ejiofor, Onwubuke, and Okafor (1987) also referred to the "draw-ability" of the soup as a factor in sensory evaluation of the soup. The chemical properties of the kernels have been the subject of earlier studies (Giami, Okonkwo, & Akusu, 1994; Oke & Umoh, 1978), but relatively little is known about the physical properties affecting the food-thickening capacity of dika nut kernels. Consequently, this study investigates aspects of the analytical methodology. Furthermore, the literature gives only limited information to indicate how the de-fatted meal can be used to thicken soups and stews and what would constitute good eating texture. Agbor (2000), however, has indicated the desirability of a high degree of thickening, together with a slimy consistency, best preserved by only short term cooking of the dika nut meal. Nigerian recipes for dika nut meal in soup included salt, at ≈0.1 M (Spindler & Akosionu, 1985). It has been reported that this reduces the food-thickening properties of dika nut meal (Ndjouenkeu et al., 1996).

Fat is the most abundant component of kernels (51–72%) (Leakey, 1999) and fatty acid profiles of 39.2% myristic and 51.1% lauric acid have been reported from Cameroon (Hellyer, unpublished), 33.5% myristic, 58.6% lauric from Sierra Leone, and 50.6% myristic, 38.8% lauric from Nigeria (Okolo, 2000). Earlier work (Ejiofor et al., 1987; Joseph, 1995; Okolo, 2000) had shown that the de-fatted residue from dika nuts maintained much of the culinary thickening power. The main component of the de-fatted meal is carbohydrate in the form of a water-soluble polysaccharide, about 35–45% (estimated from Ejiofor et al., 1987; Giami et al., 1994; Oke & Umoh, 1978; Onyeike et al., 1995) as total carbohydrate (assumed to be carbohydrate in the form of insoluble cell wall polysaccharides) minus crude fibre. A detailed rheological study (Ndjouenkeu et al., 1996) of a sample of the purified soluble polysaccharide demonstrated that this component had properties consistent with a dominant contribution to the thickening quality of dika nut meal. The second most abundant component of de-fatted dika nut meal is protein, with reported values over the range 15–30% (calculated from Ejiofor et al., 1987; Giami et al., 1994; Oke & Umoh, 1978; Onyeike et al., 1995). It is not known if this protein plays any role in the thickening quality of dika nut meal.

Based on the characterization of *I. gabonensis* fruit, nut and kernel morphology, Atangana et al. (2002) developed fruit and kernel ideotypes to aid a tree domestication programme in West and Central Africa. The kernel ideotype was based on kernel mass and shell brittleness. The present study of phenotypic variation in the physical properties of the food-thickening properties of dika nut meal and its oil content allows the refinement of this kernel ideotype to include these food-thickening traits of importance to the food industry internationally and to consumers locally.

2. Materials and methods

2.1. Materials

To refine the methodology most appropriate for this study, a bulk sample of kernels bought in Yaoundé market was used. A quite extensive series of preliminary experiments was conducted with both the full-fat and the de-fatted meal of the bulk sample in order to arrive at a protocol that it was felt would give a discriminating view of the ability of the different dika nut accessions to thicken soup (Greenwell & Hall, 2000).

The 151 kernel samples for the main study, which examined the tree-to-tree variation in physical and chemical properties of the kernels were obtained from

Table 1
Location of *Irvingia gabonensis* populations used in this study

	Elig-Nkouma (Cameroon)	Nko'ovos II (Cameroon)	Ugwuaji (Nigeria)
Latitude (°N)	4°07′	2°56′	6°25′
Longitude (°E)	11°24	11°21′	7°32′
Altitude (m)	461	610	175
No. of samples from individual trees	29	22	100

three villages in Cameroon and Nigeria (Table 1). They were the kernels extracted from fruits that have been characterised morphologically in a study of the phenotypic variation of the same *I. gabonensis* populations (Anegbeh et al., 2003; Atangana et al., 2001, 2002).

2.2. Milling

The nuts were chilled in a refrigerator at about 7 °C to make them more brittle, and broken into pieces less than about 0.5 cm across by gentle tapping with a hammer onto the bag on a hard surface. The fragmented nuts were re-chilled prior to milling in a domestic electric coffee-grinder (Braun, type 4-041). The entire sample was tipped into the metal bowl of the grinder; which had been pre-chilled with a polythene bag of crushed ice. The mill was run for 20 s and the contents brushed onto a 20 cm diameter Endecott metal mesh sieve with 710 μm aperture. A metal spatula was used to scrape out any meal that had re-aggregated on the bottom and edges of the bowl. Re-aggregated lumps were subsequently broken down by gently brushing them across the surface of the mesh. The meal was sieved for 20 s on an electric vibrating shaker (Octagon, setting No. 6) until 95% or more of the meal passed through the sieve. The overs were returned to the mill for 20 s and the resultant meal sieved for a further 20 s. The combined through were weighed and the overs retained in separate self-seal bags. The pale yellow meals were stored in self-seal polyethylene bags at 7 °C until further use. Between samples, the bag of crushed ice was replaced in the bowl of the grinder to cool it.

2.3. De-fatting

Ground dika nut meal (20.0 g) was stirred magnetically (Variomag Multipoint HP15 stirrer) with 150 ml distilled grade 40–60 °C petroleum spirit (Rathburn Chemicals Ltd., Scotland, EH45 6AU) for 30 min at room temperature, sufficiently fast to keep the solid in suspension. Four samples were processed in parallel in 210 ml MSE glass centrifuge bottles sealed with aluminium foil. If only lesser amounts of meal were available, they were carried through the procedure with the same amount of solvent. The four bottles were then centrifuged in an MSE Mistral 4L centrifuge at 1800*g* for 5 min, sufficient to form firm pellets and clear yellow supernatants. The latter were decanted onto fluted filter papers (Whatman No. 1, 24 cm diameter) in conical filter funnels and filtered to waste. The drained pellets were re-suspended in a further 150 ml of solvent and stirred for 30 min as before. The slurries were then poured into the filter papers and allowed to drain. The bottles and stirrers were rinsed onto their filters with a further 50 ml portion of solvent, and the filter cakes were allowed to drain to firmness under a loose foil cover to minimise evaporative losses. The filter papers were then opened out on a flat surface and the lightly crumbled filter cakes were allowed to dry in a gentle air current for at least 2 h. Finally, the dried filter cake was carefully scraped off the paper with a spatula and weighed into a polythene bag. The dried de-fatted meals were weighed to the nearest 0.1 g and the % weight loss was taken as an estimate of the fat content of the meal.

