Protein Quality and Physical Characteristics of Kisra (Fermented Sorghum Pancake-like Flatbread) made from Tannin and Non-tannin Sorghum Cultivars

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ABSTRACT

Kisra is a naturally lactic acid bacteria- and yeast-fermented sorghum thin pancake-like flatbread, produced in Sudan. Kisra has considerable potential as the basis for development of a gluten-free sandwich wrap. To help direct cultivar selection for commercial production of these products, two white, tan plant non-tannin Type I, one white Type II tannin and one red Type III tannin sorghum cultivar were evaluated with respect to kisra protein quality and physical characteristics. Kisra from the non-tannin sorghums were flexible and had an open textured structure with many regular gas cells, whereas those from the tannin sorghums were more brittle, dense in structure and contained far fewer and smaller gas cells. Kisra from the tannin sorghums had the lowest reactive lysine content, in vitro protein digestibility and Protein Digestibility Corrected Amino Score (PDCAAS), with values being lowest for the Type III sorghum. PDCAAS of kisra from the Type III sorghum was only 0.12, less than half of that from the Type I sorghums. As the tannins in tannin sorghums adversely affect kisra protein quality and physical characteristics, white tan plant, non-tannin sorghum cultivars are the most suitable for kisra production and for development of wrap-type sorghum-based baked goods.

INTRODUCTION

Kisra is a naturally lactic acid bacteria- and yeast-fermented sorghum pancake-like flatbread (Hamad et al 1992, Mohammed et al, 1991). Kisra is baked in round, thin sheets, approx.1-1.5 mm thick and 30-45 cm in diameter (Ejeta 1982, Badi et al 1988). Ideally it should be supple, soft and moist in texture, but not spongy. Kisra is known as the staple food of Sudan (El Tinay et al 1979, Ejeta 1982). Today, with growing urbanization, kisra is becoming a commercial home-based industry in Sudan. Internationally, because of the apparent increase in the incidence of celiac disease and intolerance to wheat, interest in gluten-free cereal products is increasing rapidly (Kelly et al 2008). Kisra appears to have considerable potential as the basis for development of a gluten-free sandwich wrap.

Kisra research has been limited. Recently, it has been shown that Lactobacillus and Saccharomyces cultures can be used to reduce the fermentation time from 19 to 4 hr (Ali and Mustafa 2009), which would be useful for commercial production. In view of the particularly attractive textural characteristics of the kisra pancake, selection of suitable sorghum cultivars for commercial production and for similar wrap type products is also a critical issue. Ejeta (1982) used an expert sensory panel to evaluate the kisra making quality of eight cultivars and concluded that some pearly yellow sorghum cultivars and cultivars with a white chalky pericarp made the best kisra. El Tinay et al (1979) found that sorghum cultivar influenced kisra protein quality.

Probably the major distinguishing characteristic among sorghum cultivars is whether or not they contain tannins. Tannins have several highly beneficial effects with regard to sorghum cultivar agronomic attributes, including protection against bird predation, insects and weathering (Waniska et al 1989). The presence of condensed tannins in sorghum cultivars also greatly affects the functional and nutritional quality of sorghum food products. For example, Yetneberk et al (2004) showed that tannin sorghums imparted a bitter taste and aftertaste to injera, a fermented flatbread similar to kisra but thicker and spongy in texture. On the other hand, the condensed tannins in sorghum are excellent antioxidants and exhibit several potentially health-promoting actions (reviewed by Dykes and Rooney 2006), and there are tannin sorghums cultivars that do not seem to impart objectionable sensory characteristics to foods (Kobue-Lekalake et al 2009).

The objective of this limited study was therefore to determine the effect of tannins in sorghum on the protein quality and physical characteristics of kisra, in order to help direct the selection of cultivars for commercial production of kisra and development of gluten-free wrap-type, sorghum-based products.

