Starch
www.starch-journal.com

In vitro Fecal Fermentation of Indigestible Residues from Heat-Moisture Treated Maize Meal and Maize Starch with Stearic Acid

*Isaac Kwabena Asare, Ayyappan Palaniappan, Thaisa Moro Cantu Jungles, Bruce R. Hamaker, and Mohammad Naushad Emmambux**

The effect of resistant starch type 5 (amylose-lipid complex, ALC) from maize starch and maize meal on short-chain fatty acids (SCFAs) production by in-vitro human faecal fermentation are determined. The maize starch and meal are modified using heat-moisture treatment (HMT), stearic acid (SA), and combination treatment (SA+HMT) and digested to obtain indigestible residues. The results showed the production of SCFAs (acetate, propionate, and butyrate) from indigestible residues containing amylose-lipid complexes during the process of in vitro faecal fermentation. The concentrations of three SCFAs are lower than fructooligosaccharides (control) in most cases. In regard to the indigestible residues, the combination treatment has a significantly higher concentration of the total SCFAs than the individual SCFAs produced by different treatments and the control. Reduction in pH with increased gas production is observed. Acetate and butyrate levels are higher than propionate values of RS 5 from maize starch and meal. A positive correlation between the gas produced and SCFAs (acetate, propionate, and butyrate) is noticed, however it resulted in negative correlation with pH. In conclusion, indigestible residues containing ALC (or RS 5) produced SCFAs during in vitro faecal fermentation, suggesting that ALC are suitable substrates for fermentation in the lower gut.

1. Introduction

Resistant starch (RS) is the starch that escapes digestion in the small intestine and gets into the large intestine with the potential to be fermented by the gut microbiota.[1] RS is categorized into five classes: RS 1, a physically unavailable starch to be digested; RS 2 is composed of native granules with structures making the starch slow to digest[2]; RS 3 is a retrograded starch[3]; RS 4 are chemically modified starches,[4] and RS 5 can be considered as starch with ALC).[5] The potential health benefits of RS include controlling postprandial glycemic and insulinemic responses and prevention of colonic cancer.[6]

ALC is an interaction between the fatty acid and hydrophobic core of the amylose helix.[7] The aliphatic chain of the fatty acid lies within the amylose helix,[8] and the helix turn is stabilized by intra and intermolecular van der Waals forces and hydrogen bonds. The carboxylic head of the fatty acid is located outside the amylose helix. This carboxylic head is prevented from entering the helix by the stearic hindrance and electrostatic repulsion.[9] According to Singh et al.,[10] ALC reduced the accessibility of enzymes to hydrolyze the starch, thus preventing the starch molecules from fitting to the enzyme binding site to be hydrolyzed. The α-1-4 glycosidic bond appears to be found inside the amylose helix and is made inaccessible to enzymes. Thus, ALC reaches the large intestine, and their fate in lower Gastrointestinal tract (GIT) is not well understood. Therefore, ALC is considered dietary fiber and is an option to increase dietary fiber without compromising the sensory properties of cereal-based foods.[11] The fermentation of dietary fiber by gut microbiota results in gas generation and SCFAproduction. It can also provide selective substrates for the growth of specific groups of bacteria that may enhance the intestinal health of the host.

HMT is a technique that involves treatment of starch at low moisture levels (<35% moisture w/w) for a specified period (15 min–16 h) and at temperatures (84—120 °C) above its glass transition temperature but below the gelatinization temperature and our previous work suggests that such conditions did not gelatinize the starch, but changes the crystalline structure.[12] HMT

I. K. Asare, A. Palaniappan, M. N. Emmambux
Faculty of Natural and Agricultural Sciences
Department of Consumer and Food Sciences
University of Pretoria
Private Bag X20, Hatfield, Pretoria 0028, South Africa
E-mail: naushad.emmambux@up.ac.za
T. M. C. Jungles, B. R. Hamaker
Whistler Center for Carbohydrate Research
Food Science Department
Purdue University
West Lafayette, IN 47907, USA

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/star.202100238

DOI: 10.1002/star.202100238

Table 1. Effect of stearic acid and heat moisture treatment alone and in a combination of the upper GI digestion and thermal properties of maize starch and maize meal.

Sample	Treatment	Percentage unhydrolyzed [%]	DSC results			
			1st endothermic peak		2nd Endothermic peak	
			Tp [°C]	Delta H [J g^{-1}]	Tp [°C]	Delta H [J g^{-1}]
Maize starch	Control	7	106.5	1.98	–	–
	SA	29	116.8	4.64	–	–
	HMT	32	101.1	9.12	–	–
	SA + HMT	37	110.4	10.43	–	–
Maize meal	Control	8	102.9	3.57	–	–
	SA	33	106.2	8.05	–	–
	HMT	33	100.1	6.04	122.2	3.21
	SA + HMT	38	111.5	10.63	–	–

Control indicates without treatment; SA, stearic acid was at 1.5% (w/w); HMT, heat moisture treatment, 20% moisture at 110 °C for 16 h; SA+ HMT, combinationtreatment; -, not detected.

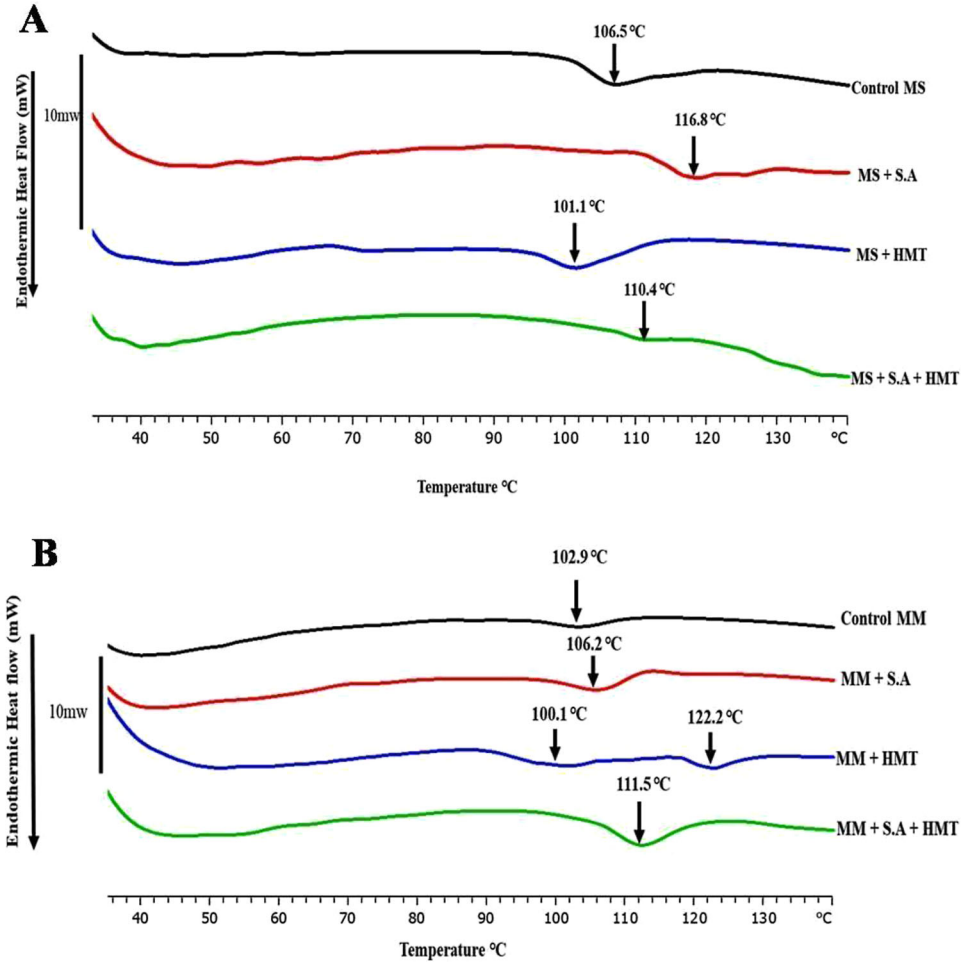

Figure 1. Effects of stearic acid and heat moisture treatment alone and in combination on the thermal properties of unhydrolyzed A) maize starch (ms) B) maize meal (mm). HMT indicates heat moisture treatment, 20% moisture at 110 °C for 16 h; SA, stearic acid was 1.5% (w/w) as db of the starch content; SA + HMT, combination treatment.

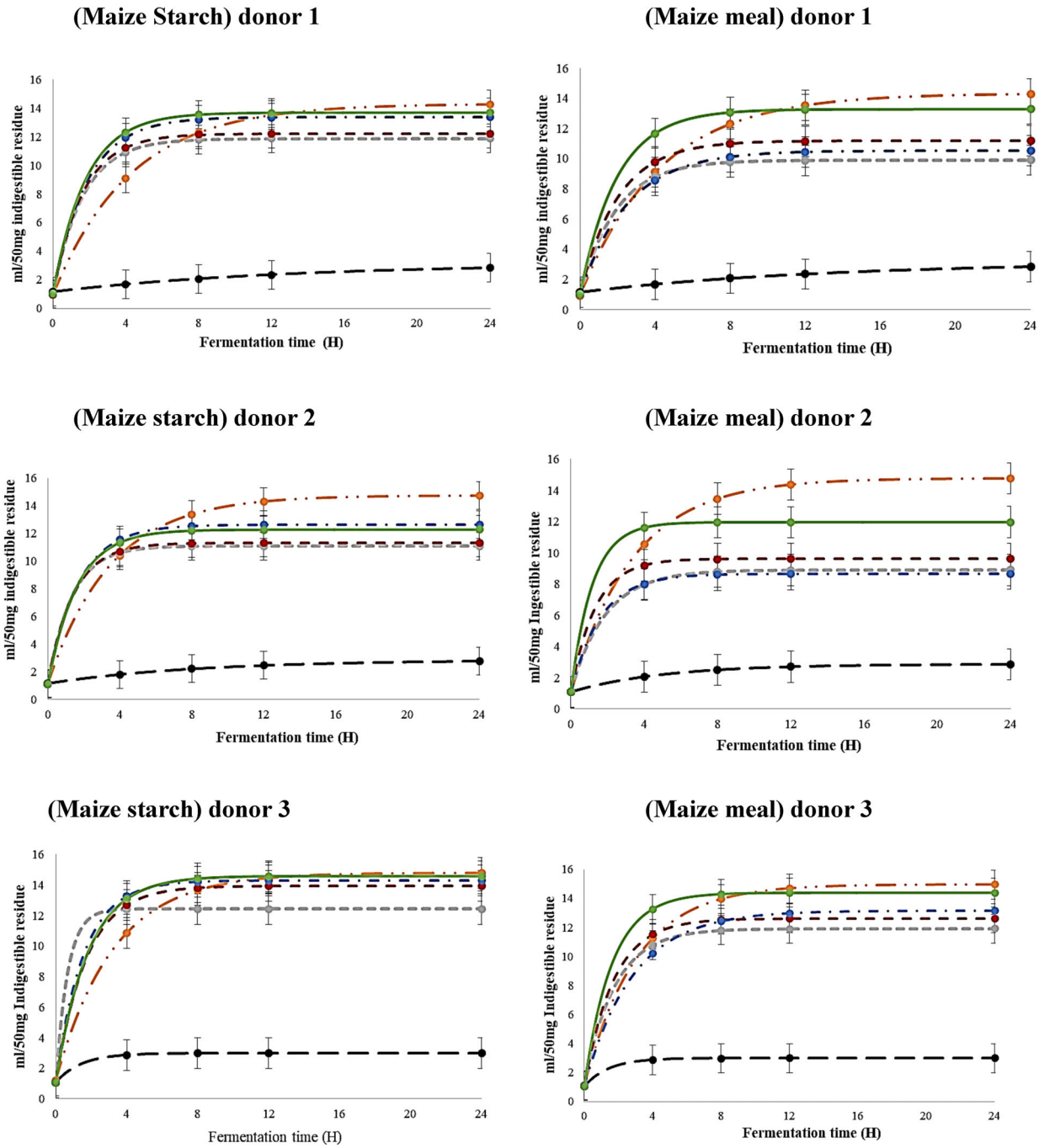

Figure 2. Effects of unhydrolyzed residue from unmodified and modified maize starch and maize meal on gas production from the three donors during their 24 h in vitro fecal fermentation. Key: Blank black (— —), Control grey (————), SA red (– – –), HMT blue (– · – · –), SA + HMT green (————), FOS orange (— · · —). FOS indicates fructooligosaccharides; HMT, heat moisture treatment at 20% moisture at 110 °C for 16 h; SA, stearic acid addition at 1.5% (w/w); SA + HMT = combination treatment.

