IN VITRO STARCH DIGESTIBILITY OF BY-PRODUCTS OF SORGHUM STARCH EXTRACTION

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ABSTRACT

The aim of the present study was to assess the nutritive value of by-products of sorghum starch extraction, as dietary starch from feed grains. Five by-products differing in their particle sizes and starch contents were collected. The mean values of fraction yields and starch contents, for first, second and third fraction of sorghum gluten feed were respectively 16.13 – 52.63 %, 01.97 – 44.08 % and 04.99 – 56.75 %, while for sorghum gluten meal the values were 22.27 – 71.13 %. The substrates from whole grain meal, prepared by dry milling, and from by-products differed in their in vitro starch digestion. The mean values for kinetic parameters ranged from 0.0066 to 0.0147 min^{-1} for the rate constant (k), from 53.66 to 98.58 % for the starch hydrolysis at infinite time (C_∞) and from 6.06×10^3 to 8.47×10^3 %.min for the area under the hydrolysis curve (AUC). Generally, a high digestibility of by-products of sorghum starch isolation with a great potential for sorghum in livestock and animal feeds are considered in this work.

Keywords: Sorghum, Starch extraction, By-products, Starch digestion, Animal feed

INTRODUCTION

The wet-milling process is industrially used for the separation of the main cereal components and involves physical, chemical and biochemical operations. The sorghum starch production by wet milling process on industrial scale has been previously reviewed (Eckhoff and Waston, 2009) and the optimal condition has been investigated in several laboratories (Yang and Seib, 1995; Beta *et al*., 2001; Higiro *et al*., 2003; Pérez Sira and Amaiz, 2004; Belhadi *et al*., 2013).

The by-products are usually recombined and dried to produce the sorghum bran, gluten feed, gluten meal and the germ meal. These feed ingredients, largely used for the animal feed (Göhl, 1981; Svihus *et al.,* 2005; Heuzé *et al.,* 2015), may find wider applications in the food and non-food products (Wronkowska, 2016) and can be used for ruminants, pigs, poultry and rabbits (Wall and Paulis, 1978; Göhl, 1981; Hamid and El Zubeir, 1990; El Zubeir and Mustafa, 1992; Onifade *et al*., 1999). In this respect, various strategies have been suggested to improve feed values by increasing the digestibility of both protein and starch. The need to improve and optimize the efficiency of starch digestion in an important research focuses in the animal nutrition (Selle *et al.,* 2010; Liu *et al*., 2013).

The starch digestion is therefore crucial for the dietary energy of compound diets based

on cereals (Wiseman, 2006). Several works has been conducted to investigate the starch digestion kinetic from different ground grains by alpha-amylase (Goñi *et al*., 1997; Slaughter *et al*., 2001; Frei *et al*., 2003; Hu *et al*., 2004; Ezeogu *et al*., 2005; Chung *et al*., 2006; Mahasukhonthachat *et al*., 2010; Souilah *et al.,* 2014). Various factors have been suggested as the cause of variation in the starch digestibility. Giuberti *et al*. (2014) and Zhu (2014) has reviewed the structure, physicochemical properties, modifications and uses of sorghum starch and the factors affecting the starch utilization in large food livestock. Lipids and proteins are the most abundant non-starch components in the feed ingredients that may affect the physical state and susceptibility to amylolysis of starch in livestock (Baldwin, 2001), and grain processing conditions (e.g. milling, soaking, pelleting, extrusion, expanding and mixing) are affected on the alteration of cereal grain starch, starch digestibility and utilization (Theurer *et al.,* 1986; Mahasukhonthachat *et al.,* 2010; Al-Rabadi *et al.,* 2012; Koa *et al.,* 2017; Sopade, 2017).

The aim of the present study was to assess the nutritive value of by-products obtained after sorghum starch extraction, as dietary starch from feed grains. Small-scale laboratory steeping in NaOH, and wet-milling process of starch extraction from sorghum whole grain was done. The fraction yield, starch yield and starch recovery in by-products, starch isolates were determined, and the kinetic of the starch digestion in sorghum meal and by-products were studied.

MATERIALS AND METHODS

Materials Preparation: Four grain samples of local sorghum [*Sorghum bicolor* (L.) Moench] cultivars were harvested from Tidikelt region in the Algerian Sahara. Samples with white color and non-tannin are labeled SB10AS, SB11AS, SB12FE and SB13FE. The sorghum grains were cleaned and stored at 4 °C until use. The small-scale laboratory steeping and wet-milling procedure for isolation of starch from sorghum grains were performed as described by Pérez Sira and Amaiz (2004), Beta *et al.* (2000), Beta *et al.* (2001) and Belhadi *et al*. (2013). A schematic view of the procedure is presented in Figure 1.

