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Enrichment of Extruded Snack Products with Coproducts from Chestnut Mushroom (*Agrocybe aegerita*) Production: Interactions between Dietary Fiber, Physicochemical Characteristics, and Glycemic Load

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ABSTRACT: Mushrooms are a common vegetable product that have also been linked to pharmaceutical and medicinal uses. However, the production of the fruiting bodies of mushrooms results in a large quantity of food waste in the form of spent compost. Hyphae and the base of fruit bodies from *Agrocybe aegerita* were retrieved from spent mushroom compost and refined as a freeze-dried powder. This fiber-rich ingredient was used in the manufacture of ready-to-eat extruded cereal snack products. Inclusions rates were 0, 5, 10, and 15% w/w replacement levels for wheat flour from a control recipe. Inclusion of mushroom coproduct material (MCM) was significantly correlated to increased product expansion ($r = 0.848$) and density ($r = 0.949$) but negatively correlated to water absorption index (WAI; $r = -0.928$) and water solubility index (WSI; $r = -0.729$). Fiber content could not be correlated to differences in pasting properties of extruded snacks even though snack products with MCM showed significantly lower final viscosity values compared to the control. The potential glycemic response of foods was significantly lowered by including MCM ($p < 0.05$) with a negative correlation between fiber content and overall AUC following a standardized in vitro digestion method ($r = -0.910$). Starch content, WAI, and WSI were positively correlated to AUC of extruded snacks ($r = 0.916$, 0.851, and 0.878. respectively). The results illustrate a reduction in the potential glycemic response from including 5% (w/w) of MCM in extruded snacks exceeds 20%. Thus, the incorporation of MCM in ready-to-eat snack foods may be of considerable interest to the food industry in trying to regulate the glycemic response of foods.

KEYWORDS: *mushroom, nonstarch polysaccharides, extrusion, glycemic response, food waste*

INTRODUCTION

Commercially, the edible form of chestnut mushrooms (*Agrocybe aegerita*) is the fruiting body harvested aboveground. These edible fractions are processed in a range of forms (minimally processed, canned, dried, or powdered) before human consumption. Recent attention has been given to the nutritional properties of mushroom both as dietary supplements or ingredients in the food industry and as pharmaceutical products.[1−3] Unprocessed mushrooms tend to have a high moisture content of between 70 and 80 g/100 g fresh weight. On a dry matter basis they form a rich source of dietary fiber (up to 80 g/100 g) as well as a significant portion of protein (6−10 g/100 g dry matter basis).[2,4] Much of the recent research on the nutritional quality of mushrooms has focused on this dietary fiber content and, in particular, the role of β-glucan components in lowering blood cholesterol and glycemic responses.[5−7] During the production of fresh mushrooms the hyphae and basal material remaining in the compost after the collection of the fruiting bodies is regarded as a waste product. This waste material may account for up to >20% of the weight of a crop and contributes significantly to production costs due to disposal expenses.[8] Although the material is regarded as a byproduct, it is considered to be safe for human consumption once cleaned and separated from residual compost. Thus, finding a novel food production use for this waste material could lead to substantial reductions in disposal costs as well as the development of an added-value coproduct rich in dietary fiber components.

Extruded snack products are part of the ready-to-eat food sector and have experienced considerable growth in recent years due to consumers' requirements for convenient food snacks associated with a reduction in preparation time for lunch and between-meal food products.[9] These extruded snack products tend to be starch based and generally high in caloric content.[10] As consumers demand healthier food products the snack industry has endeavored to improve the nutritional content of extruded products by increasing their composition of bioactive phytochemicals.[11] Dietary fiber from a number of commercial and noncommercial sources has been introduced to starch-based snack products with the attempt to lower the potential glycemic effect of these products, as well as rebalancing their nutritional profile.[9,12,13]

The use of vegetable waste material in the food industry as added-value ingredients has been investigated for vegetable resources such as apple, pear, onion, carrot, olive, pumpkin, tomato, and cauliflower.[14−18] Our previous research has shown the potential nutritional benefits of including fiber-rich plant fractions into food material but has illustrated that not all

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dietary fibers behave in a similar ways in terms of altering the physical and nutritional properties of foods.[12,13,17,18] There is a paucity of published research available regarding the nutritional quality and potential food use of the hyphae or basal material of *Agrocybe aegerita* and *Agaricus bisporus*, with the most recent publication reporting the potential use of stalk material in the food industry.[8] The aim of the current research was to evaluate the potential use of waste stream material from mushroom growth as a functional ingredient for extruded snack foods. Particular attention was focused on the potential glycemic modulating effects the waste product ingredients may possess in terms of fiber-rich ready-to-eat snack food products.