of starches has been examined to increase the percentage of RS due to the altered crystalline nature, making the glycosidic bonds inaccessible for enzyme hydrolysis.[13] HMT causes amylose-amylose interaction (AM-AM) within the amorphous domain. The interaction between the side-chain of amylose-amylopectin (AM-AMP), a molecular rearrangement, and the complex formation between the amylose helix and the endogenous lipid form amylose-lipid complexes.[14,15] It also induced side-chain interactions of amylopectin-amylopectin (AP-AP) and has further been shown to have effects on the levels of rapidly digestible starch (RDS), slowly digestible starch (SDS), and RS.[13] Sievert and Pomeranz[16] prepared RS from normal and waxy starches by

HMT at 18% moisture and temperature of 110 °C. They reported that HMT reduced enzyme susceptibility of the ordinary and waxy starches.

Chung et al.[13] showed that the RS levels of corn, pea, and lentil starches had increased from 4.6%, 10.0%, 9.1–12.3%, 14.5%, 14.7% respectively after HMT (120 °C, 1 h, and 30% moisture content). Sang and Seib[17] reported that subjecting Hylon V maize starch (about 50% amylose) to concurrent HMT (45% moisture, 110 °C, 4 h) and phosphorylation (sodium trimetaphosphate/sodium tripolyphosphate) increased RS by 19% and decreased SDS and RDS levels by 12% and 6%, respectively.

Research shows that RS reaches the large intestine and is fermented by the gut microflora to produce SCFA, such as acetate, propionate, and butyrate,[18–20] which has biological significance to human health. It was proposed that depending on specific structural features of RS, a distinct fermentation profile could be observed.[21] Besides, the consumption of RS increased the fecal bulk, which can dilute potential carcinogens and reduce their exposure to the colon.[22,18] These physiological effects of RS have been related to its improvement in colon health and prevention of colorectal cancer.[22,19] Hence, it is necessary to maintain a balance in the composition of colonic microbiota for improved health.

To the best of our knowledge, there has been no systemic study to measure the potential of ALC as carbon sources for the utilization of human gut microbiota and production of SCFA. In this study, human feces (from three different donors in the age group of 30–35) was used as a model for colonic composition. The objective was framed to quantify the fermentation patterns and products (SCFA) of unhydrolyzed residues of modified maize starch and maize meal modified with heat-moisture treatment and stearic acid compared with the rapidly fermentable substrate, fructooligosaccharides (FOS).

2. Results and Discussion

Residues from upper gastrointestinal (GI) tract samples were used for thermal property analysis (**Table 1**). The control maize starch and maize meal had 7% and 8% RS values. Stearic acid alone increased RS to 29% for maize starch and 33% for maize meal. Heat-moisture treatment alone also increased RS further to 32% and 33% for both samples, and their combination treatment had RS values of 37% and 38% for both maize starch and maize meal, respectively. These values were the percentage RS that potentially entered the large intestine for fermentation. The increase observed was due to the addition of stearic acid to starch during pasting leading to the formation of amylose–lipid (mostly stearic acid) complexes as shown in **Figure 1**, thus limiting the hydrolysis of α-1,4 glycosidic bond located in the amylose. Heat-moisture treatment also resulted in fractions of amylose and amylopectin to form a double-helical structure during the process.[23] This caused an increase in the overall stability of the granule to disruption and thus lowered the digestibility. The percentage of unhydrolyzed maize starch and maize meal from Table 1 is in agreement with our previous work on in vitro starch digestibility[24] of maize starch and maize meal where the percentage of resistant starches were also calculated.

Figure 1 shows the thermal properties of unhydrolyzed maize starch and maize meal treated with stearic acid alone and heat-

Table 2. Effects of unhydrolyzed residue from unmodified and modified maize starch and maize meal on gas production from three donors during their 24 h in vitro fecal fermentation.

Sample	Treatment	Donor 1				Donor 2				Donor 3			
		Yo [mL 50 mg^{-1}]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mL 50 mg^{-1}]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mL 50 mg^{-1}]	Time [h]	K [h^{-1}]	Tau [h]
Maize starch	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	11.0 ± 0.6^{a}	1.5 ± 0.2^{a}	0.7 ± 0.1^{b}	0.8 ± 0.2^{a}	11.1 ± 0.9^{a}	2.0 ± 0.9^{a}	0.5 ± 0.1^{c}	1.7 ± 0.2^{a}	11.9 ± 0.2^{a}	1.4 ± 0.3^{a}	0.7 ± 0.1^{a}	1.2 ± 0.2^{a}
	SA	11.8 ± 0.3^{a}	1.8 ± 0.4^{a}	0.6 ± 0.1^{b}	1.0 ± 0.1^{a}	12.1 ± 0.3^{a}	2.5 ± 1.0^{a}	0.4 ± 0.2^{c}	1.7 ± 0.2^{a}	13.5 ± 0.2^{b}	2.2 ± 0.4^{b}	0.45 ± 0.2^{b}	2.1 ± 0.1^{b}
	HMT	12.9 ± 0.6^{b}	2.1 ± 0.1^{b}	0.4 ± 0.1^{b}	1.7 ± 0.1^{b}	13.5 ± 0.1^{b}	3.2 ± 1.2^{b}	0.3 ± 0.3^{b}	2.3 ± 0.1^{b}	13.6 ± 0.1^{b}	2.2 ± 0.1^{b}	0.45 ± 0.1^{b}	2.2 ± 0.1^{b}
	SA + HMT	13.4 ± 0.9^{b}	2.8 ± 0.4^{b}	0.3 ± 0.2^{b}	2.2 ± 0.1^{b}	13.8 ± 0.2^{b}	4.9 ± 0.2^{b}	0.2 ± 0.1^{b}	3.0 ± 0.1^{c}	14.2 ± 0.4^{c}	3.4 ± 0.4^{c}	0.29 ± 0.2^{c}	3.0 ± 0.1^{c}
	FOS	14.6 ± 0.1^{c}	3.5 ± 0.2^{c}	0.2 ± 0.1^{a}	2.8 ± 0.2^{c}	14.7 ± 0.4^{c}	5.5 ± 0.2^{c}	0.18 ± 0.1^{a}	3.2 ± 0.2^{c}	14.7 ± 0.2^{c}	3.5 ± 0.2^{c}	0.28 ± 0.1^{c}	3.1 ± 0.2^{c}
Maize meal	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	8.5 ± 0.3^{a}	1.2 ± 0.2^{a}	0.6 ± 0.1^{d}	1.4 ± 0.1^{a}	8.9 ± 0.1^{a}	1.1 ± 0.4^{a}	0.9 ± 0.1^{d}	1.2 ± 0.1^{a}	10.9 ± 0.1^{a}	1.8 ± 0.1^{a}	0.6 ± 0.1^{c}	1.2 ± 0.1^{a}
	SA	11 ± 0.2^{b}	2.5 ± 0.2^{b}	0.35 ± 0.1^{c}	2.4 ± 0.1^{b}	9.6 ± 0.2^{b}	2.3 ± 0.1^{b}	0.43 ± 0.1^{c}	2.2 ± 0.1^{b}	12.6 ± 0.3^{b}	3.2 ± 0.4^{b}	0.3 ± 0.1^{b}	3.1 ± 0.1^{b}
	HMT	10.9 ± 0.8^{b}	2.4 ± 0.2^{b}	0.4 ± 0.1^{c}	2.2 ± 0.2^{b}	8.7 ± 0.1^{a}	1.0 ± 0.2^{a}	1.0 ± 0.1^{d}	1.1 ± 0.1^{a}	13.2 ± 0.5^{b}	3.0 ± 0.5^{b}	0.3 ± 0.2^{b}	3.1 ± 0.2^{b}
	SA + HMT	13.4 ± 0.1^{c}	4.0 ± 0.1^{c}	0.25 ± 0.2^{b}	3.4 ± 0.1^{c}	12.2 ± 0.4^{c}	3.4 ± 0.2^{c}	0.29 ± 0.2^{b}	3.0 ± 0.1^{c}	14.4 ± 0.1^{c}	4.6 ± 0.4^{c}	0.2 ± 0.2^{a}	4.0 ± 0.1^{c}
	FOS	14.7 ± 0.4^{d}	5.5 ± 0.2^{d}	0.18 ± 0.1^{a}	4.1 ± 0.1^{c}	14.7 ± 0.2^{d}	4.5 ± 0.2^{d}	0.2 ± 0.1^{a}	3.4 ± 0.2^{d}	14.9 ± 0.6^{c}	5.0 ± 0.5^{c}	0.2 ± 0.1^{a}	4.1 ± 0.2^{c}

Mean ± standard deviation of three independent replicates for three donors. Different alphabetical letters in the same column are significantly different ($p \leq 0.05$). This table was derived using this equation $Y = Yo + A_1 e^{-x/t}$ Yo = Maximum gas production; t_1 = time (h); tau = half-life; k = exponential decay constant (derived from the equation). ND = not determined as fitting curve FOS indicates fructooligosaccharides; HMT, heat moisture treatment was at 20% moisture at 110 °C for 16 h; SA, stearic acid was added at 1.5% (w/w); SA + HMT, combination treatment.

