JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Cooking Has Variable Effects on the Fermentability in the Large Intestine of the Fraction of Meats, Grain Legumes, and Insects That Is Resistant to Digestion in the Small Intestine in an in Vitro Model of the Pig's Gastrointestinal Tract

Christine Poelaert,[†,‡] Xavier Despret,[†] Marianne Sindic,[#] Yves Beckers,[†] Frédéric Francis,[⊥] Daniel Portetelle,[‡] Hélène Soyeurt,[Δ] André Théwis,[†] and Jérôme Bindelle*[,†]

[†]Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

[‡]Microbiology and Genomics Unit, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

[#]Laboratory of Agro-food Quality and Safety, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

[⊥]Laboratory of Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

[Δ]Applied Statistics, Computer Science and Mathematics Unit, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium

ABSTRACT: This study aimed to evaluate the fermentation in the large intestine of indigestible dietary protein sources from animal, insect, and plant origin using an in vitro model of the pig's gastrointestinal tract. Protein sources were used raw and after a cooking treatment. Results showed that the category of the ingredient (meats, insects, or grain legumes) exerts a stronger impact on enzymatic digestibility, fermentation patterns, and bacterial metabolites such as short-chain fatty acids (SCFA) and hydrogen sulfide (H_2S) than the cooking treatment. The digestibility and the fermentation characteristics of insects were more affected by the cooking procedure than the other categories. Per gram of consumed food, ingredients from animal origin, namely, meats and insects, were associated with fewer fermentation end-products (gas, H_2S, SCFA) than ingredients from plant origin, which is related to their higher small intestinal digestibility.

KEYWORDS: *in vitro method, pig, protein fermentation, short-chain fatty acid, hydrogen sulfide*

INTRODUCTION

The human diet contains a wide range of protein sources, which differ in protein content, amino acid (AA) profile, and digestibility. In Western countries, animal and plant proteins are major protein sources in the human diet, whereas, in many tropical countries, human diets also include insects, besides mainly vegetable protein. Animal food products are under the fire of critics for their possible impact on human health,[1] in contrast to plant proteins,[2] and their environmental footprint.[3] In this context, insects are suggested as a potential alternate source of proteins for livestock and humans, including in Western countries, due to (1) their low environmental impact in terms of limited greenhouse gas production, land requirement, and water consumption; (2) their high protein content (50−82% of dry matter (DM)); and (3) their well-balanced AA profiles.[4,5]

Whatever the source, proteins that escape digestion by host enzymes and acids in the upper gastrointestinal tract (GIT) reach the large intestine, where they undergo fermentation by the resident microbiota. In Western diets, approximately 12−18 g of protein from dietary origin and from endogenous origin, such as pancreatic enzymes, mucus, and exfoliated epithelial cells, reach the large intestine each day.[6,7] The amount of protein from dietary origin entering the large intestine depends on the total protein intake and its digestibility and represents at least 50% of the total pool of protein of endogenous and dietary origin that is present in the colon and becomes available for fermentation.[6,8]

With digestibility rates as high as 90%, animal proteins are, in their natural form, more susceptible to enzymatic degradation in the upper GIT than their plant counterparts, which are usually digested at a rate of 70−90%.[9] The lesser susceptibility to proteolytic breakdown of the plant proteins can be explained by (i) their compact structure, reducing access to enzymes; (ii) the presence of insoluble fiber and tannins; and (iii) the presence of antinutritional factors such as lectins, protease inhibitors, and trypsin inhibitors.[10,11] Heat treatments can induce various changes in the structural properties and in the physicochemical state of proteins, with a potential impact on digestibility, including oxidation and denaturation processes, protein aggregation, changes in hydrophobicity and solubility,[12] inactivation of antinutritional factors and subsequent appearance of novel interactions between proteins or with others components, formation of Maillard reaction products, and racemization of AA to D-enantiomers.[11]

In the large intestine, nonhydrolyzed or partially hydrolyzed proteins are fermented by microbiota as an energy source. This proteolytic fermentation occurs mainly in the distal colon,

Received: October 14, 2016
Revised: December 14, 2016
Accepted: December 20, 2016
Published: December 20, 2016

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1. Chemical Composition (g/kg DM) and Gross Energy Content (MJ/kg DM) of the Raw and Cooked Ingredients

ingredient (treatment)	ash	crude protein[a]	fat	NDF[b]	ADF[c]	starch	gross energy
house crickets							
raw	47.8	669	151	227	84.9		24.4
oven at 150 °C	47.1	667	147	309	94.7		24.3
oven at 200 °C	47.1	668	147	281	88.4		24.4
autoclaved	46.9	668	152	332	93.9		24.5
mealworm larvae							
raw	45.5	535	240	174	68.8		26.4
oven at 150 °C	45.5	535	253	236	69.2		26.7
oven at 200 °C	44.6	534	251	253	70.1		26.6
autoclaved	44.2	534	259	288	72.2		26.6
beans							
raw	39.4	195	17.2	200	61.4	279	18.4
vapor-cooked	35.6	198	20.8	202	88.6	370	18.7
lentils							
raw	26.7	220	7.03	157	56.1	374	18.6
vapor-cooked	26.7	225	14.5	178	69.9	445	18.7
peas							
raw	27.4	204	12.2	119	16.1	422	18.5
vapor-cooked	22.2	209	16.3	88.1	36.9	530	18.7
soybeans							
raw	53.2	396	239	66.0	30.2		24.5
vapor-cooked	37.8	436	258	83.8	18.5		25.7
beef muscle							
raw	43.1	799	53.6	83.8	52.8		23.4
water bath	34.5	803	59.7	88.1	44.7		24.1
oven-cooked	37.3	806	54.8	119	69.9		23.9
pan-cooked	36.3	799	59.3	66.0	53.1		23.8
chicken breast							
raw	52.1	826	59.4	202	69.7		23.1
water bath	48.7	839	44.8	178	33.1		23.7
oven-cooked	46.6	827	46.1	157	36.1		23.2
pan-cooked	45.8	829	46.0	200	54.4		23.2

[a]Nitrogen was converted into protein using conversion factors reported by Mariotti et al.[50] [b]NDF, neutral-detergent fiber. [c]ADF, acid detergent fiber.

where fermentable carbohydrates get depleted.[13] As with carbohydrate fermentation, short-chain fatty acids (SCFA) are the major end-products of the proteolytic fermentation. In addition to acetate, propionate, and *n*-butyrate, branched-chain fatty acids (BCFA), mainly isobutyrate and isovalerate but also *n*-valerate, are produced from the fermentation of branched-chain AA as valine, leucine, and isoleucine. According to the type of substrate, BCFA represent 16−23% of total SCFA produced from fermented proteins.[14,15] Other metabolites produced during protein fermentation include CO_2, CH_4, H_2, ammonia, phenolic and indolic compounds, biogenic amines, and sulfur metabolites, mainly hydrogen sulfide (H_2S). Conversely to carbohydrate fermentation, protein fermentation is usually considered as detrimental to the host's epithelial health[16,17] and has been associated with various bowel diseases.[18] As recently reviewed by Yao et al.,[19] the toxic effects on the colonic epithelium, such as a thinning of the mucous barrier and an alteration of the structure and of the metabolic functions of the epithelial cells, are mainly attributed to ammonia[20,21] and H_2S[22] and, to a lesser extent, phenols.[20,23] Moreover, a high availability of dietary nitrogen in the large intestine was associated with a low microbial diversity and a poor host−microbiome association.[24] Therefore, although animal proteins are criticized for their impact on health, it is worth questioning if other sources of protein such as grain legumes and insects potentially induce similar shifts in the intestinal ecophysiology.

The aim of the present study was thus to understand the link between the protein source (animal proteins, including insects vs plant proteins), the cooking procedure (raw vs cooked), and the fermentation of the indigestible fraction in the large intestine using an in vitro model of the pig GIT as model for the human GIT.[25] Various protein sources consumed by humans were included in this study: animal proteins (beef and chicken meat), grain legumes (lentils, peas, beans, soybeans), and insects (house crickets and mealworm larvae), raw or after undergoing a cooking procedure.