MATERIALS AND METHODS

Sorghum Cultivars

Four sorghum cultivars: Dabar, Orbit, Feterita and NS5511 were used. Dabar and Orbit are tan plant Type I (non-tannin) sorghums (Table 2). Feterita is a white colored Type II tannin sorghum and NS5511 a red Type III tannin sorghum, containing 1.7 and 4.5 g/100 g catechin equiv. respectively. . Dabar and Feterita, which strictly speaking are open-pollinating landraces, were obtained from the Food Research Centre, Shambat, Sudan. Orbit and NS5511, which are hybrids, were cultivated in the North West and Free State Provinces, respectively, of South Africa. The four cultivars were selected for two reasons. They represented a wide range of sorghum types (Table I). Also, Dabar and Feterita were chosen because they are popular sorghum cultivars in Sudan and NS5511 because despite it being a Type III tannin sorghum it has similar bitterness and astringency characteristics to some non-tannin sorghum cultivars (Kobue-Lekalake et al 2007).

Preparation of Kisra

The sorghums were milled into whole grain flour using a laboratory hammer mill fitted with a 500 μm opening screen. Kisra was prepared essentially as described by Ejeta (1982). In brief, a pH 4.0 "mother culture" was prepared as described by Taylor and Taylor (2002) by back slopping a slurry of Orbit flour in water. Flour (235 g) of each cultivar was weighed into 1 L buckets. Tap water (300 mL) at 30˚C was added to each bucket. The resulting slurry was mixed and 20 ml of the "mother culture" was added and incubated at 25˚C until pH 4.0 was obtained. This varied with cultivar. For Dabar, Orbit and Feterita it was 48-60 hr, and for NS5511 80 hr. Kisra was made by carefully pouring the fermented batter onto an electrically heated clay hot plate, in a circular motion, and cooking it until it set to the desired consistency. The setting time also varied with cultivar. For Dabar, Orbit and Feterita it was 30 s, and for NS5511 38-40 s. Samples of the fermented batter and kisra were freeze dried for chemical analysis and air-dried for light microscopy and scanning electron microscopy (SEM). Air-drying resulted in all the kisra becoming brittle and breaking into pieces.

Physical Analyses

Sorghum grain color, pericarp color and texture were assessed visually as described by Rooney and Miller (1982). Endosperm texture was assessed visually according to ICC Draft

Standard 176 (ICC, 2008). Grain size was assessed according to Gomez et al (1997). Light micrographs of pieces of kisra were obtained using a digital camera fitted with a macro lens. For SEM, pieces of dried kisra were mounted on a stub with double sided tape and sputter coated with gold. They were viewed using a Joel JSM-840 SEM (Tokyo, Japan).

Chemical Analyses

Condensed tannins were determined by the modified Vanillin HCl method of Price et al (1978), where the tannins are extracted with acidified methanol. The absorbances of the sample blanks were subtracted to compensate for non-tannin pigments and tannin content was expressed in catechin equiv. Protein content (N x 6.25) was determined by a Dumas combustion method, AACC Method 46-30 (AACC International 2000). Total lysine content was measured by the Pico-Tag reverse phase HPLC procedure (Bidlingmeyer et al, 1984) after acid hydrolysis. Reactive lysine was determined using a rapid dye-binding lysine (DBL) method of Kim et al (2007) using Crocein Orange G dye (70% dye content) (Fluka grade 27965: Sigma-Aldrich, Buchs, Switzerland. In vitro protein digestibility (IVPD) was determined by a pepsin digestion method (Hamaker et al, 1987). Accurately weighed samples (200 mg) were digested with P700-100G pepsin, activity 863 units/mg protein (Sigma-Aldrich, St. Louis, MO) for 2 h at 37^{o}C. Residual protein was determined by the Dumas combustion method and IVPM was calculated as the percentage of total N that was solubilized under the conditions of the assay.