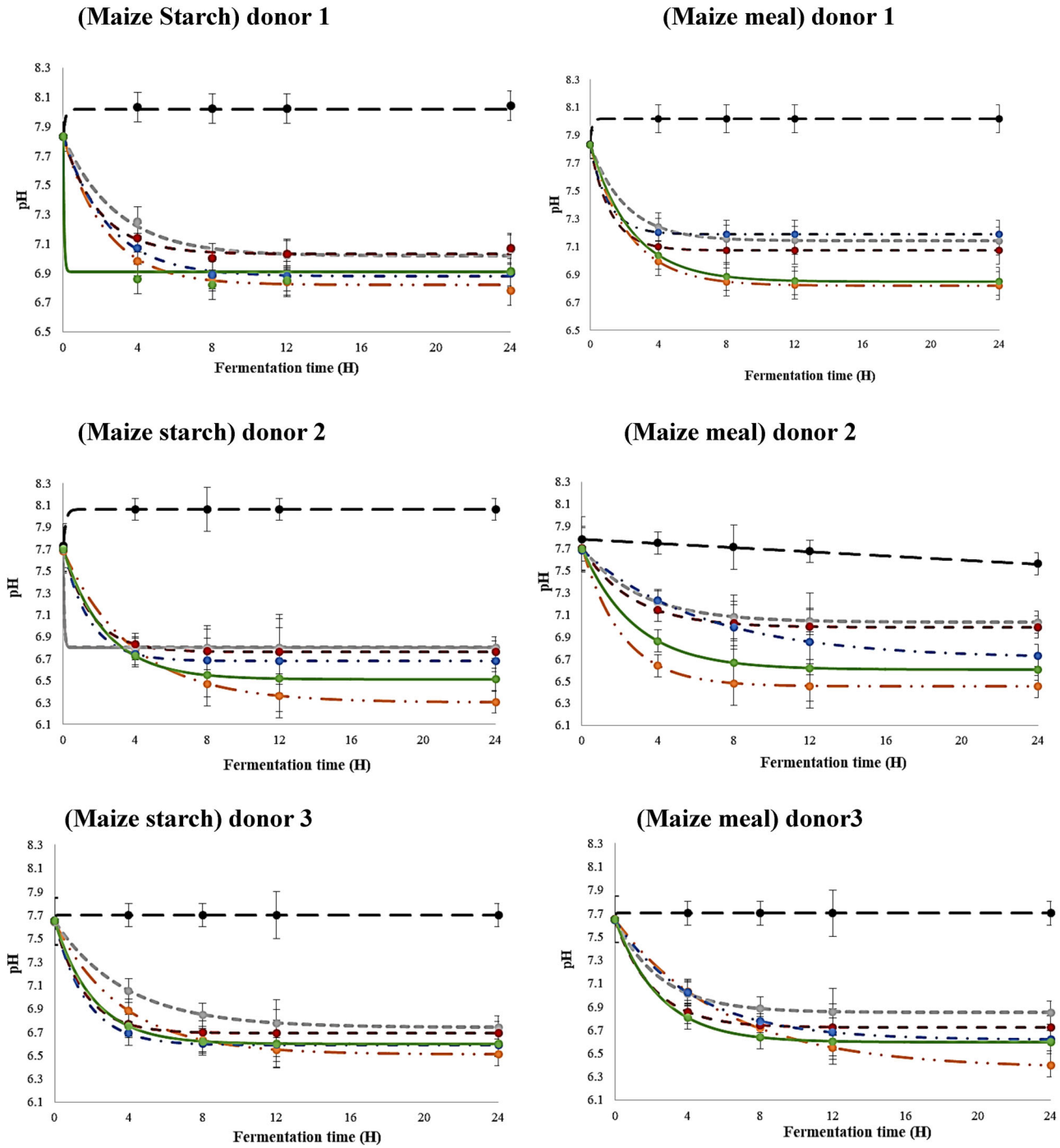

Figure 3. Effects of unhydrolyzed residue from unmodified and modified maize meal and maize starch on pH from the three donors during their 24 h in vitro fecal fermentation. Key: Blank black (— —), Control grey (————), SA red (– – –) HMT blue (– · – ·), SA + HMT green (————), FOS orange (— · · —). FOS indicates fructooligosaccharides; HMT, heat moisture treatment at 20% moisture at 110 °C for 16 h; SA, stearic acid was added at 1.5% (w/w); SA + HMT, combination treatment.

moisture treatment alone and their combinations in Table 1. The melting endotherms of the unhydrolyzed control maize starch had Tp (peak temperature) of 106.5 °C and ΔH as 1.98 J g^{-1} dry residues as observed from Table 1. Addition of stearic acid alone, heat-moisture treatment alone, and their combination showed one endothermic peak with Tp as 116.8, 101.1, and 110.4 °C, while their corresponding ΔH was 4.64, 9.12, and 10.43 J g^{-1} dry residues respectively (Table 1) compared to the melting endotherm of the control unhydrolyzed residues. Unhydrolyzed control maize meal showed Tp as 102.9 °C and its ΔH as 3.57 J g^{-1} (Table 1). The addition of stearic acid alone had Tp as 106.2 °C, and its ΔH as 8.05 J g^{-1}, heat-moisture treatment alone showed

two endothermic peaks with Tp as 100.1, 122.2 °C with their corresponding ΔH as 6.04 and 3.21 J g^{-1} respectively. The combination treatment had one endotherm with Tp as 111.5 °C and enthalpy of 10.64 J g^{-1} (Table 1) respectively as compared to the control. Endotherms with dissociation temperatures of about (98–105), (106–109), and (110–120) correspond to type I, type IIa, and type IIb amylose-lipid complexes (ALC).[24] Thus, the results showed that both types I, and IIa and IIb ALC were present in the unhydrolyzed residues. More ALC was formed when stearic acid was added to the pasted maize starch. Pasted maize starch treated with HMT showed type I, but maize meal showed type I and type IIb ALC as seen in Figure 1. The pasted maize starch and meal with stearic acid followed by HMT form more type IIa and IIb ALC, respectively (Figure 1). It is also noted that the indigestible residues of treated materials with stearic acid did not show any endotherm of the free fatty acid.

Production of gas from three donors during 24 h in vitro fecal fermentation from unhydrolyzed residues of modified and unmodified maize starch and maize meal are shown in **Figure 2**. There was an increase in the gas production of both maize starch and maize meal, as observed in Figure 2, from all three donors. The initial gas produced was 1 mL for all the donors (Figure 2); after 4 h, the production of gas increased significantly ($p \leq 0.05$) until it reached 8 h, where it plateaued until the end of the fecal fermentation as observed in Figure 2. The data were also fitted into equation $Y = \mathrm{Yo} + \mathrm{A_1}e^{-x/t1}$, to determine the reaction rate, half-life, and total gas produced with R^2 greater than 0.99 except for blank. The negligible fermentation of the blank was expected as there was no substrate for fermentation by the fecal microbes.

The addition of fecal slurry to FOS had seen a significant ($p < 0.05$) higher total gas production, but lower exponential decay constant, and higher half-life than indigestible residues from treated and untreated maize meal and maize starch observed in **Table 2**. This shows that FOS fermented faster than the indigestible residues, however, the final gas production was only 1 mL more than the indigestible residues. Comparing the indigestible residues of maize meal and maize starch, HMT with stearic acid seems to show the highest gas production in some donors of both maize meal and maize starch. Indigestible residues from maize starch appear to have an averagely higher gas production compared to maize meal. Donors 2 and 3 produced slightly higher gas than Donor 1.

Production of a large amount of gas in vivo can result in gastrointestinal discomfort, particularly in patients with visceral hypersensitivities, such as individuals with irritable bowel syndrome.[25–27] Though the treated RS samples were fully fermented, their fermentation was surprisingly rapid. This shows that α-amylases and glucoamylases were highly efficient at digesting the starch from ALCs that our enzymes could not. Some individuals may not be able to create equilibrium by flatulence when more gases are produced, which could lead to gastrointestinal intolerance.[28]

The blank fecal sample without any added substrate showed a slight increase in the mean pH value after the initial inoculum value for some samples and donors, though afterwards did not observe any change till the end of fermentation (**Figure 3**). Kaur et al.[29] also reported an increase in the pH of the blank by 0.1 units at the end of in vitro fecal fermentation. A general

Table 3. Effects of unhydrolyzed residue from unmodified and modified maize starch and maize meal on pH from three donors during their 24 h in vitro fecal fermentation.

Sample	Treatment	Donor 1				Donor 2				Donor 3			
		Yo	Time [h]	K [h^{-1}]	Tau [h]	Yo	Time [h]	K [h^{-1}]	Tau [h]	Yo	Time [h]	K [h^{-1}]	Tau [h]
Maize starch	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	7.07 ± 0.1^{c}	1.1 ± 0.3^{c}	0.91 ± 0.1^{d}	0.8 ± 0.2^{c}	6.76 ± 0.1^{d}	1.41 ± 0.9^{d}	0.71 ± 0.7^{d}	0.70 ± 0.7^{c}	6.80 ± 0.2^{c}	3.86 ± 0.5^{b}	0.26 ± 0.5^{c}	2.67 ± 0.5^{c}
	SA	6.99 ± 0.1^{c}	2.2 ± 1.2^{ab}	0.45 ± 0.2^{c}	1.0 ± 0.5^{ab}	6.73 ± 0.2^{d}	1.72 ± 0.4^{d}	0.58 ± 0.1^{c}	1.20 ± 0.3^{c}	6.72 ± 0.1^{c}	4.1 ± 0.2^{a}	0.24 ± 0.1^{c}	3.1 ± 0.1^{c}
	HMT	6.86 ± 0.2^{b}	3.0 ± 0.3^{b}	0.33 ± 0.1^{b}	1.7 ± 0.4^{bc}	6.66 ± 0.1^{c}	2.52 ± 0.5^{c}	0.39 ± 0.1^{b}	2.05 ± 0.4^{b}	6.59 ± 0.1^{b}	8.76 ± 0.1^{a}	0.11 ± 0.1^{b}	5.22 ± 0.1^{b}
	SA + HMT	6.82 ± 0.1^{b}	3.5 ± 0.4^{b}	0.29 ± 0.2^{b}	1.81 ± 0.3^{a}	6.50 ± 0.1^{b}	3.45 ± 0.9^{bc}	0.29 ± 0.2^{a}	3.1 ± 0.6^{a}	6.59 ± 0.1^{b}	8.08 ± 0.3^{a}	0.12 ± 0.1^{b}	5.44 ± 0.2^{b}
	FOS	6.79 ± 0.1^{a}	3.9 ± 0.2^{a}	0.26 ± 0.1^{a}	2.8 ± 0.1^{bc}	6.38 ± 0.1^{a}	4.33 ± 0.9^{c}	0.23 ± 0.1^{a}	3.31 ± 0.6^{a}	6.2 ± 0.1^{a}	10.6 ± 2.0^{c}	0.09 ± 0.1^{a}	8.08 ± 1.4^{a}
Maize meal	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	7.14 ± 0.1^{c}	1.40 ± 0.4^{a}	0.71 ± 0.1^{c}	0.82 ± 0.3^{c}	7.08 ± 0.1^{c}	1.28 ± 0.5^{c}	0.78 ± 0.1^{d}	1.58 ± 0.3^{a}	6.9 ± 0.1^{d}	2.61 ± 0.3^{c}	0.38 ± 0.1^{c}	2.51 ± 0.2^{c}
	SA	7.03 ± 0.1^{b}	2.77 ± 0.2^{a}	0.36 ± 0.1^{b}	1.82 ± 0.1^{b}	6.81 ± 0.2^{c}	1.80 ± 0.4^{c}	0.56 ± 0.1^{c}	2.25 ± 0.3^{ab}	6.71 ± 0.1^{c}	2.96 ± 0.3^{c}	0.33 ± 0.1^{c}	2.91 ± 0.2^{c}
	HMT	7.14 ± 0.1^{c}	1.41 ± 0.3^{a}	0.71 ± 0.1^{c}	0.67 ± 0.2^{c}	6.69 ± 0.1^{b}	2.45 ± 0.7^{b}	0.41 ± 0.1^{b}	3.16 ± 0.5^{c}	6.60 ± 0.1^{b}	4.92 ± 0.5^{b}	0.20 ± 0.1^{b}	3.72 ± 0.4^{b}
	SA + HMT	6.81 ± 0.1^{a}	3.80 ± 1.3^{a}	0.26 ± 0.1^{a}	2.90 ± 0.8^{a}	6.61 ± 0.1^{b}	2.80 ± 0.4^{b}	0.36 ± 0.1^{b}	3.94 ± 0.3^{ab}	6.60 ± 0.1^{b}	4.91 ± 0.4^{b}	0.20 ± 0.1^{b}	3.88 ± 0.3^{b}
	FOS	6.79 ± 0.1^{a}	3.85 ± 0.3^{a}	0.25 ± 0.1^{a}	2.91 ± 0.2^{a}	6.38 ± 0.1^{a}	3.39 ± 0.8^{a}	0.29 ± 0.1^{a}	4.35 ± 0.6^{b}	6.2 ± 0.1^{a}	6.2 ± 2.1^{a}	0.16 ± 0.1^{a}	5.52 ± 1.0^{a}

Mean ± standard deviation of three independent replicates for three donors. Different alphabetical letters in the same column are significantly different ($p \leq 0.05$) This table was derived from equation $Y = \mathrm{Yo} + \mathrm{A_1}e^{-x/t1}$Yo = minimum pH; t_1 = time (h); tau = time at halflife; k = exponential decay constant (derived from the equation). FOS indicates Fructooligosaccharides; HMT, heat moisture treatment at 20% moisture at 110 °C for 16 h; ND, not determined as fitting curve; SA, stearic acid added at 1.5% (w/w); SA + HMT, combination treatment.

