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PII: S0924-2244(17)30110-3

DOI: 10.1016/j.tifs.2017.05.012

Reference: TIFS 2014

To appear in: *Trends in Food Science & Technology*

Received Date: 2 March 2017

Revised Date: 6 April 2017

Accepted Date: 22 May 2017

Please cite this article as: Annor, G.A., Tyl, C., Marcone, M., Ragaee, S., , Why do millets have slower starch and protein digestibility than other cereals?, *Trends in Food Science & Technology* (2017), doi: 10.1016/j.tifs.2017.05.012.

Why do millets have slower starch and protein digestibility than other cereals?

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Abstract

Background

Millet and millet based products are known to have lower starch and protein digestibility rates when compared to other cereals. Understanding, why millets are slowly digestible and how they are affected by processing is important in maintaining their lower starch and protein digestibilities when processed.

Scope and Approach

This review explores the factors that contribute to the lower starch and protein digestibilities of millets and their underlying mechanisms. The effects of different processing methods on millet starch and protein digestibility rates are also discussed.

Key Findings and Conclusions

Factors such as starch structural characteristics, starch-protein-lipid interactions, fiber and polyphenols present in millets play significant roles in their hypoglycemic property. The amount and type of fatty acids present in millets significantly affect their starch hydrolysis rates. Unsaturated fatty acids are more effective in reducing starch hydrolysis rates than their saturated counterparts. In-vitro protein digestibility (IVPD) of millets appears to be mostly affected by polyphenols and processing. Simple processing steps such as decortication, germination and fermentation which are mostly applied to millets significantly affect both starch digestibility and IVPD of millets. The adoption of processes that maintain low starch hydrolysis rates and increases protein digestibility in millets should be encouraged.

Keywords: glycemic index, millet, starch digestibility, protein digestibility, processing

Dedication

This publication is dedicated to the memory of Koushik Seetharaman (1966-2014)

1. Introduction

The hardy nature of millets, their inherent biodiversity and the relatively lower agricultural inputs needed for their cultivation make millet a crop of choice for many farmers in India, Africa and China. In areas where they are cultivated, millets provide the much-needed energy and to some extent the protein requirements of these populations. With the first reports of the cultivation of millets dating back to about 5,550 BC (Crawford, 2006), millets arguably are the first grains cultivated by man. In terms of production, India is the world's foremost producer of millets in the world, followed by China. Per the Food and Agriculture Organization (FAO) of the United Nations, in 2014, 12.49, 0.31, 14.83, and 0.79 million tons of millet were produced in Africa, the Americas, Asia and Europe respectively (FAOSTATS, 2016). Pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*) and finger millet (*Eleusine coracana*) are the major species. Figure 1 shows pictures of some millet types. These different types of millets are cultivated in different parts of the world. While China cultivates mainly foxtail millets, pearl millets are cultivated in India, Nepal and Africa (Obilana, 2003). Proso millets on the other hand are mainly cultivated in North America (FAO, 1995). Nutritionally, millets contain as much as 60–70% dietary carbohydrates, 6–19% protein, 1.5–5% fat, 12–20% dietary fiber, 2–4% minerals, and several phytochemicals (Hadimani et al., 1995). The nutritional quality and potential health benefits of millet have been extensively reviewed by Saleh et al., (2013). Apart from the fact that millets do not contain gluten, making them suitable for people with coeliac disease, millets can also be exploited in the management of type II diabetes due to their hypoglycemic property, as reported by several studies on millets and millet based foods (Geetha & Easwaran, 1990; Anju & Sarita, 2010; Shukla & Srivastava; 2014, Ugare et al., 2014; Ren et al., 2016). The other side of the coin is protein digestibility, which is lower in

millets compared to many other grains (Mertz et al., 1984). This is particularly concerning given the fact that millet forms the basis for staple foods in many developing countries, which would make it one of the primary protein sources. In addition, processing methods that involve hydrothermal treatments may lower the protein digestibility of certain millet types (Gulati et al., 2017).

Understanding the factors that contribute to millets' hypoglycemic property and protein digestibility is important, as it will allow for the development and processing of healthier millet-based food products. This paper consists of three sections. The first discusses the factors that contribute or may contribute the hypoglycemic property of millet and millet-based products. In the second part, *in vitro* protein digestibility (IVPD) will be discussed. The final part will review the role of treatments/processes for improving and maintaining the nutritional benefits of millets in terms of starch and protein digestibility.

2. Hypoglycemic property of millet

One of the early accounts on the hypoglycemic property of millet can be traced to 1957 when Ramananthan and Gopalak fed finger millet and four other cereals to six normal men between the ages of 25-40 years and a man and woman who had glycosuria. They reported a significantly lower increase in blood glucose of the individuals fed with finger millet when compared to the cereals. Interestingly, they also reported that starch from rice and finger millet fed to these individuals gave increases in blood glucose levels that were similar. This study thus showed that the characteristics of millet starch on its own may not be a factor contributing to the hypoglycemic property of millets but in the presence of lipids, proteins and phenolic compounds may be the contributing factors. Pathak, Srivastava, & Grover (2000) fed five normal females between the ages of 22-25 year and five non-insulin-dependent diabetes males between the ages

of 57 to 70 years with Indian traditional snacks made from foxtail millet, barnyard millet, legumes and fenugreek seeds and observed significantly lower blood glucose levels compared to when subjects were administered with glucose. The snacks used were Dhokla (55% foxtail millet and barnyard millet, 35% legumes and 10% fenugreek seeds), Uppuma (60 % foxtail and barnyard millet, 20% legumes and 10% fenugreek seeds) and Laddu (50% amaranth and foxtail millet, 25% legumes and 25% fenugreek paste). The lowest glycemic index was observed for uppuma, followed by laddu and then dhokla in both normal and diabetic subjects. Even though this observed trend seems to be consistent with the amount of legumes added, Uppuma, which had the lowest glycemic index, contained the most millet. Shobana et al., (2007) after administering food formulations prepared from wheat, decorticated finger millet, popped and expanded rice and blended with legumes to five normal male and female subjects between the ages of 25 to 52 years observed significantly lower rates of digestion of the wheat and millet based food formulations compared to the rice based food formulations. They also reported that the wheat based formulations were digested significantly slower than the formulations made from millet. They attributed this observation to gluten-starch interactions as suggested by Jenkins et al. (1987). The glycemic index of refined wheat noodles incorporated with 30% finger millet was significantly lower (45.1) than refined wheat noodles (62.6). These noodles were fed to ten normal female subjects between the ages of 24 to 27 (Shukla and Srivastava 2014). After feeding thirteen healthy females between the ages of 22 to 27 years with refined wheat flour biscuits substituted with 45% foxtail millets and barnyard millets, Anju & Sarita (2010) reported glycemic index values of 50.8 and 68 for biscuits prepared from foxtail millets and barnyard millets respectively. Several other studies (Thathola et al., 2011; Neelam et al., 2013; Ugare et al., 2014; Patil et al., 2015) also indicated the hypoglycemic properties of millet and millet based

products. It is important to note that all these aforementioned studies involved the use of humans. Even though it may be argued that the number of subjects used in these studies in most cases is small, they still to some extent indicate the hypoglycemic property of millet and millet based foods.