RESULTS AND DISCUSSION

Kisra made from the two white tan plant sorghums Dabar and Orbit was cream in color, whereas that that made from the two white type II tannin sorghum Feterita was light brown and that made from NS5511 the red type III tannin was dark brown (Fig. 1). The kisras made from Dabar and Orbit were flexible to the extent that they could be folded without breaking, whereas that made NS5511 was brittle. Kisra made from Feterita was intermediate in texture. Also of note was the fact that the NS 5511 kisra batter resisted spreading when poured onto the hot plate to bake it.

The fine structure of the kisra also differed with cultivar. Kisra from Dabar and Orbit had the desirable open textured, regular cellular-type structure, associated with kisra (Ejeta 1982, Badi 1988). The cells were of approx. 200 µm diam. (Fig. 2), caused by gas bubbles resulting from fermentation by yeasts such as *Candida krusei* (Hamad et al 1992), *C. intermedia* and *Debaryomyces hansenii* (Mohamed et al 1991), and possibly heterofermentative lactic acid bacteria such as *Lactobacillus fermentum, L. amylovorus* (Hamad et al 1992) and *Pediococcus pentosaceus*, *L. confusus* and *L. brevis* (Mohamed et al 1991. In contrast, the structure of kisra from Feterita and NS5511 was much more dense, containing very few gas cells, were also considerably smaller, approx. 100 µm diam. The lack of gas cells in the tannin sorghum kisra and the fact that the fermentation was much slower with the NS5511 cultivar indicates that the tannins were inhibiting fermentation. This is consistent with the fact that there were measurable tannins remaining after the Feterita and NS5511 flours were fermented (Table II). These findings with respect to the influence of cultivar on kisra quality contrast somewhat with the report by Ejeta (1982) that apart from its color, the quality of kisra made from Feterita sorghum was good. The differences in findings may be due to the fact that as stated, Feterita is a landrace and thus the quantity of tannins in different samples may be highly variable. Ejeta did not report the tannin the contents of the sorghum cultivars.

Concerning the effects on protein quality, there was little change in total lysine content or amino acid score with fermentation, or with kisra making (Table III). This agrees with the report of El Tinay et al (1979). It, however, appears to contradict work that has shown improvement in the lysine content of sorghum with fermentation. Kazanas and Fields (1981) and Asiedu et al (1993) reported a 40% and 25% increase in lysine in sorghum gruels with lactic acid fermentation, respectively. The most likely reason for the difference is a result of different microorganisms being involved. In this regard, as mentioned above it is notable that yeasts have been identified as fermentative organisms in kisra fermentation, as well as lactic acid bacteria. Concerning the effect of cultivar, the protein lysine content of the Feterita grain and kisra was much lower than that of the other cultivars. This is most likely related to the fact that the protein content of the sample of Feterita was much higher than that of the other sorghum cultivars. Amino acid score in sorghum is based on lysine, since lysine is the first limiting indispensable amino acid in sorghum (reviewed by Klopfenstein and Hoseney 1995). Amino acid score therefore directly followed lysine content. The amino acid score of kisra was on average 42% compared to the requirements of 3-10 year old children (WHO/FAO/UNU Expert Consultation 2007).

Concerning reactive lysine (Table IV), the absolute values were higher than those for total lysine (Table III). This is probably related to the lack of specificity of the Crosein Orange G dye binding with respect to basic amino acids (Hurrell et al 1979). Notwithstanding this, it is clear that both sorghum cultivar and processing affected the level of reactive (chemically available) lysine. Overall, NS5511 had the lowest reactive lysine content, presumably as a result of tannin-protein binding. Cooking the fermented flour into kisra resulted in a substantial reduction in reactive lysine in cultivars, on average a 30% reduction. This was probably due to late Maillard type reactions that can occur during baking (Michalska et al 2008), which render the lysine nutritional unavailable (Hurrell and Carpenter 1981).