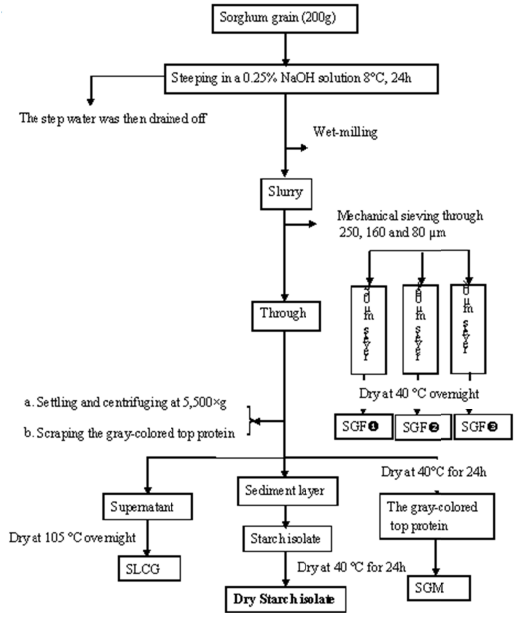

Figure 1: Laboratory small-scale scheme to isolate starch from sorghum grains

200 g of sorghum grain was steeped at 8 °C for 24 hours in 400 mL NaOH solution of concentration 0.25 % (w/v) in a batch reactor (Beta *et al*., 2001). Steeped sorghum grains were wet-milled by a grinder and mechanically sieved through 250, 160 and 80 µm on a shaker (Model RETSCH AS 200 Basic, France). Fractions that were held up by each sieve were washed with 75 mL of distilled water in order to recover more starch granules. According to the terminology used by Göhl (1981), the by-products held up by the sieves 250, 160 and 80 µm were named sorghum gluten feeds (SGF) and labeled SGF1, SGF2 and SGF3 as first, second and third sorghum gluten fraction respectively. The filtrate was centrifuged at 5,500×g, for 10 minutes using refrigerated centrifuge (Jouan E96). After each centrifugation, the supernatant was eliminated and a top protein layer was scrapped off and named sorghum gluten meal (SGM). Approximately 2 – 3 litre of water process was decanted and precipitate was dried in air oven at 105 °C. The recovered dried precipitate was named steep liquor condensed gluten (SLCG). The starch slurry was dried overnight in oven at

40 °C and recovered as dry starch isolate. The sorghum whole grains were ground to meal in IKA Labortechnik A10 sample mill. The obtained meals were manually sieved over a 500 µm sieve. The milling characteristics of sorghum grain for wet-milling can be evaluated by fraction yield, starch yield and degree of recovery. The fraction yield (FY %) was calculated using equation 1.

$$\text{FY }\% = \left[\frac{m_F(100-H_F\,\%)}{m_g(100-H_g\,\%)}\right] \times 100 \quad (1)$$

Where m_F = by-product fraction or starch isolate mass in (g), H_F = by-product fraction or starch isolate moisture, m_g = sorghum grain mass in (g) and H_g = sorghum grain moisture. The starch yields (SY %) was calculated using equation 2.

$$\text{SY }\% = \frac{(\mathbf{FY}\,\%)\times(\mathbf{TS_F}\,\%)}{\mathbf{100}} \quad (2)$$

Where TS_F = Total starch of fractions (%). The degree of recovery (DR %) was calculated using equation 3.

$$\text{DR }\% = \text{FY }\% \left[\frac{TS_F\,\%}{TS_g\,\%}\right] \quad (3)$$

Where TS_g = Total starch of sorghum grain (%).

Chemical Analysis: The moisture content was determined according to AACC methods 44-15A (AACC, 2000) while the total starch (TS) was determined by the enzymatic method (Goñi *et al.,* 1997). All the reagents were of analytical grade.

In-Vitro Starch Digestion: The nutritional values of starch in by-products obtained from sorghum starch extraction were assessed by enzymatic hydrolysis method. Among the twenty substrates, five of each sample of the four sorghum grain samples were investigated. Samples of the by-products (SGF1, SGF2, SGF3 and SGM) and meal of whole grain substrates was prepared by dry milling and sieved through 500 µm.