Table 1 outlines the expected glycemic index (eGI), rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) of various millet based products determined with in-vitro starch digestibility methods. Food products from the different types of millets and foods processed differently had different starch hydrolysis parameters. Millet porridges generally had higher eGI compared to the other food products. Millet couscous had the lowest eGI when compared to the other products, followed by millet muffins. These results confirm the important role the food matrix plays in determining the glycemic index of foods (Singh et al., 2010).

2.1 Factors contributing to the hypoglycemic properties of millet and millet based foods

The presence of proteins, lipids, α-amylase inhibitors, antinutrients, and starch characteristics affect starch hydrolysis kinetics (Singh et al., 2010). Table 2 summarizes the effects of these factors on starch hydrolysis kinetics and the mechanisms involved.

Effects of starch characteristics on millet starch hydrolysis

Starch is the major component in millet and typically ranges from 56-65% of the total seed weight though up to about 80% starch has been reported for proso millet (Casey & Lorenz, 1977). Normal millet starches have amylose contents ranging from 20-32% (Hoover et al., 1996). Amylose content of up to 34% was reported for foxtail, finger, proso and pearl millets (Annor et al., 2014). The amylose contents reported in some millet species may be linked to their hypoglycemic properties. The inverse relationship between amylose and glycemic index is

154 known, with studies showing that the addition of high amylose starch to diets modulates
155 glycemic response (Hoebler et al.,1999). The nature of millet starch architecture has also been
156 mentioned as one of the reasons for their hypoglycemic property. Millets generally have
157 polygonal and a few spherical starch granules as shown in Figure 2. Finger millet however has
158 only polygonal starch granules. The granules also appear to have pores or pinholes on the
159 polygonal starch granules. Again, these pinholes are absent on the granules of finger millet. The
160 presence of these pores on the millet starch granules facilitate the entry of starch hydrolyzing
161 enzymes into the starch granules (Tester et al., 2006; Kaur et al., 2007). The starch hydrolysis
162 index of these millet types is in the order finger millet < pearl millet < Proso < foxtail.
163 Interestingly, finger millet which had no pores on its granules had the least enzymatic starch
164 hydrolysis index. The pinholes become more prominent on the millet starch granules when they
165 are hydrolyzed as shown in Figure 3. It can be observed that the starch hydrolyzing enzymes
166 hydrolyzes the Kodo millet starch from the inside out.

167 Other factors such as the molecular weights and degree of crystallinity of starches have been
168 reported to also affect the enzymatic starch hydrolysis rates of millets. The molecular weight and
169 degree of crystallinity of residues from finger millet starch hydrolyzed with an enzyme mixture
170 of α-amylase, β-amylase and amyloglucosidase have been reported to be significantly higher
171 than those of rice, suggesting that finger millet starch was much more resistant to enzymatic
172 hydrolysis than rice starch (Mohan et al., 2005). The resistance of finger millet starch to
173 digestive enzymes could be due its rigid starch granule architecture compared to rice. The in-
174 vitro starch hydrolysis of various starches by α-amylase in order of decreasing resistance was
175 reported as follows; finger millet > potato > chickpea > rice > sorghum > green gram > wheat >
176 tapioca > waxy rice > maize (Singh et al., 2006).

177 ***Effects of lipids on millet starch hydrolysis***

178 Starch-lipid complexes influence the susceptibility of starch to starch degrading enzymes,
179 resulting in slower digestion (Hasjim et al., 2010; Ai et al., 2013; Kawai et al., 2012; Annor, et
180 al., 2013; Annor et al., 2015). The degree of enzymatic hydrolysis of amylose-lipid complexed
181 superstructures and the degree of organization of their helices into larger domains of ordered
182 chains in aggregated structures have been reported to be inversely related (Seneviratne &
183 Biliaderis, 1991). The enzymatic hydrolysis of amylose-lipid complexes usually involves an
184 initial step of rapid hydrolysis of the amorphous areas of the complex, and then a slower
185 degradation of the amylose inclusion complex (Godet et al., 1993; Jane et al., 1994). These
186 amylose-lipid complexes are eventually hydrolyzed with time or with the addition of excess
187 enzymes, even though there is a reduction in the rate of hydrolysis of the lipid-amylose
188 complexes. The rate of in-vitro hydrolysis of potato amylose complexed with lipids to α-amylose
189 was significantly reduced, although the addition of excess enzymes resulted in the complete
190 hydrolyses of the complex after 3 hours (Holm et al., 1983). The main fatty acids present in
191 millets are palmitic, oleic and linoleic acids. These main fatty acids constitute about 85% of the
192 total fatty acids in millets (Bora, 2014). Complexation with oleic and lauric acid has been
193 reported to be very effective in reducing starch hydrolysis rates, whilst enzymatic hydrolysis
194 rates of starch-linoleic acid complexes are not significantly lower than that of the native starch,
195 due to the instability of the complex (Kawai et al., 2012). The effects of corn oil, soy lecithin,
196 palmitic acid, stearic acid, oleic acid, and linoleic acid on the enzymatic hydrolysis of normal
197 corn, waxy corn, tapioca and high-amylose corn starches have been investigated. The study
198 reported significant decreases in starch-hydrolysis rates of all the starches except waxy corn
199 when cooked with the lipids. Lipids with different degrees of unsaturation showed different

effects on starch-hydrolysis rates of starch-lipid complexes (Ai et al., 2012). In addition to significant reductions in starch hydrolysis rates when lipids were complexed with rice starch, it has also been reported that long-chain saturated emulsifiers reduced starch digestibility more than short-chain saturated and unsaturated emulsifiers (Guraya et al., 1997).