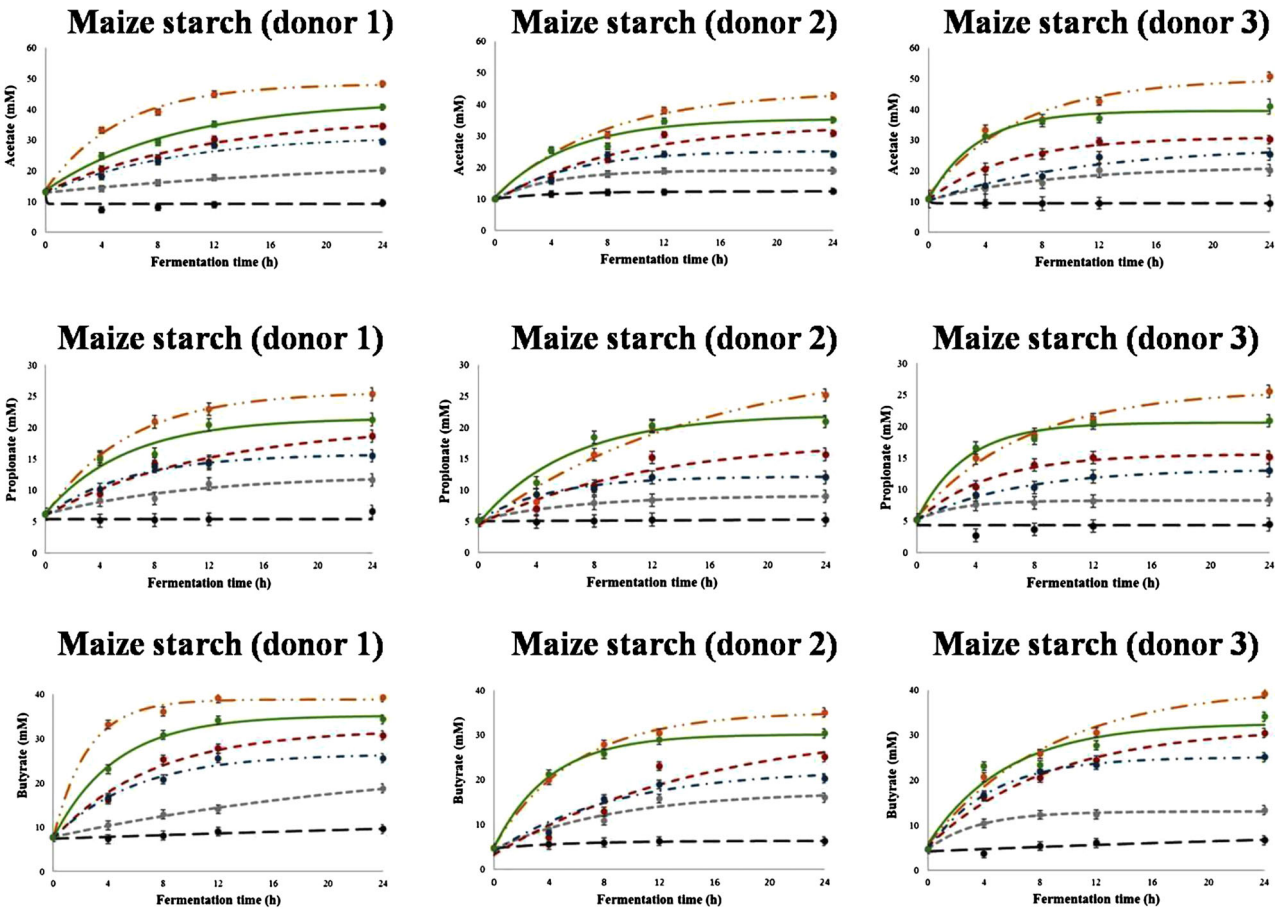

Figure 4. Effects of unhydrolyzed residues from unmodified and modified maize starch on SCFA production from the three donors during their 24 h in vitro fecal fermentation. Key: Blank black (— —), Control grey (————), FOS orange (– – –), SA red (– · – · –), HMT blue (————), SA + HMT green (— · · —), FOS indicates fructooligosaccharides; HMT, heat moisture treatment was at 20% moisture at 110 °C for 16 h; SA, stearic acid was added at 1.5% (w/w); SA + HMT, combination treatment.

reduction in the mean pH values was observed in all three donors for maize starch and maize meal treatments and FOS (Figure 3). Duncan et al.[30] also followed a similar trend when working on fermentable carbohydrates that produce SCFAs to reduce pH (**Table 3**).

Colonic pH value may be associated with reducing the risk of colonic cancer.[31] In African ethnic populations, a low colon-cancer risk has been associated with a low fecal pH.[31] A lower pH may reduce the number of pathogenic bacteria in the intestine without influencing the quantity of *Bifidobacteria*.[32] Low pH can also increase the absorption rate of minerals like calcium, magnesium, and sodium.[33,34] Reduction in the mean pH value also decreased the activity of co-carcinogenic enzymes such as glucuronidases, glycosidases, and 7a-hydroxylases.[35]

Figures 4 and **5** show the effect of unhydrolyzed residue from unmodified and modified maize starch and maize meal on SCFA production from the three donors during 24 h in vitro fecal fermentation. The data obtained were fitted into the equation with R^2 higher than 0.99 except for the blank since there was no substrate for human fecal microbiota to ferment. Unhydrolyzed maize meal can also contain non-starch polysaccharide and indigestible proteins and this can affect the fecal fermentation by reducing the SCFA values. However, it is also noted that the higher amount of SCFA was correlated with the higher amount of amylose lipid complexes (discussed later). FOS showed the increased production of acetate, propionate, and butyrate in all the three donors at the end of overall fermentation, as observed in Figure 4 and **Tables 4–6**.

Likely, acetate production was dominant and higher than propionate and butyrate in maize starch and maize meal (Figures 4 and 5 and Tables 4–6). Among the donors, the acetate production was significantly ($p < 0.05$) higher in Donor 1 compared to other donors. FOS resulted in significantly ($p < 0.05$) higher production of acetate, about 50% of the total SCFA compared to the indigestible residues of maize starch and meals in Table 4. FOS had the highest fermentation rate suggesting that the fermentation was as rapid with the maximum acetate production, lower K value and significantly higher tau value. K value signifies the fermentation rate of the substrates.

Regarding the maize starch and meal of indigestible residues, the combination treatment had a significant ($p < 0.05$) higher acetate production when compared to the stearic acid (SA) and heat-moisture treatment alone. The combination treatment also had a low K value but not as low as FOS, and even higher time

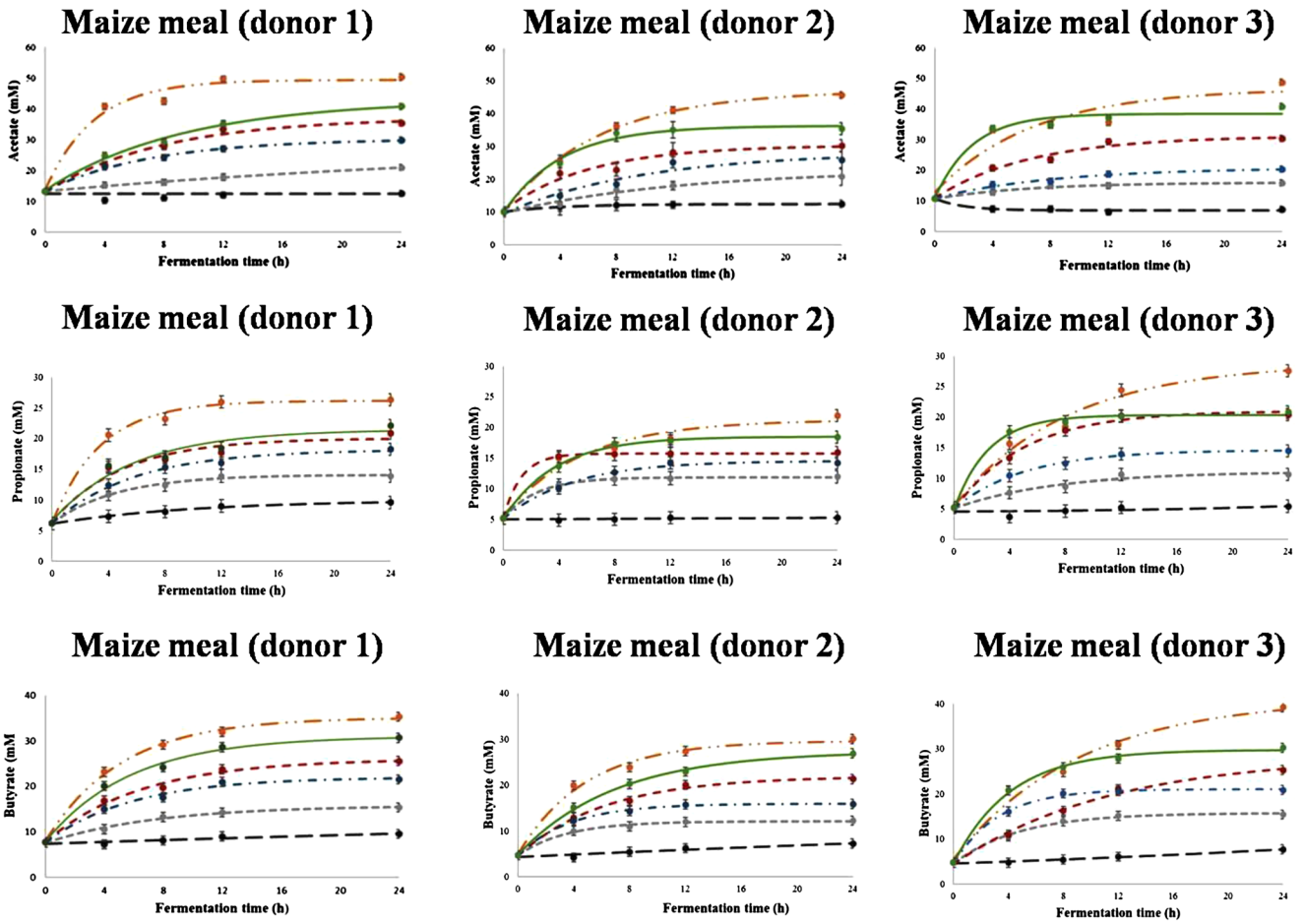

Figure 5. Effects of unhydrolyzed residues from unmodified and modified maize meal on SCFA production from the three donors during their 24 h in vitro fecal fermentation. Key: Blank black (— —), Control grey (————), FOS orange (– – –), HMT blue (– · – · –), SA red (————), SA + HMT green (— · · —) FOS indicates fructooligosaccharides; HMT, heat moisture treatment was at 20% moisture at 110 °C for 16 h; SA, stearic acid added at 1.5% (w/w); SA + HMT, combination treatment.

and higher tau in all the three donors (Table 4). The chemical structure and physical form of dietary fibers are the critical factors that determine the fermentation rate.[36] No significant difference was observed between the maize starch and meal in acetate production amongst all the substrates for donor 1. A synergistic effect was also found for combination treatment in acetate production from fermentation of maize starch and meal indigestible residues.