The *in vitro* starch digestion was determined according to the modified method described elsewhere (Goñi *et al.,* 1997; Mahasukhonthachat *et al.,* 2010; Souilah *et al.,* 2014). According to the percentage of total starch in each substrate, a mass of substrate containing 300 mg of starch was weighed. The masses were transferred in large tubes, to which 25 mL of phosphate buffer solution pH 6.9 were added. To start starch hydrolysis, 5 ml of α-amylase (2 mg/mL), type VI.B from porcine pancreas (A3172, Sigma-Aldrich) was added. The prepared mixture was incubated at 37 °C for 3 hours with constant shaking, aliquots of 0.2 ml were withdrawn at 0, 20, 40, 60, 90, 120, and 180 minutes, α-amylase was inactivated immediately by placing the tubes in boiling water bath for 5 minutes. Then, 2.6 ml of 0.4 M sodium-acetate buffer solution (pH 4.75), and 0.2 mL of an enzyme solution of amyloglucosidase from *Aspergillus niger* (3260 U/mL, Megazyme, 9032-080), 1 %(V/V) were added. In order to hydrolyze digested starch into glucose, the sample was incubated at 60 °C during 45 min. Finally, the volume was adjusted with distilled water and glucose concentration in the digesta which was measured within the range (25 – 100 µg/mL), using the oxidase-peroxidase assay kits.

Modelling of Starch Digestograms: The first-order exponential model in the kinetic study has been used to evaluate the starch hydrolysis or glycemic indices in the food and feed (Goñi *et al.,* 1997; Ezeogu *et al.,* 2005; Souilah *et al.,* 2014). Starch amylolysis data was fitted into equation 4.

$$C_t = C_\infty\,(1\text{-}\exp\,[-kt]) \quad (4)$$

Where C_t corresponds to the percentage of starch hydrolysis at time t, C_∞ is the percentage of starch hydrolyzed at infinite time, recorded in 180 minutes, k the rate constant and t the time (minutes). The area under the hydrolysis curve (AUC), which is obtained by integrating equation 4 between times t_0 = 0 minutes and t_f = 180 minutes led to equation 5.

$$AUC = C_\infty\ t_f - C_\infty / k\,(1\text{-}\exp\,[-k\,t_f\,]) \quad (5)$$

Statistical Analysis: All parameters of sample characterization were measured in three replicates, and expressed as mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA) and mean differences were assessed by Tukey's test significant difference test at the level of $p<0.05$ with the SPSS Software Version 17. The analyses of kinetic data were performed using Sigma Plot Version 10.0 (Systat Software Incorporated, Chicago, Illinois, USA) for the windows.

RESULTS

Sorghum By-Products Fractions: Five by-products were produced by small-scale laboratory steeping and wet-milling procedures for the isolation of starch from four sorghum grains, including three gluten feed fractions, gluten meal and steep liquor condensed gluten (Table 1). The results, mean, range and coefficient of variation for the fraction yield (FY %), starch yield (SY %), degree of recovery of starch (DR %), moisture (H %) and total starch (TS %) of sorghum meal, starch isolate and by-products of sorghum starch isolation are given in Tables 1 and 2. The mean degree of recovery of starch values were respectively 12.08 ± 4.05 %, 01.26 ± 0.67 % and 03.98 ± 0.99 % for first, second and third fraction of SGFs and 22.42 ± 6.53 % and 0.0037 ± 0.0014 % for SGM and SLCG respectively. This result indicated that the starch content in by-products is a significant proportion from whole sorghum grain.

In-Vitro Kinetic Starch Digestion: The curves of Figure 2 demonstrate the susceptibilities of starches in all substrates in *in vitro* digestion with pancreatic α-amylase. The curves exhibit a mono-phasic digestogram. Generally, it can be seen from Figure 2 the differences in values of rate and percentage of starch hydrolysis, at same times among substrates of each grain. The computed digestibility curves provided a very good fit to all experimental data, with a regression coefficient $R^2 > 0.9$ and standard error of estimate (SEE) < 6 % for most substrates. The model-fit analysis of digestibility data is particularly well-suited to study and the first-order kinetic model is suitable for all substrate digestions. The comparison the values of k, C_∞ and the percentage of starch hydrolysis in the different stages of the curves, indicated that there was an inverse relationship between k and C_∞ values and a positive relationship between the values of k and the reaction rate in the first rate period [0 – 20 min]. The results showed difference in the starch digestion between primary period and intermediate and last periods of the reaction.

The values of the three kinetic parameters: C_∞, k and AUC are reported in Tables 3 and 4. The analysis of the variance amongst kinetic parameters values (Table 5) revealed that the differences in k, C_∞ and AUC are significant ($p<0.05$) between substrates in these cases: between meal and the SGFs and between SGFs and SGM in k values, between meal and SGFs and between SGM and SGF1 and SGF2 in C_∞ values, and between meal and SGM in AUC values. The differences between the three SGF fractions are no significant in kinetic parameters.