Effects of proteins on millets starch hydrolysis

The effects of protein on the starch hydrolysis rates are however related more to their ability to form a physical barrier between the starches and their degrading enzymes. Protein fractions such as albumins, globulins and glutenins, combine protein bodies into a matrix surrounding starch granules, which acts as a barrier to amylases (Hamaker & Bugusu, 2003). A decrease in glycemic response due to the interaction of starches with proteins was observed after studies on the effects of starch-protein interactions on the starch digestibility of wheat were done (Jenkins et al., 1987). Annor et al., (2013) also reported an increase in glycemic response with the removal of proteins from Kodo millet.

Effects of Polyphenols on millet starch hydrolysis

Known for their health promoting properties, polyphenols are abundant in millets (Taylor & Duodu, 2015). These polyphenols are a diverse class of compounds, and mainly found in plant seed coats. The types and composition of polyphenols vary in different varieties of millets (Chandrasekara & Shahidi, 2012). The main polyphenols present in cereals are phenolic acids, with flavonoids present in smaller quantities (Subba & Muralikrishna, 2002). The phenolic and flavonoid contents of some millet varieties in terms of their soluble and bound phenolics fractions have been reported (Chandrasekara & Shahidi, 2010). Soluble phenolic contents in ferulic acid equivalents of 411-610 mg/100 g, 168 mg/100 g, 140 mg/100 g were reported for

finger, pearl and proso millets respectively. Bound phenolic values of 62-74 mg/100 g, 178 mg/ 100 g and 43 mg/100 g were also reported for finger, pearl and proso millets respectively. Reported as catechin equivalents in soluble phenolic fraction, total flavonoid contents of 203-228 mg/100 g, 49 mg/100 g and 140 mg/100 g for finger, pearl and proso millets were reported respectively, whilst values of 10-30 mg/100 g, 8 mg/100 g and 13 mg/100 g were reported in the bound fraction. It should be noted that while all millet varieties contain phenolics, finger millet has been reported to contain higher levels of flavonoids (Taylor et al., 2015; Taylor et al., 2014). Condensed tannins are usually found in (brown) pigmented (Devi et al., 2014), but not in white varieties (Siwela et al., 2007). Polyphenols in millets are known to have health promoting properties, such as reduction and/or prevention of oxidative stress, anti-cancer, anti-diabetic, anti-inflammatory, and cardiovascular disease prevention and antihypertensive (Taylor et al., 2015). In addition, millet polyphenols may be exploited in the management of type 2 diabetes due to their inhibitory effects on starch digestive enzymes. Inhibitory effects of different classes of phenolic compounds on α-glucosidase and pancreatic amylase have been reported (Tadera et al., 2006; Kim, Hyun, & Kim, 2011). Extracts from finger millet seed coat containing phenolics such as protocatechuic acid, gentisic acid, caffeic acid, vanillic acid, and ferulic acid, showed strong inhibitory effects on α-glucosidase and pancreatic α-amylase, resulting in reduced postprandial hyperglycemia (Shobana et al., 2009). However, the contributions of individual phenolics and tannins to this inhibition, as well as possible synergistic effects, are not fully understood. After investigating the effects of phenolic extracts from finger millet on rat intestinal α-glucosidase and pancreatic α-amylase, millet seed coat phenolics were observed to inhibit both pancreatic amylase and α-glucosidase in a dose dependent manner, and the velocity of reaction catalyzed by α-glucosidase and amylase was inversely proportional to the concentration of

phenolic compounds in the reaction mixture. In another study, a dose response effect of the aqueous extract from foxtail millet on the fasting blood glucose up to a dose of 300 mg/kg body weight in diabetic rats was reported (Sireesha et al., 2011). About 14-26% α-amylase inhibition of methanol extracts from raw and processed finger millet has also been reported (Kunyanga et al., 2012). It has been suggested that polyphenols, especially flavonoids, inhibit α-glucosidase and pancreatic amylase non-competitively and in some cases by competitive inhibition (Kim et al., 2011). The mode of inhibition also depends on the substrate specificity of the enzymes (Devi et al., 2014). The inhibitory effects of millet polyphenols on α-glucosidase and pancreatic amylase have been reported to be similar to drugs such as acarbose, miglitol and voglibose (Bailey, 2003). Amount or composition millets polyphenols may be affected by processes such as malting (Subba & Muralikrishna, 2012), fermentation (El Hag et al., 2002), germination (Opoku, Ohenhen, & Ejiofor, 1981), thermal treatment and decortication (Shobana & Malleshi, 2007). Any effects on the millet polyphenol contents or composition may result in the loss of their inhibitory effects on starch digestive enzymes. Further research is needed to determine if processing treatments negatively affect inhibition of starch digestibility.

Effects of Fiber on millet starch hydrolysis

Whole-grain millets are important sources of fiber and contain considerably more fiber than many other cereals. Dietary fiber contents of between 7-21% have been reported (Devi et al., 2014) with about 2.5% and 19.5% soluble and insoluble fiber contents respectively (Shobana & Malleshi, 2007). Barnyard, kodo, foxtail and little millets have been reported to have insoluble fiber content of 18-30% and soluble fiber contents of 0.6-2% (Geervani & Eggum, 1989). An increase in the relative proportion of soluble fiber content of finger millet was observed after decortication, though a decrease in the total dietary fiber (to levels <4%) was reported. The

increase in soluble fiber content has special nutritional significance due to its physiological advantages in terms of hypoglycemic and hypocholesterolemic characteristics. Furthermore, the formation of resistant starch in millet during processing contributes to dietary fiber content, which complemented the health benefits of finger millet (Shobana & Malleshi, 2007). A synergy between phenolics and dietary fiber may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of type 2 diabetes (Saito et al., 1998; Toeller, 1998). The viscous property of some soluble dietary reduces the postprandial blood glucose level concentrations in humans (Onyango et al., 2004).

