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Effects of the particle size and cooking conditions on *in vitro* digestibility of sorghum (*Sorghum bicolor*) (L.) Moench) flour starch

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Abstract

The improvement of starch digestibility of sorghum flour, involves a thorough understanding of the effects of processing technologies applied to sorghum flour. The objective of this study was to determine effects of the particle's size and cooking conditions on the *in vitro* digestibility of starch from different sorghum flours. The factorial designs method coupled with a multiple linear regression was used for the study. The results showed that the 4.48, 3.21, 0.42, 1.35 coefficients referring to the pure effect of the particle's size on *in vitro* digestibility of starch, were positive. The particle size has a beneficial effect on *in vitro* digestibility of starch of the investigated Flour obtained from non treated grains (NT), Flour obtained from germinated grains (GM), Flour obtained from soaked grains (T), Flour obtained from fermented grains (FM). However, the coefficients corresponding to the pure effects of cooking temperature and cooking time were negative. The cooking temperature and cooking time have antagonistic effects on *in vitro* digestibility of the starch from the flour. In addition, the influence of the particle's size and cooking conditions on the *in vitro* digestibility of the starch, in the case of flours NT and T, was significant at 5%.

Introduction

The improvement of digestibility of food is one of the main ways to ensure food security. It involves bioavailability's optimization of food nutrients. Improving the digestibility of food nutrients cannot be carried out without a full understanding of the effects of processing technologies applied to the food. In others words, optimization processes in the long term, involves controlling and monitoring factors that influences nutrient digestibility. In this way, identification and knowledge of the effects of factors influencing nutrient digestibility of food are critical. Improvements in sorghum digestibility are important for its utilisation and, consequently, maximisation of its nutritional benefits (Mahasukhonthachat *et al.*, 2010b) and constituted a major challenge for research on cereal in West Africa. Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal in the world after wheat, rice, maize and barley, in terms of production (FAO, 2005) and is a major crop worldwide (FAO, 2009). Sorghum is widely used for animal feed and human food, and its nutritionally bioactive components might increase its food uses (Awika *et al.*, 2005). It showed that 35% of the sorghum were cultivated for human consumption directly (FAO, 1995). In several countries of West Africa (Burkina Faso, Mali, Niger), sorghum used alone, 50% of total cereal crop land (Dicko *et al.*, 2006). As a cereal, sorghum is rich in starch, and the characteristics of its major proteins (kafirins) have been the subject of various studies to understand digestibility properties (Oom *et al*., 2008). Sorghum is also rich in phytochemicals, making it a potential ingredient in the food health, nutraceutical or specialty markets (Awika *et al*., 2005; Rooney and Awika, 2005).

Although sorghum is a staple food in Africa and offers great potential for use in the manufacturing of complementary food for infants its starch and protein digestibility is low (Mbofung and Fombang, 2003). There are many causes which are responsible for the low digestibility of sorghum flour starch. These causes can be divided into two categories. The endogenous causes which are the first category, were the variety of sorghum, the presence of anti-nutrients such as tannins, phytic acid (Mbofung and Fombang, 2003), the composition of starch (Wong *et al*., 2009), the nature of the proteins and their organization

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within the seed (Ezeogu *et al*., 2005; Belton *et al*., 2006). In the 2nd category, external causes can be summarized into processing technologies such as milling techniques, baking conditions, or methods for preparing the flour.

The particle size and cooking conditions were subject to several studies (Ezeogu *et al*., 2008). Brou *et al.* (2008) have shown that the millet flour's particle's size influences the starch's digestibility. These findings go along with those of Sauvant (2000) who observed that fine grind flours improves digestibility. In another setting, Ezeogu *et al*. (2005) showed that the digestibility of sorghum flour is related to the cooking temperature, the cooking time and the texture of the endosperm of the grain. However, they don't highlight the interaction effects of temperature and time of cooking on the digestibility.

