Biochemical characteristics of composite flours: influence of fermentation

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Abstract

The purpose of this study was to introduce yam in the development of two new composite flours containing soy and cassava. Two composite flours were obtained after fermentation of yam, soybean, and cassava in respectively 60, 30, and 10% proportions. Two varieties of yam were used: *Dioscorea alata* (variety "Bete bete") and *Dioscorea cayenensis* (variety "Lokpa"). Proximate composition, mineral content, some anti-nutritional factors (oxalates, phenols), microbiological quality, and α-amylase digestibility were determined for the fermented and unfermented composite flours. The results indicated that for the composite flours made of *D. alata* and *D. cayenensis*, fermentation increased ash and titrable acidity. Carbohydrates, pH, and energy decreased. Crude fat content was not affected by the fermentation process. Anti-nutritional factors such as oxalates and phenols were found to decrease significantly after the fermentation of the composite flours. Fermentation increased the mineral content (Mg, K, Fe, and Ca) of the composite flours. A decrease in P and Na was observed after fermentation. The microbiological study showed that safety flours contain no potential pathogenic germs. The in vitro α-amylase digestibility of the composite flours was significantly improved after fermentation. The biochemical characteristics and good hygienic quality of the obtained flours suggest that these flours can be considered as a feeding alternative for children in poor areas where yam is produced.

Keywords: infant food; tropical tuber; nutritional analysis; safety.

1 Introduction

Yams are members of the genus Dioscorea which contain about 600 species, of which only six are important in the tropics (COURSEY, 1969; HAHN et al., 1993). The economically important species grown are *Dioscorea rotundata, D. alata, D. bulbifera, D. esculenta* and *D. dumenterum*. Over 90% of world's yam production is derived from West Africa, especially from five yam zones, namely: Nigeria, Benin, Togo, Ghana, and Côte d'Ivoire. The crop is also widely cultivated in other parts of the world such as Asia, South America, South Pacific etc. (FOOD..., 1994).

The tubers of various species of *Dioscorea* constitute one of the stable carbohydrate foods for people in many tropical countries (AKISSOE et al., 2003). Many different forms and cultivars of the edible yam species are available in different areas, and it is likely that they differ in composition and nutritional values (BHANDARI; KAWABATA; KASAI, 2003).

Because of the low energy content of root crops compared to cereals on a wet basis, it is often assumed that root crops are not suitable for use as infant formula. This is not necessarily true if their energy density is increased by drying. Tapioca, for instance, is used in a number of commercial baby foods in industrialized countries. Composite flours prepared from root crops and cereals could also be used in baby food formulas if appropriately supplemented. Infants and young children, pregnant, and lactating women are among the most nutritionally vulnerable people. Their nutrient requirements are specifically higher in order to meet the increased physiological demand for growth and lactation (PICCIANO, 1998; ARMAR-KLEMESU et al., 1991).

Soybean has been widely used in human and animal nutrition because of its favourable agronomic characteristics, relatively low price, and high quantity and quality of its protein and oil (LIU, 2000). Moreover, consumers' awareness of the beneficial effects reported on health has increased its consumption (ALBERTAZZI, 2002; BUS; WORSLEY, 2003). Soybean has been widely used to improve the protein content of weaning foods in developing countries. Cassava (*Manihot esculenta,* Crantz) is largely used in human and animal nutrition, as well as raw material for several industrial products; the most important are the cassava flour, the cassava starch and the sour cassava starch (AVANCINI et al., 2007). Cassava root contains high levels of energy and has been used as a source of readily fermentable energy in ruminant rations (WANAPAT, 2003).

The edible, mature, cultivated yam does not contain any toxic principles. However, bitter principles tend to accumulate in immature tuber tissues of *D. rotundata* and *D. cayenensis*. They may be polyphenols or tannin like compounds (COURSEY, 1983). Several anti-nutritional factors are present in root and tuber crops. Enzyme inhibitors, e.g. against amylase and protease, occur in many tubers. The presence of these inhibitors could impair the digestion of starch and protein, thereby reducing the nutritional value of tubers and limiting their utilization as food (PRATHIBHA; BALA NAMBISAN; LEELAMMA, 1995). Oxalates and phytate are well known anti-nutrients of plant food, and they are associated with a decrease in bioavailability of nutritionally significant mineral elements. These organic substances can bind essential minerals to form insoluble or indigestible complexes in the lumen of intestinal

