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Feeding a high oleic acid (C18:1) diet improves pleasing flavor attributes in pork

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Abstract

One of the main drivers in consumer meat choice is flavor with some pleasing flavor attributes associated with high oleic acid (C18:1) content in the intramuscular fat. This project aimed to produce pork with a distinctively pleasant bouquet by feeding C18:1 rich macadamia oil compared to corn oil. The project also tested three feed formulations based on cereals and pulses representing different pork producing areas: corn/soy (CS), sorghum-lupins (SL), or wheat/canola (WC). Compared to corn oil, feeding macadamia oil resulted in a significant increase in the C18:1 content in pork loin (*longissimus dorsi*) with the CS showing a higher impact than the WC diets. Pork loins from the two CS-based groups (CS-Corn and CS-Macadamia) were selected for a consumer sensory study involving 82 panelists (39 of Chinese background). Across ethnic backgrounds the taste of high C18:1 pork resulted in the highest hedonic values.

Keywords: *pork; longissimus dorsi; oleic acid; MUFA; sensory attributes*

1 Introduction

Flavor is one of the main drivers in consumer preferences for meats. Tenderness, juiciness, and roast flavor positively influence customer preference (Aaslyng et al., 2007). All these characteristics are closely linked to the meat composition, especially the intramuscular fat abundance and degree of fatty acid (FA) saturation (Song et al., 2017). Tikk and co-workers established a link between specific FA profiles and flavor attributes in pork (Tikk, Tikk, Aaslyng, Karlsson, Lindahl & Andersen, 2007). In particular, the degree of unsaturation of FA appears to have a major influence on taste characteristics (Mottram, 1998). A high level of the monounsaturated fatty acid (MUFA) oleic acid (C18:1), together with a low level of polyunsaturated fatty acid (PUFA) linoleic acid (C18:2) affected the production of volatile compounds and the resulting pork ham flavor (Benet, Guàrdia, Ibañez, Solà, Arnau, & Roura, 2015). In brief, a high MUFA/PUFA ratio improved the release of pleasant aromatic notes from Maillard reactions in cooked ham (Benet, Guàrdia, Ibañez, Solà, Arnau & Roura, 2016).

The FA profile in pork adipose tissue and muscle can be modified by changing the fatty acid profile of the pig diet (Cava et al., 1997). Previous research showed that levels of the PUFA linoleic acid (C18:2) in pork tissues increased linearly with dietary inclusion (Wood, 1984). In contrast, dietary MUFAs (i.e. C16:1 and C18:1) were hardly affected by dietary concentrations (Teye, Sheard, Whittington, Nute, Stewart & Wood, 2006). The traditional Spanish Iberico breed has a high gastronomic reputation due to its tender and juicy meat which is associated with rearing practices that include foraging on oleic acid-rich acorns (Daza, Rey, Ruiz & Lopez-Bote, 2005). Overall, little is known about the efficiency of transfer of dietary C18:1 from C18:1-rich oils to fat and muscle tissues in pork.

High levels of PUFA are common in cereal-based pig commercial diets. While corn-soybean diets are widely used globally, in some geographical areas pig feed formulations might be based on other locally available grains and pulses mainly wheat, barley, sorghum, lupins or canola to mention only a few (Boye, Zare & Pletch, 2009; Sevillano, Nicolaiciuc, Molist, Pijlman & Bergsma, 2018). Little has been reported on how specific cereals and pulses may impact the amount and composition of fat deposits in pork.

The aim of this study was to produce pork with a distinctively pleasant bouquet signature associated with a high C18:1 content transferred from feed. In particular, the study assessed the impact of high dietary C18:1 in pigs on C18:1 in pork longissimus dorsi (LD) using three different feed formulations representing standard Australian, European, or North American feeds and two oils selected based on high (macadamia) or low (corn) C18:1 content. In addition, the project studied the impact of high C18:1 levels in pork on the physicochemical characteristics of the meat and on the sensory attributes of the roasted pork, with especial attention to potential differences in taste preferences with Chinese-background consumers, the main non-Western ethnic community in Australia.

It was hypothesized that C18:1-rich feeds would result in increased C18:1 levels in pork LD improving the hedonic value perceived by consumers across feed formulations relevant to Australia, Europe and North America. In addition, it was hypothesized that the improved hedonic value related to C18:1 will be independent of ethnic background.

