Journal of Cereal Science 64 (2015) 76–81

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Effects of the amount and type of fatty acids present in millets on their *in vitro* starch digestibility and expected glycemic index (eGI)

George Amponsah Annor [a, *], Massimo Marcone [a], Milena Corredig [a], Eric Bertoft [b], Koushik Seetharaman [b]

[a] Department of Food Science, University of Guelph, Guelph, ON, N1G2W1, Canada
[b] Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, 55108-1038, USA

ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form
11 May 2015
Accepted 15 May 2015
Available online 16 May 2015

Keywords:
Millet
Expected glycemic index
In vitro starch digestibility
Fatty acids
Complex index

ABSTRACT

This study investigated whether the amounts and types of fatty acids present in millet plays a role in its known hypoglycemic properties. In a two part study, the first part involved complexing excess amount (2 mmol/g of starch) of palmitic, oleic and linoleic acids to cooked pearl, finger, proso and foxtail millet starches, subjecting the complexes to *in vitro* starch digestibility and calculating their expected glycemic index (eGI). The second part of the study consisted of complexing the millet starches with the fatty acids in the amounts present in their respective millet flours. Elaidic acid in equal amounts to oleic acid was also used to ascertain the effects of the cis or *trans* configuration of the fatty acid on millet starch digestibility. The complex index (CI) of the fatty acids with millet starch increased with increasing level of unsaturation. Significant ($p < 0.05$) reductions in the *in vitro* starch digestibility and eGI of the millet starch-fatty acid complexes were observed. Reductions in the starch hydrolysis of the samples were found to be significantly linked to the amounts of the fatty acids added. The presence of unsaturated fatty acids generally resulted in less starch being hydrolyzed. Oleic acid seemed to be a very effective fatty acid in reducing the amount of starch hydrolyzed. *Trans* oleic acid (elaidic acid) showed to be less efficacious compared to oleic acid in *cis* configuration. The amount and type of fatty acids interacting with starch plays a significant role in the hypoglycemic property of millet.

1. Introduction

The digestibility of starch is known to vary for different foods. This variability results in different glycemic responses when various foods are consumed (Jenkins et al., 1988). Based on the *in vitro* digestibility of starch, three different starch fractions have been defined by Englyst et al. (1992). These fractions are: rapidly digestible starch (RDS), which corresponds to the amount of starch hydrolyzed after 20 min; slowly digestible starch (SDS), corresponding to the amount of starch hydrolyzed between 20 min and 120 min; and finally, resistant starch (RS), which is the total starch of the food minus the amount of starch hydrolyzed within 120 min.

The glycemic index concept was introduced to help classify foods on the basis of the extent to which they release glucose into the blood stream when they are consumed. Glycemic index is defined as the postprandial incremental glucose increase after a test meal, expressed as the percentage of the corresponding area after an equi-carbohydrate portion of a reference food such as glucose or white bread (Goni et al., 1997; Jenkins et al., 1987). Aside the nature of the starch itself (Thorne et al., 1983), various factors has been reported to affect the rate of starch digestion and glycemic index of starchy foods. Among these factors are: processing such as cooking (Bravo et al., 1998); dehulling (Alonso et al., 2000); popping (Capriles et al., 2008), viscosity of the food matrix (Kaur and Singh, 2009; Slaughter et al., 2002), the amount and type of dietary fiber (Jenkins et al., 1987), presence of anti-nutritional factors (Yoon et al., 1983), the presence of proteins (Hamaker and Bugusu, 2003), and the presence of lipids (Ai et al., 2012; Crowe et al., 2000; Kawai et al., 2012).

The ability of lipids to form inclusion complexes with starch, especially with amylose, is well known. Free fatty acids (Fanta et al., 1999; Kawai et al., 2012; Tufvesson et al., 2003b) and monoglycerides (Tufvesson and Eliasson, 2000; Tufvesson et al., 2003a) are examples of lipids that form helical complexes with amylose. The formation of starch-lipid complexes and their effects on the

* Corresponding author.
E-mail address: georgeannor@gmail.com (G.A. Annor).

http://dx.doi.org/10.1016/j.jcs.2015.05.004
0733-5210/

properties of starch has been found to be related to the concentration of the lipid. According to Tang and Copeland (2007), a critical micellar concentration of the lipid is required for the formation of maximum complexes between the starch and the lipid, beyond which some of the lipid tends to self-associate, rather than forming complexes with the starch.

The susceptibility of amylose–lipid complexes to starch degrading enzymes has been studied. Seneviratne and Biliaderis (1991) reported an inverse relationship between the rate and extent of hydrolysis of amylose–lipid complexes and the degree of organization of helices into larger domains of ordered chains. The degree of organization of the amylose–lipid complexes is also inversely linked to the length of both the fatty acid and the amylose chains (Godet et al., 1996; Tufvesson and Eliasson, 2000). Ai et al (2012) reported significant decreases in starch hydrolysis rates when corn oil, soy lecithin, palmitic acid, stearic acid, oleic acid and linoleic acids were added to corn, tapioca, and high amylose corn starch. Hasjim et al. (2010) produced a new resistant starch type 5 (RS 5) by complexing debranched high maize starch with palmitic acid. Crowe et al. (2000) reported a 35% reduction in the hydrolysis when lauric, myristic, palmitic and oleic acids were complexed with amylose. Kawai et al. (2012) also reported a decrease in the *in vitro* digestibility of potato starch when complexed with fatty acids.