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tracts, thereby preventing their absorption (DAVIS; OLPIN, 1979). Nevertheless yam makes a significant contribution to diets of people due to its good nutritional value (BHANDARI; KAWABATA; KASAI, 2003). A simple process such as fermentation significantly reduces most of the anti-nutritional factors (REDDY; PIERSON, 1994; IHSAN et al., 2003; INYANG; ZAKARI, 2008). It is also one of the crops available throughout the year (SHIWACHI et al., 2008). Therefore, yam can be proposed as an interesting dietary supplement ingredient, particularly for young feeding. With regard to developing countries, the use of composite flours can bring the following advantages: promotion of high-yielding native plant species; a better supply of protein for human nutrition; a better overall use of domestic agriculture production (BUGUSU; CAMPANELLA; HAMBER, 2001)

The objective of this study is to develop new composite flours made of yam supplemented with cassava and soybean. Some of their biochemical characteristics will be determined.

2 Materials and methods

2.1 Material

For the experiments, two varieties of yam tubers from the species *D. cayenensis rotundata* and *D. alata* coming from different regions of Côte d'Ivoire (West Africa) were used. These varieties were "bete bete" for *D. cayenensis rotundata* and "lokpa" for *D. alata.* Cassava (*Manihot esculenta Crantz*) and white soybean (Glycine max) are also used as ingredients.

2.2 Preparation of composite flours

Yam tuber and cassava root was peeled and cut in pieces (4×4×4 cm) that were boiled separately for 30 minutes, and 200 g of the boiled pieces were processed in a blade mixer until dough like paste was formed. While yam past was immediately dried at 45 °C for 48 hours, the obtained cassava past was pre-fermented for one day using a traditional starter (spontaneous fermented cassava flour) before drying. The typical microorganisms of traditional starters are the lactic acid bacteria with predominance of the genre Lactobacillus (*L. plantarum, L. fermentum, L. delbrueckii and Lactobacillus L. manihotvorans*) and yeasts and moulds (MORLON-GUYOT et al., 1998; LACERDA et al., 2005). A calibrated flour of cassava and yam was obtained after sieving with a 250 µm sieve. Soybeans were cleaned, washed, soaked, and milled to obtain soy flour.

2.3 Formulation of composite flours and fermentation process

The composite flours are obtained by the mixing of flours made of yam, soybean, and cassava in respectively 60, 30, and 10% proportions in 100 mL of distilled water. The proportions 60/30/10 were found to be the most equilibrated based on the preliminary sensory evaluation. The fermentation of the composite flour is initiated by inoculation with 8% (w/w) of traditional starter (spontaneous fermented cassava flour). The fermentation process was done in anaerobic conditions for 48 hours at 30 °C. The fermented paste dried for 48 hours at 45 °C gives the definitive yam based flour. Both fermented and unfermented yam based-flours were analysed for nutritional and safety value determination.

2.4 Proximate analysis

The moisture content and ash were determined in accordance with the standard methods of the AOAC (ASSOCIATION..., 1984). Titrable acidity and pH were determined using the AFNOR method (ASSOCIATION..., 1974). Crude fat was determined using the BIPEA method (BUREAU..., 1976) by exhaustively extracting samples in a Soxhlet apparatus using hexan as the solvent. Crude protein determination involved the use of routine Kjeldhal (BUREAU..., 1976) nitrogen assay (N × 6.25). Total sugars were removed by homogenizing compound flour in 80% ethanol (v/v). The homogenate was refluxed over a water bath and centrifuged. The residue was extracted twice more with 80% ethanol. The supernatant liquids were combined and used for estimation of soluble sugars by the method of Dubois et al. (1956). Calories were obtained by the summation of multiplied mean values for protein, fat and carbohydrate by their respective Atwater factors, 4, 9, and 4 (UDOSEN, 1995). All results for proximate composition are recorded on the basis of edible portion of the uncooked sample as g/100 g dry weigh.

2.5 Mineral analysis

The minerals, such as calcium, copper, iron, magnesium, manganese, sodium, potassium, and zinc were analyzed after nitric acid digestion using an atomic absorption spectrophotometer (Model No. 560, Elmer Corp, Norwalk/United States). Phosphorus was estimated colorimetrically (UV-visible spectrophotometer, Model DR 2800/United States). All results for mineral composition are recorded on the basis of edible portion of uncooked sample as mg/100 g dry weight.

