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Towards the use of undervalued African truffle and pearl millet to modulate glucose release in crackers

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Abstract
Indigenous and underutilised food resources can play a role in nutrition. Pearl millet, an underutilised cereal, has slowly digestible starch. However, it hardly features in many diets possibly due to its limited diversified use in high value convenient food products. Some mushrooms also improve postprandial glycaemic responses. This study investigated the effect of compositing Kalahari truffle (KT) with fermented pearl millet and bread wheat flour on the nutrient and *in vitro* starch digestibility of the crackers. Fermented whole grain pearl millet flour was mixed with bread wheat flour (1:1). KT powder was added to comprise 5, 10 and 15% of the composite flours. KT incorporation significantly increased the crackers' protein and significantly reduced their *in vitro* starch digestibility.

Key words: mushroom, diabetes, glycemic, indigenous, digestibility

Introduction
Generally, cereal grains are responsible for the huge part of human carbohydrates intake, thus the alteration of carbohydrate especially starch quality in cereal products has a potential positive effect on nutrition. In-vitro starch digestibility assist in the description of the carbohydrate component of a meal (Englyst and Englyst, 2005). Starch that is hydrolysed within 20 minutes is regarded as rapidly digestible starch, whereas starch that is digested between 20 and 120 minutes is called slowly digestible starch, beyond 120 minutes, it is regarded as resistant starch (Englyst et al., 1992). Rice, barley, maize, wheat, sorghum, oat, rye and millets are classified as major cereals in terms of their contribution to people's diets (Adebiyi et al., 2018). Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is cultivated in India, West and Central Africa, southern Africa, Brazil and the United States of America (Taylor, 2016). It is a staple food for over half of the Namibian human population. Pearl millet is gluten free, making it one of the low-cost alternative cereal for people suffering celiac disease (Dias-Martins et al., 2018). Additional health benefits include the management of blood glucose levels, as well as anti-cancer and prebiotic properties (Dias-Martins et al., 2018). On the other hand, millets contain less than the recommended lysine content required by adults (only 17% of the 1.93 g) (Taylor, 2016). This impediment may be overcome by incorporating mushrooms in pearl millet products. Edible mushrooms are known to be an excellent source of protein, with exceptional amounts of essential amino acids (Islam et al., 2016). Additionally, mushrooms contain β-glucans which are known to positively influence postprandial glycaemic response in humans (Ng et al., 2017).
Namibia is home to indigenous and or endemic species of edible mushrooms such as Kalahari truffles (*Kalaharituber pfeilii*), *Termitomyces schimperi*, *Termitomyces sagittiformis*, and Ganoderma mushroom species, which are barely utilised commercially. With an increased consumer interest in functional foods and biscuits and crackers can be excellent products for functional ingredients due to their popular consumption (Pasqualone et al., 2015). Crackers are significant in the baking industry due to their low moisture and

sugar contents which contribute to a better shelf life and they also offer variety in taste, texture and aroma (Reddy et al., 2019). Crackers belong to a group of crisp, chemically leavened or fermented bakery products that may contain up to 30% fat (Reddy et al., 2019). Crackers can either be fermented (soda, saltine and cream crackers) or chemically leavened (snack crackers) (Reddy et al., 2019). The aim of this study was to develop mushroom incorporated pearl millet-wheat crackers and evaluate the mushroom's influence on the resultant crackers' nutrients content and starch digestibility.

Materials and Methods

Pearl millet grains were purchased from subsistence farmers around Omuthiya gwIipundi in Oshikoto region. Grains were cleaned to remove foreign objects and fermented at a ratio of 1:2 (w/v) for 24 hours at 37 °C as outlined by Onweluzo and Nwabugwu (2009). Fermented grain was dried at 40 °C in an oven (Scientific, Model 222, South Africa) for 48 hours before milling using a hammer mill (Trapp TRF 4000, Metalúrgica Trapp Ltda. Brazil) and passed through a 250 μm screen. Bread wheat flour was purchased from a local retail shop in Windhoek, Namibia. Kalahari truffles were purchased from informal vendors at Omuthiya gwIipundi Informal (open) Market in Oshikoto region. Truffles were sliced and dried at 40 °C for 48 hours. The mushroom was milled using a blender (Waring Commercial 7011HS 2-Speed Food Blender) before passing through a 250 μm screen.