MATERIALS AND METHODS

Preparation of the Ingredients. Three types of protein sources were used in this experiment: meat, grain legumes, and insects (Table 1). These protein sources were used raw or after a cooking process. Beef muscle (Longissimus dorsi) and chicken breast (Pectoralis major) were chosen as animal protein and cut into small pieces of approximately 60 g (6 × 3 × 2 cm). Some samples were directly frozen (−20 °C) to be used as raw meat, and the rest were subjected to one of the following cooking methods: (1) immersion of meat pieces placed in individual plastic bags (polyamide/polyethylene; 15 × 20 cm; 90 μm) in a water bath at 70 or 85 °C for beef and chicken samples, respectively (mean cooking time = 40 min); (2) cooking in an oven at 175 °C with turning every 2 min (mean cooking time = 17

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

and 39 min for beef and chicken samples, respectively); (3) frying in a pan without addition of fat with turning every 2 min (mean cooking time = 7 and 15 min for beef and chicken samples, respectively). For all cooking methods, the meat temperature was monitored using a temperature probe (Testo 926, Lenzkirch, Germany), and the cooking was stopped when a core temperature of 70 or 85 °C was reached, for beef and chicken samples, respectively, according to the reference method of Boccard et al.[26]

Moreover, meat color was measured on the inside and outside of meat pieces by using a Miniscan Spectrocolorimeter (Hunterlab, Reston, VA, USA) to compare cooking methods and control the absence of excessive burning (Table 2).

Table 2. Means and Standard Error of the Means for Color Values of Lightness (L^*) for Beef Muscle and Chicken Breast

	lightness (L^*)[a]	
ingredient (treatment)	external	internal
beef muscle		
raw	37.7 ± 3.6	37.7 ± 3.6
water bath	48.7 ± 4.8	56.9 ± 2.3
oven-cooked	48.6 ± 2.5	56.7 ± 2.2
pan-cooked	40.5 ± 7.0	55.8 ± 1.8
chicken breast		
raw	53.9 ± 2.7	53.9 ± 2.7
water bath	78.8 ± 1.2	82.0 ± 0.9
oven-cooked	74.1 ± 2.6	81.4 ± 0.6
pan-cooked	63.3 ± 4.4	81.6 ± 0.9

[a]Color was expressed according to the CIELAB (the International Commission on Illumination) trichromatic system. Lightness (L^*) values vary from 100 for perfect white to 0 for black.

Two types of insects were used: adult house crickets (*Acheta domesticus*) and mealworm larvae (*Tenebrio molitor*). Mealworm larvae were reared in a dark incubator at 25 °C and were fed a mixture of wheat flour (50%), wheat bran (30%), and beer yeast (20%). The house crickets were fed the same diet and were reared at 25 °C in ventilated containers with a photoperiod of 10 h of light per day. After freeze killing, insects were used in several forms: raw, autoclaved (120 °C for 25 min), and oven-cooked (150 °C for 30 min or 200 °C for 10 min).

White pea beans (*Phaseolus vulgaris*), green lentils (*Lens culinaris*), green peas (*Pisum sativum*), and soybeans (*Glycine max*) were used raw and vapor-cooked (steaming at low pressure in a domestic vapor-cooker) for 30 min (lentils, peas) to 60 min (beans, soybeans).

After cooking, raw and cooked meats, insects, and grain legumes were freeze-dried and ground to pass a 1 mm mesh screen (Pulverisette 14, Fritsch, Idar-Oberstein, Germany).

In Vitro Enzymatic Digestion and Fermentation. Because of similarities in intestinal digestive processes,[25] the two-step in vitro model of the pig's GIT described by Bindelle et al.[27] was used as a model to mimic intestinal digestive and fermentation processes in humans. In the first step, all ingredients were predigested following the protocol of Boisen and Fernandez.[28] Briefly, 1 g ingredient samples were incubated with 50 mg of porcine pepsin (600 U/mg; Sigma-Aldrich, St. Louis, MO, USA) (pH 2, 2 h, 39 °C) and 100 mg of porcine pancreatin (8 × USP specifications; Sigma-Aldrich) (pH 6.8, 4 h, 39 °C) to simulate digestion in the upper digestive tract. The residues were centrifuged (2000*g*, 15 min, 4 °C) and freeze-dried. In vitro dry matter (IVDMD), crude protein (IVCPD), and starch (IVStarchD) digestibility were calculated by difference between the DM, protein, and starch content in the ingredient before hydrolysis and in the residue. Each ingredient was tested 15 times (3 replicates × 5 periods).

To simulate the fermentation occurring in the large intestine, the hydrolyzed residues were incubated with a fecal porcine inoculum. Fresh feces from sows were diluted 1:20 with the buffer solution of Menke and Steingass[29] with two modifications: (i) the reducing agent Na_2S was omitted[30] and (ii) the buffer was N-free by the replacement of the N source ($(NH_4)_2CO_3$) by an equimolar quantity of $NaHCO_3$.

Fermentation (39 °C) was initiated by mixing 200 mg of each ingredient (protein source × cooking procedure) with 30 mL of the inoculum and was repeated over three periods. For each period, the experimental scheme was as follows: 24 ingredients × 3 replicates + 10 blanks (containing only incubation medium). For periods 1 and 2, the fermentation was performed in 140 mL glass bottles equipped with a rubber stopper, and the pressure inside each bottle was recorded using a manometer (Bailey and Mackey Ltd., Birmingham, UK) at regular intervals over 72 h of incubation.[31] After 12, 24, and 72 h of fermentation, an aliquot of the fermentation broth was centrifuged (13000 *g*, 15 min, 4 °C) for further SCFA analysis. For period 3, the fermentation was performed in 100 mL glass syringes. After 24 h, fermentation was stopped and a 5 mL gas sample was taken from the headspace of the syringe to measure H_2S production.

Kinetics of Gas Production during in Vitro Fermentation. Gas production curves of periods 1 and 2 were modeled according to Groot et al.[32]

$$G = \frac{A}{1 + \frac{B^C}{t^C}} \quad \text{if } t > 0$$

where G (mL/g of DM) denotes cumulative gas production versus time expressed per gram of ingredient before enzymatic digestion; A (mL/g of DM) is the maximal gas volume for $t = \infty$; B (h) is the time at which 50% of A is reached; and C is a constant determining the shape of the curve. From this equation, two other parameters were calculated: the maximum rate of gas production (R_{max}, mL/g of DM × h) and the time to reach R_{max} (T_{max}, h).

Kinetics of SCFA Production during in Vitro Fermentation. Fermentation supernatants collected after 12, 24, and 72 h of fermentation were filtered using a 0.45 μm nylon HPLC syringe filter (Novolab, Geraardsbergen, Belgium) and analyzed for lactate and SCFA (acetate, propionate, *n*-butyrate, isobutyrate, *n*-valerate, isovalerate) production with a Waters 2690 HPLC system (Waters, Milford, MA, USA) fitted with an Aminex HPX 87 H column (300 mm × 78 mm; Bio-Rad Laboratories, Hercules, CA, USA) combined with an UV absorbance detector (Waters 486 tunable absorbance detector) set at 210 nm.

Measurement of H_2S Production. Production of H_2S after 24 h of fermentation was evaluated according to the procedure of Leibovich et al.[33] Briefly, 5 mL of gas was removed from the headspace of each glass syringe using a 5 mL plastic syringe equipped with a needle, and these gas samples were bubbled into 5 mL of alkaline water placed inside an 11 mL vacuum tube (BD Vacutainer, Plymouth, UK). Then 500 μL of *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride sulfate reagent and 500 μL of acidified ferric chloride solution (Sigma-Aldrich) were added. After 30 min at room temperature, the absorbance was read at 665 nm (Shimadzu UV-1650 PC, Kyoto, Japan) against a calibration curve (RAD171, Supelco, Bellefonte, PA). The concentration of H_2S was expressed in micromoles per 24 h by multiplying the H_2S concentration by the total gas production after 24 h of in vitro fermentation. Finally, IVDMD coefficients and total SCFA productions were used to express the results as micromoles of H_2S per gram of ingredient before enzymatic digestion or per gram of SCFA produced.