The hypothesis test is that the effects of particle size and cooking conditions used could significantly improve the starch digestibility of sorghum. Firstly, the investigation was carried out to determine the effect of the particle size and cooking conditions on the starch digestibility of different types of sorghum flour. Then, on the different flours, pure effects of particle size and cooking conditions and their interaction effects have been determined using the method of factorial design coupled with a multiple linear regression.

Materials and Methods

Sorghum

This study was performed on sorghum grain (*Sorghum bicolor*), bought at the market of Yamoussoukro, Côte d'Ivoire. The average major and minor diameters and the thickness of the grain were respectively 4.6, 3.7 and 2.7 (mm), while the 1000-grain weight 27 g.

Sample preparation of different types of flour

Sorghum grain (*Sorghum bicolor*) were winnowed, sorted, washed and dried in an oven at 45°C for 24 hours. Then the grains were divided into four equal batches of one kilogram each.

- The first portion of grains called NT (Non Treated) was milled using a hammer mill (TD Africa, Côte d'Ivoire), after drying. It had not been subjected to other pretreatments and was used as a control for the study of digestibility.
- The 2nd batch called T (soaking) was soaked in water with a ratio of 1: 3 (w/v) for 24 h. Then, soaked grains were dried in an oven at 45°C for 48 h on aluminium foil and then ground.
- The third batch of seeds called GM (Germinated Grains) was soaked into water with a ratio of 1: 3 (w/v) for 24 h and then, they were spread on a wet tissue at room temperature (25°C) for 72 h for germination. The humidity control was done by spraying water.

After germination, the seeds were ground after dried at 45°C in an oven for 24 h.

- The fourth batch of grains FM (Fermented Grain) was immersed into water with a ratio of 1: 4 (w/v) and submitted to natural fermentation for 48 h. After fermentation, beans were dried in an oven at 45°C for 24 h and then were ground.

The two particle sizes were obtained by grinding completely grain to pass through those sets of sieves. Different flours obtained after grinding were stored in plastic bottles for further analysis.

Physicochemical analysis

The dry matter and ash contents were determined according to the method of AOAC (1990). Ethanol-soluble sugars were extracted with ethanol 80°GL (Gay Lussac) or 80% ethanol chilled. Of this extract, total sugars were assayed according to the method of Dubois *et al.* (1956). The reducing sugar content was determined according to the method of Bernfeld (1955). The determination of total carbohydrates was performed according to the method proposed by Rao and Pattabhirama (1982). The total starch content of the samples was analyzed using a method derived from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland) as described before (Mahasukhonthachat *et al*., 2010b; Sopade and Gidley, 2009). About 50 mg of the sample was wetted with ethanol before it was heated in a boiling water bath in the presence of dimethyl sulphoxide. The solubilized and gelatinized starch was digested with thermostable α-amylase in MOPS before sodium acetate buffer and amyloglucosidase were added prior to incubation at 50°C. The glucose, and hence, starch content, were determined using an enzymatic glucose reagent and measuring absorbance at 505 nm against a blank reagent.

Digestibility of the starch

For studies relating to starch digestion, α-amylase from *B. licheniformis* (E.C.3.2.1.1; Megazyme, Wicklow, Ireland) supplied at a concentration of 3000 UmL^{-1} was added to the gel of sorghum flour containing. Glucoamylase from *Aspergillus niger* (E.C. 3.2.1.3; A7095, Sigma, St Louis, MO) was obtained at a concentration of 300 UmL^{-1}, where a single unit of enzyme is defined as that amount which hydrolyzes the α (1,4) linkage of maltose at a rate of 1 mMol min^{-1}, at 25°C. After, appropriate dilution of either one of enzymes was added to a flour suspension. The rate of hydrolysis of starch were measured. Both were pretreated with a "cocktail"

Table 1. Matrix of the experimental design

Type of flour	Particle size (µm)	Cooking Temperature (°C)	Cooking time (min)
N°	X1	X2	X3
1	O1 (500µm-250µm)	75	15
2	O1 (500µm-250µm)	75	30
3	O1 (500µm-250µm)	95	15
4	O1 (500µm-250µm)	95	30
5	O2 (1000µm-500µm)	75	15
6	O2 (1000µm-500µm)	75	30
7	O2 (1000µm-500µm)	95	15
8	O2 (1000µm-500µm)	95	30
R1			
R2			