Composite flours were prepared by mixing equal amounts of wheat and pearl millet flours to make a pearl millet-wheat composite flour (control). The composite crackers were formulated with 51.4% pearl millet-wheat flour (1:1) for control sample. The pearl millet wheat flour was then replaced by 5%, 10% and 15% Kalahari truffle flours for the other samples. The ingredients at constant inputs in all samples were baking powder (0.9%), sugar (1.4%), salt (1.7%), vegetable oil (17.8%) and water (26.8%). The dough was rested for 20 minutes followed by sheeting and cutting. The crackers were baked at 180 °C in a conventional oven (Macadams Convecta 7, Cape Town, South Africa) for 8 minutes.

Crackers were ground using a blender (Waring Commercial 7011HS 2-Speed Food Blender) and passed through a 500 μm screen. Moisture, ash, crude fat, crude protein and crude fibre contents were determined following AACC methods 44-15.02 A, 08-01, 30-10.01, 46-30.01 and 32-10.01 respectively. Total starch was determined using the Megazyme Total Starch Assay Kit. *In vitro* starch digestibility was determined using a modified Englyst method (Englyst et al., 1992). About 100 mg of dry sample was suspended in 2 mL 0.1 M sodium acetate buffer (pH 5.2) and incubated at 37 °C for 5 minutes before the addition of 100 μL of diluted α-amylase and 100 μL of diluted amyloglucosidase. Samples were incubated in a shaking water bath at 37 °C for 120 minutes. 50 μL aliquots were pipetted into 2 mL micro-centrifuge tubes containing 400 μL of cold ethanol after 20, 60 and 120 minutes of digestion. Glucose released at the specified time intervals was determined using the Megazyme D-Glucose Assay Procedure (GOPOD).

The experiment (baking) was done twice. All analyses were done in duplicates and results represent the average of duplicates, presented as means ± standard deviation. Data obtained was subjected to an analysis of variance (ANOVA) and Duncan's least significant differences (LSD) test using SPSS Statistics Software, Version 25 (IBM, USA).

Results and discussion

Results for the chemical characteristics of mushroom supplemented pearl millet-wheat crackers are presented in Table 1. Kalahari truffle crackers' moisture content decreased as the percentage of Kalahari truffle powder increased, except at 5% mushroom inclusion. These results are also consistent with the findings of Ng et al., (2017). In contrast, Gadallah and Ashoush, (2016) reported that the moisture content of biscuits fortified with desert truffle powder increased as the truffle powder percentage was increased. The fibre in the mushrooms' powders absorbs water and that could be the reason for the inverse relationship between moisture content and mushroom incorporation (Ng et al., 2017).

Table 1. Chemical characteristics of mushroom supplemented pearl millet-wheat crackers.

Crackers	Moisture (%)	Ash (%)	Protein	Total Starch (%)	Crude Fibre	Crude Fat
Control	3.44 ±0.01[d]	3.77 ±0.02[a]	8.72 ±0.26[a]	58.99 ±3.03[b]	0.93 ±0.19[a]	25.50 ±0.47[a]
5% KTC	4.27 ±0.00[f]	3.83 ±0.00[a]	9.13 ±0.09[b]	50.04 ±3.13[a]	1.06 ±0.18[ab]	25.79 ±1.23[a]
10% KTC	3.18 ±0.04[c]	3.96 ±0.05[b]	9.67 ±0.14[c]	48.97 ±4.77[a]	1.51 ±0.12[abc]	26.11 ±0.97[a]
15% KTC	2.22 ±0.01[a]	4.19 ±0.00[c]	10.55 ±0.47[d]	48.62 ±2.56[a]	1.68 ±0.29[c]	26.21 ±0.29[a]

*Values are means (n = 4) ± standard deviation, values with the same letter in a column are not significantly different ($p > 0.05$). Values were adjusted to dry weight conditions. KTC = Kalahari truffle cracker