3. In-vitro Protein digestibility of millets

In contrast to starch digestibility, less work has been performed to evaluate millet protein digestibility and mechanisms that lower or enhance it. Plant storage proteins, such as those in cereal grains or legumes, often have lower digestibility than most animal proteins (Becker & Yu, 2013). This can be the result of various factors, such as the inhibition of digestive enzymes by protease inhibitors or tannins, low protein solubility, protein organization into protein bodies, and lower enzyme accessibility due to rigid cell walls and/or seed coats (Becker & Yu, 2013). In addition, disulfide-mediated protein cross-linking has been shown to occur in sorghum upon heating and to lower is protein digestibility (Duodu et al., 2003). The digestibility of cooked millet proteins is lower than for some other cereals such as wheat or corn (Mertz et al., 1984), and has, in some cases, been found to be higher after cooking (Ravindran, 1992; Pawar & Machewad, 2006) or only slightly lowered (Ejecta et al., 1987). Raw finger, foxtail and proso millet were reported to have IVPD levels of 72.3, 77.1 and 71.3%, which increased to 85.5, 91.6, and 88.6% after cooking (Ravindran, 1992). Another study reported an increase in IVPD when cooking was combined with soaking or dehulling in foxtail millet, from 62.3% in untreated

291 foxtail millet to 83% after dehullilng, soaking and cooking (Pawar & Machewad, 2006). Soaking
292 alone only changed the value to 76%, while all treatments that included dehulling or cooking
293 raised values to > 80%.

294 However, other work has shown a decrease in proso millet protein digestibility after
295 hydrothermal treatments (Gulati et al., 2017). A similar result has been reported for sorghum:
296 sorghum proteins experience a structural change during heating that lead to lower digestibility,
297 and have been studied more extensively in this regard (Hamaker et al., 1987; Elkin et al., 1996;
298 Duodu et al., 2002; El Hag et al., 2002). Sorghum IVPD is not necessarily directly related to
299 polyphenol content (Elkin et al, 1996; Duodu et al., 2002), but markedly improved in the
300 presence of reducing agents (Hamaker et al., 1987). However, the amount of proteins extractable
301 with aqueous alcohol containing a reducing agent was shown to be six times higher in sorghum
302 than in pearl millet (Ejecta et al., 1987). Recently, it was shown that the IVPD loss caused by
303 cooking proso millet was not reverted by addition of reducing agent, but by chaotropes,
304 indicating that hydrophobic interactions among proteins are responsible for the drop in proso
305 IVPD (Gulati et al., 2017).

306 As the addition of chaotropes such as urea is not a feasible strategy for food production, more
307 research needs to be undertaken to investigate appropriate processing methods for increasing
308 millet protein digestibility in general, and proso millet protein digestibility in particular.

309 In addition, the presence of tannins, i.e. polyphenols that bind to proteins, has been shown to
310 reduce millet IVPD in some work (Geetha et al., 1977). Tannins levels in whole grain millets can
311 be as high as 0.87% (d.b.) for Kodo millet, while French, Italian, Barnyard and little millet had
312 levels between 0.21-0.36% (Geervani & Eggum, 1989), and proso millet <0.2% (Lorenz, 1983).
313 Pigmented millets generally contain higher tannin levels (Geetha et al., 1977; Lorenz, 1983).

Whole finger millet was estimated to contain between 0.03 and 3.47% tannins, and tannin levels above 2% markedly reduced the IVPD, from 80-90% for low-tannin varieties to <55% for high tannin varieties (Geetha et al., 1977). Interestingly, some millet varieties with intermediate tannin levels still had IVPD > 80%, indicating a possible threshold above which tannins exert this effect. Sieving flour decreased the phenolic content of finger millet, which coincided with higher IVPD (Oghbaei & Prakash, 2012). In work performed on Italian millet, tannin levels were < 0.1% and did not appear to interfere with IVPD, as it was > 90 if pepsin was used as the digestive enzyme (Monteiro et al., 1988). In contrast, IVPD with trypsin was much lower (<37%). Other studies also indicate that tannins are not solely responsible for low millet IVPD. The IVPDs of a red and white finger millet variety were similarly low at 61.4 and 65.7% (Antony & Chandra, 1999). However, while the red finger millet contained 0.74% tannins, no tannins were detected in the white variety. In pearl millet, the IVPD was significantly lower in a variety with higher polyphenol levels (El Hag et al., 2002). However, while the polyphenol contents were 444 and 304 mg/100g, the difference in IVPDs was relatively small (70.4 and 72.7%). Table 3 states the IVPD of millet varieties at different processing stages, and Table 4 summarizes the proposed mechanisms.

4. Effect of processing on millet starch and protein digestibility

The effects of processing on chemical constituents of millets have been widely investigated (Devi et al., 2014; Taylor & Duodu, 2015). As would be expected, processing has also an influence on starch and protein digestibility and this is of great interest in view of potential health benefits provided by the finished product (Singh et al., 2010). Therefore, the effect of various food-processing methods on digestibility in millets and millet-products has become an important area of research. The effects of four main processes, i.e. decortication, germination, fermentation

and thermal processing, on millet-based food and beverage digestibility are discussed in the following section (Table 4).

4.1 Decortication

The first step of dry milling is termed decortication or dehulling whereby the outer layers of the grain and the pericarp are removed. This step fractionates the seed caryopsis into its three basic components (germ, pericarp and endosperm). By removing the germ and pericarp, decortication reduces anti-nutrients, but also fiber, lipid, minerals and phenolic acids (Lestienne et al., 2007; Shobana & Malleshie, 2007). Annor et al., (2013) showed how the removal of lipid, protein, or both, increases the in vitro starch digestibility of kodo millet. As most lipids and proteins are concentrated in the millet germ and pericarp, the removal of the outer layers can be expected to lead to an increase in starch digestibility. Kodo millet showed a substantial increase in eGI by 42% after decortication while other millet types showed an increase less than 6%. The increase in the eGI of decorticated millets may be due to reductions in insoluble dietary fiber, phenolics and lipid contents (Bora, 2014).

Decortication is found to increase IVPD of pearl millet (El Hag et al., 2002), likely due to the decrease in anti-nutrients that reduce IVPD (Hulse et al.,1980). In Foxtail millet, a higher IVPD was observed after dehulling, a combination of dehulling and soaking or cooking treatments, coinciding with a decline in phenolics content (Pawar & Machewad 2006). While the combination treatments led to the highest IVPD, dehulling seemed to be the biggest contributor to the observed increase.