EXPERIMENTAL PROCEDURES

Recovery and Preparation of Mushroom Waste Material. Stalks and basal hyphae clumps of chestnut mushrooms were obtained from Oakland Farms (Evesham, U.K.). The material was recovered from spent mushroom compost after harvesting of the mushroom cap had occurred. The mushroom coproduct material (MCM) was collected, separated manually, and cleaned of any compost/growing medium by hand removal of clumps of soil, followed by careful brushing of the stalks and basal sections. The cleaned material was then freeze-dried for 2 days using an Edwards Super Modulyo freeze-dryer (Bristol, U.K.). The freeze-dried stalks were hermetically sealed and stored at ambient temperature. Cleaned, dried stalks were milled to produce a powder using a Retsch ZM100 with a no. 2 screen (Retsch Gmbh, Haan, Germany). The resulting powdered mushroom waste material was hermetically sealed in foil bags and stored at room temperature for future use.

Extrusion Processing. White culinary wheat flour was obtained from Smith's Flour Mills (Worksop, Notts, U.K.), oatmeal was obtained from Morning Foods Ltd. (Crewe, U.K.), and fine maize grits were supplied by Dacsa, Seaforth Corn Mill (Liverpool, U.K.). These flours were used as the major constituent of the base recipe (Table 1) to which MCM was added as a replacement for the wheat flour component of the recipe at 5, 10, and 15% (w/w).

Table 1. Recipes Used To Determine Best Fiber/Waste To Use in Extruded Product

substitution level	wheat flour (g/100 g)	maize grits (g/100 g)	oatmeal (g/100 g)	coproduct (g/100 g)
control	65	20	15	0
5%	60	20	15	5
10%	55	20	15	10
15%	50	20	15	15

Snack products were made by extruding the recipes in Table 1 using a Werner and Pfleiderer (Stuttgart, Germany), Continua 37, corotating, self-wiping, twin-screw extruder. The L/D (length to diameter) ratio of the extruder was 27:1, the screw diameter was 37.4 mm, and a Werner and Pfleiderer screw profile number 2054 was used while maintaining the screw speed at 175 rpm throughout the extrusion process. Extrusion was conducted at 9 kg/h, and the feed rate of the samples was determined by calibrating each sample through the feed hopper (Rospen Twin Screw Volumetric Feeder, Gloucester, U.K.) as a dry mix prior to extrusion and recording of the actual mass passing through the hopper as a factor of time (data not shown). A 4 mm diameter twin die was used for all samples. An automated product cutter was placed at the die face and set at 300 rpm to obtain pelleted snack product cereal. The expanded snack products were allowed to air-dry and cool for 1 h before they were sealed in polyethylene bags for storage.

Physicochemical Characteristics. The product density of the extruded snack samples and the expansion ratio of samples were calculated in duplicate following the formula described by Brennan et al.[12] Moisture determinations of extruded products were conducted in duplicate according to the AACC methodology (moisture − air-oven methods, method 44-15).[19] Total starch was determined in duplicate using the Total Starch assay kit from Megazyme International (Wicklow, Ireland); total dietary fiber (TDF) content was determined in duplicate using a Total Dietary Fiber assay kit (Megazyme Internationa) with determinations for soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) fractions being recorded as by Brennan et al.[12] The water absorption index (WAI) and water solubility index (WSI) were determined in duplicate as by Anderson et al.[20]

A Rapid Visco Analyzer (RVA-4; Newport Scientific, Warriwold, Australia) was used to determine the pasting properties of the extruded food products. Extruded samples were milled to achieve a fine particle according to the manufacturer's guidelines. Briefly, 25 mL of water was added to an aluminum canister, a sample suspension was prepared by placing 2.5 g of dry milled sample, and the mixture was inverted and dispersed by shaking vigorously for 10 s with a rubber stopper over the open end of the canister to avoid loss of product from the canister; the sample was then placed in the RVA apparatus with a plastic molded paddle. Experiments were conducted at a paddle speed of 160 rpm, and samples were subjected to the heating and cooling procedure of the standard 1 profile.[21] The software Thermocline for Windows (Newport Scientific) was used to analyze the pasting profiles of the graphs obtained. The peak viscosity (maximum viscosity of the sample during the heating and holding phase of the procedure) as well as the final viscosity (viscosity readings at the end of the test profile) were recorded for all samples. Analysis of samples was conducted in duplicate.