With regard to IVPD, there were also effects of both cultivar and processing. The two tannin sorghums, and their fermented flours and kisra had lower IVPD than the non-tannin sorghums (Table V). Further, the IVPD of the NS5511 the type III tannin sorghum was lower than the type II tannin sorghum Feterita. In fact, the IVPD of NS5511 was only just over 50% of that of Dabar and Orbit kisra. Since Feterita kisra had essentially no measurable tannins, it appears that the reduction in digestibility was caused by the tannins rendering the sorghum proteins less digestible through binding with them, rather than the tannins reacting with the pepsin. Tannins have been shown to bind strongly with the sorghum kafirin storage proteins, due to their relatively high proline content (Butler et al 1984, Emmambux and Taylor 2003, Taylor et al 2007).

As reported by Taylor and Taylor (2002), fermentation slightly improved the IVPD of the sorghum (Table V). These authors attributed the improved IVPD to changes in the conformational structure of the sorghum proteins brought about by fermentation. However, IVPD was reduced somewhat when the fermented flour was cooked into kisra. The reduction in sorghum IVPD on wet cooking is considered to be primarily due cross-linking of the kafirin proteins through disulfide bonding (reviewed by Duodu et al 2003).

Protein Digestibility Corrected Amino Acid Score (PDCAAS) was slightly reduced when sorghum was processed into Kisra (Table V). PDCAAS is the officially accepted measure of protein quality (WHO/FAO/UNU Expert Consultation 2007) and predicts the protein quality of both dietary mixtures and individual protein food sources. The reduction in PDCAAS was a result of the reduction of IVPD due to wet cooking. The PDCAAS of kisra made from the tannin sorghums was rather lower than that from the non-tannin sorghums. The protein quality of kisra made from NS5511 the Type III tannin sorghum was only 12% with respect to the requirements of 3-10 year old children of that of a food with nutritionally complete protein, and less than half of that of the kisra made from the two Type I, non-tannin, sorghums.. The lower PDCAAS of the kisra from NS5511 and Feterita sorghums was a result of their lower IVPD. As stated, this was probably caused by tannin-sorghum protein binding.

The fact that kisra made from tannin sorghums had a more dense and brittle texture and that tannin-sorghum protein interactions were strongly indicated (Table V) suggests that the kisra fermentation process modifies the sorghum proteins so that they play a functional role in kisra texture. This is consistent with the observation that the similar sourdough process modifies sorghum proteins improving the pan bread making quality of sorghum (Schober et al 2007) and sorghum-wheat composites (Hugo et al 2000).

CONCLUSIONS

The protein quality and physical characteristics of kisra are strongly affected by sorghum cultivar. The tannins in tannin sorghums adversely affect these properties through inhibition of microbial fermentation and tannin-protein binding. White tan plant, non-tannin sorghums appear to be the best for production of kisra and development of gluten-free sandwich wrap-type sorghum-based products.

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LEGENDS TO FIGURES

Fig 1. Light micrographs of pieces of the kisra made from different sorghum cultivars. (a) Dabar, (b) Orbit, (c) Feterita, (d) NS5511. Bar = 10 cm

Fig 2. SEM of pieces of the kisra made from different sorghum cultivars. (a) Dabar, (b) Orbit, (c) Feterita, (d) NS5511

TABLE I

General Characteristics of Sorghum Cultivars Investigated for Kisra Making

Cultivar	Grain size	1000 kernel weight (g)	Overall colour	Glume colour	Pericarp texture	Testa (subcoat)	Endosperm texture	Protein content (N x 6.25 db)
Dabar	Small	27.3	White	Straw	Intermediate	Non-pigmented	Intermediate	10.21[b1] ± 0.09[2]
Orbit	Small	20.0	White	Red	Intermediate	Non-pigmented	Intermediate	7.36[c] ± 0.06
Feterita	Medium	29.5	White	Purple	Thick and chalky	Pigmented	Floury	13.44[a] ± 0.11
NS5511	Medium	30.3	Red	Purple	Thick	Pigmented	Floury	9.13[d] ± 0.09