4.2 Germination

The terms “sprouting”, “malting” and “germination” are used interchangeably to refer to the process of soaking grains in water until saturated, and then germinating them under controlled conditions. Sprouted millet flour is often added as an ingredient in porridge making. This imparts a sweeter taste by action of the β-amylase (i.e. production of maltose and thus increase of sweetness) to the porridge and also reduces its viscosity due to starch hydrolysis by α-amylase. Moreover, malted millet is used in the production of opaque beer in many countries in sub-Saharan Africa and increasingly in lager beer and malt beverages across the world (Taylor & Duodu, 2015).

During germination, hydrolytic enzymes lead to biochemical changes, structural modification and the synthesis of new compounds, some of which have high bioactivity and can increase the nutritional value and stability of the grains. Comprehensive reviews of the effects of germination on the nutrient composition of cereals have been published elsewhere (Mbithi-Mwikya et al., 2000; Hübner & Arendt, 2013). Beside the increase in B vitamins and the improvement of mineral bioavailability and essential amino acid composition, it has been found that germination of pearl millet improved the in vitro protein (Mbithi-Mwikya et al., 2000; Hejazi & Orsat, 2016) and starch (Mbithi-Mwikya et al., 2000; Sehgal & Kawatra, 2001) digestibility. The magnitude of changes varies among studies, likely due to differences in soaking practices, germination duration and temperature, and millet species.

Various mechanisms have been proposed to account for these effects of germination on starch and protein digestibility. Perhaps most importantly, anti-nutrients such as phytic acid, tannins and other phenolics, as well as amylase and protease inhibitors, are reduced during germination

(Sehgal and Kawatra, 2001; Deshpande & Cheryan, 1984; Thompson & Yoon, 1984). Increase in protein digestibility may be also attributed to the degradation of storage protein commonly occurring during sprouting and may be more easily available to pepsin hydrolysis (Mbithi-Mwikya et al., 2000; Sehgal & Kawatra, 2001).

Lipid hydrolysis during germination (Choudhury et al., 2011) should also be taken into consideration, in view of the results of Annor et al. (2013), who found an increase in starch digestibility when samples were defatted.

Germination followed by fermentation appeared to be more effective in improving protein and starch digestibility than germination alone (Khetarpaul & Chauhan, 1991). Therefore, combinations of germination and fermentation offer unique nutritional approaches for making starch and protein in pearl millet more digestible.

4.3 Fermentation

Many traditional millet foods and beverages, especially in Africa, are fermented either by lactic acid bacteria alone or in combination with yeasts. The processing and the characteristics of these fermented products, which comprise flatbreads, doughs and dumplings, porridges, gruels, non-alcoholic beverages, opaque and cloudy beers, are reported elsewhere (Hübner & Arendt, 2013). Traditionally, the fermentation may be spontaneous (i.e. performed by intrinsic bacteria) or performed by selected starter cultures. Another possibility is to use a portion of the fermented food product or intermediate, such as dough, as inoculum for the next fermentation (Hübner & Arendt, 2013).

Raw pearl millet IVPD was reported as 68-76% (depending on cultivar) and improved to 82-87% after being fermented in dough form for up to 14 hours (El Hag et al., 2002; Ali et al., 2003).

These findings are supported by other work where fermentation was shown to have a positive effect on pearl millet, which improved from 51% IVPD to 80-90%, depending on the bacteria/yeast combination employed (Khetarpaul & Chauhan, 1990). Lactic fermentation brings about several nutritional improvements in the grain, including the improvement in protein and starch digestibility (Khetarpaul & Chauhan, 1990; Shama & Kapoor, 1996; Elyas et al., 2002; El Hag et al., 2002; Ali et al., 2003). Interestingly, the combination that led to the highest increase in IVPD, i.e. *Saccharomyces cerevisiae* and *Lactobacillus fermentum*, caused the least increase of *in vitro* starch digestibility, and vice versa (Khetarpaul & Chauhan, 1990). Even higher IVPD improvements could however be seen when the fermentation was combined with soaking, debranning or germination, with the latter leading to highest IVPD (Sharma & Kapoor, 1990).

Enhanced proteolytic activity during fermentation is generally associated with improved protein digestibility. This phenomenon could be attributed to the partial degradation of complex storage proteins to more simple and soluble products and to the degradation of tannins, polyphenols and phytic acid by microbial enzymes.

A combination of enzymatic pretreatment - by cellulase and hemicellulases - and directed fermentation, may provide the double advantage of accelerating the fermentation and enhancing protein availability in finger millet. The enhanced protein digestibility has been attributed to the release of protein from the seed by the enzymatic breakdown of dietary fibers, with concomitant reductions in phytate and tannins (Antony & Chandra, 1999).

Possible starch hydrolysis by microflora may account for improvement in the in vitro starch digestibility during millet fermentation. The decrease in phytic acid content during fermentation may also account for improved starch digestibility as phytic acid had a significant negative correlation with in vitro starch digestibility.

4.4 Thermal treatments

Food uses of millet are usually traditional, and processing methods may involve boiling, pressure-cooking, or roasting. Millet consumption in the Western hemisphere may be promoted by the introduction of millet-based foods more familiar to Western consumers, such as bread or pasta.

Compared to cooking in boiling water, either roasting or baking promoted a decrease in starch digestibility, likely due to the limited degree of starch gelatinization induced by the dry thermal processing (Roopa & Premavalli, 2008). On the other hand, by promoting an intense starch gelatinization, either puffing of grains or pressure-cooking of the flour improved finger millet starch digestibility (Roopa & Premavalli, 2008).

Cooking improved IVPD of foxtail, finger and common millet (Ravindran, 1992). Various mechanisms have been proposed to explain the effect of cooking on IVPD: (1) low protein digestibility in uncooked materials is largely due to the presence of heat-labile antiproteinase factors (Ravindran, 1992); (2) protein denaturation and/or decreasing resistance of protein to enzyme attack (Sathe et al., 1982); (3) during cooking, proteins may interact with non-protein components or other proteins, thereby affecting their digestibility (Duodu et al., 2003). In contrast to the findings of Ravindran (1992), more recently Pushparaj & Urooj (2011) showed that wet heat treatments (i.e. boiling, pressure-cooking) did not improve the protein digestibility of the millet. Differences in millet varieties might account for differences in results. On the other hand, roasting markedly improved IVPD of pearl millet, suggesting that dry heat treatment is more effective in this regard than wet heat treatment (Pushparaj & Urooj, 2014).