Product hardness (HAR) and crunchiness (CRU) were determined for the samples using a Stable Microsystems Texture Analyzer (TA-XT32, Stable Micro Systems, Surrey, U.K.). An aluminum cylinder probe of 35 mm diameter was used with a test speed of 1 mm/s at a trigger force of 5 g. Extruded pellets were axially compressed to 50% of their original height, and the maximum force recorded during the compression was expressed as the hardness of the product. Hardness of the product was defined as the peak force exhibited on compression of the sample, whereas crunchiness was defined as the number of fracture peaks >50 g obtained during analysis. All measurements were performed for a minimum of 20 samples.

Determination of Potential Glycemic Response via in Vitro Product Digestion. The procedure for measuring the breakdown of carbohydrates to sugars follows that reported by Brennan et al.[22] This procedure measures the breakdown of carbohydrates to sugars by the action of amylase enzymes added to the extruded product. The amount of reducing sugars released (RSR) was determined over a 120 min digestion process. Reducing sugar values were recorded at time 0, 20, 60, and 120 min of digestion and converted to available glucose released as by Woolnough et al.[23] Experimental products were compared to that of the control extrudate. Data presented are the mean of two replicates. Areas under the glucose release (in vitro) curves were calculated using the trapezoid rule and ignoring the area beneath the baseline.[24]

Statistical Analysis. Unless otherwise stated, all determinations were made in duplicate, and mean ± standard deviation (SD) values are presented. Minitab v. 16 (Minitab Pty, Sydney, Australia) was used to carry out two-tailed t tests when appropriate to establish p values ($p \leq 0.05$). Pearson's correlations were also conducted to determine significant correlations at $p \leq 0.05$,$p \leq 0.01$, and $p \leq 0.001$, respectively.

RESULTS AND DISCUSSION

The dietary fiber compositional profile of the control and MCM snack products is shown in Figure 1. The inclusion of MCM significantly increased the amount of TDF in the extruded snacks. This is to be expected on the basis of previous research indicating a content of 80 g/100 g of fiber (dry matter basis) of mushroom stalk material.[25] Figure 1 illustrates that the major component of the fiber derived from MCM was IDF, which is consistent with the earlier findings of Manzi et al.[2]

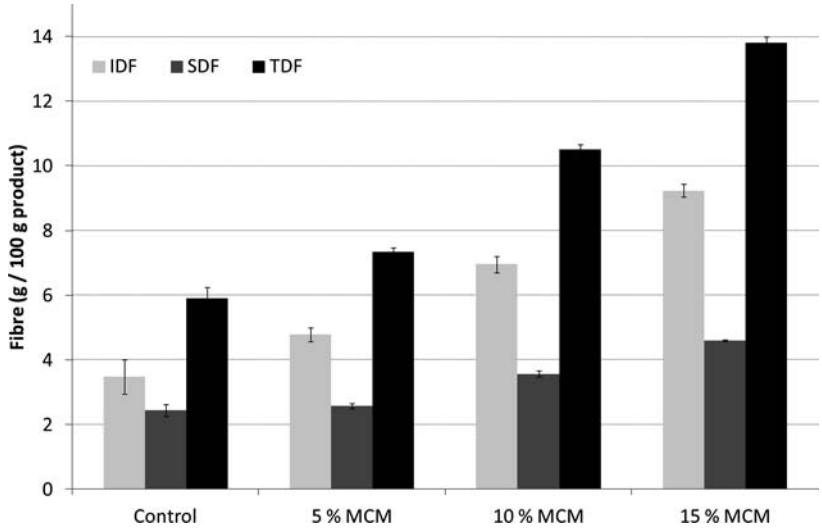

Figure 1. Fiber content of extruded snack products. Error bars represent standard deviation of replicates.

Table 2. Expansion and Textural Properties of Extruded Snack Products Containing Mushroom Coproduct Material (MCM)[a]

	expansion ratio	density (kg/m^2)	hardness (g)	crunchiness (no. of peaks < 50 g force)
control	194.82 ± 59.85a	100.18 ± 3.39a	2.86 ± 0.58a	105.27 ± 21.43a
5% MCM	245.80 ± 33.88b	107.02 ± 0.21b	0.97 ± 0.36b	115.11 ± 20.26a
10% MCM	222.15 ± 27.74ab	128.46 ± 10.56c	0.78 ± 0.38b	98.85 ± 18.99a
15% MCM	304.75 ± 31.68c	n/a	n/a	n/a

[a]Values represent means of analysis; within columns, values with different letters assigned are significantly different ($p \leq 0.05$).