2.6 Total phenols and oxalates

One gram sample was extracted with 10 mL methanol 80% (v/v) containing formic acid 8% (v/v). After 30 minutes, the mixed was centrifuged (3000 tours/min) for 10 minutes. The supernatant was diluted in a Folin-Ciocalteu solution containing one mL of sodium carbonate 17% (w/v). The total phenols were determined spectrophotometrically as described by Swain and Hills (1959). Total oxalates were determined in accordance with the standard methods of the AOAC (ASSOCIATION..., 1984).

2.7 Microbiology

The samples were subjected to a microbiological analysis to monitor the population of potential pathogenic microorganisms during fermentation. Twenty-five grams of each sample were transferred into a sterile stomacher bag, 225 mL of saline-peptone water (8g of NaCl per liter, 1g of bacterial peptone per liter from Sigma-Aldrich Inc.) was added, and the mixture was treated for 1.5 minute in a stomacher machine (Sigma-Aldrich Inc./Germany). Further decimal dilutions were made, and the following analyses were carried out on duplicate agar plates. Total aerobic mesophile flora was determined on plate count agar (Biochemika./United Kingdom) after incubation at 30 °C for 72 hours; coliforms were counted on violet red bile agar (Sigma-Genosys./France).) after incubation at 30 °C for 24 hours; *Staphylococcus aureus* were numbered on Baird-Parker medium (Sigma-Aldrich Inc./Germany).) after incubation at 37 °C for 48 hours; streptococci were counted on bile esculine azide (Biochemika/United Kingdom).) after incubation for 24 to 48 hours at 37 °C; Enteroccocci were numbered on citrate acide agar after incubation at 37 °C for 72 hours. Yeasts and molds were isolated on malt extract agar (Ovoid) supplemented with tetracycline (1 mg/ml) (Sigma/France).) and incubated at 25 °C for 72 hours. For *salmonella,* the International Organization for Standardization - ISO (1991) methods was performed. After counting, means and standard deviations were calculated.

2.8 Digestibility

The in vitro digestibility of flour was carry out using an α-amylase purified from *Periplaneta americana* according to the method of Kouame et al. (2004).

2.9 Statistical procedure

All determinations were carried out in triplicate for each nutrient analysis. For all analyses, the mean and standard deviation for each the nutrient analyzed were calculated and reported.

3 Results and discussion

3.1 Proximate composition

The proximate composition of unfermented and fermented composite flours is presented in Table 1. The moisture contents for *Dioscorea alata* based flour are 6.33 and 6.81% of dry weight respectively for unfermented (UFF*Da*) and fermented (FF*Da*) flour, respectively. The moisture contents of *Dioscorea cayenensis* based flours are 6.65% for unfermented flour (UFF*Dc*) and 6.84% of dry weight for the fermented (FF*Dc*) flour. The moisture contents of the composite flours were not affected after fermentation and drying. The values are in agreement with those generally reported for dried flours (EIMAN; AMIR; ABDEL, 2008).

The ash content of UFF*Da* is higher than that of UFF*Dc.* While fermentation does not change ash content of composite flour made of *Dioscorea cayenensis* going from 2.53 to 2.62%, it significantly increases the ash content of the one made of *Dioscorea alata* from 2.84 to 3.61%.

The crude protein content of UFF*Da and* UFF*Dc* are respectively 15.21% and 13.83%, while the fermented flours contain 15.66% and 15.30%. The protein content increased significantly at the end of fermentation. This is mainly due to the reproduction of microorganisms and their metabolic activity during fermentation, as reported by previous studies. (HAYAT et al., 2008; INYANG; ZAKARI, 2008). Enzymatic proteins are produce by the lactic bacteria that represent the dominant microflora. They hydrolyze the starch of the composite flour leading to the production of sugar, lactic acids, acetaldehyde, diacetyl, peptides, and amino acids, which are precursors of flavor (AGBOR et al., 1995)

A decrease in the carbohydrate levels of the composite flours was observed with fermentation. The carbohydrates decreased from 8.46 to 5.92% for FF*Da* and from 12.20 to 8.50% for FF*Dc*. This decrease might be due to the increase in alpha-amylase activity (LASEKAN, 1996) during fermentation. The alpha-amylase breaks down complex carbohydrates to simple sugar. Energy values, 389.67 and 398.76 kcal/100g, of dry weight obtained respectively for FF*Da* and for FF*Dc* were higher than those reported for yam (FOOD..., 1990).