Parboiling is a hydrothermal treatment widely used in rice technology, wherein the main steps consist of soaking, steaming, and drying. Studies on rice showed how starch digestibility increased owing to complete starch gelatinization or decreased owing to subsequent retrogradation upon cooling after parboiling, depending on the severity of processing and on the type or variety of rice used (Larsen et al., 2000).

An increase in carbohydrate digestibility was found in parboiled finger millet (Dharmaraj & Malleshi, 2011). On the contrary, Bora (2014) stated that the RDS values of the products from parboiled millets were significantly lower than the native millets while the SDS values were not significantly different. As expected, parboiling led to a significant increase (in the range of 4-17%, depending on variety) in RS and to a decrease in eGI of the products prepared from parboiled millets than the products from native millets (Bora, 2014). The formation of amylose-lipid complexes, and amylose and amylopectin retrogradation might have occurred during parboiling, which may have reduced the eGI and RDS in the products (Bora, 2014).

As for IVPD, Dharmaraj & Malleshi (2011) showed an increase IVPD from 79 to 98% for parboiled decorticated finger millet, mostly due to the increase in extractability of globulins and prolamin-like proteins. When parboiled millet was processed to porridge or cous-cous, IVPD decreased, compared to the products prepared from native millet (Bora, 2014). The reduction in protein extractability has been mainly attributed to the formation of di-sulphide cross-links and changes in protein secondary structure (Duodu et al., 2003). Parboiling may induce these changes to a higher extent, resulting in lower IVPD. The increase in free and bound phenolic content after parboiling (Bora, 2014) might also have reduced IVPD. The oxidation of phenolic compounds may lead to formation of peroxides which are highly reactive species and may oxidize amino acid residues and polymerize proteins (Duodu et al., 2003).

5. Conclusion

The hypoglycemic nature of millets can be related not only to the nature or characteristics of their starches, but also to other factors, such the presence of proteins and lipids, which interact with starch to reduce the rate at which glucose is released by α-glucosidases and pancreatic α-amylase. Not only are millet starches more resistant to starch digestive enzymes, the polyphenols present in millets also inhibit α-glucosidases and pancreatic α-amylase. The presence of soluble fibers presents in millets may also play a role in their hypoglycemic property. Polyphenols, especially tannins, also negatively affect protein digestibility. Processing methods that reduce their content can be employed to increase protein utilization, which is especially important for areas where millets present a staple food.

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Table 1. Starch hydrolysis indices for different varieties of millet processed differently

Type of Millet	Type of food	GI	RDS	SDS	RS	References
Foxtail	Cooked	54.3	36.9	38.3	24.9	Ren et al., 2016
	Porridge	60.7	50.7	40.5	8.8	Ren et al., 2016
	Steamed bread	60.4	46.3	44.9	8.8	Ren et al., 2016
	Pancake	59.4	39.1	45.0	15.9	Ren et al., 2016
	Porridge	69.4	38.5	39.4	22.1	Annor et al., 2015
Proso	Muffin	56.0	29.5	32.3	38.2	McSweeney et al., 2017
	Extruded snack	64.7	35.2	37.7	27.1	McSweeney et al., 2017
	Porridge	53.1	30.8	23.8	45.4	McSweeney et al., 2017
	Couscous	50.2	27.6	25.6	46.8	McSweeney et al., 2017
Finger	Roti	-	29.5	3.3	4.5	Aarathi et al., 2003
	Porridge	65.4	34.2	41.5	24.3	Annor et al., 2015
Kodo	Porridge	49.4	31.2	15.87	35.91	Annor et al., 2013
Proso	Porridge	69.3	37.2	42.6	20.2	Annor et al., 2015
Pearl	Porridge	67.6	35.6	42.9	21.5	Annor et al., 2015

Table 2: Factors affecting enzymatic starch hydrolysis and their mechanisms

Component	Effect on Starch hydrolysis	Mechanism	References
Starch morphology	Starches with large granules have lower enzymatic starch hydrolysis rates and vice versa for smaller granules.	Smaller starch granules have larger specific surface area and hence increase the extent of enzyme binding.	Lindeboom et al., 2004; Singh and Singh, 2006; Singh et al., 2007; Singh et al., 2010
	Starches with pores on their surfaces tend to have higher enzymatic starch hydrolysis rates	The presence of pores on the surface of starch granules facilitate the penetration of enzymes to the interior of the granules resulting in the endocorrosion of the starch granules	Tester et al., 2006; Kaur et al., 2007
Amylose/amylopectin ratio	Starches with higher amylose tend to have lower enzymatic starch hydrolysis rates	Amylose has a much lower surface area per molecule when compared to amylopectin resulting in lower enzymatic biding. Amylose chain are also more susceptible to retrogradation which results in the conformation of the chains and thus resulting in a much lower rate of enzymatic attack	Thorn et al., 1983; Hoover and Zhou 2003; Hu et al., 2004
Lipids	The presence of lipids results in a decrease a lower enzymatic starch hydrolysis rates	Lipids result in the formation of starch -lipid complexes, especially with amylose. These complexes result in changes in the conformation of starch chains and	Hasjim et al., 2010; Ai et al., 2012; Kawai et al., 2012; Annor et al., 2013; Annor et al., 2015

		results in their slower digestion by starch hydrolyzing enzymes.	
Protein	Presence of proteins generally results in the reduction of enzymatic starch hydrolysis rates.	The presence of proteins such as albumin, globulins and glutenins results in the formation of a matrix around the starch granules that acts as a barrier towards starch hydrolytic enzymes	Rooney and Pflugfelder, 1986; Jenkins et al., 1987; Hamaker and Bugusu, 2003; Annor et al., 2013
Fiber	Presence of fiber results in the reduction of enzymatic starch hydrolysis rates	Fibers reduce enzymatic starch hydrolysis rates by increasing the viscosity of the digestion mixture.	Jenkins et al., 1980; Singh et al., 2010.
Antinutritional factors/Phenolic compounds	The presence of antinutritional factors such as phenolic compound and tannins results in a reduction in enzymatic starch hydrolysis rates	Antinutritional factors interact with amylase proteins and thus inhibit starch hydrolytic enzymes	Subba and Muralikrishna, 2002; McDougall et al., 2005; Chandrasekara and Shahidi, 2012; Taylor et al., 2015