Table 3. Pasting, Water Absorption, and Water Solubility Properties of Extruded Snack Products Containing Mushroom Coproduct Material (MCM)[a]

	peak viscosity (cP)	final viscosity (cP)	WAI	WSI
control	149.50 ± 4.95a	51.00 ± 1.41a	5.03 ± 0.12a	20.57 ± 2.66a
5% MCM	174.00 ± 1.41ab	37.50 ± 4.95b	4.85 ± 0.19ab	16.70 ± 1.40b
10% MCM	131.50 ± 6.36a	34.00 ± 5.66b	4.64 ± 0.16b	15.19 ± 0.55b
15% MCM	106.00 ± 1.41c	25.00 ± 0.00c	4.23 ± 0.03c	15.00 ± 0.69 b

[a]Values represent means of analysis; within columns, values with different letters are significantly different ($p \leq 0.05$). WAI, water absorption index; WSI, water solubility index.

The effect of inclusion of MCM on the physical characteristics of extruded snack products is shown in Table 2. Snack products with MCM inclusion at the 5% level exhibited a significantly greater expansion ratio compared to the control snack product ($p < 0.05$). Snack products with a 10% MCM inclusion rate also exhibited a higher expansion ratio than the control samples; however, this was not statistically significant different due to the high variability in the control extruded snack products. Density values for the snacks showed that both the 5 and 10% MCM snack products were significantly denser than the control samples. These observations are interesting in that generally there is a negative correlation between expansion ratio and density properties of extruded snack products.[12,13] In the case of fiber-rich products this association is related to increased water-holding capacities of fiber components, reducing the amount of water removed from the product as steam during the extrusion process, hence leading to a denser product.[12,13] More recently, we have illustrated that this correlation is not always the case and that not all dietary fibers behave in a similar way.[26] The results from this study support this observation. However, the hardness of the products decreased with increasing MCM inclusion rate. No significant differences could be observed between the samples in terms of the number of peaks observed during the compression of samples.

Table 3 illustrates the water-holding capacity of the snack products and their pasting properties as measured by RVA analysis. Both the WAI of MCM samples (except MCM at 5% inclusion rate) and the WSI were observed to be lower than those of the control product. Similarly, the inclusion of MCM significantly decreased the final viscosity (FV) values of the extruded product ($p < 0.05$).

Significant positive correlations (Table 4) exist between TDF and both product density and expansion ($r = 0.949$ and 0.848, respectively, at $p < 0.01$), whereas negative correlations exist between the amount of TDF and both WAI and WSI ($p < 0.001$ and $p < 0.05$, respectively). Sompong et al.,[27] investigating the effect of rice and soy flour blends on extruded product characteristics, established that the WSI of extrudates was affected by the moisture content of products post extrusion; however, no significant correlation between WSI and moisture content was observed in our research. A possible explanation is that the high proportion of IDF led to an increased water-holding capacity of the product and a greater water retention postflashing off of moisture at the extruder die face. This would also explain the increased product density

Table 4. Pearson's Correlation Coefficient (r) of Physicochemical and Nutritional Properties of Extruded Snack Products[a]

	ST	IDF	SDF	TDF	MST	WAI	WSI	EXP	DEN	HAR	CRU	PV	FV	AUC	D20	D60	D120
ST		−0.947***	−0.903**	−0.941***	−0.375	0.826*	0.722*	−0.755*	−0.891*	0.780*	0.112	0.515	0.836*	0.916***	0.930***	0.895**	−0.096
IDF			0.967***	0.997***	0.591	−0.909**	−0.729*	0.847**	0.951**	−0.800	−0.102	−0.537	−0.751	−0.926***	−0.930***	−0.862**	0.275
SDF				0.983***	0.687	−0.954***	−0.712*	0.831*	0.872*	−0.595	−0.050	−0.716	−0.832*	−0.846**	−0.862**	−0.770*	0.275
TDF					0.623	−0.928***	−0.729*	0.848**	0.949**	−0.762	−0.090	−0.595	−0.788	−0.910**	−0.917***	−0.842*	0.117
MST						−0.622	−0.098	0.642	−0.500	−0.958**	−0.524	−0.203	0.445	−0.308	−0.350	−0.192	0.424
WAI							0.788	−0.855**	−0.597	0.705	−0.472	0.380	0.700	0.851**	0.874**	0.800*	−0.100
WSI								−0.675	−0.686	0.755	−0.507	0.093	0.482	0.878**	0.864**	0.883**	0.215
EXP									0.423	−0.830*	0.602	0.426	−0.148	−0.799*	−0.873**	−0.814*	−0.209
DEN										−0.637	−0.205	−0.573	−0.588	−0.838*	−0.769	−0.724	0.154
HAR											−0.367	0.012	0.557	0.939***	0.961**	0.970***	0.540
CRU												0.572	0.161	−0.232	−0.300	−0.346	−0.662
PV													0.683	0.198	0.116	0.038	−0.832*
FV														0.615	0.611**	0.566	−0.234
AUC															0.981***	0.971***	0.155
D20																0.986***	0.200
D60																	0.318