The fermentation process did not significantly change the fat content of the composite flours obtained from both *D. alata* and *D. cayenensis.* Fermented flour made of *D. cayenensis* and *D. alata* contains respectively respectively 7.32 and 6.27% of crude fat. This relative high level is known to decrease shelf-life due to oxidation as noticed by Inyang and Idoko (2006) for a fermented meal made of pearl millet flour.

Fermentation remarkably decreased pH to 4.12 and 3.88 for flours made of *D. alata* and *D. Cayenensis,* respectively. Simultaneously with the drop in pH, there was an increase in the titrable acidity for the two kinds of flour. Chavan and

Table 1. Proximate composition of the composite flour (g/100 g dry weight)[a].

Parameters	*Dioscorea alata*		*Dioscorea cayenensis*	
	UFF*Da*	FF*Da*	UFF*Dc*	FF*Dc*
Dry matter	93.37 ± 0.03	93.19 ± 0.01	93.35 ± 0.025	93.16 ± 0.02
Moisture	6.33 ± 0.011	6.81 ± 0.012	6.65 ± 0.022	6.84 ± 0.012
Ash	2.83 ± 0.02	3.61 ± 0.022	2.53 ± 0.012	2.63 ± 0.018
Crude protein	15.21 ± 0.02	15.66 ± 0.01	13.83 ± 0.00	15.30 ± 0.01
Crude fat	6.31 ± 0.003	6.27 ± 0.001	7.17 ± 0.000	7.32 ± 0.002
Carbohydrates	8.46 ± 0.02	5.92 ± 0.017	12.20 ± 0.011	8.50 ± 0.00
pH	6.5 ± 0.01	4.12 ± 0.00	6.3 ± 0.01	3.88 ± 0.02
Titrable Acidity	0.06 ± 0.011	0.28 ± 0.013	0.05 ± 0.02	0.29 ± 0.012
Energy (kcal/100 g dry weight)	476.350.01	389.67 ± 0.0	502.31 ± 0.01	398.76 ± 0.01

[a]: Values are the means of three determinations S.D. (n=3). UFF*Da*: unfermented flour made of *Dioscorea alata*; FF*Da*: fermented flour made of *Dioscorea alata*; UFF*Dc*: unfermented flour made of *Dioscorea cayenensis*; FF*Dc*: fermented flour made of *Dioscorea cayenensis.*

Kadam (1989) stated that during fermentation, pH decreases with a concomitant increase in acidity as lactic acid accumulates due to microbial activity. During fermentation titrable acidity significantly increased from 0.06 to 0.28 and from 0.05 to 0.29 for FF*Da* and FF*Dc*, respectively. These results are in agreement with those of many researchers (HAMAD; FIELD, 1997; YOUSIF; EL-TINAY, 2001).

3.2 Mineral composition

The results of the mineral estimation of the wild yam tubers are presented in Table 2. Fermentation was observed to improve the content of most minerals. This is true for potassium, iron, calcium, and magnesium with respectively 4845.74, 334.72, 113.89, and 120.5 g/100 mg of dry weight for fermented composite flours made of *D. alata* and 5380.98, 118.69, 107.45 and 257.89 for composite flours made of *D. cayenensis*. This result was consistent with those of Nnam (2000), who reported increases in iron, phosphorus, and calcium following germination of hungary rice (acha). In this study, only two mineral (phosphorus and sodium) contents decreased after fermentation, which is also in agreement with the results reported by Rendlemen (1982) and Pomeranz and Gain (1983). These authors monitored mineral composition of composite cereal flours.

3.3 Total phenols and oxalates

The unfermented composite flours of *D. alata* and *D. cayenensis* contain respectively 95.44 and 105.66 μg/g of dry matter of total phenols. The analysis of the results shows significant effect of the fermentation process. There was a decrease in total phenols levels after fermentation (Figure 1). After 48 hours of fermentation of the two composite flours, about 39.48% and 50.92% loss of total phenols was noted respectively for flour made of *D. alata* and *D. cayenensis*. A similar effect has been reported in an earlier study, in which 48% loss of phenolic compounds was found after 14 day fermentation of *D. dumetorum* hardened tubers (MEDOUA et al., 2008).