Table 3: Percent *in vitro* protein digestibility of different millet varieties at different processing stages

Variety	Raw	Cooked	Soaked	Dehulled	Sieved	Germinated	Fermented	Parboiled	After combination treatments
Finger	67.4-74.7[a] 55.4-85.1[b] 38.8[c] 61.4 (red); 65.7 (white)[d] 33.9[e] 74[f] 79.0[g]	84.7-86.3[a]		91.0-93.7[b]	43.9[c]	55.4[e] up to 92%[f]	71.2-83.7[d]	91.0[g]	74.5-89.5 (fermentation & enzymatic cell wall degradation) [d] 98.0 (parboiling & dehulling) [g]
Italian	90.5-96.9[h]								
Foxtail	75.5-79.3[a] 62.3[i]	90.4-93.8[a]	76.6[i]	81.1[i]					80.6 (dehulling & soaking) [i] 82.4 (dehulling & cooking) [i] 82.7 (dehulling, soaking & cooking) [i]
Pearl	70.4-72.7[j] 69.0-76.9[k] 51.0[l] 51.8[m]			78.6-79.1[j]		77.2[l] 59.3-65.7[m]	up to 81.6-83.6[j] 77.5-86.6[k]		90.1 (fermentation & germination) [l]
Proso	68.4-72.9[a]	86.4-89.4[a]							

References

[a] Ravindran, 1992
[b] Geetha et al., 1977
[c] Oghbaei & Prakash, 2012
[d] Antony & Chandra, 1999
[e] Mbithi-Mwikya et al., 2000
[f] Hejazi & Orsat, 2016
[g] Dharmaraj & Malleshi, 2011
[h] Monteiro et al. 1988
[i] Pawar et al. 2006
[j] El Hag et al., 2002
[k] Ali et al., 2003
[l] Khetarpaul & Chauhan, 1990
[m] Sehgal & Kawatra, 2001

Table 4. Effects of processing on starch and protein digestibility and related mechanisms

		Decortication/Dehulling	Germination	Fermentation	Parboiling
Starch digestibility	Effect	Increase	Increase	Increase	Decrease
	Mechanism	changes in insoluble dietary fiber, phenolics and lipid contents	(i) removal of antinutrients; (ii) lipid hydrolysis	(i) starch hydrolysis; (ii) degradation of anti-nutrients by microbial enzymes	(i)formation of amylose-lipid complex; (ii) amylose and amylopectin retrogradation
	References	Bora, 2014	Mbithi-Mwikya et al., 2000; Sehgal & Kawatra, 2001	Khetarpaul & Chauhan, 1990; Sharma & Kapoor, 1996	Bora, 2014
Protein digestibility	Effect	Increase	Increase	Increase	Increase
	Mechanism	decrease in the anti-nutrients that interfere with the IVPD	(i) decrease in the anti-nutrients by enzymatic activities or leaching; (ii) degradation of storage proteins	(i) partial degradation of complex storage proteins; (ii) degradation of anti-nutrients by microbial enzymes	(i)increase in extractability of globulins and prolamin-like proteins; (ii) oxidation of phenolic compounds
	References	El Hag et al., 2002	Mbithi-Mwikya et al., 2000; Hejazi & Orsat, 2016; Sehgal & Kawatra, 2001.	El Hag et al., 2002; Ali et al., 2003; Khetarpaul & Chauhan, 1990	Dharmaraj & Malleshi, 2011; Bora, 2014; Duodu et al., 2003

Highlights

Exploring the factors that contribute to the slow starch digestibility of millets

Understanding how these factors reduce millet starch and protein digestibility

Effect of processing on the in-vitro starch and protein digestibility of millets

Figure Captions

Figure 1: Different millet types (a: Finger millet, b: Pearl millet, c: Proso millet, d: Foxtail millet

Figure 2: Scanning Electron Micrographs of Millet Starches (a: Foxtail millet, b: Proso millet, c: Finger millet, d: Pearl millet)

Figure 3: Scanning Electron Photomicrographs of enzymatically hydrolyzed Kodo millet starch