Chemical Analyses. Raw and cooked insects, meats, and grain legumes were analyzed for their contents in DM (105 °C for 24 h; method 967.03[34]), ash (550 °C for 8 h; method 923.03[34]), crude protein (CP; N × 6.25; method 981.10[34]), ether extract with the Soxhlet method by using diethyl ether (method 920.29[34]), ash-corrected neutral detergent fiber (NDF) using Termamyl (Novo Nordisk, Bagsværd, Denmark), and ash-corrected acid detergent fiber (ADF) with the Fibercap system (Foss Electric, Bagsvaerd, Denmark).[35] Gross energy was measured by means of an adiabatic oxygen bomb calorimeter (1241 adiabatic calorimeter, PARR Instrument Co., Moline, IL, USA). Starch content was analyzed colorimetrically using a

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 3. In Vitro Dry Matter (IVDMD) and Crude Protein (IVCPD) Digestibility during Enzymatic Hydrolysis (n = 15), Gas Production Parameters (A, B, C, R_{max}, T_{max}) Modelled According to Groot et al.[32] (n = 6), and Short-Chain Fatty Acid (SCFA) and H_2S Production (n = 3) during in Vitro Fermentation by Pig Fecal Bacteria of Beef Muscle and Chicken Breast Used Raw or after a Heat Treatment[a]

	beef muscle				chicken breast					*P* values		
	raw	WB[b]	oven[c]	pan[d]	raw	WB	oven	pan	SEM[e]	I[f]	C[g]	I × C
enzymatic predigestion												
IVDMD (%)	89.2 a	84.6 bc	84.6 bc	84.2 c	85.9 b	84.8 bc	85.6 bc	84.4 c	0.22	0.184	<0.001	0.001
IVCPD (%)	73.0 a	74.4 a	69.3 ab	72.2 ab	73.2 a	69.4 ab	66.3 b	66.0 b	0.83	0.030	0.074	0.519
gas fermentation kinetics												
A (mL/g DM)	6.00 c	10.1 ab	9.94 ab	9.99 ab	8.68 b	10.3 ab	11.5 a	11.8 a	0.33	0.004	<0.001	0.392
B (h)	7.61	8.50	8.04	9.27	9.07	9.76	9.58	9.08	0.37	0.196	0.869	0.840
C (−)	2.14	1.91	1.72	1.82	1.81	1.94	1.71	1.83	0.04	0.395	0.181	0.477
R_{max} (mL/g DM × h)	0.571	0.791	0.798	0.735	0.648	0.746	0.790	0.886	0.03	0.483	0.106	0.669
T_{max} (h)	4.73	4.45	3.58	4.52	4.44	5.15	4.15	4.52	0.24	0.624	0.594	0.889
total SCFA production and molar ratios after 24 h of in vitro fermentation												
total SCFA (mg/g DM)	30.6 e	39.3 cd	40.6 bcd	44.4 bc	38.9 d	45.1 b	41.3 bcd	52.2 a	1.31	<0.001	<0.001	0.155
acetate (%)	50.9	48.1	49.4	48.2	50.6	50.6	48.2	49.7	0.41	0.463	0.345	0.392
propionate (%)	15.1 c	16.9 a	16.9 a	16.4 ab	15.7 bc	17.0 a	17.2 a	16.5 ab	0.17	0.274	<0.001	0.850
n-butyrate (%)	14.3	14.5	14.0	14.6	14.5	13.7	13.8	13.0	0.24	0.253	0.840	0.608
BCFA (%)	19.7	20.4	19.7	20.7	19.2	18.7	20.7	20.8	0.36	0.723	0.601	0.657
H_2S production after 24 h of in vitro fermentation												
H_2S (μmol/24 h × g DM)	4.30 f	6.49 e	8.16 cd	7.45 de	6.36 e	9.46 b	10.6 a	9.26 bc	0.31	<0.001	<0.001	0.520
H_2S (μmol/24 h × g of SCFA)	140 e	165 de	201 bc	168 d	163 de	210 b	257 a	178 cd	5.85	<0.001	<0.001	0.074

[a]Within a row, means without a common letter differ (p < 0.05). [b]Ingredients were cooked by immersion in a water bath at 70 or 85 °C for beef and chicken samples, respectively. [c]Ingredients were cooked in an oven at 175 °C until reaching a core temperature of 70 or 85 °C for beef and chicken samples, respectively. [d]Ingredients were cooked on a pan until reaching a core temperature of 70 or 85 °C for beef and chicken samples, respectively. [e]SEM, standard error of the mean. [f]I, ingredient. [g]C, cooking treatment.

Table 4. In Vitro Dry Matter (IVDMD) and Crude Protein (IVCPD) Digestibility during Enzymatic Hydrolysis (n = 15), Gas Production Parameters (A, B, C, R_{max}, T_{max}) Modelled According to Groot et al.[32] (n = 6), and Short-Chain Fatty Acid (SCFA) and H_2S Production (n = 3) during in Vitro Fermentation by Pig Fecal Bacteria of Insects Used Raw or after a Heat Treatment[a]

	house crickets				mealworms larvae					*P* values		
	raw	O150[b]	O200[c]	auto[d]	raw	O150	O200	auto	SEM[e]	I[f]	C[g]	I × C
enzymatic predigestion												
IVDMD (%)	56.1 d	46.1 e	47.3 e	48.7 e	76.2 a	69.8 b	69.4 b	63.8 c	1.08	<0.001	<0.001	<0.001
IVCPD (%)	65.5 b	59.3 d	61.1 bcd	59.5 cd	72.5 a	64.1 b	63.9 bc	59.5 cd	0.70	0.002	<0.001	0.166
gas fermentation kinetics												
A (mL/g DM)	23.0 b	33.1 a	34.2 a	32.7 a	21.1 bc	16.5 c	18.8 bc	16.6 c	1.25	<0.001	0.204	0.002
B (h)	24.0 b	13.7 cd	15.4 c	14.6 c	34.2 a	10.3 cd	15.2 cd	7.30 d	1.42	0.925	<0.001	0.005
C (−)	1.43 ab	1.16 b	1.13 b	1.19 b	1.82 a	1.19 b	1.10 b	1.67 a	0.06	0.032	0.002	0.197
R_{max} (mL/g DM × h)	0.856 bc	2.17 a	1.72 ab	2.10 a	0.42 c	1.25 abc	1.02 bc	1.65 ab	0.14	0.012	0.003	0.863
T_{max} (h)	7.90 b	1.49 c	1.29 c	2.13 bc	15.8 a	1.35 c	0.967 c	2.65 bc	1.01	0.204	<0.001	0.187
total SCFA production and molar ratios after 24 h of in vitro fermentation												
total SCFA (mg/g DM)	55.4 b	77.9 a	84.3 a	89.9 a	27.9 c	49.9 b	51.2 b	60.7 b	4.33	<0.001	<0.001	0.946
acetate (%)	49.2	47.5	49.0	48.4	51.8	47.8	48.1	49.7	0.56	0.514	0.476	0.776
propionate (%)	15.3 d	18.6 bc	19.3 abc	18.3 bc	17.7 c	19.6 abc	20.5 a	19.7 ab	0.39	0.003	<0.001	0.637
n-butyrate (%)	14.2	13.7	11.5	10.7	11.6	11.4	13.6	11.3	0.51	0.624	0.616	0.358
BCFA (%)	21.3 a	20.2 ab	20.1 ab	22.6 a	19.0 ab	21.2 ab	17.8 b	19.2 ab	0.44	0.044	0.306	0.290
H_2S production after 24 h of in vitro fermentation												
H_2S (μmol/24 h × g DM)	3.13 d	11.5 ab	10.8 ab	11.9 a	1.32 d	6.02 c	7.28 c	10.0 b	0.58	<0.001	<0.001	0.038
H_2S (μmol/24 h × g of SCFA)	56.5 d	148 ab	128 bc	133 bc	47.4 d	121 c	142 abc	165 a	6.61	0.744	<0.001	0.019

[a]Within a row, means without a common letter differ (p < 0.05). [b]Ingredients were cooked in an oven at 150 °C for 30 min. [c]Ingredients were cooked in an oven at 200 °C for 10 min. [d]Ingredients were autoclaved for 25 min. [e]SEM, standard error of the mean. [f]I, ingredient. [g]C, cooking treatment.

total starch assay kit (Megazyme Ltd., Wicklow, Ireland) following the manufacturer's instructions. Residues obtained after pepsin−pancreatin hydrolysis were also analyzed for their DM, CP, and starch contents.