2.4. Hot-pasting on Rapid Visco-Analyser

Viscosity measurements were conducted on dispersions of de-fatted dika nut meal in glass-distilled water, whilst undergoing controlled heating and cooling in the RVA (Model 3D) developed by Newport Scientific Pty Ltd., New South Wales 2102, Australia (Walker, Ross, Wrigley, & McMaster, 1988; Wrigley, Booth, Bason, & Walker, 1996). Since the dika nut meal pastes were clearly seen to be complex rheologically, the measured viscosities were operational values as dictated by the mixing conditions prevailing in the sample can at any given time.

The sample was placed in a cylindrical, flat-bottomed aluminium can and dispersed by hand to wet the solid in 25 ml of water, using the two-bladed, disposable, plastic paddle used to stir the mixture in the RVA. The paddle and can were then mounted on the stirring head and the latter pressed down to start the run by lowering the can into the heating chamber. Copper blocks, electrically heated and water cooled, were clamped firmly around the can, which was then heated or cooled as desired using the proprietary Thermocline MS-DOS software run on a PC computer linked to the RVA.

In the RVA, the paddle was stirred at high speed (960 rpm for 10 s) to homogenise the dispersion, and then at a steady 160 rpm through the rest of the run. The electrical energy needed to maintain the constant speed during the heating and cooling profile was used as a measure of the viscosity of the can contents using Thermoview software. Measurements of the operational viscosity in Stirring Number Units (SNU) and block temperature were taken at 4 s intervals. Viscosity and temperature profiles against time were presented as a chart, or as a data file for import into Microsoft Excel. The conversion factor into SI units was 1 SNU = 12 mPa s as stated by the instrument manufacturers. The temperature of the heating blocks, not of the can contents, was monitored and maintained. The contents may lag in temperature during rapid heating and cooling ramps, and catch up asymptotically (Deffenbaugh & Walker, 1989). The maximum heating and cooling rate is about 14 °C min^{-1}, and the maximum attainable temperature is limited to 95 °C (to avoid boiling).

Mean operational viscosity was assessed over the final 30 time points (2 min). Additionally, the standard deviation of the viscosity over the last 30 points was calculated as a convenient measure of the spikiness of the trace. The spikiness was taken to be a measure of the drawability of the dika nut sample although this needs further verification by direct comparison of RVA data to consumer testing of soups.

The RVA instrument was calibrated each day according to the manufacturer's instructions by pasting a standard starch sample and a sample of the bulk sample de-fatted dika nut meal at least once each day to gain an estimate of the experimental variability in the measured values for sample viscosity and drawability. This gave an indication of the significance of any differences between sample values. Six other meal samples were run three or four times, and about 30 were run twice, to gain further evidence of the significance of the results. The remainder of the sample set was run only once because of time constraints, and lack of meal in some samples.

2.5. Preliminary studies with a bulk meal sample

(1) To examine the effects of the fat component of dika meal on the viscosity curve, two experiments were done using 4.0 g of whole meal and 1.33 g of de-fatted meal (these samples contained approximately equal amounts of the non-fat residue). In the first experiment, the samples were run through the RVA in a 30–95–30 °C profile (Table 2(a)), in which the ramp portions of the profile, during which thickening was taking place, were extrapolated down to 30 °C, such that they would pass through the melting range of the fat. This was carried out in order to indicate any change in viscosity as a result of the fat melting.

In the second experiment, full-fat and de-fatted meal bulk samples were run in a set of six profiles (Table 2(b)) to explore further the effects of temperature.

The profiles at 25, 35 and 45 °C, were designed to observe further the effect of the fat content below, near and above the melting range, while the profiles started at 55, 75 and 95 °C, tested were selected to mimic the different ways in which dika nut meal may be used. All samples were cooled down (with the same rate of cooling) to a final temperature of 50 °C at 14 min. The latter temperature was chosen as one at which an ogbono soup might be consumed, whereas the higher starting temperatures of was seen as simulating the hot soup into which the dika nut meal would be sprinkled, as a thickener and condiment, a few minutes before consumption.

(2) To test the effects on the food-thickening properties of dika meal, de-fatted samples were run through the RVA at a 95–50 °C profile, in the presence and absence of 0.1 M sodium chloride (analytical grade from Merck, UK).

(3) To test the effects of protein-disruptive agents on RVA hot-pasting, de-fatted dika nut meal from the bulk sample was used with the 15 min 95–50 °C temperature profile for simulation of cooking to generate pasting curves in the presence and absence of reagents that might be expected to disrupt the conformational integrity of the dika nut proteins. The reagents might thereby alter the viscosity profile if the native proteins were contributing to the viscosity. The reagents, chosen on the basis that they should be able to exert an effect on protein structure during the time course of the run, were 0.1 M sodium sulphite, 10 mM DTT, 2% w/w SDS, and 10 mM DTT, combined with 2% SDS.

Sodium dodecyl sulphate (SDS), an anion which has low affinity for negatively charged carbohydrates is known to bind rapidly in fixed ratio to most proteins (Hames, 1981), and sufficient SDS was used to saturate all the dika protein present in the RVA runs. The other two reagents, sulphite anion and dithiothreitol (DTT), were used on the grounds that they rapidly cleave disulphide bonds by reduction (Means & Feeney, 1971,

Table 2
Rapid Visco-Analyzer profiles used in these studies

(a) 30–95–30° Profile						
Time (min)	0	2	7.5	9.5	15	16
Temperature (°C)	30	30	95	95	30	30
(b) Variable profiles						
Time	*25 °C Profile*		*35 °C Profile*		*45 °C Profile*	
0–16 min	25 °C		35 °C		45 °C	
	55–50 °C Profile		*75–50 °C Profile*		*95–50 °C Profile*	
0–9.5, 11.5, or 13.5 min	55 °C until 13.5 min		75 °C until 11.5 min		95 °C until 9.5 min	
14–16 min	50 °C		50 °C		50 °C	
(c) 95–50 °C Profile						
Temperature (°C)	95	95		50	50	
Time (min)	0	7.5		12	15	

Chap. 8) and would therefore, assist thermal denaturation of the dika nut proteins. Dika nut proteins have an unusually high cystine content (Oke & Umoh, 1978) and therefore, almost certainly have conformations dependent on intramolecular disulphide bonds. Neither DTT nor sulphite would be expected to react rapidly with a polysaccharide. All three reagents were incubated with the bulk sample meal through two successive cycles in the RVA, as also was the combination of SDS + DTT, and water alone as a control. To enlarge any effects, the mixtures were each put through the RVA pasting profile for a second time, immediately after the first run. Bulk sample meal in water alone was run as a control. Limitations of time and resource meant that only single runs were done with these exploratory experiments.

2.6. Characterization study

Using the same general methods as described above for the preliminary study the following modifications were specific to the characterization of the 151 individual tree samples of dika nut.