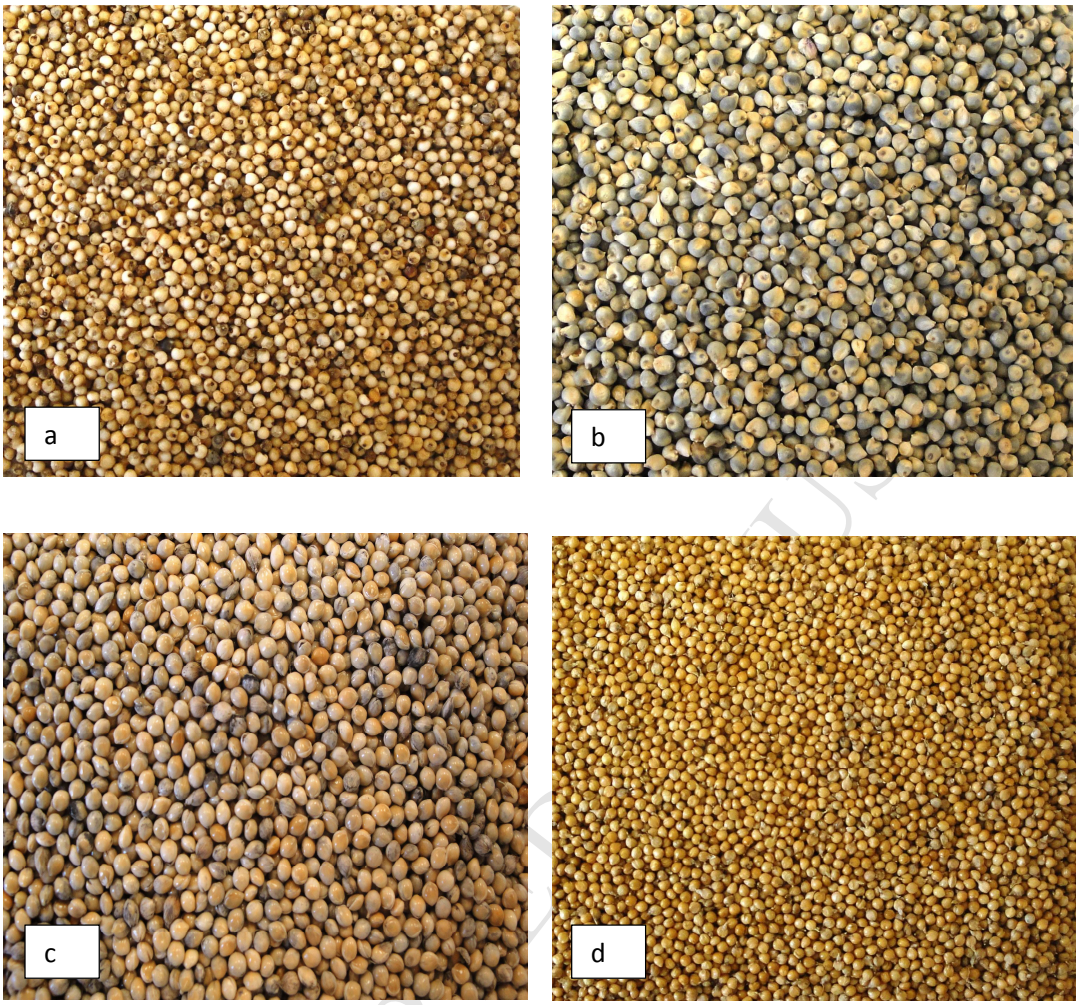
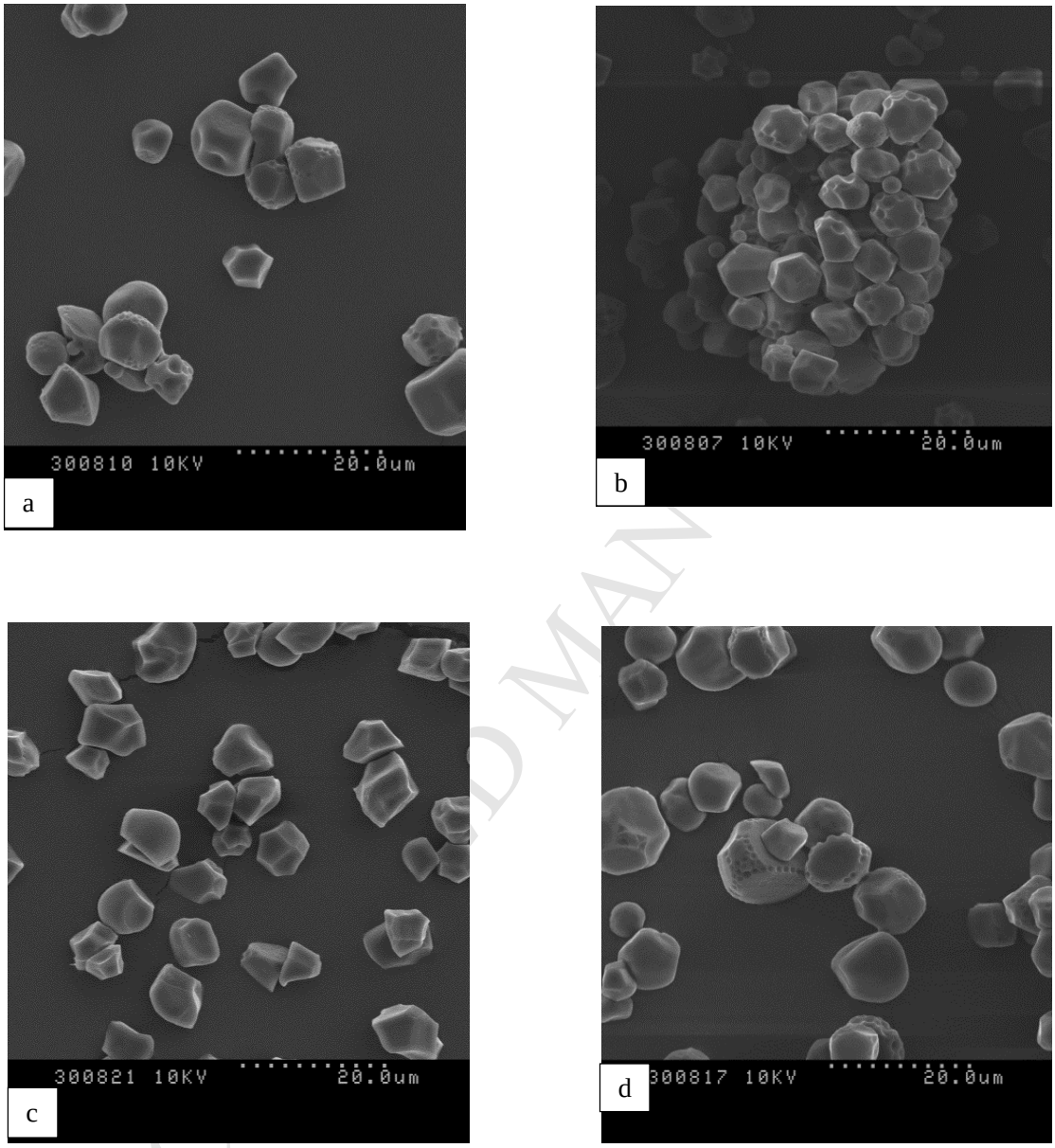


Figure 1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d

Figure 2

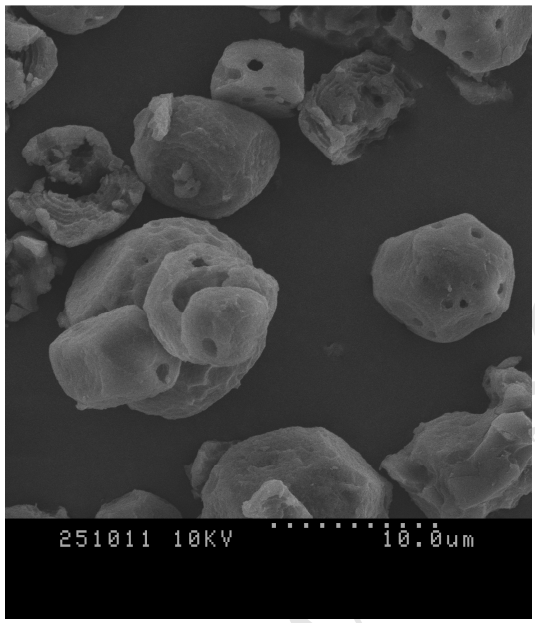

Figure 3