Production of propionate for modified and unmodified maize starch and maize meal is presented in Figures 4 and 5 and Table 5. FOS had the overall highest significant ($p < 0.05$) concentration in all the three donors compared to the indigestible residue. The indigestible residues of SA + HMT showed significantly ($p < 0.05$) higher production than the control in all donors in maize starch and maize meal treatments (Table 5). FOS had the highest time to reach the maximum propionate production with a lower K value and highest tau value, which was significantly different ($p < 0.05$) from the maize starch and undigested meal residues. Considering the indigestible residues, a combination of stearic acid and heat-moisture treatment resulted in the highest propionate production with corresponding higher time, low K value, and high tau value, significantly ($p < 0.05$) different from the addition of SA alone and HMT alone and the control for both maize starch and maize meal amongst the three donors (Table 5). The combination showed a synergistic effect regarding the maize starch and indigestible meal residues in all the donors.

Butyrate showed similar observations as acetate and propionate, graphically represented in Figures 4 and 5 and Table 6 for indigestible residues. Maize starch, maize meal, and FOS showed maximum production in all donors, followed by indigestible residues from SA + HMT. Indigestible residues from SA + HMT maize starch and meal resulted in increased butyrate production compared to the undigested residue of SA addition alone and HMT alone and residues from the control (Table 6). Butyrate produced by SA + HMT indigestible residue was almost similar to FOS, especially in Donors 2 and 3. This could be attributed to the fermentation rate of the combination treatment.[37]

From the above results (Figures 4 and 5) and (Table 4–6), the rate and amount of acetate, propionate, and butyrate production depend on both the treatments and the donors. The combination of stearic acid and heat-moisture treatment amongst all

Table 4. Effects of unhydrolyzed residue from unmodified and modified maize starch and maize meal on acetate production from three donors during their 24 h in vitro fecal fermentation.

Sample	Treatment	Donor 1				Donor 2				Donor 3			
		Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]
Maize starch	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	20.1 ± 0.2^{a}	5.0 ± 1.7^{a}	0.2 ± 0.03^{a}	3.9 ± 1.2^{a}	18.9 ± 0.9^{a}	3.0 ± 0.8^{a}	0.33 ± 0.1^{a}	2.4 ± 0.6^{a}	20.1 ± 1.1^{a}	2.9 ± 1.1^{a}	0.34 ± 0.03^{a}	2.2 ± 0.8^{a}
	SA	34.5 ± 0.2^{c}	10.4 ± 0.9^{c}	0.1 ± 0.02^{b}	6.1 ± 0.4^{c}	30.8 ± 2.9^{c}	6.4 ± 0.3^{c}	0.16 ± 0.1^{c}	4.4 ± 0.2^{c}	30.3 ± 1.0^{c}	6.0 ± 0.6^{c}	0.17 ± 0.05^{c}	6.7 ± 0.4^{c}
	HMT	29.4 ± 0.3^{b}	8.5 ± 2.4^{b}	0.1 ± 0.02^{b}	5.4 ± 0.6^{b}	24.2 ± 1.3^{b}	4.3 ± 1.6^{b}	0.23 ± 0.1^{b}	3.4 ± 1.1^{b}	25.4 ± 0.4^{b}	4.0 ± 1.0^{b}	0.25 ± 0.04^{b}	4.3 ± 0.7^{b}
	SA + HMT	$40.8 \pm 0.5^{d*}$	12.2 ± 2.8^{d}	0.08 ± 0.02^{c}	7.9 ± 1.7^{d}	$35.1 \pm 1.0^{d*}$	8.5 ± 0.4^{d}	0.1 ± 0.02^{d}	5.2 ± 0.2^{d}	$40.9 \pm 0.4^{d*}$	8.2 ± 0.5^{d}	0.12 ± 0.1^{d}	8.4 ± 1.0^{d}
	FOS	50.3 ± 0.4^{e}	13.7 ± 0.5^{e}	0.07 ± 0.01^{c}	10.8 ± 1.2^{e}	42.5 ± 1.8^{e}	10.8 ± 0.5^{e}	0.09 ± 0.1^{e}	6.1 ± 0.3^{d}	50.6 ± 0.4^{e}	10.0 ± 0.7^{e}	0.1 ± 0.02^{d}	9.0 ± 0.5^{e}
Maize meal	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	20.3 ± 1.7^{a}	5.5 ± 1.0^{a}	0.2 ± 0.2^{a}	1.7 ± 0.7^{a}	20.8 ± 0.6^{a}	2.5 ± 0.4^{a}	0.4 ± 0.1^{a}	1.7 ± 0.3^{a}	15.8 ± 0.2^{a}	2.8 ± 1.6^{a}	0.36 ± 0.1^{a}	2.3 ± 1.1^{a}
	SA	35.4 ± 0.5^{c}	8.6 ± 0.3^{c}	0.1 ± 0.1^{b}	5.1 ± 0.2^{c}	30.3 ± 0.6^{c}	5.1 ± 0.2^{c}	0.2 ± 0.2^{c}	4.1 ± 0.2^{c}	30.3 ± 0.2^{c}	6.3 ± 1.0^{c}	0.16 ± 0.2^{c}	5.9 ± 1.1^{c}
	HMT	29.8 ± 0.6^{b}	7.2 ± 0.3^{b}	0.1 ± 0.1^{b}	3.2 ± 0.2^{b}	25.8 ± 0.4^{b}	4.0 ± 0.8^{b}	0.25 ± 0.3^{b}	2.8 ± 0.5^{b}	20.3 ± 1.0^{b}	$4.3. \pm 1.1^{b}$	0.23 ± 0.1^{b}	3.5 ± 0.8^{b}
	SA + HMT	$40.8 \pm 0.5^{d*}$	10.9 ± 1.4^{d}	0.09 ± 0.1^{d}	7.1 ± 1.0^{d}	$35.4 \pm 1.1^{d*}$	7.2 ± 0.7^{d}	0.13 ± 0.1^{d}	5.9 ± 0.9^{d}	$40.8 \pm 1.0^{d*}$	8.1 ± 0.3^{d}	0.12 ± 0.1^{d}	6.9 ± 0.9^{d}
	FOS	50.5 ± 0.8^{bc}	13.3 ± 2.0^{e}	0.07 ± 0.1^{e}	9.0 ± 1.4^{e}	45.6 ± 1.9^{e}	9.7 ± 0.3^{e}	0.1 ± 0.1^{e}	8.2 ± 0.2^{e}	49.6 ± 1.0^{e}	11.8 ± 0.7^{e}	0.08 ± 0.1^{e}	9.5 ± 0.5^{e}

Mean ± standard deviation of three independent replicates for three donors. Different alphabetical letters in the same column are significantly different ($p \leq 0.05$). This table was derived using this equation $Y = Yo + A1e^{-x/t1}$; Yo = maximum acetate; t1 = time to reach maximum; tau = time at half-life; k = exponential decay constant (derived from the equation); ND = not determined as non-fitting curve. FOS indicates fructooligosaccharides; HMT, heat moisture treatment at 20% moisture at 110 °C for 16 h; SA, stearic acid at 1.5% (w/w); SA+HMT, combination treatment; *, synergistic effect.

Table 5. Effects of unhydrolyzed residue from unmodified and modified maize starch and maize meal on propionate production from three donors during their 24 h in vitro fecal fermentation.

Sample	Treatment	Donor 1				Donor 2				Donor 3			
		Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]
Maize starch	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	11.2 ± 4.3^{a}	3.0 ± 0.3^{a}	0.33 ± 0.1^{a}	4.7 ± 0.3^{a}	8.9 ± 1.0^{a}	5.1 ± 1.5^{a}	0.2 ± 0.1^{a}	3.7 ± 1.1^{a}	8.4 ± 1.0^{a}	3.1 ± 1.7^{a}	0.32 ± 0.1^{a}	2.6 ± 0.3^{a}
	SA	20.9 ± 0.2^{bc}	5.5 ± 0.2^{c}	0.18 ± 0.1^{c}	7.3 ± 0.2^{c}	15.7 ± 1.1^{c}	8.8 ± 1.8^{c}	0.11 ± 0.1^{c}	6.4 ± 1.0^{c}	15.1 ± 1.1^{c}	5.3 ± 0.9^{c}	0.19 ± 0.2^{b}	4.5 ± 1.3^{c}
	HMT	18.2 ± 0.3^{b}	4.5 ± 0.2^{b}	0.22 ± 0.1^{b}	5.8 ± 0.2^{b}	12.1 ± 1.1^{b}	6.5 ± 1.2^{b}	0.15 ± 0.1^{b}	4.5 ± 0.5^{b}	12.9 ± 2.3^{b}	4.9 ± 0.3^{b}	0.20 ± 0.1^{b}	3.2 ± 0.2^{b}
	SA + HMT	22.4 ± 1.0^{c}	6.7 ± 0.4^{c}	0.14 ± 0.1^{d}	9.8 ± 0.4^{d}	20.9 ± 1.2^{d}	10.5 ± 1.1^{d}	0.1 ± 0.1^{d}	8.5 ± 0.8^{d}	20.9 ± 1.8^{d}	6.0 ± 0.6^{d}	0.7 ± 0.1^{c}	6.2 ± 0.2^{d}
	FOS	25.3 ± 0.8^{d}	12.8 ± 1.2^{d}	0.08 ± 0.1^{e}	11.3 ± 0.1^{e}	25.4 ± 1.0^{e}	12.2 ± 1.6^{e}	0.08 ± 0.1^{e}	10.2 ± 1.1^{e}	25.5 ± 1.9^{e}	8.5 ± 1.3^{e}	0.1 ± 0.1^{d}	8.1 ± 0.3^{e}
Maize meal	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	12.2 ± 0.7^{a}	2.5 ± 0.3^{a}	0.4 ± 0.1^{a}	1.8 ± 0.2^{a}	11.6 ± 1.0^{a}	3.1 ± 0.9^{a}	0.32 ± 0.2^{a}	2.5 ± 2.0^{a}	10.6 ± 1.8^{a}	2.5 ± 0.6^{a}	0.4 ± 0.1^{a}	1.8 ± 2.3^{a}
	SA	18.4 ± 1.0^{c}	4.7 ± 0.6^{c}	0.21 ± 0.2^{c}	4.6 ± 0.4^{c}	15.9 ± 1.0^{b}	5.8 ± 0.5^{b}	0.17 ± 0.1^{b}	5.0 ± 0.4^{b}	18.4 ± 1.4^{c}	6.9 ± 0.5^{c}	0.14 ± 0.1^{c}	5.4 ± 1.4^{c}
	HMT	15.5 ± 0.5^{b}	3.5 ± 0.9^{b}	0.29 ± 0.1^{b}	3.4 ± 0.2^{b}	14.2 ± 0.5^{b}	5.4 ± 1.2^{b}	0.18 ± 0.1^{b}	4.5 ± 0.8^{b}	14.4 ± 1.5^{b}	4.2 ± 0.6^{b}	0.24 ± 0.1^{b}	3.0 ± 1.2^{b}
	SA + HMT	21.1 ± 1.2^{d}	6.0 ± 0.8^{c}	0.17 ± 0.1^{d}	5.3 ± 0.6^{d}	20.9 ± 1.0^{c}	7.4 ± 0.9^{c}	0.14 ± 0.1^{c}	6.0 ± 0.6^{c}	20.9 ± 1.5^{c}	7.7 ± 0.5^{c}	0.13 ± 0.1^{c}	5.8 ± 1.8^{c}
	FOS	25.2 ± 0.4^{e}	12.6 ± 0.2^{d}	0.08 ± 0.1^{e}	6.4 ± 0.3^{e}	25.2 ± 0.8^{d}	11.6 ± 3.6^{d}	0.09 ± 0.1^{d}	7.9 ± 0.5^{b}	27.6 ± 1.8^{d}	9.6 ± 0.5^{d}	0.1 ± 0.1^{d}	7.7 ± 1.7^{d}

Mean ± standard deviation of three independent replicates for three donors. Different alphabetical letters in the same column are significantly different ($p \leq 0.05$). This table was derived using equation $Y = Yo + A_1e^{-x/t1}$; Yo = maximum propionate; t_1 = time to reach maximum (h); tau = time at half-life; K = exponential decay constant (h^{-1}), ND = not determined as non-fitting of curve. FOS indicates fructooligosaccharides; HMT, heat moisture treatment was at 20% moisture at 110 °C for 16 h; SA, stearic acid was added at 1.5% (w/w); SA + HMT, combination treatment.