2 Material and methods

2.1 Production of high-oleic acid pork

2.1.1. Diets and animals

Three iso-energetic and iso-nitrogenous diets (14.1 MJ/kg and 17.4 % CP) were formulated based on current standard diets used in Australia, Europe, and North America. A diet based on corn (cereal) and soybean meal (pulse) was chosen as representative of diets used in North America. A diet based on wheat and canola oil was chosen as likely to be used in parts of Europe (and potentially Western Australia). Finally, a diet with sorghum and lupins aimed to represent a uniquely Australian formulation. Corn oil was used as a source of PUFA while macadamia oil was used as a source of uniquely Australian MUFA, particularly C18:1. During the first stage of the animal experiment (approximately from 50 to 70 kg of body weight) the two experimental oils (macadamia or corn) were added at 1.5% of the diet following current commercial recommendations on energy/protein ratios. From 70kg to slaughter, when the oil supplements were anticipated to have a higher impact on carcass composition, the oils were added at 3% of the diet.

The 12 experimental feeds (3 diets, 2 oils, 2 levels of oil -1.5, and 3%-) were manufactured by Riverina Pty Ltd (Warwick, Australia). Diet composition and nutritional value are are shown in the supplementary material. Corn oil (Soon Oilmill SDN BHD, Penang, Malaysia) was sourced from Queensland Produce Distributors (Toowoomba, Australia). Macadamia oil (cold-pressed) was manufactured and directly sourced from Proteco Gold Pty. Ltd. (Kingaroy, Australia).

Two hundred grams of each of the 12 diets were collected for analysis at arrival to the piggery. One hundred grams of the feed samples were destined for macro

and micro-nutrient content following standard procedures (AOAC, 2019), and 100 grams were destined to obtain the fatty acid profile. One liter of each of the oils included in the feeds (corn or macadamia oils) was sourced from the mill for fatty acid profile analysis.

The pig feeding study was performed at the SunPork Group pig research farm in Westbrook (Queensland, Australia) with animal ethics approval from the University of Queensland Animal Ethics Committee (certificate CHM/SAFS/256/17). Thirty-six post-weaning immuno-castrated by intramuscular injection of Improvac® (Zoetis, Parsippany, USA) on week 13 and 17, male pigs (Large White X Landrace) were individually penned for 6 weeks, from 50 kg to 110kg of body weight (BW). Pigs were stratified based on similar body weights into 6 blocks of 6 pigs each. The blocks were co-located in adjacent pens and accounted for potential live weight differences and shed locations. After an adaptation period of 7 days pigs were assigned to the experimental diets following a randomized complete block design. During the adaptation period, the pigs received a standard commercial feed (13.2 MJ/Kg of energy and 16.5% crude protein). After the adaptation, each block had one pig receiving one of the 6 diets resulting in a final replication of n=6. Bodyweight and feed disappearance were recorded weekly. In adherence to animal welfare recommendations by the Animal Ethics Committee, the pigs had daily periods of exercise outside of the individual pen.

At the end of the finishing period the animals were sent to the SunPork Group abattoir (Swickers, Kingaroy, Australia). Meat pH, carcass temperature, and back fat depth (P2) data were collected 45 minutes after the pigs were slaughtered. Pork samples were collected 24h post-slaughter. The ribs containing the LD were

cut from both sides of the carcass with one 2-rib wide cut from the right side stored separately. The three samples from each pig were vacuum packaged and labeled individually for meat quality (the 2-rib cut) and sensorial analysis (the LD from both sides). The 2-rib cuts were immediately sent to the meat laboratory to perform the meat physicochemical measures. The two pieces for sensorial analysis were aged for 7 days at 4ºC and then frozen at -20ºC until required.

2.1.2 Pork physicochemical analysis.

2.1.2.1 pH

Loin pH was determined using a Eutech2700 pH meter (Thermo Fisher Scientific, Waltham, USA) at 24, 48, and 72 hours after slaughter (n=36, in triplicates). The procedure was repeated in frozen samples to determine the effect of freezing on muscle pH.

2.1.2.2 Drip loss

Drip loss (% relative to the initial sample weight) at 48 hours was measured for each pork sample (n=36, in triplicates) using the drip bag method adapted from Honikel (1998).

2.1.2.3 Meat color

Color measurements were performed after the loin surface was exposed to air at room temperature for 10 min. Meat color was determined using a Chroma Meter Model CR-400 (Konica Minolta,Tokyo, Japan), which was set on the L*, a* and b* system, where L* denotes relative lightness, a* relative redness, and b* relative yellowness using D65 illumination and a 2° standard observer.