Substrates analyzed ranked follows the following sequence for their rate constants k (min^{-1}): SGM > meal > SGF3 > SGF2 ≥ SGF1. The starch hydrolysis at infinite time C_∞(%) in substrates ranked as follows: SGF2 ≥ SGF1 > SGF3 > SGM > meal, and ranged from the lowest in the meal (49.22 %) to the highest in first sorghum gluten feed (112.20 %) from landrace (SB10AS). While, the area under the hydrolysis curve AUC (%.min) ranked as follows: SGM > SGF2 ≥ SGF3 ≥ SGF1 > meal.

DISCUSSION

Sorghum By-Products Fractions: The starch yield (SY %) in starch isolate, obtained from four sorghum samples, ranged from 26.54 to 44.38 % with a mean value 34.90 %. This result indicated that the starch yield obtained from sorghum samples was lower than those determined by Buffo *et al.* (1998) (59.21 %) from twenty-four grain sorghum hybrids (1993 crop year) grown in USA and by Xie *et al.* (2006) (38.7 – 58.9 %) from sixteen grain sorghum. Moreover, the starch yield remains higher than those found by Yang and Seib (1995) (14.1 – 20.6 %), from nine samples and by Pérez Sira and Amaiz (2004) (27.73 – 30.0 %), from dark and white sorghum varieties from FUSAGRI, Venezuela.

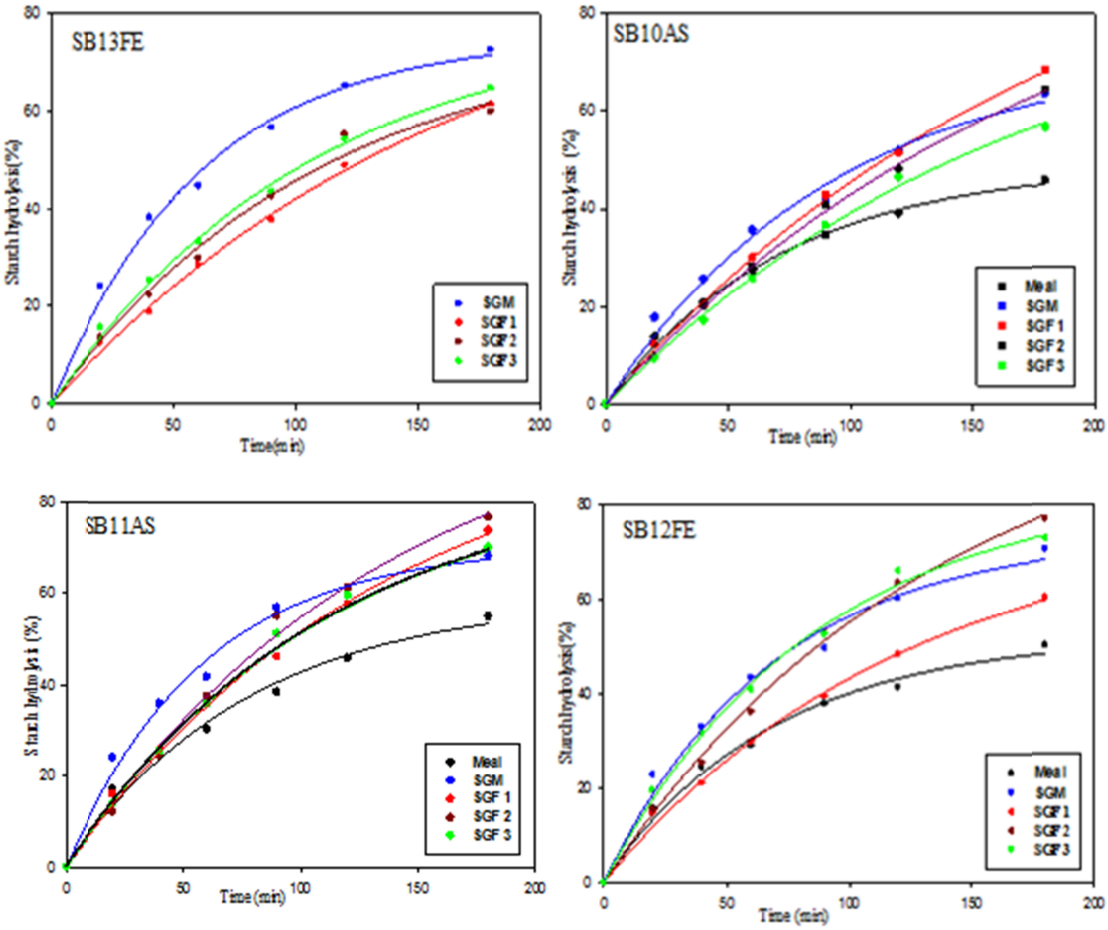

Figure 2: Starch digestibility curves obtained for sorghum flour and by-products after small-scale laboratory steeping and wet-milling procedures