[a]ST, starch; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber; MST, moisture content of extruded samples; WAI, water absorption index; WSI, water solubility index; EXP, expansion; DEN, product density; HAR, peak force/hardness of product; CRU, number of peaks; PV, pasting peak viscosity; FV, pasting final viscosity; D20, in vitro digestion reading after 20 min; D60, in vitro digestion reading after 60 min; D120, in vitro digestion reading between 60 and 120 min; AUC, in vitro area under the curve value. *, significant at $p < 0.05$. **, significant at $p < 0.01$. ***, significant at $p < 0.001$.

observed in the MCM samples as well as the reduced WAI of the samples. What is of added interest is that there was a negative correlation between WAI and the expansion ratio of the products ($r = 0.855$; $p < 0.01$). WAI has been shown previously to be related to the ability of starch to disperse in excess moisture, in that WAI is increased by the degree of starch gelatinization and fragmentation.[27] Table 4 illustrates a positive correlation between starch content of the extruded samples and both WAI and WSI ($r = 0.826$ and 0.722, respectively; $p < 0.05$) The correlation of starch to WAI and WSI as well as the correlation of WAI with product expansion indicates that in these MCM samples the fiber composition of the material has a major role in determining the physicochemical characteristics of the extruded products. One can speculate that the results suggest that in the fiber-rich extrudate starch may be less gelatinized, or fragmented, compared to the starch in the control products, potentially leading to a lower starch digestibility of MCM-containing samples.

In vitro digestibility values for the extruded products are shown in Figure 2. MCM exerts a significant reduction in terms of glucose released throughout the 120 min starch digestion process ($p < 0.05$). For instance, the 5% MCM extruded samples exhibit a 22% reduction in glucose released during the time 0−20 min (D20) and a 25% reduction of glucose released up to 60 min (D60). This indicates that the MCM samples are indeed restricting the amount of readily digestible carbohydrates in the extruded samples. The degree of restriction exhibits a greater response than what would be expected if we were to consider a simple 5% replacement of available carbohydrate as a response to MCM inclusion. Higher inclusion rates of MCM led to greater reductions in glucose released at all time points so that at 120 min the glucose released from the 15% MCM extruded sample was reduced by 29%. This fact is further illustrated when the averaged AUC values for the extruded samples are examined (Figure 3), which in turn demonstrates that the inclusion of MCM significantly reduced overall AUC of the extruded samples in a dose (but not linear) response.

Table 4 further demonstrates the strong negative correlation between TDF and D20 ($r = -0.917$; $p < 0.001$), between TDF and D60 ($r = -0.842$; $p < 0.05$), and between TDF and AUC ($r = -0.910$; $p < 0.0$). Our previous research has illustrated the fact that fiber-rich products can exert significant reductions in starch digestibility and glucose release values using both in vitro and in vivo digestion procedures.[12,13,26,28,29] If we hypothesize regarding the positive correlation between WAI and starch gelatinization and fragmentation as alluded to by Sompong et al.,[27] we can suggest that in our results the MCM fiber is exhibiting a protective role in reducing the amount of starch gelatinization/denaturation. This in turn reduces the ease of enzyme accessibility to the starch granules within the product. Certainly the negative correlations observed between TDF and WAI and WSI suggest this is the case. In addition, Table 4 illustrates positive correlations between WAI and D20 ($r = 0.874$; $p < 0.01$), D60 ($r = 0.800$; $p < 0.05$), and AUC ($r = 0.874$; $p < 0.01$). Positive correlations were also observed between WSI and D20 ($r = 0.864$; $p < 0.01$), D60 ($r = 0.883$; $p < 0.01$), and AUC ($r = 0.878$; $p < 0.01$). This observation is consistent with that made by Sompong et al.,[27] who suggested that starch degradation was reduced in extruded samples exhibiting lower WSI values.