[1]Means with different superscript letter in the same column are significantly different ($p \leq 0.05$)

[2]Standard deviation of two replicate experiments

TABLE II
Effects of Cultivar and Kisra Processing on Sorghum Measurable Tannin Content (g/100 g db)

Cultivar	Flour	Fermented flour	Kisra (fermented then cooked)	Mean cultivar
Dabar	0.09[a1] ± 0.00[2]	0.06[a] ± 0.00	0.03[a] ± 0.00	0.06
Orbit	0.11[a] ± 0.01	0.07[a] ± 0.01	0.02[a] ± 0.00[c]	0.07
Feterita	1.72[b] ± 0.01	1.29[b] ± 0.03	0.05[b] ± 0.01	1.02
NS5511	4.50[c] ± 0.07	3.93[c] ± 0.05	2.47[c] ± 0.07	3.63
Mean processing step	1.61	1.34	0.64	

[1]Means with different superscript letter in the same column are significantly different ($p \leq 0.05$)

[2]Standard deviation of two replicate experiments

TABLE III

Effects of Cultivar and Kisra Processing on Sorghum Lysine Content (g/100 g protein) and Amino Acid Score (%)

Cultivar	Flour	Fermented flour	Kisra (fermented then cooked)	Mean cultivar
Dabar	2.30[c1] ± 0.07[2] (48)[3]	2.01[b] ± 0.00 (42)	2.08[b] ± 0.06 (43)	2.16 (45)
Orbit	2.45[d] ± 0.06 (51)	2.14[c] ±0.03 (45)	2.03[b] ± 0.08 (42)	2.21 (46)
Feterita	1.58[a] ±0.09 (33)	1.54[a] ±0.09 (32)	1.67[a] ± 0.06 (35)	1.60 (33)
NS5511	2.19[b] ± 0.05 (46)	2.05[b] ± 0.07 (43)	2.20[c] ± 0.06 (46)	2.15 (45)
Mean processing step	2.13 (44)	1.94 (40)	2.00 (42)	

[1]Means with different superscript letter in the same column are significantly different ($p \leq 0.05$)

[2]Standard deviation of two replicate experiments

[3]Calculated from WHO/FAO/UNU Expert Consultation (2007) lysine requirement for 3-10 year old children of 48 mg/g protein

TABLE IV
Effects of Cultivar and Kisra Processing on Sorghum Reactive (Chemically Available) Lysine content (g/100 g protein)

Cultivar	Flour	Fermented flour	Kisra (fermented then cooked)	Mean cultivar
Dabar	$2.72^{b1} \pm 0.13$	$2.63^{b} \pm 0.17$	$1.86^{b} \pm 0.09$	2.40
Orbit	$2.58^{b} \pm 0.05$	$2.61^{b} \pm 0.04$	$2.22^{c} \pm 0.12$	2.47
Feterita	$2.59^{b} \pm 0.13$	$2.26^{a} \pm 0.06$	$1.53^{a} \pm 0.09$	2.05
NS5511	$2.00^{a} \pm 0.04$	$2.38^{a} \pm 0.27$	$1.31^{a} \pm 0.20$	1.90
Mean processing step	2.47	2.47	1.73	

[1]Means with different superscript letter in the same column are significantly different ($p \leq 0.05$)

TABLE V
Effects of Cultivar and Kisra Processing on Sorghum In Vitro Protein Digestibility (%) and Protein Digestibility Corrected Amino Score (PDCAAS)