Table 6. Effects of unhydrolyzed residue from unmodified and modified maize starch and maize meal on butyrate production from three donors during their 24 h in vitro fecal fermentation.

Sample	Treatment	Donor 1				Donor 2				Donor 3			
		Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]	Yo [mM]	Time [h]	K [h^{-1}]	Tau [h]
Maize starch	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	15.8 ± 0.3^{a}	3.1 ± 0.4^{a}	0.32 ± 0.1^{a}	2.3 ± 1.7^{a}	13.9 ± 1.1^{a}	3.1 ± 2.3^{a}	0.32 ± 0.1^{a}	1.5 ± 1.6^{a}	13.3 ± 0.5^{a}	3.4 ± 0.1^{a}	0.29 ± 0.1^{a}	1.5 ± 0.8^{a}
	SA	25.7 ± 0.5^{b}	5.8 ± 0.1^{c}	0.17 ± 0.1^{c}	4.4 ± 0.8^{c}	25.1 ± 1.0^{c}	7.0 ± 1.1^{c}	0.14 ± 0.1^{c}	5.0 ± 0.8^{c}	30.4 ± 1.0^{c}	5.7 ± 0.6^{c}	0.18 ± 0.1^{c}	3.6 ± 1.1^{c}
	HMT	20.6 ± 0.8^{c}	4.1 ± 0.8^{b}	0.24 ± 0.1^{b}	3.4 ± 1.9^{b}	18.3 ± 0.4^{b}	5.6 ± 0.2^{b}	0.18 ± 0.1^{b}	3.9 ± 1.6^{b}	25.1 ± 0.8^{b}	4.2 ± 0.4^{b}	0.24 ± 0.1^{b}	2.5 ± 0.3^{b}
	SA + HMT	35.2 ± 1.0^{c*}	7.9 ± 0.6^{d}	0.12 ± 0.1^{d}	6.6 ± 0.4^{d}	30.3 ± 0.2^{d*}	8.7 ± 0.2^{d}	0.11 ± 0.1^{d}	6.6 ± 0.8^{d}	35.1 ± 1.0^{d*}	7.8 ± 2.0^{d}	0.13 ± 0.2^{d}	6.6 ± 1.4^{d}
	FOS	40.3 ± 0.4^{d}	10.7 ± 0.6^{e}	0.09 ± 0.1^{e}	7.3 ± 0.4^{e}	35.0 ± 0.9^{e}	10.8 ± 0.5^{e}	0.09 ± 0.1^{e}	8.5 ± 1.7^{e}	39.2 ± 1.0^{e}	10.8 ± 1.2^{e}	0.09 ± 0.1^{e}	7.5 ± 0.9^{e}
Maize meal	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control	12.3 ± 1.1^{a}	2.3 ± 0.3^{a}	0.43 ± 0.2^{a}	1.2 ± 1.6^{a}	12.2 ± 0.6^{a}	2.0 ± 3.1^{a}	0.50 ± 0.1^{a}	2.9 ± 0.1^{a}	15.4 ± 0.4^{a}	3.1 ± 0.2^{a}	0.32 ± 0.1^{a}	2.1 ± 0.3^{a}
	SA	25.6 ± 0.3^{c}	6.0 ± 0.2^{c}	0.17 ± 0.1^{c}	3.5 ± 0.2^{c}	21.3 ± 1.0^{c}	5.0 ± 0.9^{b}	0.2 ± 0.1^{c}	6.0 ± 0.6^{c}	25.2 ± 0.9^{c}	6.3 ± 0.9^{c}	0.16 ± 0.1^{c}	5.3 ± 0.6^{c}
	HMT	18.6 ± 0.7^{b}	4.0 ± 0.7^{b}	0.25 ± 0.1^{b}	2.8 ± 0.8^{b}	16.9 ± 1.0^{b}	3.7 ± 0.3^{b}	0.27 ± 0.1^{b}	4.4 ± 0.2^{b}	20.8 ± 1.6^{b}	4.1 ± 1.0^{b}	0.24 ± 0.1^{b}	3.3 ± 1.2^{b}
	SA + HMT	30.7 ± 1.0^{d*}	8.5 ± 0.6^{d}	0.11 ± 0.1^{d}	7.9 ± 1.8^{d}	26.9 ± 1.1^{d*}	7.5 ± 0.3^{d}	0.13 ± 0.1^{d}	8.2 ± 0.3^{d}	30.2 ± 2.6^{d*}	7.6 ± 0.9^{d}	0.13 ± 0.1^{d}	6.2 ± 0.6^{d}
	FOS	35.3 ± 1.0^{e}	10.2 ± 1.1^{e}	0.1 ± 0.1^{d}	9.7 ± 1.1^{e}	35.0 ± 0.4^{e}	12.7 ± 0.6^{e}	0.08 ± 0.1^{e}	10.5 ± 0.5^{e}	39.2 ± 0.9^{d}	10.2 ± 1.0^{e}	0.09 ± 0.1^{e}	8.6 ± 0.7^{e}

Mean ± standard deviation of three independent replicates for three donors. Different alphabetical letters in the same column are significantly different ($p \leq 0.05$). This table was derived using this equation $Y = Yo + A_1 e^{-x/t1}$; Yo = maximum butyrate; t_1 = time to reach maximum (h); tau = time at half-life; k = exponential decay constant (derived from the equation), ND = not determined as non-fitting curve. FOS indicates fructooligosaccharides; HMT, heat moisture treatment was at 20% moisture at 110 °C for 16 h; SA, stearic acid was added at 1.5% (w/w); SA+HMT, combination treatment; *, synergistic effect.

the donors in acetate and butyrate showed a synergistic effect (Tables 4 and 6) in regards to the indigestible residues of maize starch and meal. This suggests that the indigestible residues from treated and untreated maize meal and maize starch served as suitable substrates for in vitro fermentation by human feces. Results from literature are in sync with the current research for other resistant starches,[38] using in vitro fermentation system with human feces as inoculum, resulting in total SCFA production of 7.6 and 7.7 mM g^{-1} organic matter with substrates such as retrograded tapioca starch and retrograded maize starch (i.e., RS3).

In a study by Kalmokoff et al.,[39] the use of high amylose maize starch (RS3) feedings in rats showed increased propionate, acetate, and butyrate, while Sarbini and Rastall[40] showed an increase in the quantity of propionate with high amylose maize starch (RS2). Besides, the production of organic acids, gas, and enzymes has also been used as markers to monitor the stimulation of bacterial activity.[41] Gas production had a positive correlation with SCFA production (**Tables 7** and **8**) and (Figures 2, 4, and 5). While gas production is inherent in the fermentation process, which results in SCFA production, it is also responsible for the adverse effects of prebiotics in humans (i.e., abdominal discomfort). Therefore, the ratio of total SCFA production to gas output may be used to estimate tolerability when given in vivo. The fermentation of the indigestible residues produced these gases,[41] which are used by the microbial community to help produce SCFA. For instance, Bacteroidetes are part of a community, stabilized by mutual cross-feeding, where other community members consume these gasses. For example, Archaea produces CH_4 from CO_2 and H_2, while acetogens convert CO_2 into acetate.

Significantly ($p < 0.05$) high concentrations of acetate followed by butyrate were observed in fermentation end products of the indigestible residues of maize starch and maize meal compared to propionate (Tables 4–6). Low propionate production could be due to non-functional propionyl-CoA carboxylase; this makes the gut microbiota (e.g., *Prevotella* spp.) to produce minimal propionate concentration.[42] This estimated the propionate production rate as lower than the reported acetate and butyrate production.

Concerning the indigestible residues of maize starch and meal, the combination treatment (SA + HMT) of maize starch and maize meal had the lowest K value and showed a slow fermentation rate for all the donors. This suggests that the complex structure formed from heat-moisture treatment combined with stearic acid was slowly fermented by saccharolytic bacteria in the feces. This complex structure is most likely to be mostly amylose-lipid complexes, as shown by DSC (Figure 1).

In general, the overall effect of the SA + HMT of both maize starch and meal had the higher production of SCFAs, since increased production of SCFAs has a positive impact on our system. This slow but ultimately high fermentation of the ALCs could be beneficial since the metabolites produced are evenly distributed and contribute to the energy requirement of the entire colon.[23,43] Our findings corroborate with work done by Goñi-Urriza et al.[44] who reported that fermentability was slow for retrograded starch samples (RS3). They suggested that the fermentation rate of RS3 was associated with the crystallinity level and crystalline type. Wang et al.[45] also reported that B-type polymorph of potato and high-amylose maize starch (RS2) also

Table 7. Correlation coefficient between gas, pH, and short chain fatty acid production of modified and unmodified maize starch.