2.1.2.4 Texture

Pork texture attributes (raw and cooked) were measured using a Warner-Bratzler shear forcer (WBSF) with a V-shaped blade attached to an Instron Universal Testing Machine (Instron Corp., New York, USA) following the method reported by King et al. (2009). In the measurement of WBSF, six pieces of the LD muscle

were sliced from each sample (raw and cooked) parallel to the muscle fiber using a core borer of 1.27 cm of diameter.

2.1.2.5 Cooking losses

Cooking losses were determined after the pork (2 cm thick deboned steak) was grilled at 185 °C for 2 minutes and 30 seconds without any added seasoning. The steaks were weighed before and after cooking and the juice from each steak was collected and weight. Juice samples were kept in a crystal vial until FA profile analysis.

2.1.2.6 Fatty acids profile

Lipid content from oils, feed, and raw pork were extracted using an adaptation of a method from Hara and Radin (1978). The samples were then derivatized and the fatty acid methyl esters (FAME) and extracted following the method of Ma et al. (2018). The samples were run on a GCMS (Shimadzu, Kyoto, Japan) using a Restek Stabilwax column (Restek, Bellefonte, USA) with an id of 0.25 mm and a 0.25 µm film thickness. Individual fatty acids were identified by their mass spectrum, and by comparison to a Restek mixed fatty acid standard (Restek, Bellefonte, USA).

2.1.2.7 Lipid oxidation

Thiobarbituric acid reactive (TBAR) contents in the meat samples (triplicates of 5 g) were determined by colorimetric analysis using an adaptation of the method Sørensen & Jørgensen (1996). Absorbance in each sample was measured at 510 nm using a spectrophotometer FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany). TBARS values were expressed as mg of Malondialdehyde per kg of meat.

2.1.3 Sample preparation

A pre-trial was performed to assess and adopt the optimal sample preparation and cooking conditions. Whole pork loin samples (bone-in) were thawed over 48h

169 at 4°C, deboned, and portioned accurately into 1 cm thick loin steaks (fat on)
170 using an ES9600 Cafe Series® 17cm Food Slicer (Newell Australia, Botany,
171 Australia). Loin steaks were stored at 4°C until used (within 3 days of
172 preparation). On the day of the sensory trial, the steaks were removed from the
173 refrigerator and kept at room temperature until they reached 12°C. The grill, Silex
174 GTT-10-10 Titan High-Speed Grill (Silex Elektrogerate GmbH, Hamburg,
175 Germany), was preheated to 185°C for 10-15 minutes before cooking. Steak
176 temperature was measured before and after cooking using a JXB-188 Infrared
177 Thermometer (Berrcom, Guangzhou, China).
178 Samples were grilled at 185°C for 50 seconds without salt, spices, or additional
179 oils or fats. Cooked steaks were placed on an aluminum tray covered with
180 aluminum foil for 2 minutes. After two minutes of resting, the excess of
181 subcutaneous fat was then removed, and the loins were sliced in 1 cm pieces,
182 with one fatty and 2 lean meat slices in each foil tart tray. Fat was not removed
183 before cooking, to prevent the meat from drying out and to preserve all flavors.
184 The pieces of sliced pork were placed into small aluminum trays, labeled with a
185 three-digit number for future identification, and covered by aluminum foil, and
186 placed in a buffet food warmer TARBFS310 (Target, Williams Landing, Australia)
187 set at 55-60°C, to be kept heated until served to panelists. The grill was cleaned,
188 and all the fat released was removed with a paper towel between each sample.

189 *2.2 Sensory analysis*

190 The study was approved by the University of Queensland Human Ethics
191 Committee (2017000444). The volunteers for the sensory trial were recruited
192 using the University of Queensland (St Lucia Campus) online and paper platforms
193 in English and Chinese. Following the completion of a recruitment questionnaire