2.6.1. Milling

A kernel sub-sample for each tree accession, either 25 g to the nearest nut or nut fragment (or the whole sample if less than 25 g), was weighed into a small self-seal polythene bag of sufficient area to contain the nuts as a monolayer, prior to chilling and crushing. In four samples (Elig Nkouma 19, 24, 29 and 37), which had high fat content, the concretion of the meal was so extensive that the overs were re-chilled, milled and sieved for a third time to boost the recovery of meal.

2.6.2. Hot-pasting

All the de-fatted samples of dika nut meal from different trees were pasted in the RVA according to a 15 min 95–50 °C temperature profile (Table 2(c)) to simulate culinary use, as developed in the preliminary studies (see profile below). Meal (1.33 g) was dispersed in distilled water (25.0 g) and run on the RVA 3D instrument in the following profile.

2.6.3. Fatty acid profiles with tree samples

Dika nut samples were selected for fatty acid analysis. The selection was made to represent the three village origins in Cameroon and Nigeria (EN, Elig Nkouma; Nk, Nko'ovos II; Ug, Ugwuaji) and cover the highest and lowest total fat content samples:

EN41	37.5% fat	Nk31	43.0% fat	Ug16	52.0% fat
EN19	75.5% fat	Nk19	69.5% fat	Ug26	68.0% fat

The method used for the analysis of the fatty acid is based on the IUPAC Standard (Method for the Analysis of Oils, Fats and Derivatives, 7th ed., 1987, Method 2.301). The fat was extracted from the milled samples using the Bligh and Dyer fat extraction method with chloroform and methanol (Bligh & Dyer, 1959). The extracted fat was then saponified to break down glycerides. Liberated fatty acids were then esterified in the presence of methanol and boron trifluoride. Fatty acid methyl esters were then extracted with heptane and analysed by gas chromatography with flame ionisation detection. Results for fatty acid composition were expressed as % w/w of the total fatty acids recovered.

2.6.4. Protein analysis

Six 200 mg de-fatted dika nut meals of diverse viscosity (Table 5) were subjected to nitrogen analysis by the Dumas combustion technique on a LECO FP-528 instrument, according to the detailed protocol of CCFRA method FTWG 0019. For convenience, in the absence of a known protein conversion factor for dika nuts, the factor of 5.7 was used, as for wheats.

2.6.5. Protein electrophoresis

Total dika nut protein from the same six samples as above were extracted as reduced, denatured, SDS–protein complexes by heating de-fatted meal samples (10 mg) in sample loading buffer (500 μl) at 100 °C for 3 min. The buffer contained DTT (Sigma–Aldrich Co. Ltd., Poole, UK) to reduce cystine disulphide bonds, and sodium sulphite and SDS (Merck Ltd., Poole, UK) to bind, denature and solubilise the proteins. The extracts, clarified by centrifugation, were analysed for the relative amounts and molecular sizes of the solubilised proteins by SDS polyacrylamide gel electrophoresis (SDS–PAGE; see Hames (1981) for review). This was done with portions (5 μl) loaded into adjacent sample wells of a CleanGel 10% polyacrylamide electrophoresis slab and associated buffers, provided in the kit, for electrophoretic analysis of proteins by SDS–PAGE, (Pharmacia Biosystems GmbH), close to a mixture of marker proteins of known molecular weight (MW). Electrophoresis was conducted according to the manufacturer's instructions on a Pharmacia Multiphor II flatbed electrophoresis apparatus to yield parallel tracks containing the protein profiles of the samples. These patterns, separated according to protein molecular size, were fixed and stained with Coomassie Blue dye and recorded on a light box with a video camera. The recorded digital image was processed by Phoretix GelWorks software to enable printing of a photographic image of the gel slab and calculation of the apparent MW of the dika nut proteins (by comparison of their migration distances with those of the standard proteins). The intensity of staining of the bands was used as a qualitative guide to the relative abundance of the dika nut proteins.

3. Results

3.1. Preliminary study with a bulk sample

1. The development of viscosity in an aqueous dispersion of de-fatted bulk sample dika nut meal, as generated by stirring and heating in the RVA was affected by:
   (a) Temperature: the transient peak of viscosity seen for full-fat meal at 25–35 °C (Fig. 1) was due to the effect of unmelted meal particles.
   (b) Stirring: at fixed temperatures ranging from 25 to 95 °C: with de-fatted meal generated successively lower viscosity curves, which were established increasingly quickly after initiation of dispersion.
   (c) A 15 min RVA 95–50 °C temperature profile, with 7.5 min at 95 °C, followed by ramping down to 50 °C over 4.5 min, is appropriate for the examination of the hot-water pasting profiles of dika nut samples under simulated cooking conditions.
2. Salt: dissolved salt at the level of culinary usage made only a slight decrease in the viscosity in distilled water.

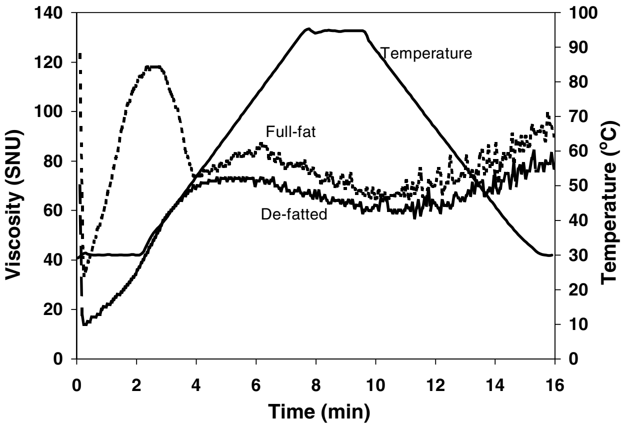


Fig. 1. Effect of de-fatting *I. gabonensis* kernels (bulk sample from Yaoundé market, Cameroon) on the viscosity of meal across a temperature profile simulating cooking (1 SNU = 12 mPa s).

3.2. Characterization study with tree samples

3.2.1. Milling

The yields of meal varied from 62.3% to 96.9%, with a mean of 91.4%. This recovery is not quite as high as the 93–95% achieved from the bulk sample in the preliminary experiments. The Elig Nkouma trees gave the lowest average yield of meal at 86.4%, compared to 91.4% for Nko'ovos II and 92.9% for Ugwuaji.

3.2.2. Fat content

Fat content ranged widely about the overall mean of 60.9% (37.5–75.5%) and was greatest in trees from Elig Nkouma (Table 3), with continuous variation between the different tree samples (Fig. 2).

3.2.3. Hot-pasting

Repeatability was assessed in a few samples. The most extensive replication was the 15 RVA runs done with the bulk sample, since this was run every day during processing of the main set of 153 dika nut samples. Seven additional samples were each run on three or four occasions (Table 4). In an additional 32 cases, the sample was run in duplicate. The RVA Viscosity values had standard deviations of 1.7–3.3 SNU for the eight samples, with an average of 2.3 SNU. This is the same as found for the 15 runs with the bulk sample, which had a viscosity of 61 SNU, not far from the median of the range found, 21–124 SNU. A difference of three standard deviations signifies a 95% confidence level.