Oxalates appear as end-metabolism products in many vegetable tissues. When they are consumed, oxalates can link calcium and other minerals (NOONAN; SAVAGE, 1999). UFF*Da* and UFF*Dc* contained 2.28 and 3.31 mg/100 g of dry matter of oxalates. A decrease in oxalate levels was noted after the fermentation process (Figure 2). Around 58.08% and 66.17% loss of oxalates was noted after 48 hours of fermentation of flours made of *D. alata* and *D. Cayenensis*, respectively. The decreases in oxalate levels observed in this study can be imputed to their solubility in the soak water since it is known that soaking decreases oxalate levels in food by leaching (NOONAN; SAVAGE, 1999; MEDOUA et al., 2008).

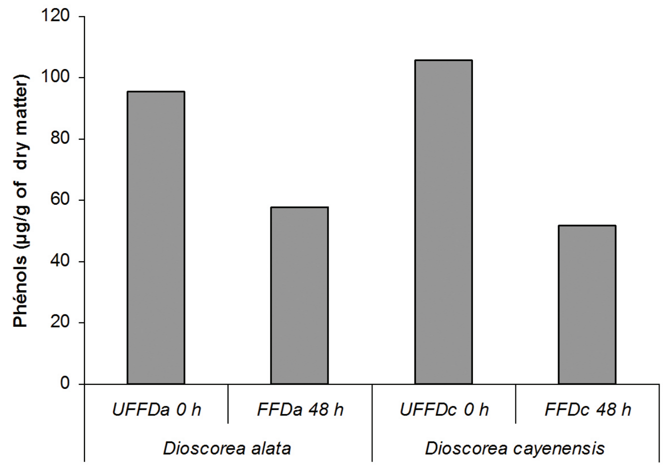

Figure 1. Influence of fermentation on total phenols content. UFF*Da*: unfermented flour made of *Dioscorea alata*; FF*Da*: fermented flour made of *Dioscorea alata*; UFF*Dc*: unfermented flour made of *Dioscorea cayenensis*; FF*Dc*: fermented flour made of *Dioscorea cayenensis*.

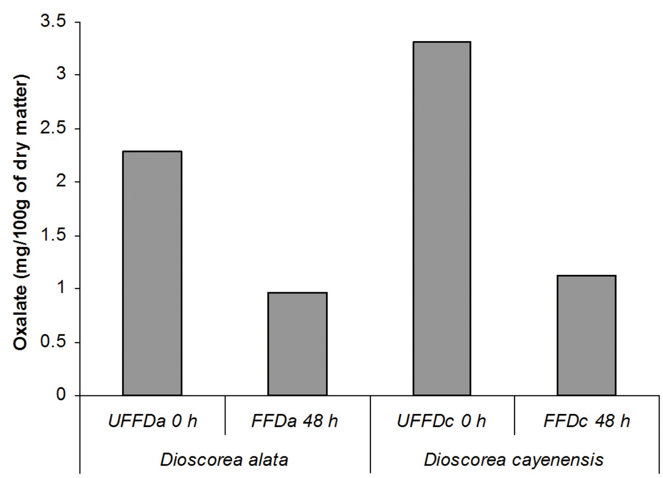

Figure 2. Influence of fermentation on total oxalates content. UFF*Da*: unfermented flour made of *Dioscorea alata*; FF*Da*: fermented flour made of *Dioscorea alata*; UFF*Dc*: unfermented flour made of *Dioscorea cayenensis*; FF*Dc*: fermented flour made of *Dioscorea cayenensis*.

Table 2. Mineral composition of the composite flours (mg/100 g dry weight)[a].

Minerals	*Dioscorea alata*		*Dioscorea cayenensis*	
	UFF*Da*	FF*Da*	UFF*Dc*	FF*Dc*
Mg	18.17 ± 0.02	120.50 ± 0.01	157.82 ± 0.012	257.89 ± 0.02
K	4699.25 ± 0.01	4845.74 ± 0.01	4525.49 ± 0.01	5380.98 ± 0.01
Na	51.440.003	28.390.0012	76.250.0025	55.840.0031
P	15.14 ± 0.014	4.18 ± 0.012	13.43 ± 0.02	12.33 ± 0.017
Fe	113.39 ± 0.045	334.72 ± 0.063	102.48 ± 0.055	118.69 ± 0.074
Ca	121.17 ± 0.14	133.89 ± 0.22	65.76 ± 0.27	107.45 ± 0.17

[a]: Values are the means of three determinations_S.D. (n=3). UFF*Da*: unfermented flour made of *Dioscorea alata*; FF*Da*: fermented flour made of *Dioscorea alata*; UFF*Dc*: unfermented flour made of *Dioscorea cayenensis*; FF*Dc*: fermented flour made of *Dioscorea cayenensis*.