Statistical Analyses. The data set was split in three, one for each food ingredient category (insects, meats, and grain legumes) and analyzed separately for each category to meet the conditions of normality of distributions and homogeneity of variances. IVDMD and IVCPD during enzymatic hydrolysis, fermentation parameters (A, B, C, R_{max}, and T_{max}), and H_2S production as well as total SCFA production and molar ratios after 24 h of fermentation were analyzed using the GLM procedure of SAS 9.4 software (SAS Institute Inc.,

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 5. In Vitro Dry Matter (IVDMD) and Crude Protein (IVCPD) Digestibility during Enzymatic Hydrolysis (n = 15), Gas Production Parameters (A, B, C, R_{max}, T_{max}) Modelled According to Groot et al.[32] (n = 6), and Short-Chain Fatty Acid (SCFA) and H_2S Production (n = 3) during in Vitro Fermentation by Pig Fecal Bacteria of Grain Legumes Used Raw or after a Heat Treatment[a]

	beans		lentils		peas		soybeans			P values		
	raw	vapor[b]	raw	vapor	raw	vapor	raw	vapor	SEM[c]	I[d]	C[e]	I × C
enzymatic predigestion												
IVDMD (%)	27.2 g	46.9 d	32.6 f	60.2 b	37.1 e	63.4 a	49.6 c	46.2 d	1.13	<0.001	<0.001	<0.001
IVCPD (%)	68.5 c	68.8 bc	68.8 bc	72.5 abc	74.1 ab	76.0 a	60.8 d	76.3 a	0.81	0.003	<0.001	<0.001
IVStarchD (%)	−1.71	57.3	14.7	73.3	4.34	75.8						
gas fermentation kinetics												
A (mL/g DM)	218 a	142 c	192 b	93.8 d	186 b	88.4 d	83.1 d	84.6 d	7.82	<0.001	<0.001	<0.001
B (h)	18.6 a	9.19 c	14.1 b	11.2 c	14.2 b	9.65 c	11.2 c	9.00 c	0.56	0.003	<0.001	0.002
C (−)	2.48 ab	1.85 cd	2.16 bc	1.71 d	2.04 c	1.64 d	2.48 ab	2.66 a	0.06	<0.001	<0.001	0.007
R_{max} (mL/g DM × h)	8.66 ab	10.1 a	9.40 a	5.45 d	8.75 ab	5.89 cd	5.60 d	7.34 bc	0.30	<0.001	0.018	<0.001
T_{max} (h)	13.2 a	4.83 cd	8.80 b	5.16 cd	8.43 b	4.13 d	7.91 b	6.54 bc	0.48	0.011	<0.001	0.001
total SCFA production and molar ratios after 24 h of in vitro fermentation												
total SCFA (mg/g DM)	298 a	251 c	273 b	153 e	285 ab	159 e	198 d	200 d	11.35	<0.001	<0.001	<0.001
acetate (%)	52.8 a	55.2 a	54.0 a	55.1 a	52.8 a	54.7 a	52.5 a	56.6 a	0.49	0.914	0.028	0.739
propionate (%)	33.2 b	34.5 ab	32.7 b	35.5 a	34.2 ab	35.9 a	27.4 c	27.7 c	0.69	<0.001	0.011	0.450
n-butyrate (%)	9.11 a	5.43 c	9.57 a	5.19 c	8.77 ab	4.94 c	8.79 ab	7.19 b	0.42	0.245	<0.001	0.092
BCFA (%)	4.19 c	4.83 c	3.68 c	4.13 c	4.28 c	4.48 c	11.3 a	8.56 b	0.57	<0.001	0.208	0.002
H_2S production after 24 h of in vitro fermentation												
H_2S (μmol/24 h × g DM)	23.2 b	35.7 a	36.9 a	19.9 b	36.2 a	24.2 b	37.6 a	34.7 a	1.21	0.003	0.003	<0.001
H_2S (μmol/24 h × g of SCFA)	78.1 d	142 c	135 c	130 c	127 c	152 bc	190 a	174 ab	5.56	<0.001	0.017	<0.001

[a]Within a row, means without a common letter differ (p < 0.05). [b]Ingredients were vapor-cooked for 30 (lentils, peas) to 60 (beans, soybean) min. [c]SEM, standard error of the mean. [d]I, ingredient. [e]C, cooking treatment.

Cary, NC, USA) as this fermentation time corresponds to transit time in the human colon. Means were separated using the LSMEANS statement with a general linear model using two criteria of classification (ingredient, cooking procedure) and their interaction. The hydrolysis or fermentation flask was used as experimental unit. Post hoc pairwise comparisons were done using Student's t test. Significance was judged at P < 0.05.

In addition, a principal component analysis (PCA) was performed on the whole data set using the PRINCOMP procedure of SAS 9.4 software (SAS Institute Inc.) to analyze the relationships between digestibility, gas production parameters, SCFA after 12, 24, and 72 h, and H_2S production. The correlation among the variables was determined with the loading plots of the first two eigenvalues.

RESULTS

Chemical Composition of the Ingredients. As shown in Table 1, ingredients differed greatly in terms of chemical composition (CP, fat, fiber, starch, and ash). In this study, fiber was measured by NDF and ADF without correction for the nitrogen content, which explains that the sum of CP, fat, fiber, and ash exceeded 100% for some protein-rich ingredients. Although it is well established that NDF and ADF fractions contain mainly structural carbohydrates embedding some nutrients in grain legumes, the exact compositions of these fractions resistant to neutral and acid detergent digestion are unknown in insects and meats.

In Vitro Enzymatic Hydrolysis of the Ingredients. The highest IVDMD values were obtained for the meats (89.2 and 85.9% for beef muscle and chicken breast, respectively; Table 3), followed by mealworm larvae (76.2%) and crickets (56.1%) (Table 4). For raw grain legumes, IVDMD values were below 50% (27.2−49.6%; Table 5). Cooked insects and, to a lesser extent, cooked meats were less susceptible (P < 0.05) to enzymatic degradation in the upper GIT (IVDMD) than their raw counterparts. For meats, the significant interaction (P = 0.001) between the ingredient (beef or chicken) and the cooking treatment reflected the more pronounced impact of cooking on beef muscle than on chicken breast in terms of IVDMD. In contrast to meats and insects, IVDMD of grain legumes rich in starch (beans, lentils, peas), as expected, was improved (P < 0.001) after vapor-cooking, which reduced the quantity of dietary components available as substrate fermentation in the large intestine. In terms of IVCPD of raw ingredients, mealworms (72.5%) equaled the best grain legumes (60.8−74.1%) and meats (73.0−73.2%), whereas crickets were less digestible (65.5%; P < 0.01). Although IVCPD was unaffected (P > 0.05) by cooking for meats and for grain legumes rich in starch, IVCPD decreased (P < 0.001) after heat treatment for both tested insects.

Comparison of the Fermentability of Raw Ingredients. Among the tested ingredients, grain legumes generated the highest gas production after 72 h of in vitro fermentation expressed per gram of food ingredient before the in vitro enzymatic hydrolysis (83.1−218 mL/g of raw ingredient), followed by raw insects (21.1−23.0 mL/g) and finally by raw meats (6.00−8.68 mL/g).