Drawability was considerably less precise than viscosity. The average values of the spikiness of the final part of the hot-pasting curves, postulated as a measure of the elasticity and drawability of the dika mucilage, ranged from 0.6 to 2.9 SNU for this small subset of samples. The standard deviations averaged 0.2 SNU, but with a 6-fold range; when expressed as percentages, they averaged 14% with a 5-fold range. Given that the full set of dika samples yielded a 17-fold range of values, it was more interesting to use three times the % standard deviation as a basis for estimating significant difference. Thus a difference of 42% of the average value between two grab factor values was judged to give usefully high confidence of real biological difference in this parameter.

Table 3
Summary of fat content for the dika nut populations from Cameroon and Nigeria

Sample origin (village)	No. of samples	Fat content (% w/w)			
		Lowest	Highest	Mean	Standard deviation
Elig Nkouma	29	37.5	75.5	63.9	8.0
Nko'ovos II	22	43.0	69.5	59.8	5.8
Ugwuaji	100	52.0	68.0	56.5	3.6
Total	153	37.5	75.5	60.9	5.2

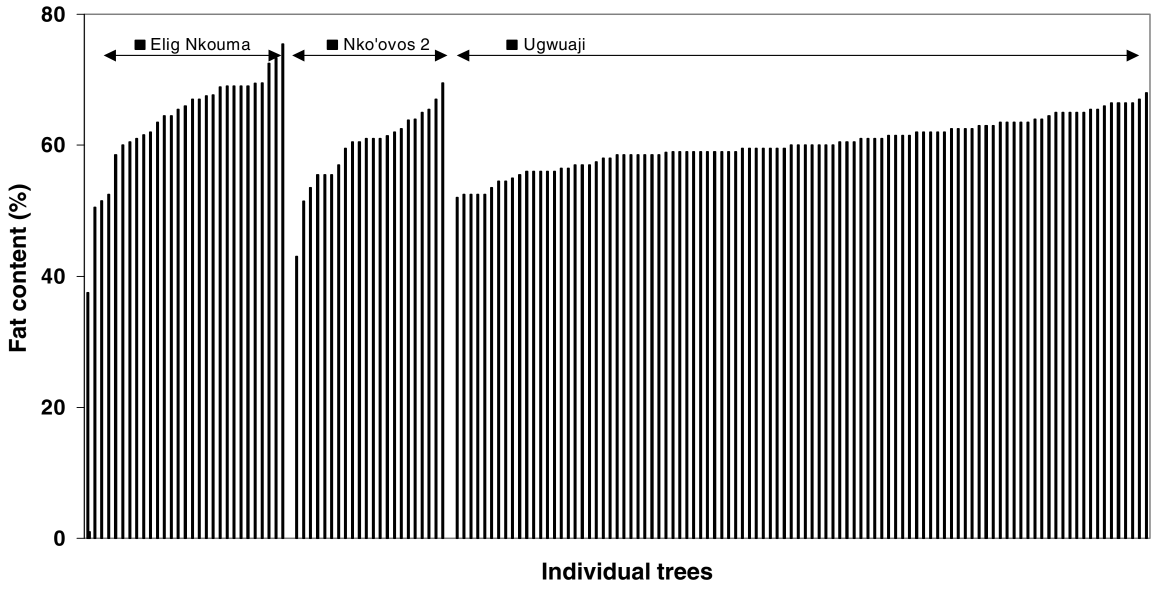


Fig. 2. Tree-to-tree variation in percentage oil content of *I. gabonensis* kernels from trees in three villages in Cameroon and Nigeria.

Table 4
Summary of statistical quality of results from RVA pasting curves (1 SNU = 12 mPa s)

Sample code	No. of RVA runs (SNU)	Mean viscosity (SNU)	Standard deviation (SNU = %)	Mean drawability (SNU)	Standard deviation (SNU = %)
Bulk sample	15	61.3	2.3 = 3.8%	2.92	0.5 = 17.1%
EN27	3	72.7	2.5 = 3.4%	1.43	0.08 = 5.6%
EN47	3	64.7	2.2 = 3.4%	0.70	0.08 = 11.4%
Ug51	3	35.6	1.7 = 4.8%	0.73	0.11 = 15.1%
Ug52	4	29.8	1.8 = 6.0%	0.61	0.15 = 24.6%
Ug53	3	42.2	2.6 = 6.2%	1.93	0.21 = 10.9%
Ug61	3	26.6	1.7 = 6.4%	1.36	0.14 = 10.3%
Ug75	3	45.7	3.3 = 7.7%	1.68	0.32 = 19.0%

Both viscosity and drawability varied continuously and significantly between trees (Figs. 3 and 4) and between villages (Table 5), with trees from Nko'ovos II having the greatest mean drawability and those from Elig Nkouma having the greatest mean viscosity. However, the viscosity profiles, produced for each defatted dika nut meal with the 15 min 95–50 °C temperature profile, revealed that they could be separated into two qualitatively different subtypes (Fig. 5). Each of these subtypes was found to occur over a range from high to low viscosity values, i.e., the averaged viscosity over the final 2 min.

The distinguishing characteristics of these two subtypes were:

1. A maximum viscosity soon after initiation of the run, and then the viscosity declined during the stirring and heating to 95 °C (as expected from the small viscometric study by Joseph (1995)). Finally, the curve rose a little during the cooling to 50 °C and flattened out in the 50 °C plateau. This was the most common type. This subtype had both spiky curves (= high drawability) and smooth curves (= low drawability).
2. Slowly developing viscosity in the early stages, rising through cooling to yield maximum viscosity through the 50 °C plateau. This late maximum curve type was seen definitely in only eight of the 154 samples, all from Elig Nkouma village (EN7-A, 7-B, 19, 20, 24, 29, 40, and 44), with Nk23, Ug39 as possible, marginal, examples from Nko'ovos II and Ugwuaji. This subtype had only smooth curves.

Some samples showed minor variations on the above profiles. For example, some samples developed 'spikiness', while others were continuously spiky. In other words, spikiness showed variations in temperature sensitivity between samples. Another minority population from Elig Nkouma trees (e.g., EN32, 35 and 37) all had smooth curves with a particularly pronounced decline in viscosity at 95 °C, but then an unusually abrupt start to a strong recovery at the start of the cooling period. Their viscosity was particularly responsive to temperature changes.