Main effects	ΔH	Gas			pH			Acetate			Propionate			Butyrate		
		Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
ΔH		1.0**	1.0**	0.89	−1.0**	−0.89	−1.0**	0.75	0.64	0.72	0.73	0.69	0.78	0.71	0.63	0.75
Gas D1	1.0**		1.0**	0.91	−1.0**	−0.90	−1.0**	0.79	0.69	0.76	0.77	0.73	0.81	0.75	0.68	0.78
Gas D2	1.0**	1.0**		0.90	−1.0**	−0.86	−1.0**	0.75	0.65	0.71	0.76	0.76	0.77	0.70	0.63	0.76
Gas D3	0.89	0.91	0.90		−0.89	−0.78	−0.88	0.94	0.88	0.86	0.96*	0.86	0.91	0.85	0.86	0.96*
pH D1	−1.0**	−1.0**	−1.0**	−0.89		0.89	0.99*	−0.75	−0.65	−0.73	−0.74	−0.70	−0.78	−0.71	−0.64	−0.75
pH D2	−0.88	−0.90	−0.86	−0.79	0.89		0.81	−0.79	−0.74	−0.88	−0.68	−0.84	−0.88	−0.86	−0.76	−0.74
pH D3	−1.0**	−1.0**	−1.0**	−0.88	0.99*	0.81		−0.70	−0.58	−0.64	−0.72	−0.62	−0.71	−0.63	−0.56	−0.72
Ace D1	0.75	0.79	0.75	0.94	−0.75	−0.79	−0.70		0.99*	0.96*	0.97*	0.98*	0.98*	0.97*	0.98*	0.99*
Ace D2	0.64	0.69	0.65	0.88	−0.65	−0.74	−0.58	0.99*		0.97*	0.95	0.98*	0.97*	0.97*	1.0**	0.97*
Ace D3	0.72	0.76	0.71	0.86	−0.73	−0.88	−0.64	0.96*	0.97*		0.88	1.0**	0.99*	1.0**	0.98*	0.92
Prop D1	0.72	0.77	0.76	0.96*	−0.74	−0.68	−0.72	0.97*	0.95	0.88		0.90	0.92	0.88	0.93	0.99*
Prop D2	0.69	0.73	0.76	0.86	−0.70	−0.84	−0.62	0.98*	0.98*	1.0**	0.90		0.99*	1.0**	0.99*	0.94
Prop D3	0.78	0.81	0.77	0.91	−0.78	−0.88	−0.71	0.98*	0.97*	0.99**	0.92	0.99*		0.99*	0.98*	0.96*
But D1	0.71	0.75	0.70	0.85	−0.71	−0.86	−0.63	0.97*	0.97*	1.0**	0.88	1.0**	0.99*		0.98*	0.93
But D2	0.63	0.68	0.63	0.86	−0.64	−0.76	−0.56	0.98*	1.0**	0.98*	0.93	0.99*	0.98*	0.98*		0.96*
But D3	0.75	0.78	0.76	0.96*	−0.75	−0.74	−0.72	0.99**	0.97*	0.92	0.99*	0.94	0.96*	0.93	0.96*	

** indicates significant with $p \leq 0.001$; *, significant with $p \leq 0.05$. ΔH indicates Delta H; Gas D1 D3, Gas donor 1, Donor 2 and Donor 3; pH D1 D3, pH Donor 1, Donor 2 and Donor 3; Ace D1 – D3, Acetate Donor 1, Donor 2 and Donor 3; Prop D1 – D3, Propionate Donor1. Donor 2 and Donor 3; But D1 – D3, Butyrate Donor1, Donor 2 and Donor 3.

Table 8. Correlation coefficient between gas, pH, and short chain fatty acid production of modified and unmodified maize meal.

Main effects	ΔH	Gas			pH			Acetate			Propionate			Butyrate		
		Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
ΔH		0.97*	0.87	0.90	−0.92	−0.83	−0.74	0.99*	0.99**	0.98*	0.99**	0.98*	0.99*	0.99**	0.99**	0.99**
Gas D1	0.97*		0.84	0.97*	−0.87	−0.93	−0.86	0.97*	0.96*	0.92	0.99*	0.98*	0.99	0.94	0.96*	0.96*
Gas D2	0.87	0.84		0.73	−0.99*	−0.59	−0.46	0.81	0.87	0.93	0.84	0.94	0.82	0.84	0.88	0.86
Gas D3	0.90	0.97*	0.73		−0.76	−0.98*	−0.94	0.92	0.89	0.82	0.88	0.90	0.86	0.86	0.89	0.89
pH D1	−0.92	−0.87	−0.99*	−0.76		0.63	0.50	−0.87	−0.91	−0.96*	−0.89	−0.96*	−0.87	−0.89	−0.93	0.90
pH D2	−0.83	−0.93	−0.59	−0.98*	0.63		0.99*	−0.87	−0.82	−0.73	−0.82	−0.81	−0.80	−0.80	−0.81	−0.83
pH D3	−0.73	−0.86	−0.46	−0.94	0.50	0.99*		−0.80	−0.73	−0.62	−0.74	−0.71	−0.72	−0.71	−0.72	−0.75
Ace D1	0.99*	0.97*	0.81	0.92	−0.87	−0.87	−0.80		0.99*	0.96*	1.0**	0.96*	0.99*	0.99*	0.99*	1.0**
Ace D2	0.99**	0.96*	0.87	0.89	−0.91	−0.82	−0.73	0.99*		0.99*	1.0**	0.98*	0.99*	1.0**	1.0**	1.0**
Ace D3	0.98*	0.92	0.93	0.82	−0.96*	−0.73	−0.62	0.96*	0.99*		0.98*	0.98*	0.97*	0.98*	0.99*	0.98*
Prop D1	0.99**	0.99*	0.84	0.88	−0.89	−0.82	−0.74	1.0**	1.0**	0.98*		0.97*	1.0**	1.0**	1.0**	1.0**
Prop D2	0.98*	0.98*	0.94	0.90	−0.96*	−0.81	−0.71	0.97*	0.98*	0.98*	0.97*		0.95	0.96*	0.98*	0.98*
Prop D3	0.99*	0.99	0.82	0.86	−0.87	−0.80	−0.72	0.99*	0.99*	0.97*	1.0**	0.95		1.0**	0.99*	0.99*
But D1	0.99**	0.94	0.84	0.86	−0.89	−0.80	−0.71	0.99*	1.0**	0.98*	1.0**	0.96	1.0**		0.99*	1.0**
But D2	0.99**	0.96*	0.88	0.89	−0.93	−0.81	−0.72	0.99*	1.0**	0.99*	1.0**	0.98*	0.99*	0.99*		1.0**
But D3	0.99**	0.96*	0.86	0.89	0.90	−0.83	−0.75	1.0**	1.0**	0.98*	1.0**	0.98*	0.99*	1.0**	1.0**	

** indicates significant with $p \leq 0.001$; *, significant with $p \leq 0.05$. ΔH indicates Delta H; Gas D1 D3, Gas donor 1, Donor2 and Donor 3; pH D1 – D3, pH Donor 1, Donor 2 and Donor 3; Ace D1 – D3, AcetateDonor 1, Donor 2 and Donor 3; Prop D1 – D3, Propionate Donor1. Donor 2 andDonor 3; But D1 – D3, Butyrate Donor1, Donor 2 and Donor 3.

showed slow and completely fermentable within a 24 h fermentation period. There is little work on the effects of ALC on fecal fermentation. In the present study, there was a significant ($p < 0.05$) increase in total SCFAs, acetate, and butyrate production for indigestible residues of both maize starch and maize meal during fermentation of fecal inocula from all three donors. These changes show high fermentability and SCFA production by human fecal inocula of the RS type 5, particularly indigestible residues rich in ALC. The molar ratios obtained for all the substrates confirmed the findings that, in three donors, acetate is by far the dominant SCFA produced during fermentation. Notably, combination treatment (SA + HMT) of maize starch and meal increased the butyrate concentrations in donors 2 and 3, similar to the butyrogenic FOS used as control.

Tables 7 and 8 show the correlation coefficient between the melting endotherm, gases, pH, and SCFA of both modified and unmodified maize starch and maize meal. There was a positive correlation between the melting endotherm, gas production, and the SCFA but a negative correlation with the pH for both maize starch and maize meal. pH also showed a higher negative correlation with gas and SCFA. This means that the higher melting endotherms as amylose-lipid complexes are partly responsible for increased production of SCFA.

3. Conclusion

Upper GIT indigestible residues from maize meal and maize starch modified with HMT and SA alone and in a combination of SA + HMT were shown to probably function as prebiotics, like FOS, due to the production of SCFA during in vitro fecal fermentation. Acetate and butyrate appear to be the dominant SCFAs fermentation metabolites of these indigestible residues. Hence, resistant starch type 5 (ALC), the most abundant RS in the residues from treated HMT + SA treated maize meal and maize starch could potentially promote health benefits for different metabolic conditions. However, further, studies are required to elucidate the potential of RS 5 as a substrate in in vivo studies, the microbiome analyses, and its mechanism in the gut needs to be assessed.

4. Experimental Section

Materials: Commercially available super fine sized maize meal was purchased from the local supermarket (Pretoria, South Africa), which contained 15% moisture, total starch of about 81%, ash content of 0.65%, fat content of 1.1%, and dietary fiber of 4.7%. Commercial maize starch, Amyral with 12.9% moisture, total starch of 95%, ash content of 0.08%, fat content of 0.31%, and dietary fiber of 0.9% was obtained from TongaatHulett Starch (Edenvale, South Africa) [data were on dry basis except moisture]. Stearic acid with CAS number 57–11-4 was purchased from Sigma–Aldrich (St. Louis, MO, USA) and all other analytical grade reagents from Merck Chemicals (Bremen, Germany).

Methods: *Modification of Maize Starch and Maize Meal*: Stearic acid (1.5% w/w in absolute ethanol) was added to 100 g of each sample, followed by the procedure of D'Silva et al.[46] The mixture was incubated in the shaking water bath at 50 °C for 30 min with a speed of 120 rpm. After the incubation period, the mixture was then dried in a hot air oven at 40 °C to evaporate the excess solvent, and residues were stored at 4 °C for further analysis.

The maize starch and maize meal were mixed with deionized water to give the desired moisture content of 20% for heat-moisture treatment. The starch slurry was heated above the glass transition temperature, but below the gelatinization temperature, thus a temperature of 110 °C for 16 h[47,48] in a hot air oven, and the treated residues were used for further studies. The samples (maize meal and maize starch) were also treated with stearic acid and followed by heat-moisture treatment for testing their effect on GIT microbiota.

Upper GIT Enzyme Hydrolysis: The Goni et al.[49] method was followed with slight modification. Fifty milligrams of treated residues sample was used for the upper GIT analysis. Boiling water (1 mL) was added to each sample for dispersion, followed by the addition of 10 mL HCl–KCl buffer (pH 1.5) and 0.2 mL of a solution containing 1 mg of pepsin (Sigma–Aldrich P7000-100G). The solutions samples were incubated at 40 °C for 60 min with constant agitation. After the incubation period, 10 mL of Tris-maleate buffer (pH 6.9) was added to the solutions and adjusted to 25 mL. An aliquot of 0.1 mL was taken for 0 min before the addition of 5 mL Tris-maleate buffer (pH 6.9) containing 2.6I U of pancreatic α-amylase with the activity of 19.6 units mg^{-1} (Sigma–Aldrich A-3176) followed by incubation at 37 °C with a constant shaking water bath. Aliquots of 0.1 mL were taken during the incubation period at different time intervals of 5, 30, 60, 90, 120, and 180 min. The tubes containing the solutions were placed in boiling water for 15 min to inactivate the α-amylase. Then, 1 mL of 0.4 M sodium–acetate buffer (pH 4.75) and 90 µL of amyloglucosidase with an activity of 64.7 U mg^{-1} (Megazyme E-AMGDF Bray, Ireland) was added into the tubes and incubated at 60 °C for 45 min. After incubation, the tubes containing solutions were analyzed in a UV–vis spectrophotometer at 510 nm using glucose to create the standard graph.

Differential Scanning Calorimeter (DSC) Analyses on Undigested Residues: Thermal properties of unhydrolyzed maize starch/meal samples were analyzed using a differential scanning calorimetry (DSC) system (HP DSC827e, Mettler Toledo, Greifensee, Switzerland) as described by Wokadala et al.[50] Undigested residues and untreated samples (control) (10 mg) were weighed in aluminum pans and thoroughly mixed with distilled water at a ratio of 1:3 (w/w) starch-to-water to make a homogeneous slurry. The pans were sealed and equilibrated for 24 h at room temperature before scanning. Scanning was done from 25 to 140 °C under high pressure (4 MPa using N_2) at a rate of 10 °C min^{-1}. The Indium (Tp = 156.6 °C, 28.45 J g^{-1}) was used as an internal standard to calibrate the pan, and an empty pan was the reference.