3.4 Microbiology of fermented flours

Microbiological analysis of fermented and unfermented flours was mainly focused on the search for potentially pathogenic microorganisms. *S. aureus,* coliforms, Streptococci, Enteroccocci, *Salmonella* were not detected (Table 3). These results are in good agreement with those found by Parveen and Hafiz (2003) on fermented cereal from indigenous raw materials. The manipulator and the traditional starter are potential sources of contamination of composite flours. The good hygienic quality of obtained flours highlights the safety of the traditional starter and good manufacturing practices.

3.5 Digestibility

In vitro digestibility of composite flours is affected by fermentation. α-amylase in vitro digestibility of composite flours are increased in fermented flours. Values of α-amylase digestibility of UFF*Da* and FF*Da* increased from 56.9 to 85.3% after 120 hours of digestion. For UFF*Dc* and FF*Dc,* digestibility is improved from 65.5 to 88.5%. Digestibility of fermented flour made of *D. cayenensis* is higher than that of flour made of *D. alata* (Figure 3). The fermentation process was also observed to improve in vitro digestibility of sorghum (*Sorghum bicolo*r L. Moench) flour supplemented with Cluster bean (*Cyamopsis tetragonolaba* L.) (ELBASHIR et al.*,* 2008).

Table 3. Microbiology of fermented composites fours.

	Dioscorea alata		***Dioscorea cayenensis***	
Micro	UFF*Da*	FF*Da*	UFF*Dc*	FF*Dc*
Total aerobic count < 3000/g	30 000	30 000	30 000	30 000
Coliform < 1000/g	nf	nf	nf	nf
S. aureus < 1000/g	nf	nf	nf	nf
Streptococci < 1000/g	nf	nf	nf	nf
Enteroccocci < 1000/g	nf	nf	nf	nf
Salmonella absence in 25g	nf	nf	nf	nf
Yeasts < 100/g	nc	nc	nc	nc
Molds < 1/g	nc	nc	nc	nc

nf: not found, nc: not counted; UFF*Da*: unfermented flour made of *Dioscorea alata*; FF*Da*: fermented flour made of *Dioscorea alata*; UFF*Dc*: unfermented flour made of *Dioscorea cayenensis*; FF*Dc*: fermented flour made of *Dioscorea cayenensis.*

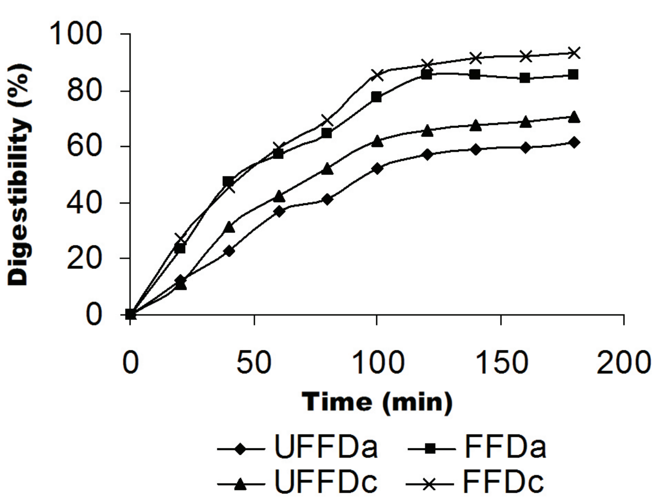

Figure 3. In vitro digestibility of composite fermented and unfermented flours made of *Dioscorea alata* and *Dioscorea cayenensis*. UFF*Da*: unfermented flour made of *Dioscorea alata*; FF*Da*: fermented flour made of *Dioscorea alata*; UFF*Dc*: unfermented flour made of *Dioscorea cayenensis*; FF*Dc*: fermented flour made of *Dioscorea cayenensis.*

4 Conclusion

This study shows that yam can be successfully introduced in the formulation of composite flours, which are low cost and easy to prepare. This is a new method of valuing this very important crop in the diets of vulnerable people such as children in poor regions but which produce large quantities of yam. The stability of the obtained flours should be further studied.

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