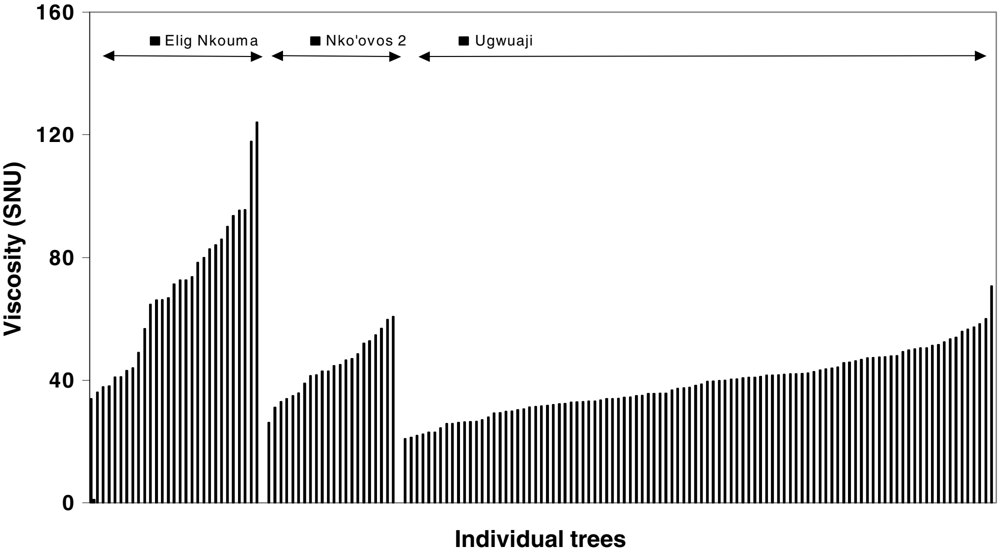


Fig. 3. Tree-to-tree variation in viscosity of *I. gabonensis* kernel meal from trees in three villages in Cameroon and Nigeria across a temperature profile simulating cooking (1 SNU = 12 mPa s).

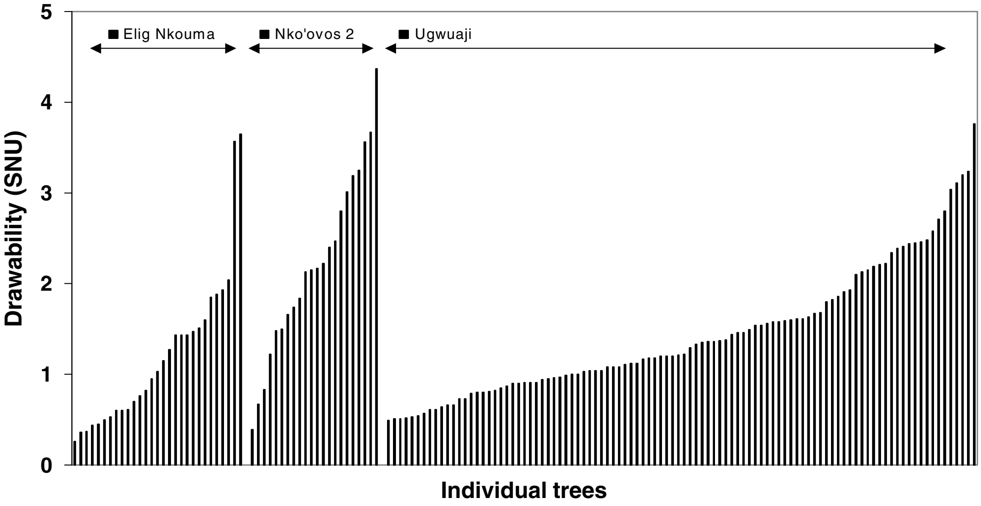


Fig. 4. Tree-to-tree variation in drawability of *I. gabonensis* kernel meal from trees in three villages in Cameroon and Nigeria across a temperature profile simulating cooking (1 SNU = 12 mPa s).

Table 5
Summary of viscosity (V) and drawability (D) values (SNU) for the dika nut populations (1 SNU = 12 mPa s)

Sample origin (village)	No. of samples	Lowest		Highest		Mean		Standard deviation	
		V	D	V	D	V	D	V	D
Elig Nkouma	29	34.0	0.26	124.1	3.65	69.1	1.21	24.3	0.85
Nko'ovos II	22	26.2	0.39	60.7	4.37	44.2	2.21	9.5	1.02
Ugwuaji	100	20.9	0.49	70.1	3.76	38.8	1.44	9.8	0.72
Overall	153	20.9	0.26	124.1	4.37	45.3	1.51	17.9	0.84

3.2.4. Interactions

The interactions between viscosity and fat content (Fig. 6) were very weak ($R^2 = 0.051–0.223$) in samples from all three villages, but had a similar slope, while there were no relationships between drawability and fat content. By contrast, the relationships between viscosity

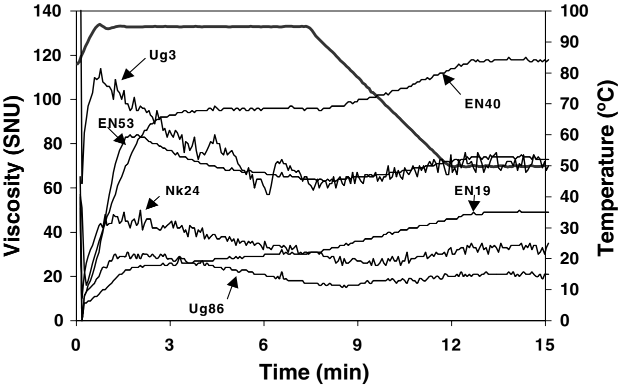


Fig. 5. Examples of tree-to-tree variation in the profiles of viscosity of *I. gabonensis* kernel meal from trees in three villages in Cameroon and Nigeria across a temperature profile simulating cooking. Smooth curves have low drawability, while 'spiky' curves have high drawability (1 SNU = 12 mPa s).

and drawability were stronger (Fig. 7), but very different between villages (Elig Nkouma: $R^2 = 0.037$; Nko'ovos: 2 $R^2 = 0.647$ and Ugwuaji: $R^2 = 0.336$).

3.3. Mechanistic studies

3.3.1. Protein analysis

Samples selected for their variability in viscosity and drawability, varied significantly in protein content, but there was no relationship between protein content and viscosity or drawability (Table 6).

3.3.2. Protein electrophoresis

The electrophoretic patterns of the proteins from the six selected dika nut samples all showed very similar protein bands. Two faint but broad bands of relatively high MW (≈66 and 52 kDa) were seen. The most prominent feature of the patterns was a heavily stained triplet of bands, ≈32, 30 and 28 kDa, and below that was a strong doublet of bands with MW ≈21 and 20 kDa.

3.3.3. RVA hot-pasting with protein-disruptive agents

(a) Double RVA incubation with water. The bulk sample de-fatted meal had an early maximum, quite high viscosity, and quite high drawability after cooling. The second incubation gave a lower trace, with an absence of the initial maximum and a lower final viscosity. There was also a lesser, but still evident, build-up of spikiness (drawability) on cooling.