In concordance with final gas production, higher SCFA and H_2S productions were obtained after 24 h of in vitro fermentation of raw grain legumes (198−298 mg of SCFA/g of raw ingredient and 23.2−37.6 μmol of H_2S/g) than with meats (30.6−38.9 mg of SCFA/g and 4.30−6.36 μmol of H_2S/g). Insects generated relatively low SCFA (27.9−55.4 mg/g of raw ingredient) and H_2S (1.32−3.13 μmol/g) productions after 24 h of in vitro fermentation, which could be related to the slower fermentation (B values) of crickets and mealworm larvae compared with meats and grain legumes. With regard to SCFA, both insect and meat sources displayed higher BCFA

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

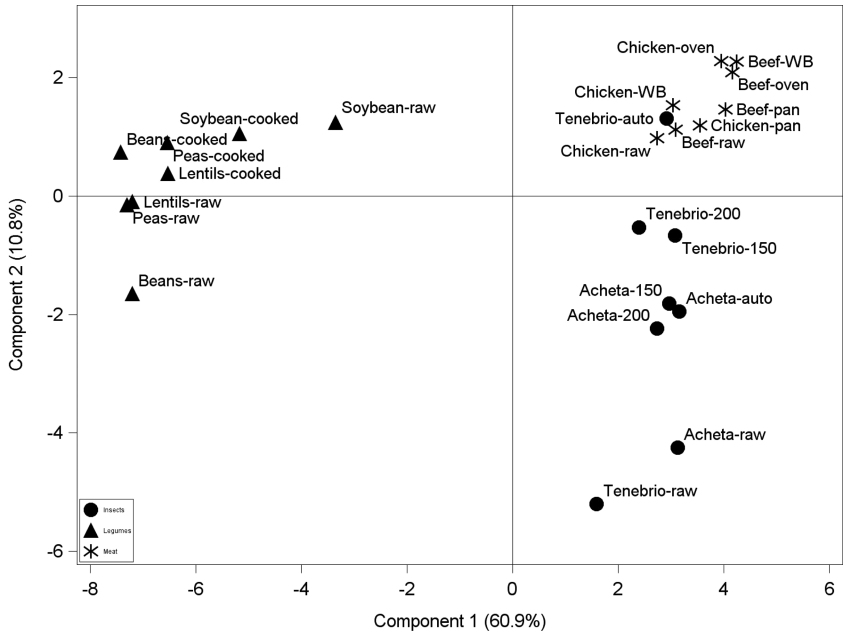

Figure 1. Score plot from the first two principal components (PC1, PC2). Different symbols indicate the scores of the ingredient according to the category (●, insects; ▲, grain legumes; ∗, meat).

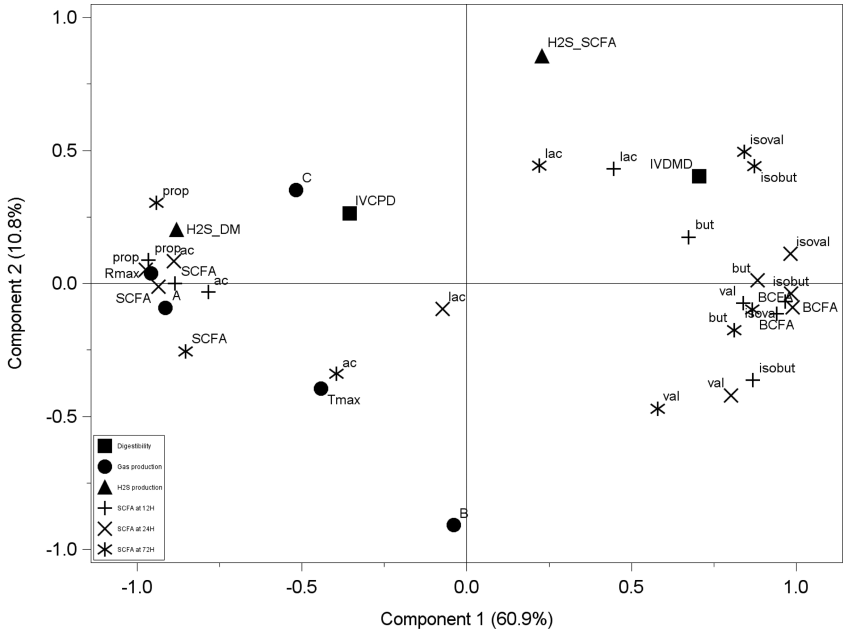

Figure 2. Loading plot from the first two principal components (PC1, PC2) describing the relationships between digestibility and fermentation parameters, H_2S production after 24 h of fermentation, and kinetics of short-chain fatty acid (SCFA) production. Abbreviations: IVDMD, in vitro dry matter digestibility; IVCPD, in vitro crude protein digestibility; *A*, maximal gas volume; *B*, time to reach 50% of *A*; *C*, constant; R_{max}, maximum rate of gas production; T_{max}, time to reach R_{max}; H2S_DM, H_2S produced by grams of ingredient consumed; H2S_SCFA, production of H_2S expressed by grams of SCFA; SCFA, total SCFA production expressed by grams of ingredient consumed; lac, lactate; ac, acetate; prop, propionate; but, *n*-butyrate; isobut, isobutyrate; val, *n*-valerate; isoval, isovalerate; BCFA, branched-chain fatty acids.

proportions (19.0−21.3%) than beans, lentils, and peas (3.68−4.28%), partially offset by a lower propionate ratio (15.1−17.7% vs 32.7−34.2%). Soybeans differed from other grain legumes by yielding a higher BCFA molar ratio (11.3%) counterbalanced by a reduction in propionate (27.4%).

Influence of Cooking on the Fermentability of Meats. As cooked meats were less digestible ($P < 0.05$), a higher amount of dietary components was available as energy source for microbiota during in vitro fermentation for each gram of ingredient ingested. This was reflected by the higher productions ($P < 0.001$) of all fermentation products (total gas (A), SCFA, H_2S correlation coefficients of −0.85, −0.80, and −0.64, respectively, compared to IVDMD) measured in this experiment when cooked meats were fermented. Among SCFA, the propionate molar ratio was increased ($P < 0.001$) as a result of the thermal treatment. By comparison of thermal treatments, a higher ($P < 0.05$) H_2S production was observed when meats were previously oven-cooked.

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Influence of Cooking on the Fermentability of Insects. The fermentation of cooked insects led to higher ($P < 0.001$) SCFA and H_2S productions (correlation coefficients of −0.93 and −0.72, respectively, compared to IVDMD) compared to raw ones. It also yielded higher ($P < 0.001$) propionate molar ratios. Gas production kinetics were also influenced by the thermal treatment. The maximum rate of gas production (R_{max}) was increased ($P < 0.01$) when insects were previously oven-cooked or autoclaved. Moreover, hydrolyzed residues of cooked insects fermented more quickly (B, T_{max}; $P < 0.05$) than raw ones.

Influence of Cooking on the Fermentability of Grain Legumes. Soybeans differed from the other three grain legumes, explaining that statistical analysis revealed significant interactions between the ingredient (beans, peas, lentils, soybeans) and the cooking treatment (raw, vapor-cooked) for most parameters. The increase of IVDMD ($P < 0.001$) by cooking for grain legumes rich in starch was reflected by the reduced ($P < 0.01$) gas (A) and SCFA productions with cooked beans, peas, and lentils compared to raw ones (correlation coefficients of −0.88 and −0.94, respectively, compared to IVDMD). Cooking lentils and peas reduced ($P < 0.001$) H_2S production, whereas an increase ($P < 0.001$) in the H_2S production was observed with vapor-cooked beans compared to raw ones. In addition, hydrolyzed residues of cooked grain legumes, except for soybeans, fermented more quickly (B, T_{max}; $P < 0.05$) than their raw counterparts. For soybeans, all fermentation products (total gas (A), SCFA, H_2S) measured in this experiment were unaffected ($P > 0.05$) by cooking. Consequences of the thermal treatment were also higher ($P < 0.05$) propionate and acetate molar ratios, which were counterbalanced by less ($P < 0.001$) butyrate.