Cultivar	Flour	Fermented flour	Kisra (fermented then cooked)	Mean cultivar
Dabar	76.7[d1] ± 0.3[2] (0.37)[3]	80.7[c] ± 0.8 (0.34)	65.3[c] ± 1.9 (0.28)	74.2 (0.33)
Orbit	72.4[c] ± 0.5 (0.37)	81.1[c] ± 1.4 (0.36)	67.2[c] ± 1.9 (0.28)	73.6 (0.34)
Feterita	61.0[b] ± 2.2 (0.20)	70.2[b] ± 0.7 (0.22)	58.0[b] ± 2.1 (0.20)	63.1 (0.21)
NS5511	42.1[a] ± 1.5 (0.19)	47.3[a] ± 0.7 (0.20)	26.3[a] ± 1.0 (0.12)	38.6 (0.17)
Mean processing step	63.1 (0.28)	69.8 (0.28)	54.2 (0.23)	

[1]Means with different superscript letter in the same column are significantly different ($p \leq 0.05$)

[2]Standard deviation of two replicate experiments

[3]Protein Digestibility Corrected Amino Acid Score calculated as protein digestibility x amino acid score for 3-10 year old children (based on lysine) using the WHO/FAO/UNU Expert Consultation (2007), using lysine values in Table III