In vitro Fecal Fermentation: Batch fecal fermentation was performed according to the methodology of (Lebetet al.[51]; Rose et al.[52]). Fifty milligrams (50 mg) of each substrate (indigestible residue from modified and unmodified maize starch and maize meal—Section 4.2.1) was weighed in three test tubes for triplicate analysis. Fecal samples were obtained from three healthy volunteers (age 35–40) who fed on unspecified and varied diets and had not taken any antibiotics for the past 6 months. Fecal samples were collected in plastic bags that were sealed after removing the air and immediately placed inside the anaerobic chamber (10% H_2, 5% CO_2, and 85% N_2; BactronEZ, SHEL LAB, Cornelius, OR, USA), where all further procedures were performed within 2 h after collection. Fecal samples of each individual donor were used separately. The slurry was prepared by homogenization with carbonate-phosphate buffer (pH 6.8 ± 0.1) in a ratio of 1:3 (w/v) and further strained through four layers of cheesecloth. The samples (modified and unmodified maize starch and maize meal) and controls (fructooligosaccharides and blank) were hydrated with 4 mL of carbonate-phosphate buffer (pH 6.8 ± 0.1) and inoculated with 1 mL of the fecal filtrate. The tubes were sealed and incubated at 37 °C in an incubation chamber. The following was then analyzed at specific times.

Gas Production: At the incubation period intervals of 4, 8, 12, and 24 h of fermentation, assigned tubes were removed from the incubation chamber, and gas was measured using a plunger displacement of a syringe.

pH: The pH of the fecal inoculated samples was measured simultaneously during fermentation (0, 4, 8, 12, and 24 h) using a pH meter (Mettler Toledo, Columbus, OH, USA).

Short-Chain Fatty Acids (SCFAs): Aliquots (1 mL) were taken from each substrate inoculated feces fermented media for SCFA analysis using a gas chromatograph with a flame ionization detector (GC-FID). The samples were thawed and centrifuged at 13000 × *g* for 10 min. Aliquots (400 µL) from fermented supernatant samples were combined with 100 µL of

a mixture containing 50 mM 4-methyl-valeric acid No. 277827–5 G, Sigma-Aldrich Inc., St. Louis, MO, USA), meta-phosphoric acid (5%), and copper sulfate (1.56 mg mL^{-1}) used as an internal standard for SCFA analysis. The mixture was centrifuged at 1300 × *g* for 10 min. An aliquot of 0.2 µL was injected into a GC-FID (7890 A, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a fused silica capillary column (NukolTM, Supelco No. 40369-03 A, Bellefonte, PA, USA). The initial oven temperature was held at 50 °C for 2 min, ramped to 70 °C at a rate of 10 °C min^{-1}, further to 85 °C at a rate of 3 °C min^{-1}, to 110 °C at a rate of 5 °C min^{-1}, then to 290 °C at a rate of 30 °C min^{-1}, and finally held at 290 °C for 8 min. Helium was used as a carrier gas at a constant flow rate of 1 mL min^{-1} through the column. Quantification was performed based on the relative peak area of each SCFA in a fatty acid external standard mixture (Volatile Free Acid Mix, 10 mM, 46975-U, Supelco), adjusting the quantity of each compound on that of the internal standard.

Statistical Analysis: The experimental design was a 2 × 2 factorial design (stearic acid addition with two levels of 0% and 1.5% w/w and heat-moisture treatment with two levels of [0% and 20% w/w moisture]). All the experiments were carried out in triplicates. Multivariate analysis of variance (MANOVA) was used to determine significant differences due to stearic acid and heat-moisture treatment in maize starch and maize meal. The data for maize starch and maize meal were analyzed separately. Averages were compared using the Fischer's Least Significant Difference Test (LSD) at $p \leq 0.05$. Origin Pro version 2019b was used to fit the graphs for gases, pH, and SCFAs.

The equation used was

$$Y = Yo + A1e^{-x/t1} \quad (1)$$

where *Y* was the dependent variable; *x* = independent variable; *t* = time in h; *K* was derived from the graph as was the exponential decay rate constant (h^{-1}); tau = time at the half-life of product production (h) and 1 was constant.

Acknowledgements

Acknowledgement goes to the DST/NRF Centre of Excellence in Food Security, NRF, and the University of Pretoria, South Africa. They also want to thank Purdue University, the USA, for their support and the use of their Laboratory work.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The authors can provide data on request.

Keywords

amylose–lipid complexes, differential scanning calorimetry, resistant starch type 5, short-chain fatty acid

Received: September 29, 2021
Revised: March 7, 2022
Published online: May 29, 2022

[1] M. M. Murphy, J. S. Douglass, A. Birkett, *J. Am. Diet. Assoc.* **2008**, *108*, 67.
[2] H. Jiang, J. Jane, *Novel Applications of Amylose-Lipid Complex as Resistant Starch Type 5*, John Wiley & Sons, Ltd. **2013**, p. 23.
[3] C. R. Luckett, Y. Wang, *J. Agric. Food Chem.* **2012**, *60*, 4751.
[4] G. O. Rombo, J. R. N. Taylor, A. Minnaar, *J. Sci. Food Agric.* **2004**, *84*, 350.
[5] J. Hasjim, Y. Ai, J. Jane, *Novel Applications Of Amylose-Lipid Complex as Resistant Starch Type 5*, (Eds: Y. Shi, C. C. Maningat), John Wiley & Sons, Ltd. **2013**, p. 79.
[6] J. A. Higgins, *J. AOAC Int.* **2004**, *87*, 761.
[7] E. O. Arijaje, Y. J. Wang, *Starch/Stärke* **2016**, *68*, 700.
[8] W. C. Obiro, S. S. Ray, M. N. Emmambux, *Food Rev. Int.* **2012**, *28*, 412.
[9] M. C. Godet, A. Buléon, V. Tran, P. Colonna, *Carbohydr. Poly.* **1993**, *21*, 91.
[10] J. Singh, A. Dartois, L. Kaur, *Trends Food Sci. Technol.* **2010**, *21*, 168.
[11] A. Aigster, J. Annelisse, P. Duncan, S. Susan, B. Conforti, J. Frank, A. B. William, *LWT Food Sci. Technol.* **2011**, *44*, 2159.
[12] I. K. Asare, C. R. Mapengo, M. N. Emmambux, *Starch-Stärke* **2021**, *73*, 2000128.
[13] H. J. Chung, Q. Liu, R. Hoover, *Carbohydr. Poly.* **2009**, *75*, 436.
[14] A. Gunaratne, R. Hoover, *Carbohydr. Poly.* **2002**, *49*, 425.
[15] R. Hoover, G. Swamidas, T. Vasanthan, *Carbohydr. Res.* **1993**, *246*, 185.
[16] D. Sievert, Y. Pomeranz, *Cereal Chem.* **1989**, *66*, 342.
[17] Y. Sang, P. Seib, *Carbohydr. Poly.* **2006**, *63*, 167.
[18] H. J. Flint, K. P. Scott, S. H. Duncan, P. Louis, E. Forano, *Gut Microbes* **2012**, *3*, 289.
[19] D. Y. Zhao, T. Ma, J. Zeng, W. M. Yan, J. Q. Wang, R. Huang, M. Wang, *Afr. J. Microbiol. Res.* **2011**, *5*, 4820.
[20] H. D. Holscher, *Gut Microbes* **2017**, *8*, 172.
[21] W. A. Phillips, D. L. Von Tunge, *Sheep & Goat Res. J.* **1995**, *11*, 150.
[22] D. L. Topping, P. M. Clifton, *Physiol. Rev.* **2001**, *81*, 1031.
[23] R. Hoover, *Food Sci. Nutr.* **2010**, *50*, 835.
[24] C. R. Mapengo, M. N. Emmambux, *Food Chem.* **2020**, *289*, 397.
[25] S. Raphaelides, J. Karkalas, *Carbohydr. Res.* **1988**, *172*, 65.
[26] J. Serra, F. Azpiroz, *Gastroenterol.* **1998**, *115*, 542.
[27] M. Dong, R. F. Anda, V. J. Felitti, S. R. Dube, D. F. Williamson, T. J. Thompson, W. H. Giles, *Child Abuse & Neglect* **2004**, *28*, 771.
[28] C. Fassler, E. Arrigoni, K. Venema, F. Brouns, R. Amado, *Mol. Nutr. Food Res.* **2006**, *50*, 1220.
[29] P. Kaur, A. Zeh, N. Chaudhri, A. Gerdes, M. Okrusch, *Precambrian Res.* **2011**, *187*, 155.
[30] S. H. Duncan, A. Barcenilla, C. S. Stewart, S. E. Pryde, H. J. Flint, *Appl. Environ. Microbiol.* **2002**, 5186.
[31] A. R. P. Walker, B. F. Walker, A. J. Walker, *Br. J. Cancer* **2005**, *53*, 189.
[32] T. P. Trinidad, T. M. Wolever, L. U. Thompson, *Am. J. Clin. Nutr.* **1996**, *63*, 574.
[33] Y. M. Ounes, L. V. Lechago, J. R. Somoano, M. Mosharaf, J. Lechago, **1996**, *56*, 1164.
[34] J. M. Thornton, *J. Mol. Biol.* **1981**, *151*, 261.
[35] M. J. Gidley, *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 371.
[36] A. Pompei, L. Cordisco, S. Raimondi, A. Amaretti, U. M. Pagnoni, D. Matteuzzi, M. Rossi, *Anaerobe* **2008**, *14*, 280.
[37] M. C. Jonathan, J. J. van den Borne, P. van Wiechen, C. S. da Silva, H. A. Schols, H. Gruppen, *Food Chem.* **2012**, *133*, 889.
[38] J. Charrier, I. R. Scheich, A. L. Teckentrup, *SIAM J. Numer. Anal.* **2013**, *51*, 322.
[39] M. Kalmokoff, B. Zwicker, M. O'hara, F. Matias, J. Green, P. Shastri, S. P. J. Brooks, *J. Appl. Microbiol.* **2013**, *114*, 1516.
[40] S. R. Sarbini, R. A. Rastall, *Funct. Food Rev.* **2011**, *3*, 93.
[41] P. Louis, H. J. Flint, *FEMS Microbiol. Lett.* **2009**, *294*, 1.
[42] C. A. Cherrington, M. Hinton, G. C. Mead, I. Chopra, *Adv. Microb. Physiol.* **1991**, *32*, 87.

[43] B. A. Williams, D. Mikkelsen, L. le Paih, M. J. Gidley, *J. Cereal Sci.* **2011**, *53*, 53.
[44] M. Goñi-Urriza, M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, C. Quentin, *Appl. Environ. Microbiol.* **2000**, *66*, 125.
[45] S. Wang, S. Dhital, K. Wang, X. Fu, B. Zhang, Q. Huang, *Carbohydr. Poly.* **2021**, *263*, 118003.
[46] T. V. D'Silva, J. R. Taylor, M. N. Emmambux, *J. Cereal Sci.* **2011**, *53*, 192.
[47] H. Jacobs, J. A. Delcour, *J. Agric. Food Chem.* **1998**, *46*, 2895.
[48] M. Kweon, L. Haynes, L. Slade, H. Levine, *J. Therm. Anal. Calorim.* **2000**, *59*, 571.
[49] I. Goni, A. Garcia-Alonso, F. A. Saura-Calixto, *Nutr. Res.* **1997**, *17*, 427.
[50] O. C. Wokadala, S. S. Ray, M. N. Emmambux, *Carbohydr. Poly.* **2012**, *90*, 616.
[51] V. Lebet, E. Arrigoni, R. Amado, *LWT-Food Sci. Technol.* **1998**, *31*, 473.
[52] D. J. Rose, J. A. Patterson, B. R. Hamaker, *J. Agric. Food Chem.* **2010**, *58*, 493.