PCA of the Influence of the Ingredient. A PCA was performed based on the correlation matrix from the complete data set (Figures 1 and 2). The first two principal components (PC1, PC2), which explained, respectively, 60.9 and 10.8% of the variability in the data set, indicated a clear separation according to the variables that were tested. PC1 discriminated ingredients from plant origin from animal protein sources, whereas on the score plot PC2 separated ingredients according to the thermal treatment. Insects were also more dispersed along PC2 than the meats and grain legumes. On the loading plot, high proportions of acetate and propionate during the in vitro fermentation of raw and cooked grain legumes as well as a high production of gas (A and R_{max}) and H_2S by gram of ingredient ingested were translated into high negative scores on PC1. In return, high positive values on PC1 reflected high BCFA and butyrate productions, as observed with insects and meats. PC2 showed a separation according to the thermal treatment applied to insects. High values of B, as observed when raw insects were fermented, were associated with low values along PC2.

DISCUSSION

In the human diet, dietary nutrients such as indispensable AA can be provided by ingredients from plant and animal origins, including insects. In terms of nutritive value, it is preferable to consume ingredients that are highly digested in the upper GIT to allow the absorption of nutrients. In this study, ingredients, in the raw state and after cooking using several methods, were incubated with pepsin and pancreatin to assess their digestibility. The residues obtained after enzymatic digestion were incubated with a bacterial inoculum from porcine faeces to simulate the fermentation occurring in the large intestine. This fermentation leads to the production of various metabolites, with potentially beneficial (butyrate, for example) or detrimental (H_2S, for example) impacts on the host's intestinal health.

In our experiment, digestibility of ingredients in the small intestine was evaluated after incubation with porcine pepsin and pancreatin and removal of soluble components by centrifugation, according to the protocol of Boisen and Fernandez.[28] This protocol, initially developed to study the digestibility of pig's dietary ingredients, was chosen as it has been validated with in vivo results. The combination of the enzymatic hydrolysis to subsequent in vitro fermentation with porcine feces was also validated in vivo in pigs, namely, to predict the fate of dietary protein in the GIT,[36] which is strongly related to the aim of the present study. Moreover, the use of porcine enzymes for in vitro protocols has been acknowledged for human studies.[37] However, the specific protocol that was used here has some drawbacks and must in this study be considered as a preliminary step to perform the in vitro fermentation. As we chose to work with ingredients in their natural form rather than with extracts of proteins from various origins, the same quantity of enzymes was used to digest all ingredients, although these ingredients contain variable amounts of protein. However, this variable enzyme/substrate ratio is expected to have a minor effect on the results given that enzymes are used in excess in the protocol.[28] Moreover, undigested components were present in the residue of centrifugation, and consequently supernatants were assumed to contain only digested components. In reality, some potentially undigested components can still be present in the supernatants, such as short-chain peptides, oligosaccharides, and dextrins. To avoid this drawback, Kaur et al.[38] have monitored the release of ninhydrin reactive amino nitrogen during enzymatic hydrolysis to evaluate the protein digestibility. Another possibility to enhance the precision of the protein digestibility estimate could be the use of a dialysis membrane instead of centrifugation, as used by Gatellier and Sante-Lhoutellier[39] in their enzymatic microreactor. Despite the drawbacks of the in vitro enzymatic digestion protocol that was used, our results showed that, working with raw ingredients, meats were highly susceptible to pepsin and pancreatin hydrolysis, followed by insects and finally by grain legumes. As a consequence, for each gram of ingredient consumed, the quantity of dietary components available as energy source for microbiota in the large intestine was negatively correlated (correlation coefficients of −0.81, −0.83, and −0.71 between IVDMD and gas, SCFA, and H_2S productions, respectively) to the digestibility in the upper GIT. Therefore, the in vitro fermentation of raw meats resulted in low quantities of fermentation products (gas, SCFA, H_2S). In addition to the quantity of fermentation products, the kinetics of gas production are affected by the ingredient. Gas production was fast when residues of raw meat were incubated with porcine inoculum, probably because these ingredients contain more soluble peptides than other tested ingredients.[14] In the residues of grain legumes and insects, proteins are respectively embedded in cell walls or bound to the chitin in the cuticle, making them less available for microbes at the beginning of the incubation. The fermentation was particularly slow with hydrolyzed residues of insects. Coming to fermentation metabolites, SCFA are major end-products of proteolytic and carbohydrate fermentation, in addition to gases. In vivo, SCFA

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

are absorbed in the blood and by the enterocytes as fermentation takes place in the colon, and their energy content becomes available to the host as metabolizable energy. SCFA profiles reveal that different fermentation pathways were used by pig fecal bacteria when fermenting ingredients of different origins. Fermentation of grain legumes was associated with increased propionate and decreased BCFA molar ratios by comparison with meats and insects. BCFA are indicators of protein fermentation.[17] They can be produced from the deamination of branched-chain AA (valine, leucine, and isoleucine) by many gut genera such as *Bacteroides* spp., *Propionibacterium* spp., *Streptococcus* spp., and *Clostridium* spp.[40] The molar ratio of BCFA is directly related to the quantity of dietary protein available for bacterial fermentation after digestion by pepsin and pancreatin. Therefore, among grain legumes, soybean proteins showed the highest BCFA production, which is in line with the higher proportion of protein in the hydrolyzed residue of this ingredient by comparison with beans, peas, and lentils.

In the human diet, protein sources are usually cooked before consumption. This heat treatment does have consequences on the digestibility and the subsequent intestinal fermentation of these foods. In this study, cooking treatments that mimic common cooking practices in industrial and domestic kitchens were applied to meats, insects, and grain legumes. Application of a heat treatment altered the availability of dietary nutrients from insects and, to a lesser extent, from meats, as indicated by the reduction in IVDMD. The influence of heat treatment was more pronounced for insects than for meats, probably because the thermal treatment was harsher than for meats for sanitation purposes. This change in digestibility after cooking leads to a higher supply of dietary components in the large intestine, which is reflected by an increased total gas production after 72 h of fermentation except for mealworm larvae. Similarly, SCFA and H_2S productions were also higher with cooked insects and meats than with raw ones, more markedly for insects (1.6- and 4.3-fold increases for SCFA and H_2S productions with insects vs 1.3- and 1.6-fold increases with meats). Heat treatment also induces an increase in the propionate molar ratio. Limiting H_2S production is beneficial because this component is reputed as toxic to the intestinal mucosal barrier via DNA damage, alteration of the cellular respiration,[41] and inhibition of the butyrate oxidation in colonocytes.[42] As H_2S originates from the fermentation of sulfur-containing AA and of dietary and mucinous inorganic sulfur by sulfate-reducing bacteria,[43] the increase in H_2S could be explained by an alteration of methionine and cysteine at temperatures around 120 °C, making them more resistant to enzymatic hydrolysis in the small intestine and thus more available as fermentation substrate in the large intestine.[44,45] Whereas oven-cooking insects at 150 °C for 30 min and at 200 °C for 10 min had similar impacts on digestibility and intestinal fermentation, autoclaving insects seemed the most detrimental thermal treatment (reduced IVDMD and higher H_2S production). Among ingredients from animal origin, cooked meats should be preferred to cooked insects due to their higher small intestinal digestibility and their slight response to heat treatment, notably in relation to the increase in the production of H_2S after cooking. For starchy grain legumes (lentils, peas, and beans), heat treatment exerted the opposite effect compared to animal proteins and improved the availability of nutrients including starch, as highlighted by the increase in IVStarchD values. This is explained by the well-documented rupture of starch granules induced by moist heat and its gelatinization, which improves the accessibility of starch to enzymatic attack, as well as the destruction of heat-labile antinutritive compounds such as amylase inhibitors.[46,47] The higher degradation in the upper GIT is then reflected by reduced gas and SCFA productions in the large intestine per gram of ingredient consumed. Similarly to animal proteins, the molar ratio of propionate was also increased after heat treatment, which is compensated by a reduced butyrate molar ratio. As starch is known to be butyrogenic,[48] this reduced molar ratio of butyrate is explained by the higher IVStarchD when grain legumes were previously cooked. H_2S production after 24 h of in vitro fermentation was also reduced with cooked lentils and peas but, surprisingly, it increased with cooked beans compared to raw ones. Cooking also induced a faster initiation of the fermentation, possibly because insoluble cell wall components available for fermentation in grain legume hydrolyzed residues are more similar to the substrate fecal microbes are fermenting when sampled in the rectum of sows, compared to starch from raw ingredients. Among tested grain legumes, the impact of cooking on soybean differed markedly as compared to beans, lentils, and peas, reflecting this higher content of CP and lower content of starch. IVCPD of soybeans was improved by cooking, probably due to the thermal inactivation of trypsin inhibitors, the most deleterious antinutritive factor in soybeans.[49]