R1 and R2 indicate the repetition of a test
Every type of flour had a specific experimental matrix

of hydrolytic enzymes (Sopade and Gidley, 2009) including porcine pancreas α-amylase (A4268, Sigma), porcine mucosa pepsin (P7000, Sigma), porcine pancreas pancreatin (P7545, Sigma) and glucoamylase. The mixture was incubated with stirring in a water bath at 37°C for 100 min. The glucose released as a result of starch digestion was measured with an AccuCheck® Performa® glucometer (Roche Diagnostics Australia Pty. Ltd., Caste Hill NSW 2154, Australia), and digested starch (g per 100 g dry starch) at a measurement time (min) was calculated as before (Sopade and Gidley, 2009).

Method of preparation of flour gels: experimental design

The experiment was to verify the main hypothesis of this study as follows: the particle size and cooking conditions have an influence on *in vitro* digestibility of starch flour of sorghum. The experimental design used to prepare the gels, is based on that, the starch digestibility of sorghum flour is a system influenced by three independent variables, including the size (X1), the cooking temperature (X2) and the cooking time (X3). The cooking conditions in our study are represented by the temperature (X2) and the length (X3) of cooking. The experimental design consisted of a full factorial (2 × 2 × 2) with 2 levels for each factor chosen. For each type of flour, eight tests were achieved; tests were repeated to calculate the standard deviation of the experimental error. For each test, a Y response (the amount of reducing sugars) is measured. The Y response is a function of F factors. This function takes into account the effects of all factors tested. For known factors but uncontrolled and unknown factors and not controlled, the effects are combined in a single variable e, characteristic of background noise. To assess the effects of these factors, we must know the F function or model defined as:

$$Y= a_0 + a_1X1+ a_2X2+ a_3X3+ a_{12}X1X2+ a_{13}X1X3+ a_{23}X2X3+ a_{123}X1X2X3$$

The matrix of experimental design is presented in Table 1.

Statistics analysis

Results are expressed as the mean ± standard deviation of several sample with Kyplot (version 2.0 beta 15, ©1997-2001, Koichi Yoshioka) statistical software. The data were statistically analyzed by one way analysis of variance (ANOVA). Means were compared by Turkey's test. Differences were considered statistically significant at $P < 0.05$.

Results

Physicochemical properties

The moisture content average of different types of flours was determined (Figure 1). This values ranges from 8.31% for flour GM type O1 to 14.43% for flour FM type O2. The average of moisture content in flour FM (14.29%) is higher, while flours GM have the lowest moisture content (8.85%). Statistical analysis of differences, show a huge gap between the moisture content of the flour and the FM, GM, NT and T flours. For every meals, the analysis of particles size showed that type O2 meal have a higher content of moisture than the type O1 meal in the same category. The ash content of different types of flours was assessed and the results are shown in Figure 2. It varies from 3.20% for flour T-type O1 to 1% for flour GM type O2. For ash content, meals with smaller particles (type O1) have higher ash content than those with larger particles (type O2). Statistical analysis shows that the difference between the meal and flour type O1 O2 type is significant.

As far as concerned total carbohydrates, their availability in the flour has an average content ranging between 70.06% for flour FM type O2 and 79.79% for flour GM type O1 (Table 2). Statistical analysis highlights the differences and similarities between the different contents of total carbohydrates. Flour GM contains more total carbohydrates than other flours (NT, T, FM). The average rate of reducing sugars available in the flours ranges from 0.63% for flour FM type O2 to 4.54% for flour GM type O1. All in all, flour GM has the highest content of reducing sugars compared to other flours (NT, T, FM), while flour FM contains the smallest average content of reducing sugars.