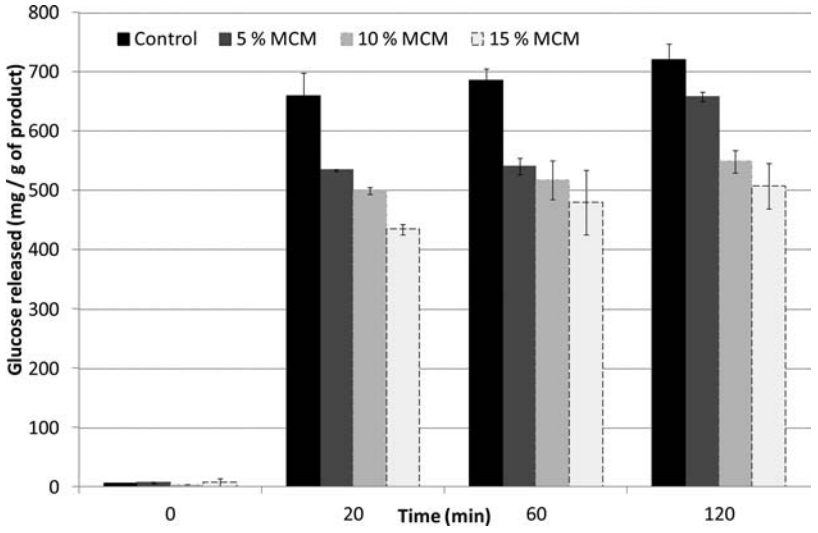

Figure 2. Glucose release during in vitro digestion of extruded samples. Error bars represent standard deviation of replicates.

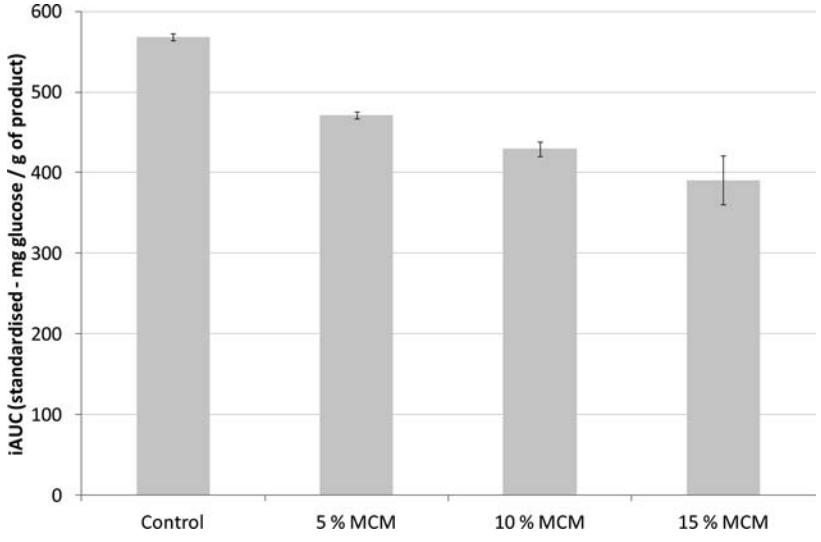

Figure 3. Average incremental area under the curve values (iAUC) for extruded samples as determined using in vitro procedures. Error bars represent standard deviation of replicates.

Recently, Butterworth et al.[30] and Roder et al.[31] have added commentary regarding the role of water enzyme mobility (and concentrations) in dictating the effectiveness of in vitro digestion systems to hydrolyze starch granules to sugar units. The mobility of water in these MCM fiber-rich products requires further investigation to establish what role the MCM plays in either reducing the starch structural disassembly during processing or how the MCM fiber components regulate free and bound water (and hence water mobility) during digestion and, hence, are responsible for manipulating the potential glycemic response of extruded snack products.

Further work is required to assess how these properties are perceived by consumers in terms of product acceptability. However, MCM has a clear benefit in terms of improving the nutritional quality of snack products. The significant reduction in glucose release during the in vitro digestion of MCM snack products compared to the control indicates that there is a great potential of using this waste stream material in the manipulation of postprandial glucose response of snack products and hence the potential manipulation of glycemic response of individuals.

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Notes

The authors declare no competing financial interest.

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