Millet is a major staple crop in the arid and semi-arid regions of the world. It thrives on soils with low fertility and severe conditions such as low rainfall or intense heat. In many African and Asian countries, it is a staple diet in low income populations providing much needed calories and proteins (Sawaya et al., 1984). One property of millets, which can be exploited in North America, is its low glycemic and insulinemic response (Shobana et al., 2009). Lakshmi Kumari and Sumathi (2002) reported significantly lower plasma glucose levels from the consumption of a millet based diet. Noodles prepared from the substitution of 20% wheat flour with millet flour resulted in significantly lower glycemic index and load compared to that from noodles prepared from 100% wheat flour (Vijayakumar et al., 2010). However, the reason why millet is slowly digestible, compared to other cereals is not clearly understood. In a recent study (Annor et al., 2013), significant increases in the *in vitro* starch digestibility and expected glycemic index were observed when lipids were removed from debranned kodo millet flour. This study investigates whether the fatty acids present in millet play a role in their hypoglycemic property.

2. Materials and methods

2.1. Materials

The seeds of four millet species, namely, proso millet, foxtail millet, finger millet, and pearl millet were obtained from the Agriculture Environmental Renewal Canada (AERC) Inc, Delhi, Canada. These seeds were kept at −20 °C and later used for the study.

2.2. Methods

2.2.1. Starch extraction

Millet starch was extracted from their flour samples according to the method reported by Annor et al. (2013).

2.2.2. Sample preparation

Millet starch samples (0.7 g) were weighed into flat-bottomed flasks in duplicate and 14 mL of distilled water added (5% slurry). The samples were then cooked for 10 min in a boiling water bath, with continuous stirring. After cooking, the samples were kept at 70 °C and palmitic acid (P0500), oleic acid (O1008), linoleic acid (L1376) or elaidic acid (E4637), purchased from Sigma–Aldrich, St Louis, MO, USA, were added to the samples based on levels reported in the original millet flours (Table 1). Table 2 summarizes the concentrations used in this study. Samples were kept at 70 °C to ensure that the fatty acids melted when added to the samples. The gelatinized starch-fatty acid complex was allowed to form for 10 min with vigorous vortexing every 2 min. They were then cooled to room temperature and used immediately for subsequent analyses. This part of the study constituted part 2. For the first part of the study, the same amount of the fatty acids (2 mmol/g of starch) was added to the cooked starches from proso, foxtail, finger, and pearl millets. A control sample, consisting of cooked millet starch without the addition of any fatty acid, was also prepared.

2.2.3. Complex index (CI) of gelatinized starch-fatty acid complexes

Millet starch (0.1 g) was weighed in duplicates into screw-capped test tubes and 5 mL of distilled water was added (2% slurry). The samples in the test tubes were cooked for 10 min in boiling water bath with vigorous vortexing every 2 min. The cooked starch samples were kept at 70 °C and 2 mmol of palmitic, oleic, linoleic or elaidic acid per gram of starch was added to the samples. The gelatinized starch-fatty acid complex was allowed to form as described above and a control sample was also prepared. The extent of iodine complexation was determined using the procedure described by Tang and Copeland (2007). Briefly, deionized water (8.6 mL) was added to the millet starch-fatty acid complex prepared in the screw-capped test tubes and mixed thoroughly by vortexing. The suspension (200 μL) was mixed with 10 mL of distilled water and 1 mL of iodine solution (2.0% (w/w) KI and 1.3% (w/w) I_2) and vortexed. A reference sample containing only starch was also prepared. The absorbance (Abs) values of the sample and a reference were measured at 690 nm. The CI was calculated as:

$$\mathrm{CI} = 100 \times (\text{Abs of reference sample} - \text{Abs of sample})/\text{Abs of reference sample}$$

2.2.4. In vitro digestibility and expected glycemic index of starch-fatty acid complexes

The *in vitro* starch digestibility of the raw and cooked samples was carried out using the method described by Englyst et al. (1992). Hydrolyzed starch was classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst et al., 1992). The calculations were as follows: RDS = glucose released at 20 min × 0.9; SDS = (glucose released at 120 min − glucose released at 20 min) × 0.9, and RS = total starch − (RDS + SDS). A non-linear first-order equation $C{=}C_\infty\,(1{-}e^{-\mathrm{kt}})$, which was established by Goñi et al. (1997), was used to describe the kinetics of hydrolysis of the samples. C is the starch hydrolyzed at a chosen time t; C_∞ is the equilibrium concentration at the final time (120 min); k is the kinetic constant. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (AUC) of the samples by AUC of white bread, which serves as a reference sample, as reported by Goñi et al. (1997). The AUC was calculated by the equation: $\mathrm{AUC} = C_\infty\,(t_f{-}t_o) - (C_\infty/k)\,[1{-}e^{-k\,(tf{-}to)}]$, where t_f is the final time and t_o is the initial time. The eGI was calculated by the equation: eGI = 8.198 + 0.862 *HI as described by Granfeldt et al. (1992).

2.2.5. Total starch

Total starch was determined by the total starch kit from Megazyme (Bray, Ireland). Total starch was reported on a dry weight basis. This was needed to calculate the expected glycemic index of the samples.