Contents of total sugars range between 1.50% for flour FM type O2 and 6.18% for flour GM type O1. In addition, statistical analysis shows at 5%, there are differences and similarities between different levels of total sugars and flours. In general, the average content of total sugars is highest in flour GM and flour FM presents the lower average content of the

Table 2. Carbohydrate profile of flours obtained from different preparations (% 100 g of dry matter)

Flours	Reducing sugars (%)	Total sugars (%)	Total carbohydrates (%)	Starch (%)
Flour **NT O1**	3,48 ± 0,18 f*	4,74 ± 0,25b	78,68 ± 0,16c	66,55 ± 0,21b
Flour **NT O2**	2,26 ± 0,09bc	4,13 ± 0,12a	74,56 ± 0,10a	63,40 ± 0,03de
Flour **GM O1**	4,54 ± 0,24g	6,18 ± 0,41e	79,79 ± 0,54e	66,24 ± 0,85ab
Flour **GM O2**	2,04 ± 0,05b	5,47 ± 0,39c	78,26 ± 1,07c	65,52 ± 1,28ab
Flour **T O1**	2,35 ± 0,02c	4,42 ± 0,13ab	75,68 ± 0,43ab	64,13 ± 0,30ef
Flour **T O2**	1,42 ± 0,04a	3,97 ± 0,50a	75,10 ± 0,67a	64,01 ± 0,67de
Flour **FM O1**	1,58 ± 0,18a	3,94 ± 0,16a	76,43 ± 0,27b	65,24 ± 0,35af
Flour **FM O2**	0,63 ± 0,05e	1,50 ± 0,06f	70,06 ± 0,98d	61,70 ± 0,84c

*Values affected to the same letter, in the same column, are statistically identical at 5% (n = 3)
NT : Flour obtained from non treated grains ; GM : Flour obtained from germinated grains; T : Flour obtained from soaked grains; FM : Flour obtained from fermented grains; O1 : Flour with a particle's size between 250 µm and 500 µm; O2 : Flour with a particle's size between 500 µm and 1000 µm

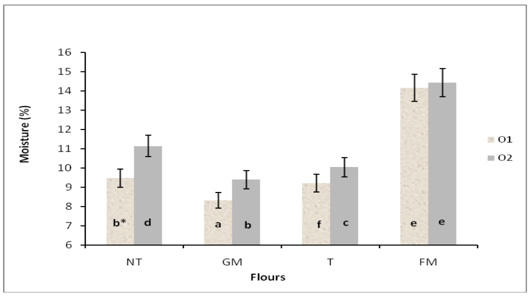

Figure 1. Moisture content of flours

* Histograms affected in the same letter show humidity levels statistically identical at 5% (n = 3)
NT: Flour obtained from non treated grains; GM: Flour obtained from germinated grains; T: Flour obtained from soaked grains; FM: Flour obtained from fermented grains; O1: Flour with a particle's size between 250 µm and 500 µm; O2: Flour with a particle's size between 500 µm and 1000 µm

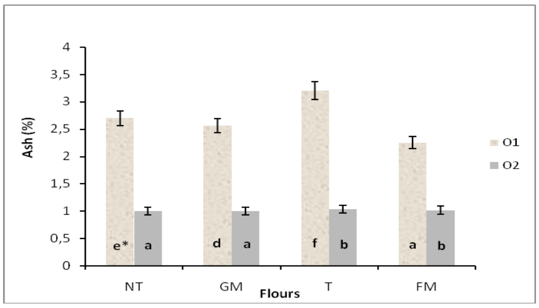

Figure 2. Ash content of flours

* Histograms affected in the same letter show ash levels statistically identical at 5% (n = 3)
NT: Flour obtained from non treated grains; GM: Flour obtained from germinated grains; T: Flour obtained from soaked grains; FM: Flour obtained from fermented grains; O1: Flour with a particle's size between 250 µm and 500 µm; O2: Flour with a particle's size between 500 µm and 1000 µm

total sugars.

The average content of starch goes up to 61.70% for the flour-type FM O2 and up to 66.55% for flour NT type O1 (Table 2). Significant differences at 5% level, and similarities were identified using the Anova test I linked to Duncan. Furthermore, the analysis based on the size of the flour, meal reveals that type O1 have higher sugar levels than the flour type O2.