(b) Double RVA incubation with 0.1 M sodium sulphite. The initial maximum at 95 °C was diminished and delayed in the first trace, but the final part at 50 °C was very similar to the water control. The repeat incubation gave a trace very similar to the water, except that the curve was smoother, particularly in the final part.

(c) Double RVA incubation with 10 mM DTT. The first trace had a slightly diminished early maximum, but the build-up of spikiness was still evident at the end. The repeat incubation gave a trace very similar to that obtained with water alone, with some build-up of spikiness.

(d) Double RVA Incubation with 2% SDS. The whole first trace was at a higher viscosity than with water, although the early maximum was less abrupt, and the spikiness of the whole trace was noticeably higher. The repeat trace was lower (but still higher than that with water) and very much less spiky than the first trace.

(e) Double RVA incubation with 2% SDS + 10 mM DTT. Both the first and second traces were very similar to those obtained with 0.1 M sodium sulphite, with a

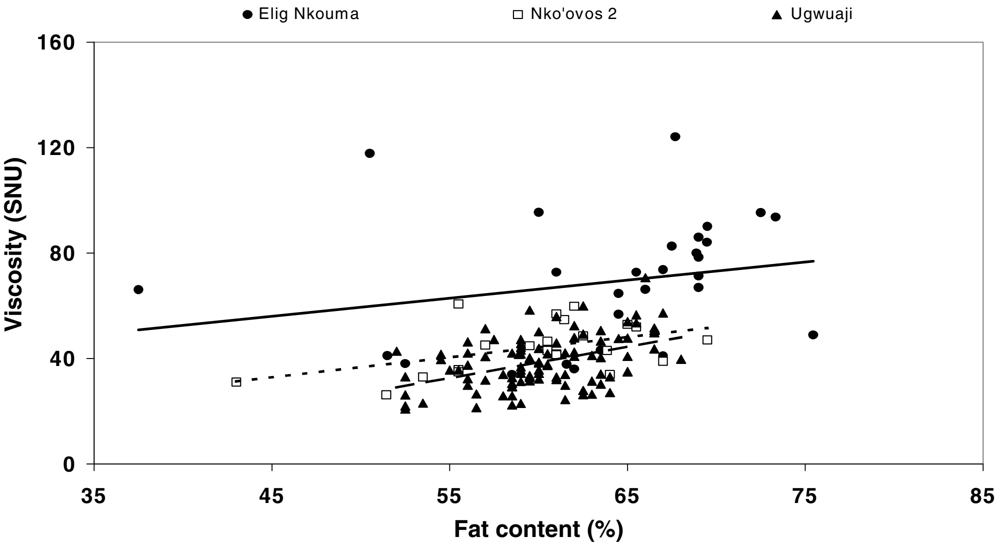


Fig. 6. The relationships between tree-to-tree variation in fat content of *I. gabonensis* kernels and the viscosity of kernel meal from fruits collected in three villages in Cameroon and Nigeria (1 SNU = 12 mPa s).

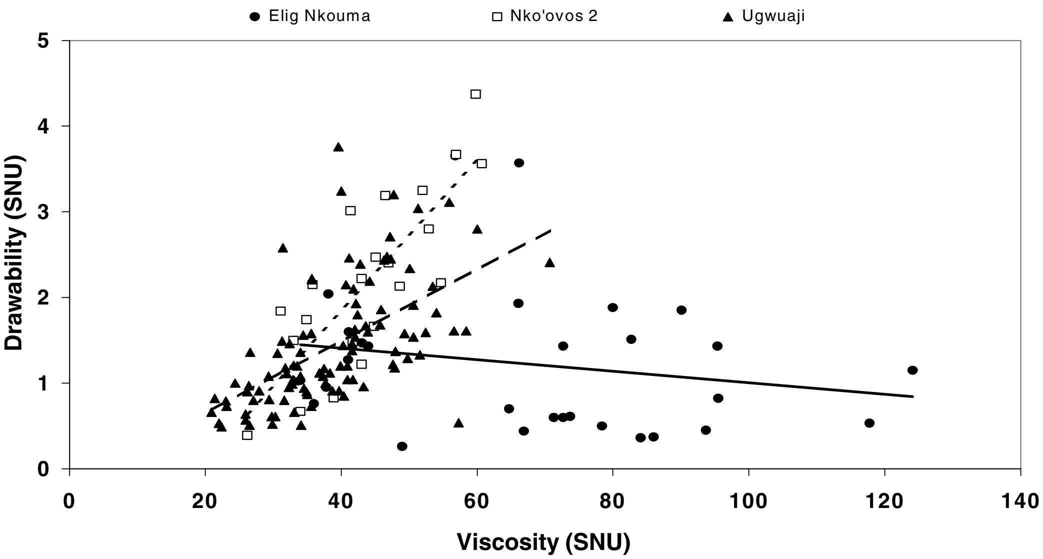


Fig. 7. The relationships between tree-to-tree variation in viscosity and drawability of *I. gabonensis* kernel meal from fruits collected in three villages in Cameroon and Nigeria (1 SNU = 12 mPa s).

Table 6
Protein content of six de-fatted dika nut meals of diverse viscosity (1 SNU = 12 mPa s)

Sample code (curve type)	Viscosity (SNU)	Protein content (%N × 5.7)	Drawability (SNU)
EN40 (late, smooth)	118	19.4	0.53
EN53 (early, smooth)	74	20.4	0.61
Ug3 (early, spiky)	71	24.1	2.41
EN19 (late, smooth)	49	20.8	0.26
Nk24 (early, spiky)	33	14.3	1.50
Ug86 (early, smooth)	21	17.78	0.66

diminished and delayed early maximum, and the second both lower and smoother than the first.

3.3.4. *Fatty acid profiles with tree samples*

The pattern of fatty acids found was generally comparable between the six samples analysed, with only minor variation between samples from different villages, and high or low fat content.

Only about 3.2% of the fat was unsaturated (Table 7). It displayed a relatively unusual profile with myristic acid and lauric acid (52.9% and 36.4%, respectively), as the major components (Table 8). The most similar fat found in searches is ucuhuba butter/oil from trees in the *Virola* genus; Myristaceae family (Gunston, Harwood, & Padley, 1986). Ucuhuba oil has been used as a cocoa butter substitute (Usher, 1974) and for the manufacture of candles and soap in South America (Vaughan, 1970).

3.4. *'Ideotypes' for tree selection*

Web diagrams of the phenotypic variation in all traits measured together with published data on fruit mass, kernel mass and shell brittleness of the fruits from the same trees indicate no patterns in the variation between trees (Fig. 8). Selection of trees with the highest value for each trait with a hypothetical 'ideal' tree with high values for all the traits of importance to different uses of dika nut kernels (the ideotype) shows that none of the trees are close to the ideals (Fig. 9(a) and (b)).