The degree of recovery (DR %) obtained from sorghum samples ranged from 37.65 to 61.73 %. with a mean value 48.89 % were lower than those obtained by Higiro *et al*. (2003) (91.6 – 96.5 %), Xie and Seib (2000) (91.9 – 95.0 %), Wang *et al.* (2000) (82.2 – 85.9 %), Buffo *et al*. (1998) (71.37 – 89.71 %), Xie *et al*. (2006) (57.8 – 83.7 %), Xie and Seib (2002) (56.8 – 88.6 %) and Belhadi *et al*. (2013) (62.8 – 80.4 %). Moreover, the starch recovery in the four studied sorghum grains is higher than that found by Yang and Seib (1995) (18.4 – 26.6 %) and the difference in the starch yield and recovery is due to steeping conditions, wet-milling process, separation efficiency and sorghum genotype Wang *et al.* (2000). The purity of starch isolate evaluated by total starch analysis lies between 97.66 and 98.99 % with a mean value 98.51 %; this value shows high purity of starch isolate which is visually white. The total fraction yields of the three sorghum gluten feed fractions: (SGF1, SGF2 and SGF3), reached 23.09 % and such value was similar to those given by Buffo *et al.* (1998) (22.71 %), but higher than those recorded by Xie and Seib (2000), Higiro *et al*. (2003), Eckhoff and Waston (2009) (13.1 – 16.25 %), and greater than 17.8 % when starch was extracted from corn (Malumba *et al*., 2015). Sorghum gluten meal yield, reached 22.27 % was higher than results from previous studies with the range [7.13 – 9.80 %]. While steep liquor condensed gluten (SLCG) yield, reaches 0.089 %, and lower than values in range [2.10 – 2.61 %] Buffo *et al*. (1998) and Higiro *et al.* (2003).

Table 1: Fraction yield (FY %), starch yield (SY %), degree of recovery of starch (DR %), moisture (H %) and total starch (TS %) of sorghum meal, starch isolate and by-Products (fractions) of sorghum starch isolation

Landraces codes	Samples	FY (%)	SY (%)	DR (%)	H (%)	TS (%)
SB10AS	**Meal**	-	-	-	11.51	70.51
	Starch isolate	27.18	26.54	37.65	11.86 ± 0.22	97.66 ± 4.92
	SGF1	16.34	08.36	11.86	10.87 ± 0.07	51.17 ± 2.90
	SGF2	01.48	00.57	00.81	11.25 ± 0.21	38.50 ± 0.11
	SGF3	05.36	03.20	04.54	11.41 ± 0.18	59.74 ± 0.40
	SGM	27.86	21.66	30.72	07.21 ± 0.25	77.76 ± 2.15
	SLCG	0.071	0.0012	0.0018	-	01.76 ± 0.01
SB11AS	**Meal**	-	-	-	11.35	73.20
	Starch isolate	34.46	33.93	46.35	09.11± 0.20	98.44 ± 0.75
	SGF1	21.24	11.50	15.71	10.24 ± 0.14	54.15 ± 4.86
	SGF2	01.32	00.58	00.79	10.00 ± 0.06	44.04 ± 1.12
	SGF3	04.11	02.32	03.16	13.57 ± 0.20	56.27 ± 0.23
	SGM	16.08	11.12	15.20	14.20 ± 0.04	69.19 ± 1.74
	SLCG	0.081	0.0033	0.0045	-	04.08 ± 0.16
SB12FE	**Meal**	-	-	-	10.65	69.70
	Starch isolate	35.11	34.74	49.84	15.08 ± 0.04	98.94 ± 3.10
	SGF1	16.86	09.95	14.27	11.07 ± 0.21	59.00 ± 0.21
	SGF2	01.85	00.84	01.20	09.48 ± 0.98	45.31 ± 0.34
	SGF3	04.03	02.19	03.14	11.08 ± 0.21	54.38 ± 0.51
	SGM	21.80	16.49	23.66	11.91 ± 0.14	75.66 ± 0.14
	SLCG	0.092	0.0023	0.0033	-	02.51 ± 0.09
SB13FE	**Meal**	-	-	-	10.35	71.89
	Starch isolate	44.83	44.38	61.73	16.67 ± 0.13	98.99 ± 1.18
	SGF1	10.09	04.66	06.49	10.06 ± 0.14	46.20 ± 0.23
	SGF2	03.21	01.59	02.22	07.41 ± 0.28	48.47 ± 7.60
	SGF3	06.46	03.66	05.09	11.12 ± 0.21	56.60 ± 2.90
	SGM	23.35	14.46	20.11	10.67 ± 0.01	61.91 ± 0.89
	SLCG	00.11	0.0036	0.0050	-	03.11 ± 0.01