Evolution of digestibility of flours as the function of preparation method of the gel

The results obtained by kinetics of digestibility of each flour, depend on how the gel is prepared (Figures 3a and 3b). The kinetics of overall digestibility have the same shape. The digestibility of flour starch increases in time. In addition, it is high when the method of preparation is characterized by a size of type O1, with a 95°C cooking temperature and 30 min cooking time (treatment 2 or T2). Otherwise, if the method of preparation of the gel is characterized by a size of type O2, with a cooking temperature at 75°C and a cooking time at 15 min (treatment 7 or T7), the digestibility of the flour is low. In addition, the amount of reducing sugar released after 100 min depends on which method of preparation of the gel is used. Statistical analysis of differences in digestibility

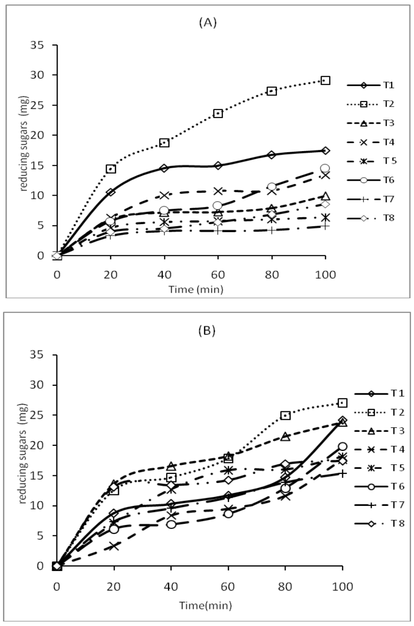

Figure 3 a. Changes in the digestibility of the flour NT (A) and flour GM (B), according to different modes of preparation of the gel.

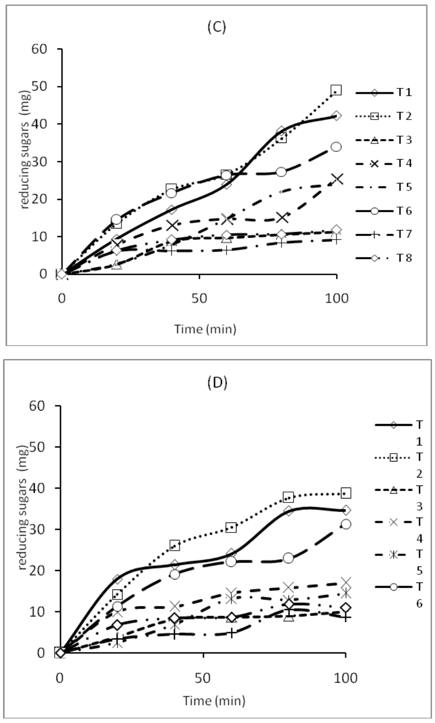

Figure 3 b. Changes in the digestibility of the flour T (C) and flour FM (D), according to different modes of preparation of the gel.

Table 3. Effect of factors on the digestibility of starch of flours

Effects of factors	Flour NT		Flour T		Flour GM		Flour FM	
	Coef	pValue	Coef	pValue	Coef	pValue	Coef	pValue
Order (amylase)	**14,53**	**0,00***	**26,10**	**0,00***	**20,83**	**0,00***	**19,33**	**0,02***
Granulometry	**4,48**	**0,02***	**3,91**	**0,02***	0,42	0,69	1,35	0,72
Cooking temperature	**- 5,31**	**0,01***	**-11,15**	**0,00***	-1,48	0,25	-7,63	0,15
Cooking time	**- 5,94**	**0,01***	**-6,36**	**0,00***	-3,12	0,07	-2,92	0,47
Granulometry *Temperature	-2,68	0,06	- 0,26	0,71	-0,68	0,54	1,00	0,79
Granulometry *Time	-1,53	0,17	-0,77	0,35	0,50	0,64	3,47	0,40
*Temperature*Time*	**3,48**	**0,04***	1,90	0,09	0,18	0,86	1,11	0,77
*Granulometry*Temperature*Time*	1,59	0,16	-1,58	0,13	0,79	0,48	-4,64	0,30

* The coefficient is significant if his p value <0,05
NT: Flour obtained from non treated grains; GM: Flour obtained from germinated grains;
T: Flour obtained from soaked grains; FM: Flour obtained from fermented grains

reveals significant differences between the various methods of preparation of the gels. Moreover, the statistical analysis of differences in digestibility based on the size, shows that meal type O1 have a higher digestibility than meal type O2.