4. Discussion

For the first time, this paper presents information on the tree-to-tree variation in properties physical and

Table 7
Saturation classes of fatty acids in dika nuts of diverse fat content

Sample	Saturated fatty acids (%)	Monounsaturated fatty acids (%)	Polyunsaturated fatty acids (%)	*Trans* fatty acids (%)
EN41	97.5	2.1	0.4	<0.1
EN19	95.3	3.1	1.6	<0.1
Nk31	96.9	2.5	0.6	<0.1
Nk19	97.4	2.0	0.6	<0.1
Ug16	96.4	3.1	0.6	<0.1
Ug26	96.8	2.6	0.5	<0.1
Means	96.7	2.6	0.6	<0.1

Table 8
Fatty acid profiles from dika nuts of diverse fat content

Fatty acid	Fatty acid name	Range (%)		Mean (%)
		Minimum	Maximum	
C10:0	Capric acid	0.7	1.15	0.9
C12:0	Lauric acid	33.5	42.1	36.4
C14:0	Myristic acid	48.7	55.5	52.9
C16:0	Palmitic acid	4.6	6.1	5.6
C16:1 *trans*	Palmitelaidic acid	<0.1	<0.1	<0.1
C16:1 *cis*	Palmitoleic acid	<0.1	<0.1	<0.1
C18:0	Stearic acid	0.6	1.0	0.8
C18:1 *cis*	Oleic acid	1.9	3.0	2.5
C18:2 *cis*	Linoleic acid	0.4	1.4	0.8
C18:3 *cis*	Linolenic acid	<0.1	0.2	<0.1
C20:0	Arachidic acid	<0.1	<0.1	<0.1

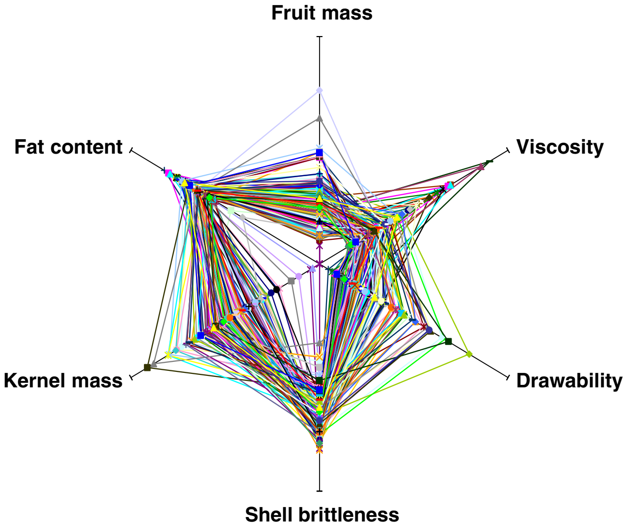


Fig. 8. Web diagram of relative values of six fruit and kernel traits in 151 *I. gabonensis* trees from three villages in Cameroon and Nigeria.

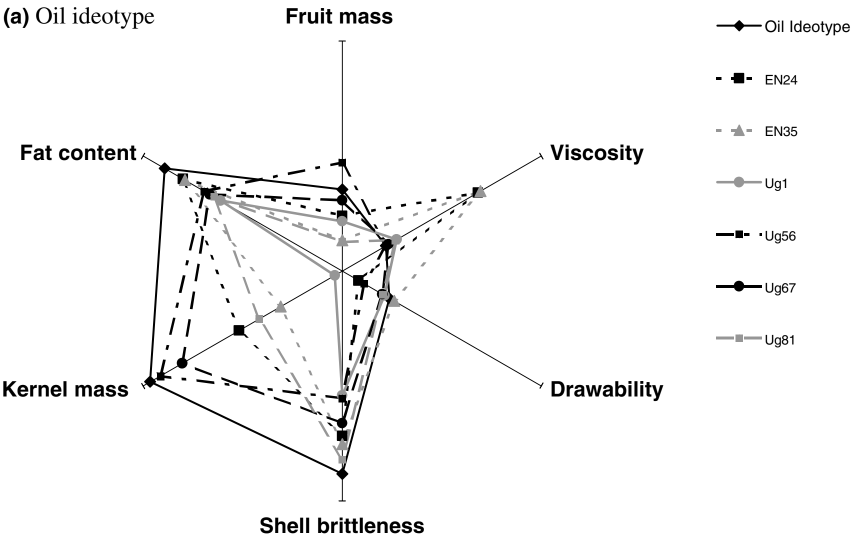
chemical properties of dika nut meal of importance to their consumption and use by the food industry. Although some of this variation could be due to environmental effects on the production of the nuts, the wide variability found in each village strongly suggests that genetic differences are substantial. The level of variation found in each of the different traits examined is in accordance with what would be expected in an outbreeding species, such as trees (Zobel & Talbert, 1984) and clearly illustrates the opportunity for the food industry to work with agroforesters domesticating tree species as new crop plants (Leakey, 1999). Particularly, the industry could assist by indicating the quality attributes required by the industry for new food products, and so to influence the ideotypes implemented in the tree selection process.

The food-thickening properties of dika nut meal are much appreciated in West and Central Africa (Ejiofor et al., 1987; Spindler & Akosionu, 1985). Prior to this study, good quality kernels for 'ogbono' or dika nut soup were considered to be those with mucilagenous polysaccharides conferring 'drawability' (e.g. Ndjouenkeu et al., 1996), but it is now clear from the form of the curves generated by the RVA that there are two separate and unrelated traits: drawability and viscosity. This discovery now requires a consumer sensory trial to determine which of these two traits is the more important to consumers in West and Central Africa, or whether it is some combination of the two which is most appreciated. The results of such a study would be of direct relevance to the refinement of the 'food-thickening ideotype' used in the tree domestication programme. In this regard, it is particularly interesting to note the differences in viscosity between the two Cameroonian tree populations (Elig Nkouma and Nko'ovos II), and the similarity among all three populations in drawability.

The total range of fat values of 37.5–75.5% (trees EN41 and EN19) found is consistent with, but somewhat broader than, previous reports. Irvine (1961) reported a range of 54–67%, Joseph (1995) found 66.4–69.7%, and Ejiofor (2000) found 51% and quoted three references reporting 71–72%. Again it is interesting that the Elig Nkouma population showed the greatest diversity in fat content. Clearly there are opportunities to develop cultivars of *I. gabonensis* for oil production, and perhaps for oil quality, although the fatty acid profile was relatively consistent within the limited numbers of samples analysed. The fatty acid profiles found suggest that the dika nut oil is somewhat unusual in its relatively high level of short chain saturated fatty acids, particularly lauric and myristic, perhaps indicating its suitability as a cocoa butter substitute, or for candle and soap production.