Although many studies like Buffo *et al.* (1998), Higiro *et al*. (2003), Wang *et al.* (2000), Malumba *et al*., (2015) have been investigated various techniques of wet-milling process for sorghum and corn grains, they have neglected the properties of residues of extractions related to their uses as feedstock or their ingredients. Our research estimates some of starch properties in residues. Results showed that we produce by-products with high starch yield and starch content among sorghum samples. In order to improve the starch for more efficient animal feed, we see the need to improve the separating process of the starch from the proteins extracted from the seed. All by-products can be classified as dietary starch from feed grains (Göhl, 1981; Svihus *et al.,* 2005).

In-Vitro Kinetic Starch Digestion: The first-order model has been demonstrated in *in vitro* starch digestion of raw and processed food and feed (Goñi *et al.,* 1997; Ezeogu *et al.,* 2005; Wiseman, 2006; Mahasukhonthachat *et al.,* 2010; Souilah *et al.,* 2014). The AUC is related with values k and C_{∞}, thus expressing the starch digestion during all reaction phases and is the

Table 2: Mean, range and coefficient of variation (CV %) of fraction yield (FY %), starch yield (SY %), degree of recovery of starch (DR %), moisture (H %) and total starch (TS %) of starch isolates and by-products of sorghum starch isolation

Variable		Mean ± SD	Range	CV (%)
Starch isolates	FY (%)	35.40±7.24	27.18-44.83	**20.45**
SGF1		16.13±4.59	10.09-21.24	**28.46**
SGF2		01.97±0.86	01.32-03.21	**45.03**
SGF3		04.99±1.15	04.03-06.46	**23.05**
SGM		22.27±4.86	16.08-27.86	**21.82**
SLCG		0.089±0.017	0.071-0.11	**19.10**
Starch isolates	SY (%)	34.90±7.32	26.54-44.38	**20.97**
SGF1		08.62±2.93	04.66-11.50	**33.99**
SGF2		00.90±0.50	00.57-01.59	**55.55**
SGF3		02.84±0.71	02.19-03.66	**25.00**
SGM		15.93±4.41	11.12-21.66	**27.68**
SLCG		0.0026±0.0011	0.0012-0.0036	**42.31**
Starch isolates	DR (%)	48.89±9.98	37.65-61.73	**20.41**
SGF1		12.08±4.05	06.49-15.71	**33.53**
SGF2		01.26±0.67	00.79-02.22	**53.17**
SGF3		03.98±0.99	03.14-05.09	**24.87**
SGM		22.42±6.53	15.20-30.72	**29.13**
SLCG		0.0037±0.0014	0.0018-0.0050	**41.18**
Starch isolates	H (%)	13.18±3.37	09.11-16.67	**25.57**
SGF1		10.56±0.49	10.06-11.07	**04.46**
SGF2		09.53±1.60	07.41-11.25	**16.79**
SGF3		11.79±1.19	11.08-13.57	**10.09**
SGM		11.00±2.92	07.21-14.20	**26.54**
Starch isolates	TS (%)	98.51±0.62	97.66-98.99	**00.93**
SGF1		52.63±5.36	46.20-59.00	**10.18**
SGF2		44.08±4.16	38.50-48.47	**09.44**
SGF3		56.75±2.22	54.38-59.74	**03.91**
SGM		71.13±7.15	61.91-77.76	**10.05**
SLCG		02.86±0.98	01.76-04.08	**34.26**

most important parameter (Sopade, 2017), which can be used to evaluate the starch digestion in meal and by-product substrates.

The parameters of the model, and hence digestion kinetic from Table 4, depended on sorghum by-products properties, the dependence was more affected by wet-milling process (Sopade, 2017); soaking and steeping (Singh *et al.,* 2010); milling (McAllister *et al.,* 1994) and particle size of by-products fraction (Mahasukhonthachat et al. 2010). The changes of wet-milling process used in this study were thought to be responsible for different digestion kinetics of the sorghum by-products samples.

The statistical analysis and comparisons the values of kinetic parameters showed variation in the starch susceptibility among some substrates. They reflect the effect of different factors on the mechanism of digestion due to differences in the physical properties and type of chemical components of substrates. To try to explain the differences in starch digestion between some substrates and to know the factors influencing the digestion reaction, we show the correlation between the kinetic parameters and some physical properties and chemical components of substrates. This helps to understand the effect of the steps of starch extraction (steeping, wet milling, centrifugation and sieving) and dry milling, on the starch digestibility in different substrates.