Effects of the particle's size and cooking conditions on the digestibility

The method of multiple linear regression showed coefficients corresponding to the effects of the studied factors (particle size, cooking temperature and cooking time) (Table 3). For flours NT and T, the coefficients corresponding, respectively, to pure effects of particle size, temperature and cooking time are significant at level 5%. This means that the particle size and cooking conditions affect the individual starch digestibility of flours NT and T. In addition, we note that, coefficients relating to cooking conditions are assigned a negative sign. This can be explained by the fact that the conditions used (temperature and cooking time) have antagonistic effects on digestibility. This implied that the particle's size has not any effects on the starch digestibility.

As far as the particle's size is concerned, the coefficient for its effect is positive. This means that the particle's size has a positive effect on the starch digestibility. As far as NT flour is concerned, analysis of the coefficient corresponding to the interaction effect "temperature and cooking time" shows that it is positive and significant at 5%. This means that the interaction between temperature and cooking time has a significant and positive influence on the digestibility of the flour starch. The coefficients corresponding to the effects of the enzymatic activity of amylase (14.53; 26.10; 20.83 and 19.33) on the digestibility of tested flours (NT, T, GM, FM) are significant (Table 4). This means that the enzymatic activity significantly influences the digestibility of flours.

Discussion

The results for the moisture content of the meals are similar to those obtained by Dicko *et al*. (2006). Analysis of moisture showed that the moisture content of flour FM is higher than the one of other flours (NT, T, GM). This is due to the method used to obtain this meal. Indeed, the duration of soaking in water for sorghum grain, would have favoured the absorption of water in the grains and the drying time was not sufficient to reduce the humidity to a value similar to the moisture content of other flours.

The grinding may explain the fact that flours type O1 have higher ash content than flours type O2. According to Akaffou (2007), Ash is closely associated with grain structures, particularly in the aleuron layer and germ (Favier, 1989). Small particles from the grinding would probably have a structure similar to the aleuron layer or germ, so the ash is close to these structures. This is not the case with large particles of flour (type O2).

The carbohydrate composition of flours type O1 and O2 could be explained by the effect of grinding, which causes a loss of water due to the duration of the operation and the temperature rise. Indeed, the water coming out of large particles (flour type O2) under the effect of pressure also causes the release of sugars (reducing and total), carbohydrates and starch (Brou *et al*., 2008). The high concentration of reducing and total sugars in the flour GM could, on the one hand, be explained by the pronounced disorganization of the structure of the starch in the grains during milling (Garcia-Alonso *et al*., 1996). This disorganization is provoked by the disruption of inter- and intra-molecular links bonds between the molecule of glucose of starch chains and the other, by enzymatic activity. Indeed, during germination, the increase of soluble sugars resulted from the amylolytic activity of endogenous amylases in the sorghum grain allowing the chains of molecule of glucose. These results are similar to those obtained by Brou *et al*. (2008) for millet and by Elmaki *et al.* (1999) for sorghum.

The similarity of the shape of the starch digestibility curve according to methods of preparation of the gel, could be explained by the increase in reducing sugars over time. This increase would result from the action of amylases on the starch. In addition, differences in digestibility kinetics observed for each group of flour, would be due to the specific method of preparation of the gel, in particular the particle size and cooking conditions used. Indeed, when the gels are made with flour with small particles (flour type O1) in conditions of high cooking (95°C, 30 min), the digestibility is higher than the gels prepared with flour with large particles (flour type O2) under conditions of lower-level cooking (75°C, 15 min). The extent of milling (i.e.

particle size) influences starch digestion in cereals and legumes. Large particles have a smaller surface area than smaller ones and therefore large particles are digested more slowly (Noda *et al*., 2008; Parada and Aguilera, 2009).