In addition to the above characterization of variability in food properties of dika nut meal originating from different trees, this study tried to gain some understanding about the underlying molecular mechanism of such variation. The study confirmed earlier reports by Ejiofor et al. (1987), Joseph (1995) and Okolo

(a) Oil ideotype



(b) Food thickening ideotype

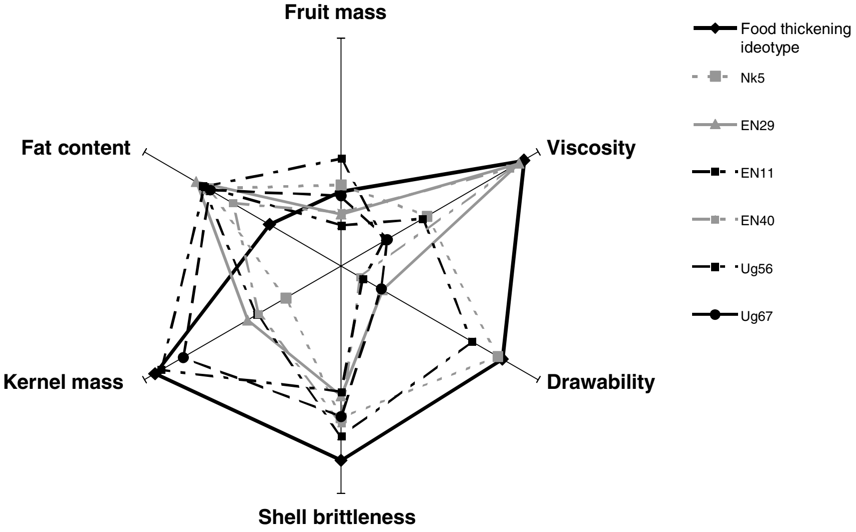


Fig. 9. Multiple combinations (ideotypes) of fruit and kernel traits in *I. gabonensis* for (a) kernel oil, and (b) kernel meal food-thickening properties.

(2000) that the de-fatted residue from dika nuts maintained much of the culinary thickening power. It can therefore be concluded that the fat, although a major component by weight, is not a major determinant of viscosity. This was confirmed by the RVA experiments carried out at temperatures above the melting point of the fat.

Additionally, it was found that the protein content of six dika nut samples of diverse viscosity did not correlate obviously with either viscosity or drawability. The same samples contained a similar pattern of eight prominent protein bands, ranging from 66 to 6 kDa in apparent MW, suggesting that protein content does not markedly affect viscosity. This conclusion was supported by the unchanged viscosity when protein-disruptive agents (the denaturing detergent SDS, and the disulphide-reducing agents DTT and sodium sulphite) were applied. These agents did not affect the RVA traces in any major way, relative to water alone, although there was an indication that they reduced the spikiness of the curves slightly and may, therefore, interact in the development of drawability.

No analysis of polysaccharide content was done with the present set of dika nut samples, but in the light of earlier published studies, and by a process of elimination, it was concluded that the polysaccharide component was dominant in determining the thickening quality of the meals. A wide range of biological diversity was present in the population of samples, but it is not known how much of this variation was due to different quantities of polysaccharide in the meal and how much to different qualities of the polysaccharide.

The separation of drawability from viscosity was concluded from the greater spikiness of some traces, particularly as the pasted mixtures cooled. This was presumably due to a rheological effect of the extracted macromolecules, an effect that was different from the one leading to the smooth traces usually seen with starches. The cyclic build-up and decline of extra power consumption by the stirrer, with an irregular amplitude and a frequency of about 5–6 cycles per min resulted in an oscillation superimposed on the main curve (not obviously related to the stirring speed of 160/min). Such spiky RVA traces are not seen in the RVA pasting of normal starches, but Li and Corke (1999) did observe such a trace with a waxy starch. Waxy starches, which lack amylose and contain only amylopectin, are known to give pastes with a "long" or stringy consistency. Amylopectin molecules are characterised by their very large MW and highly branched structure. Perhaps, therefore, the spiky behaviour in the RVA of the dika nuts with higher drawability implies that their polysaccharide molecules are of a larger and/or more branched molecular structure. The 'spikiness' of these traces varied between samples in intensity and in temperature sensitivity.

Waxy starch pastes are also known to differ from their normal counterparts in their greater ability to be drawn out into threads. This "spinnability" character was measured by Takahashi, Ojima, and Yamamoto (1969), and their technique could perhaps be applied as a direct measure of the drawability of dika nut pastes.

The relationships between drawability and viscosity of the de-fatted kernel meal were weak overall, but varied between village populations (Elig Nkouma: $R^2 = 0.037$: Uguawji: $R^2 = 0.336$; Nko'ovos II: $R^2 = 0.647$). To some extent this seems to reflect differences in the range of variation in both these food-thickening traits, which were as extensive in samples from Nko'ovos II and Uguawji, as they were in those from Elig Nkouma (Fig. 8). However, it is not known, why the levels of viscosity found in kernels form Elig Nkouma were so much greater.

It appears, therefore, that drawability and viscosity characteristics are not directly related and result from somewhat different mechanisms. To advance understanding about the importance of meal quality further, it will be necessary to carry out detailed structural studies on the polysaccharides from dika nut accessions with contrasting viscosity properties. Analysis of the amounts, MW distributions, sugar components, and sugar linkage patterns would all be appropriate. At the same time, studies should be done on the detailed physico-chemical properties of the solutions of the different samples in order to relate the chemical structures to the functional effects.

The results of this study point the way forward for domestication of this species through genetic selection and cultivar development using vegetative propagation (Leakey & Simons, 1998). Adding the results of the present study on the phenotypic variation in viscosity, drawability, and fat content to already published data on fruit mass, kernel mass and shell brittleness of the fruits from the same trees (Atangana et al., 2002), indicates that the considerable tree-to-tree variation in all these traits are weakly related or unrelated (Fig. 8), with the lines for different trees frequently crossing each other. Comparing the trees with the highest value for each trait with the 'ideotype' shows that none of the trees are close to the ideal. Indeed, even if separate ideotypes are derived for production of fat and for food-thickening agents there are no trees within these populations that combine even two of the three important traits for fat production (Fig. 9(a)) or more than two of the four traits of importance for producing food-thickening agents (Fig. 9(b)). There is insufficient data from the present study to add protein content or fatty acid content or profiles to these ideotypes, but these traits too are very variable (Tables 6–8) and apparently unrelated to viscosity or drawability.

Contributing to the domestication of species like *I. gabonensis* for the economic enhancement of subsistence farmers, in ways that are also environmentally beneficial (Leakey, 2001), is one of the means by which the food industry can support the development of more sustainable landuse in the tropics.

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