The control cracker had the least ash content (3.77%). There was a gradual increase in the cracker's ash content with every increase in Kalahari truffle incorporation since the truffle had a high ash content (5.61%). This is consistent with the findings of Farzana and Mohajan (2015) who found that the ash content of mushroom fortified biscuits increased (from 1.50% to 1.76%) with every increase in the percentage of soy flour. These findings suggest that the addition of Kalahari truffle can potentially be used in improving the mineral content of cereal based baked products. Kalahari truffle incorporation significantly increased the crackers' protein content. This could have been due to the mushrooms' high protein content than that of wheat or pearl millet. The results are in agreement with the trend of protein content found in composite biscuits by Kolawole et al. (2018). Kalahari truffle incorporation significantly reduced the cracker's starch content (59% to 49%). The decrease in the mushroom incorporated cracker's starch content could be attributed to the Kalahari truffle low starch content. The decrease in starch content is consistent with the findings of Farzana and Mohajan (2015) who recorded a reduction (from 65.62% to 56.38%) in the starch content of mushroom fortified biscuits. Similarly, Ng et al. (2017) also reported a decrease in the starch content of mushroom fortified biscuits as the mushroom percentage increased. Gadallah and Ashoush (2016) also reported similar findings on biscuits fortified with desert truffle powder. Kalahari truffle incorporation of up to 15% significantly increased the crackers' crude fibre content from 0.93 to 1.68%. A similar trend was reported by Kolawole et al. (2018). The crackers' fat content was between 25.02 and 26.21%. There was no significant difference observed between the control and all Kalahari truffle crackers.

In vitro starch digestibility results are presented in Table 2. Compared to the mushroom powder enriched crackers, the control cracker had the highest digestible starch at both G20 (16.3%), G60 (26.6%) and G120 (37.7%), respectively. It was observed that starch digestibility significantly ($p < 0.05$) decreased as Kalahari truffle powder incorporation increased. This could be a result of the low starch contents of Kalahari truffle powders and/or the effect of mushroom powder on the digestibility of starch. These results are consistent with the findings of Ng et al. (2017), who reported a similar trend in mushroom fortified biscuits. Similarly Brennan et al. (2013) recorded a decline in the amount of glucose released from mushroom enriched extruded snacks.

Table 2 *In vitro* starch digestibility of Kalahari truffle enriched crackers (%).

Crackers	G20	G60	G120
Control	16.32 ±0.55[c]	26.63 ±1.08[b]	37.71 ±1.05[d]
5% KTC	15.89 ±0.39[bc]	25.84 ±1.40[ab]	33.75 ±0.34[c]
10% KTC	15.09 ±0.04[b]	23.54 ±2.92[a]	32.78 ±1.88[bc]
15% KTC	14.16 ±0.42[a]	23.67 ±1.82[a]	30.11 ±0.86[a]

*Values are means (n = 4) ± standard deviation, values with the same letter in a column are not significantly different ($p > 0.05$). KTC = Kalahari truffle cracker. G20 = glucose measured at 20 min of hydrolysis; G60 = glucose measured at 60 min of hydrolysis; G120 = glucose measured at 120 min of hydrolysis

The decline in the release of glucose could be due to the increase in mushroom percentage, which could have in turn resulted in an increase in the crackers' dietary fibre content. Dietary fibre is especially important as it improves glucose metabolism and insulin response in patient suffering from Type 2 diabetes, by facilitating the gradual hydrolysis of carbohydrates and glucose release (Brennan, 2005). Wan Rosli et al. (2012) reported an increase in the amount of dietary fibre as the mushroom percentage increased in butter biscuits. These authors reported that butter biscuits formulated with 4% and 6% mushroom powder had high amounts of β-glucans, 0.72% and 0.79% respectively. Mushroom dietary fibre has been of interest lately, as it has been found to contain β-glucans which have positive effects on blood glucose modulation (Cheung, 2013). The soluble fraction of β-glucans is apparently responsible for their ability to lower blood glucose levels (De Paula et al., 2017). These *in vitro* starch digestibility results showed that the compositing of Kalahari truffle powder into crackers can significantly reduce the amount of glucose released. Therefore, the incorporation of the Kalahari truffle in pearl millet-wheat crackers can potentially be beneficial to individuals with the Type 2 diabetes.

Conclusions

Incorporating Kalahari truffle in pearl millet-wheat crackers increased the protein content of resultant crackers. Furthermore, Kalahari truffle can be used in the development of low *in vitro* starch digestibility foods. Pending large consumer acceptance assessments, this study can be a foundation to the development of value-added Kalahari truffles and pearl millet healthful products.

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