These results are consistent with those of Brou *et al.* (2008). According to these authors, the digestibility changes on a size basis. When the particle size is smaller, the digestibility is high. In addition, Van der Merwe *et al.* (2001) showed that the method of preparation has an effect on the digestion of starch. The results for the effects of the particle's size, temperature and cooking time, revealed a significant effect on the digestibility of starch for flours NT and T. It has no significant effect on the digestibility of starch to flours GM and FM. The significant influence of the particle's size, cooking temperature and cooking time on the digestibility of starch flour NT and T, could be explained partly by the method used to obtain the flour (Van der Merwe *et al.*, 2001). Furthermore, Caldwell *et al.* (2000) are demonstrated that the actual time needed to cook cereal products is generally a function of both moisture (interacting with shear) and temperature. Cooking increases the rate of starch hydrolysis by gelatinising the starch and making it more easily available for enzymatic attack (Roder *et al.*, 2009; Alsaffar, 2010; Singh *et al*., 2010).

On the other hand, according Ezeogu *et al.* (2005), the heat could have a great influence on the protein's structure which contains the starch granules. Indeed, in the case of flour NT and T, the action of cooking conditions particularly of the pair (temperature and duration) may be double. Firstly, the significant effect of cooking temperature on the digestibility could be explained by its action on the protein matrix surrounding the starch granules. The temperature would probably have disrupted the physical structure of the protein network. It would have generated cracks inside the starch granules resulting in the disruption of the envelope of the protein matrix. The fragmentation of the protein matrix with starch granules has been described by Parker *et al.* (1999). Secondly, the effect of cooking time could be explained by the strong presence of polymerized kafirins during cooking. Finally, the significant interaction effect of the pair: temperature and duration, in the case of flour NT would be the result of individual actions on the temperature and cooking time, which might generally favour the availability of starch on the action of amylases. Moreover, the antagonistic effect of cooking conditions on the starch digestibility could be explained by the polymerization of kafirins (Hamaker and Busugu, 2003). According to these authors, the polymerization of kafirins during cooking may hinder the gelatinization of starch granules, thus its digestibility. The significant influence of the particle's size on digestibility in the case of flours NT and T, could be explained by previous actions of cooking conditions. The cooking conditions would have favoured an increase in the contact area.

The insignificant influence of cooking conditions on the digestibility of starch flour GM and FM, may be due to the action of ions contain in water on the protein. Indeed, according to Audigier and Zonszain (1991), neutral salts (sodium chloride, ammonium sulphate, magnesium, sodium), depending on their concentration and ionic strength, influence the solubility of a protein. For a low ionic strength, solvent effect or salting-in effect is observed. Whereas for a high ionic strength, there is a release or effect of salting-out. Thus, soaking the seeds in NaCl solution at 10% would have caused disturbance in the protein structure of the grains. This disturbance would have mitigated the effect of temperature on the protein network. In addition, disruption of the protein network would not have advanced significantly the formation of polymerized kafirins during cooking.

Conclusion

This study aimed at determining the effect of the particle's size and cooking conditions on the starch digestibility of sorghum flour obtained in different ways. To achieve this goal, a physicochemical characterization and a series of experiments were performed. The results of experiments showes that for all studied flours, the particle's size has a beneficial effect on *in vitro* digestibility of starch from sorghum flour. On the other hand, temperature and cooking time have antagonistic effects on the digestibility of starch flours. In addition, the influence of the particle's size and cooking conditions on the digestibility of starch is significant at 5% for NT and T flours. However, for the GM and FM flour, the influence of the particle's size and cooking conditions on the digestibility of starch is not significant at 5%. Moreover, the variability of the effect of particle size and cooking conditions can be explained by the manufacturing processes of flour.

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