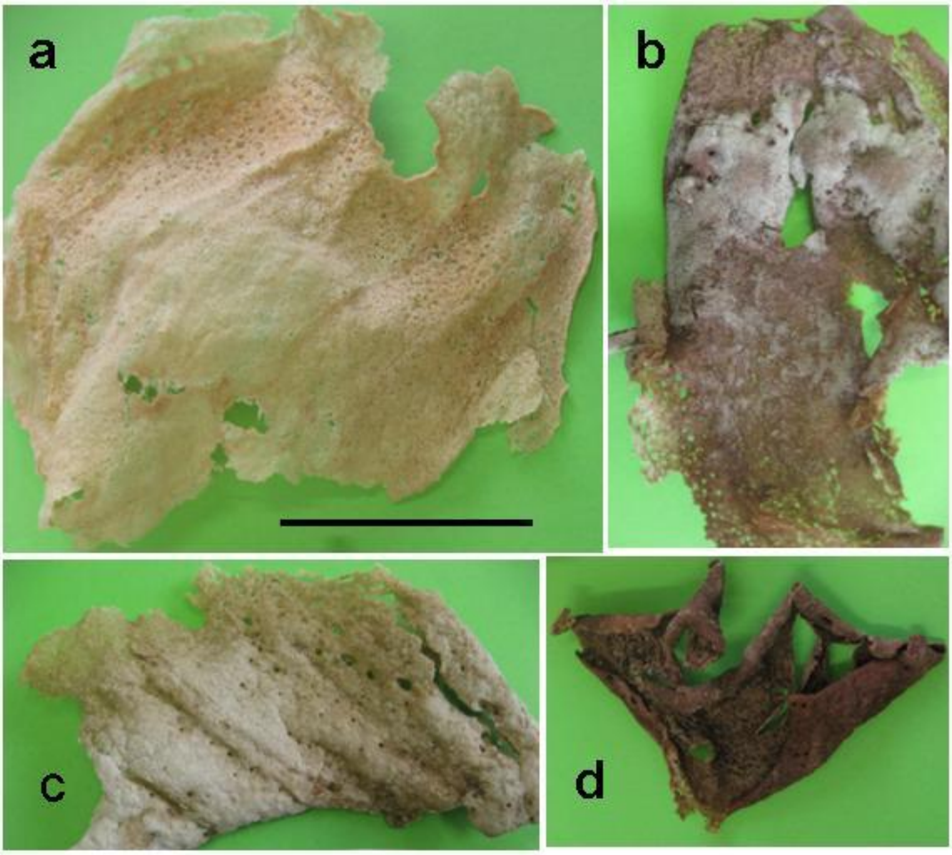
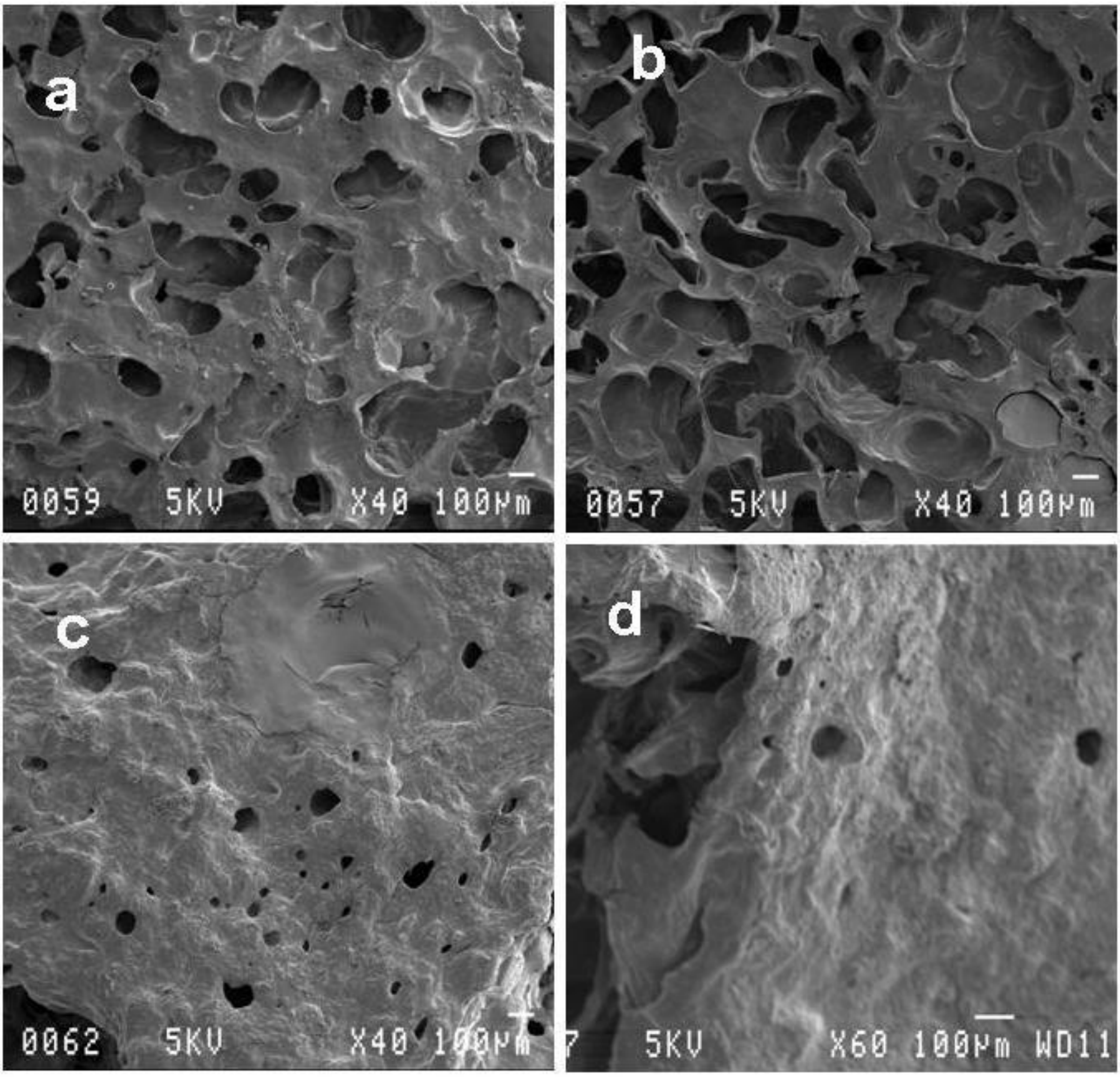


Figure 1


a
0059 5KV X40 100µm
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0057 5KV X40 100µm
c
0062 5KV X40 100µm
d
5KV X60 100µm WD11

Figure 2