Table 3: Comparisons of fraction yields and starch contents values of sorghum by-products in the present study with previous studies

	Present Study[a]	Buffo *et al.* (1998)[a]	Higiro *et al.* (2003)[a]	Eckhoff and Waston (2009)[a]	Xie and Seib (2000)[a]	Malumba *et al.* (2015)[b]
By-products	Gluten feed, 250 µm	-	Bran/germ, 1000 µm	Fibre/germ	Bran/germ, 1000 µm	Fibre/germ, 400 µm
	Gluten feed, 160 µm	-	-	-	-	-
	Gluten feed, 80 µm	Fibre/germ, 63 µm	Fine fibre, 73 µm	-	Fine fibre, 73 µm	sieved gluten, 50 µm
	Gluten meal	Gluten	Gluten	Gluten	Gluten	SLC[d]
	SLCG[c]	Washing solids	Process water solids	-	Process water solids	-
Fraction yield, %	16.13	-	5.55	15.5	8.3	9.7
	1.97	-	-	-	-	-
	4.99	21.71	10.7	-	4.8	8.1
	22.27	8.23	7.13	9.6	8.5	9.8
	0.089	2.31	2.61	-	2.1	-
Total starch, %	52.63	-	-	55.8	10.0	13.1
	44.08	-	-	-	-	-
	56.75	-	-	-	26.3	56.1
	71.13	-	-	39 .9	17.9	51.6
	02.86	-	-	-	-	-

*[a]Sorghum wet-milling process, [b]Corn wet-milling process, [c]**SLCG**: steep liquor condensed gluten, [d]**SLC**: steep liquor condensed.*

Table 4: Kinetic parameters of first order reaction model, of meal and by- products of sorghum starch isolation: (k, C_∞, AUC)

Landraces codes	Samples	k(min^{-1})[a]	C_∞(%)[a]	AUC×10^3(%.min)[a]	StdErr (%)[b]	R^{2b}
SB10AS	**Meal**	0.0138	49.22	5.59	0.097	0.997
	SGF1	0.0052	112.20	7.08	0.045	0.999
	SGF2	0.0053	104.60	6.96	0.040	0.999
	SGF3	0.0058	89.13	6.09	0.070	0.997
	SGM	0.0107	72.49	7.26	0.160	0.989
SB11AS	**Meal**	0.0128	59.45	6.52	0.180	0.987
	SGF1	0.0073	100.30	8.01	0.076	0.997
	SGF2	0.0073	106.30	8.49	0.110	0.993
	SGF3	0.0090	89.27	8.11	0.075	0.997
	SGM	0.0172	70.79	8.81	0.170	0.992
SB12FE	**Meal**	0.0145	52.31	6.07	0.170	0.990
	SGF1	0.0080	78.18	6.62	0.091	0.996
	SGF2	0.0073	106.30	8.49	0.071	0.997
	SGF3	0.0117	83.63	8.78	0.110	0.995
	SGM	0.0145	73.74	8.56	0.190	0.988
SB13FE	**Meal**	-	-	-	-	-
	SGF1	0.0059	93.81	6.48	0.068	0.997
	SGF2	0.0090	77.13	7.01	0.150	0.990
	SGF3	0.0093	79.63	7.38	0.077	0.997
	SGM	0.0164	75.51	9.23	0.150	0.994

*[a] **k** (min^{-1}): kinetic constant, **C_∞** (%): the equilibrium percentage of starch hydrolyzed after 180 min, **AUC** (%.min): area under the hydrolysis curve. [b] Values are estimated from fit to experimental data, with R^2 > 0,9 and standard error of estimate (SEE) < 6 % for most meals and fractions*

Table 5: Means of parameters with Tukey's test and standard error for flour and by-products of sorghum starch isolation

Samples	k(min^{-1})	C_∞(%)	AUC×10^3(%.min)
Meal	0.0137 ± 0.00049^b	53.66 ± 3.03^a	06.06 ± 0.27^a
SGF1	0.0066 ± 0.00064^a	96.12 ± 7.09^c	07.05± 0.35^{ab}
SGF2	0.0072 ± 0.00076^a	98.58 ± 7.16^c	07.74± 0.43^{ab}
SGF3	0.0090 ± 0.00121^a	85.42 ± 2.33^{bc}	07.59± 0.58^{ab}
SGM	0.0147 ± 0.00145^b	73.13 ± 1.00^{ab}	08.47 ± 0.42^b

Means followed by the same letters (a, b and c) are significantly different according to Tukey's test (p<0.05)

Processing of sorghum grains breaks down recalcitrant barriers such as the hull, pericarp and protein matrix (Sopade, 2017). Soaking and steeping increases the grain moisture with accompanying changes to physico-chemical and structural properties (Singh *et al.,* 2010). The milling reduces the particle of grain sizes, increasing the surface area available for enzyme attachment (McAllister *et al.,* 1994).