Comparing the in vitro digestibility, fermentation kinetics, and fermentation products (SCFA and H_2S) of various ingredients showed that globally the category of the ingredients (meats, insects, grain legumes) accounted for a large part of the variability observed as highlighted by the 60.9% explained by PC1 in the PCA. Among the three categories of ingredients, insects were more dispersed along PC2 (10.8%) than meats and grain legumes, suggesting that the digestibility and fermentation characteristics of insects were more affected by the cooking procedure than other categories. These three categories display big differences in terms of proteins (CP content and AA composition) and also in terms of other components (fiber, carbohydrates, chitin, etc.) making up the complex food matrix, which in turn affected differently their susceptibility to digestive and subsequent fermentation processes in the in vitro model.

It can be concluded that fermentation in the large intestine of the undigestible fraction of ingredients from plant and animal origins, including insects, is mainly influenced by the ingredients due to their different compositions rather than the cooking procedure. For each gram of ingredient consumed, ingredients from animal origin, namely, meats and insects, were associated with fewer fermentation end-products (gas, H_2S, SCFA) than ingredients from plant origin, which is related to their higher small intestinal digestibility. Limiting fermentation end-products could be interesting due to the potential detrimental impact of some fermentation metabolites on the host's intestinal health, as, for example, H_2S. According to our results, cooking has variable effects according to the ingredient. Therefore, consumption of cooked ingredients from animal origin could be associated with higher quantities of fermentation products, although less SCFA and gas are produced when cooked grain legumes are ingested compared to raw ones. With regard to the SCFA production, we can also note that application of heat treatment to ingredients from animal and plant origins increased the proportion of propionate by comparison with the total SCFA production. Why

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

propionate is increased by cooking is unknown and needs further investigation.

AUTHOR INFORMATION

Corresponding Author

*(J.B.) Phone: +32 81 62 26 09. E-mail: Jerome.Bindelle@ulg.ac.be.

ORCID

Christine Poelaert: 0000-0002-5337-4191

Funding

This research was supported by the Fund for Scientific Research (FNRS, Brussels, Belgium − Research Credit 1.5180.12). The research was performed within the framework of the collaborative Food4Gut excellence research program of the Walloon Government.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The expert technical and scientific assistance of the staff of the Precision Livestock and Nutrition Unit of Gembloux Agro-Bio Tech is gratefully acknowledged.

ABBREVIATIONS USED

A, maximal gas production (mL/g of DM); AA, amino acid; ADF, acid detergent fiber; *B*, time at which half of the maximal gas production is reached (h); BCFA, branched-chain fatty acids; *C*, constant; CP, crude protein; DM, dry matter; GIT, gastrointestinal tract; H_2S, hydrogen sulfide; IVCPD, in vitro crude protein digestibility; IVDMD, in vitro dry matter digestibility; IVStarchD, in vitro starch digestibility; NDF, neutral detergent fiber; PCA, principal component analysis; R_{max}, maximum rate of gas production (mL/g of DM per hour); SCFA, short-chain fatty acids; SEM, standard error of the mean; T_{max}, time at which the maximum in vitro fermentation rate occurs (h)

REFERENCES

(1) Klurfeld, D. M. Research gaps in evaluating the relationship of meat and health. *Meat Sci.* **2015**, *109*, 86−95.

(2) Rebello, C. J.; Greenway, F. L.; Finley, J. W. Whole grains and pulses: a comparison of the nutritional and health benefits. *J. Agric. Food Chem.* **2014**, *62*, 7029−7049.

(3) Gerber, P. J.; Steinfeld, H.; Henderson, B.; Mottet, A.; Opio, C.; Dijkman, J.; Falcucci, A.; Tempio, G. *Tackling Climate Change through Livestock: A Global Assessment of Emissions and Mitigation Opportunities*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2013.

(4) Sánchez-Muros, M.-J.; Barroso, F. G.; Manzano-Agugliaro, F. Insect meal as renewable source of food for animal feeding: a review. *J. Cleaner Prod.* **2014**, *65*, 16−27.

(5) Rumpold, B. A.; Schlüter, O. K. Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Sci. Emerging Technol.* **2013**, *17*, 1−11.

(6) Chacko, A.; Cummings, J. H. Nitrogen losses from the human small bowel: obligatory losses and the effect of physical form of food. *Gut* **1988**, *29*, 809−815.

(7) Cummings, J. H.; Macfarlane, G. T. Role of intestinal bacteria in nutrient metabolism. *Clin. Nutr.* **1997**, *16*, 3−11.

(8) Silvester, K. R.; Cummings, J. H. Does digestibility of meat protein help explain large bowel cancer risk? *Nutr. Cancer* **1995**, *24*, 279−288.

(9) Gilbert, J.-A.; Bendsen, N. T.; Tremblay, A.; Astrup, A. Effect of proteins from different sources on body composition. *Nutr., Metab. Cardiovasc. Dis.* **2011**, *21*, B16−B31.

(10) Carbonaro, M.; Grant, G.; Cappelloni, M.; Pusztai, A. Perspectives into factors limiting in vivo digestion of legume proteins: antinutritional compounds or storage proteins? *J. Agric. Food Chem.* **2000**, *48*, 742−749.

(11) Gilani, G. S.; Wu Xiao, C.; Cockell, K. A. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *Br. J. Nutr.* **2012**, *108* (Suppl. 2), S315−S332.

(12) Sante-Lhoutellier, V.; Aubry, L.; Gatellier, P. Effect of oxidation on in vitro digestibility of skeletal muscle myofibrillar proteins. *J. Agric. Food Chem.* **2007**, *55*, 5343−5348.

(13) Macfarlane, G. T.; Macfarlane, S. Bacteria, colonic fermentation, and gastrointestinal health. *J. AOAC Int.* **2012**, *95*, 50−60.

(14) Cone, J. W.; Jongbloed, A. W.; Van Gelder, A. H.; de Lange, L. Estimation of protein fermentation in the large intestine of pigs using a gas production technique. *Anim. Feed Sci. Technol.* **2005**, *123*, 463−472.

(15) Macfarlane, G. T.; Gibson, G. R.; Beatty, E.; Cummings, J. H. Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. *FEMS Microbiol. Lett.* **1992**, *101*, 81−88.

(16) Windey, K.; De Preter, V.; Verbeke, K. Relevance of protein fermentation to gut health. *Mol. Nutr. Food Res.* **2012**, *56*, 184−196.

(17) Pieper, R.; Villodre Tudela, C.; Taciak, M.; Bindelle, J.; Pérez, J. F.; Zentek, J. Health relevance of intestinal protein fermentation in young pigs. *Anim. Health Res. Rev.* **2016**, 1.

(18) Chao, A.; Thun, M. J.; Connell, C. J.; McCullough, M. L.; Jacobs, E. J.; Flanders, W. D.; Rodriguez, C.; Sinha, R.; Calle, E. E. Meat consumption and risk of colorectal cancer. *JAMA* **2005**, *293*, 172−182.

(19) Yao, C. K.; Muir, J. G.; Gibson, P. R. Review article: insights into colonic protein fermentation, its modulation and potential health implications. *Aliment. Pharmacol. Ther.* **2016**, *43*, 181−196.