The differences in the k values between SGM and SGF by-products substrates can be explained by small and free starch granules in SGM, while starch granules for SGF are encapsulated by particles from whole grain cells and embedded within protein matrix in endosperm particles. Thus, at the first reaction period, the starch digestion rate in SGM substrates is higher because the area that can be attacked by the enzyme in the free granules is large when the inhibitor protein effect of the reaction is negligible (or low).

The difference in the k values between meal substrates and three substrates of SGF by-products is due to the presence of a large value of rapidly digestible starch concentration in the meal, so that the digestion rate of the flour starch at first period reaction is greater. The slight difference in the k values between the substrates of three SGF by-product extracts is due to the difference in the dimensions of their particle sizes. We record an inverse correlation between the dimensions of particle size and k values, which is reached by several researchers, Mahasukhonthachat *et al.* (2010) found that the smaller the particle size (120 – 560 µm), the more digested is sorghum (*var. Buster*) from Australia. Also, the finest particles (0.16 – 0.2 and 0.315 mm) are more nutritive and have a better digestibility in sorghum (*Sorghum bicolor*) from Cote d'Ivoire (Brou *et al.*, 2013). Comparison of C_∞ values in the starch digestion showed that the differences between meal substrates and three by-product SGF substrates could be explained by the change of structure and architecture of endosperm and starch granule. During steeping and soaking processes, grains swell and lead to loss of part of their crystallinity and formed a starch voids in the endosperm (Sopade, 2017), due to the transfer of starch granules, parts of the protein matrix and other components to soak water and to starch slurry after wet milling and sieving. The earlier change in the endosperm particle structure and the starch granule structure helps the external enzyme diffusion to the surface of starch granule and the internal enzyme diffusion in granule pores and channels. Thus, the starch digestibility is high in three residues of SGF. The meal is composed from peripheral endosperm particles region, which is extremely dense, hard, with high protein content, and resist to both physical and enzymatic degradation (Rooney and Pflugfelder, 1986). The endosperm protein, associated to the type and location of protein, has been demonstrated to be responsible for many of the differences in the starch digestion between slowly digested grains substrates and those that are rapidly digested (Giuberti *et al.,* 2014).

The values of C_∞ in the three by-products of SGF are greater than their values in SGM substrates; this is due to the high free protein ratio in the SGM substrates and its inhibitory effect at the advanced stages of the reaction. This is because the endosperm protein, associated to the type and location of protein, was found to be responsible for many differences in the starch digestion between slowly digested substrates and those that are rapidly digested starch (Giuberti *et al.,* 2014). A

comparison of C_∞ values for SGF by-product substrates shows that their value in SGF3 is lower than SGF1 and SGF2. This is due to the small size of the starch grains attached to the endosperm.

The significant differences in AUC values between meal substrates and SGM substrates is due to the large area of free starch granules in the SGM substrates that can be attacked by the enzyme, while this area is small in meal. In sorghum meal particles, the endosperm cell walls surround starch granules embedded within a protein matrix and limit the access of enzymes to starch granules.

The results of this study showed that there was a wide variation in the digestibility properties (k, C_∞ and AUC), between different by-products and in comparison with meal. Generally, starches in sorghum by-products from wet-milling process were shown to vary widely in the digestibility properties. They are classified as starch sources in animal feeding.

Conclusions: In the present work, we used small-scale laboratory soaking and steeping in NaOH solution and wet-milling process to isolate starch from four white sorghum grains. Starch isolate with high purity (98.51 $\pm$ 0.62) were produced, and five by-product was collected. The fraction yield and degree of recovery in starch isolate were ranged from 27.18 to 44.83 % and 37.65 to 61.73 % respectively. These results indicate that the percentage of non-extracted starch, from kernels, reached 60 % and confirmed that the sorghum starch isolation was not an attractive process in industry. In order to valorize the by-products as dietary starch feed grains, we studied the kinetic of *in vitro* starch digestion in starches substrates from by-products and grain meals. The results showed that there is a wide variation in the digestibility properties among different by-products and between grain meals and by-products. The percentages of starch digestion reached 100 % in some by product substrates. Thus, the by-products substrates do not need additional heat moisture treatment, such as those which are subjected to dry milling or dry grind substrates to increase their digestibility. The by-products of starch separation from the sorghum grain applied in this work could be integrated differently, with high nutritional value, in the animal feeding strategies and livestock industry.

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