(20) Hughes, R.; Kurth, M. J.; McGilligan, V.; McGlynn, H.; Rowland, I. Effect of colonic bacterial metabolites on Caco-2 cell paracellular permeability in vitro. *Nutr. Cancer* **2008**, *60*, 259−266.

(21) Andriamihaja, M.; Davila, A.-M.; Eklou-Lawson, M.; Petit, N.; Delpal, S.; Allek, F.; Blais, A.; Delteil, C.; Tomé, D.; Blachier, F. Colon luminal content and epithelial cell morphology are markedly modified in rats fed with a high-protein diet. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2010**, *299*, G1030−G1037.

(22) Attene-Ramos, M. S.; Wagner, E. D.; Plewa, M. J.; Gaskins, H. R. Evidence that hydrogen sulfide is a genotoxic agent. *Mol. Cancer Res.* **2006**, *4*, 9−14.

(23) Pedersen, G.; Brynskov, J.; Saermark, T. Phenol toxicity and conjugation in human colonic epithelial cells. *Scand. J. Gastroenterol.* **2002**, *37*, 74−79.

(24) Holmes, A. J.; Chew, Y. V.; Colakoglu, F.; Cliff, J. B.; Klaassens, E.; Read, M. N.; Solon-Biet, S. M.; McMahon, A. C.; Cogger, V. C.; Ruohonen, K.; Raubenheimer, D.; Le Couteur, D. G.; Simpson, S. J. Diet-microbiome interactions in health are controlled by intestinal nitrogen source constraints. *Cell Metab.* **2016**, DOI: 10.1016/j.cmet.2016.10.021.

(25) Heinritz, S. N.; Mosenthin, R.; Weiss, E. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. *Nutr. Res. Rev.* **2013**, *26*, 191−209.

(26) Boccard, R.; Buchter, L.; Casteels, E.; Cosentino, E.; Dransfield, E.; Hood, D. E.; Joseph, R. L.; MacDougall, D. B.; Rhodes, D. N.; Schön, I.; Tinbergen, B. J.; Touraille, C. Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the commission of the European Communities' (CEC) beef production research programme. *Livest. Prod. Sci.* **1981**, *8*, 385−397.

(27) Bindelle, J.; Buldgen, A.; Boudry, C.; Leterme, P. Effect of inoculum and pepsin−pancreatin hydrolysis on fibre fermentation

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

measured by the gas production technique in pigs. *Anim. Feed Sci. Technol.* **2007**, *132*, 111−122.
(28) Boisen, S.; Fernandez, J. A. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. *Anim. Feed Sci. Technol.* **1997**, *68*, 277−286.
(29) Menke, K. H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 7−55.
(30) Poelaert, C.; Boudry, C.; Portetelle, D.; Théwis, A.; Bindelle, J. Use of medium without reducing agent for in vitro fermentation studies by bacteria isolated from pig intestine. *J. Anim. Sci.* **2012**, *90* (Suppl. 4), 387−389.
(31) Mauricio, R. M.; Mould, F. L.; Dhanoa, M. S.; Owen, E.; Channa, K. S.; Theodorou, M. K. A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. *Anim. Feed Sci. Technol.* **1999**, *79*, 321−330.
(32) Groot, J. C. J.; Cone, J. W.; Williams, B. A.; Debersaques, F. M. A.; Lantinga, E. A. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Anim. Feed Sci. Technol.* **1996**, *64*, 77−89.
(33) Leibovich, J.; Vasconcelos, J. T.; Galyean, M. L. Effects of corn processing method in diets containing sorghum wet distillers grain plus solubles on performance and carcass characteristics of finishing beef cattle and on in vitro fermentation of diets. *J. Anim. Sci.* **2009**, *87*, 2124−2132.
(34) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 1990.
(35) Van Soest, P. J.; Robertson, J. B.; Lewis, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583−3597.
(36) Bindelle, J.; Buldgen, A.; Delacollette, M.; Wavreille, J.; Agneessens, R.; Destain, J. P.; Leterme, P. Influence of source and concentrations of dietary fiber on in vivo nitrogen excretion pathways in pigs as reflected by in vitro fermentation and nitrogen incorporation by fecal bacteria. *J. Anim. Sci.* **2008**, *87*, 583−593.
(37) Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Bohn, T.; Bourlieu, C.; Carriere, F.; Boutrou, R.; Corredig, M.; Dupont, D.; Dufour, C.; Egger, L.; Golding, M.; Karakaya, S.; Kirkhus, B.; Le Feunteun, S.; Lesmes, U.; Macierzanka, A.; Mackie, A.; Marze, S.; McClements, D. J.; Menard, O.; Recio, I.; Santos, C. N.; Singh, R. P.; Vegarud, G. E.; Wickham, M. S. J.; Weitschies, W.; Brodkorb, A. A standardised static in vitro digestion method suitable for food − an international consensus. *Food Funct.* **2014**, *5*, 1113−1124.
(38) Kaur, L.; Maudens, E.; Haisman, D. R.; Boland, M. J.; Singh, H. Microstructure and protein digestibility of beef: the effect of cooking conditions as used in stews and curries. *LWT−Food Sci. Technol.* **2014**, *55*, 612−620.
(39) Gatellier, P.; Santé-Lhoutellier, V. Digestion study of proteins from cooked meat using an enzymatic microreactor. *Meat Sci.* **2009**, *81*, 405−409.
(40) Rist, V. T. S.; Weiss, E.; Eklund, M.; Mosenthin, R. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. *Animal* **2013**, *7*, 1067−1078.
(41) Davila, A.-M.; Blachier, F.; Gotteland, M.; Andriamihaja, M.; Benetti, P.-H.; Sanz, Y.; Tomé, D. Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. *Pharmacol. Res.* **2013**, *68*, 95−107.
(42) Leschelle, X.; Robert, V.; Delpal, S.; Mouillé, B.; Mayeur, C.; Martel, P.; Blachier, F. Isolation of pig colonic crypts for cytotoxic assay of luminal compounds: effects of hydrogen sulfide, ammonia, and deoxycholic acid. *Cell Biol. Toxicol.* **2002**, *18*, 193−203.
(43) Lewis, S.; Cochrane, S. Alteration of sulfate and hydrogen metabolism in the human colon by changing intestinal transit rate. *Am. J. Gastroenterol.* **2007**, *102*, 624−633.
(44) Moughan, P. J. Amino acid availability: aspects of chemical analysis and bioassay methodology. *Nutr. Res. Rev.* **2003**, *16*, 127−141.
(45) Bender, A. *Meat and Meat Products in Human Nutrition in Developing Countries*; FAO: Rome, Italy, 1992.
(46) Bravo, L.; Siddhuraju, P.; Saura-Calixto, F. Effect of various processing methods on the in vitro starch digestibility and resistant starch content of Indian pulses. *J. Agric. Food Chem.* **1998**, *46*, 4667−4674.
(47) Altay, F.; Gunasekaran, S. Influence of drying temperature, water content, and heating rate on gelatinization of corn starches. *J. Agric. Food Chem.* **2006**, *54*, 4235−4245.
(48) Dongowski, G.; Jacobasch, G.; Schmiedl, D. Structural stability and prebiotic properties of resistant starch type 3 increase bile acid turnover and lower secondary bile acid formation. *J. Agric. Food Chem.* **2005**, *53*, 9257−9267.
(49) Andrade, J. C.; Mandarino, J. M. G.; Kurozawa, L. E.; Ida, E. I. The effect of thermal treatment of whole soybean flour on the conversion of isoflavones and inactivation of trypsin inhibitors. *Food Chem.* **2016**, *194*, 1095−1101.
(50) Mariotti, F.; Tomé, D.; Mirand, P. P. Converting nitrogen into protein − beyond 6.25 and Jones' factors. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 177−184.

DOI: 10.1021/acs.jafc.6b04599
J. Agric. Food Chem. XXXX, XXX, XXX−XXX