194 volunteers with one of the following conditions were excluded from the study: a)
195 on specific medications (antibiotics, psychotropic, etc.); b) declared food allergies
196 or intolerances; c) smokers; d) pregnant or lactating women. Eighty-two non-
197 trained participants attended the sensory evaluation of the pork. The panel was
198 comprised of 24 males and 58 females aged from 18 to 79. The average age was
199 27 years old. Specific ethnic groups were sought for participation in this study:
200 Chinese (39) or Non-Chinese (43). The Non-Chinese group consisted of
201 panelists from different origins (Indian, European, Australian, etc.). The
202 volunteers recruited were invited to attend sensory test sessions, lasting one
203 hour, and were compensated with AU$20 voucher card.
204 Three (of the initial six) dietary treatments (WC and macadamia oil –WCM-, CS
205 and corn oil –CSC-, and CS and macadamia oil –CSM-) were selected to
206 evaluate the main hypothesis. To avoid the potential effect of the individual pig,
207 samples from 3 different pigs from each dietary group were used in the sensorial
208 trial (total of 9 pigs). The WCM treatment was chosen as a neutral reference to
209 compare CSC and CSM. The tests consisted of pair-comparisons where one
210 sample of the reference treatment (WCM) was compared to a test treatment
211 (CSC or CSM). Each sensory session consisted of testing 6 pork samples from
212 the testing treatments (CSC or CSM, 3 samples each) paired with 6 pork samples
213 from the reference group (WCM). The test was a single-blind study and the
214 samples were randomized. Special care was taken to offer homogeneous pork
215 cuts to avoid potential effects due to differences in thickness, form, or composition
216 (see section 2.2.1 on “Sample preparation”).

2.2.1 Sensory test presentation

The sensory session was performed in an equipped food sensory lab with 6 isolated booths, temperature control (22°C), and under day-light equivalent lighting. The evaluation was performed between meals, after breakfast, and before the midday meal. The panelists received a tray, which included the pork samples, a questionnaire, a pen, a napkin, a plastic cup with lukewarm water, and pieces of green apples (for mouth cleansing between samples).

The pork samples were provided to the participants in pairs. The participants were asked to rate each sample based on its aroma, appearance, and pork flavor pleasantness (from dislike extremely to like extremely) following a questionnaire described below in 2.2.3. This procedure was then repeated five times, totaling six sets.

2.2.2 Questionnaire

The questionnaire used for the sensory test was composed of 3 introductory questions and 12 recurring questions for the 6 sets. To determine the preferences of the participants, 10 descriptors (pork aroma pleasantness, pork appearance, juiciness, tenderness, fattiness, pork flavors pleasantness, fatty flavor, savory, caramelized, overall flavor intensity) were chosen based on previous literature (Civille & Lyon, 1996; Byrne et al., 2001; Aaslying et al., 2007; Tikk et al., 2007; Coggins, 2012; Maughan & Martini, 2012; Madeira et al., 2013). Three scales were used to score the descriptors (Lim, 2011): a 9-point hedonic labeled scale from "dislike extremely" to "like extremely" was used to evaluate the pleasantness of the aroma, the appearance, and the flavor of the pork (Lawless et al., 2010); a sensory 9-point labeled scale was used to rate the juiciness, tenderness, fattiness, fatty flavor, savory (umami) flavor, and caramelized notes (sweet

roasted flavor); and the pork flavor intensity was rated with a sensory gLMS (labeled magnitude scale) from "barely detectable" to "strongest imaginable". The two last questions in the questionnaire were intended to find out if participants believed the samples were different, and if they had a preference between the two samples.

2.3 Statistical analysis

All data were analyzed using the R statistical language (Version 3.4.4, R Core Team 2020). Animal production data and objective measurements of pork were averaged across duplicate or triplicate measurements as appropriate. A randomized block factorial model was fitted with effects for the base diet, oil type, and their interaction. Where an F-test was significant ($p<0.05$), means were compared using a simple least significant difference (LSD) test. Residual plots were used to check model adequacy and detect outliers. Sensory scale data were analyzed using a linear mixed model (lme4 library) with random effects for pig and panelists. Tasting set (order) was included as a fixed effect along with diet, ethnicity, and their interaction. Marginal (least-squares) means and their standard errors were estimated, and pair-wise comparisons carried out when the ANOVA indicated a significant effect. The ability of panelists to detect a difference between samples as well as their preference was analyzed using logistic regression models. For the sample preference, only individuals who correctly detected a difference were included.

3 Results and discussion

Future trends in pork market are heavily influenced by consumer demands towards healthier, safer, and better-quality meat (Yang & Lien 2016). Pork eating quality has been associated with tenderness and flavor (Dunshea et al. 2005).

267 An iconic pork flavor for succulence has been referred to as the Spanish Iberico
268 breed typical of Western Spain (Ventanas et al., 2007). Compared to more
269 prevalent commercial crossbreeds, the traditional Iberico shows slower growth
270 rates associated with unique grazing-based rearing practices that include
271 foraging on oleic acid-rich acorns (Lopez-Bote, 1998). The result is a tender and
272 juicy meat with abundant marbling (i.e. intramuscular fat –IMF-) rich in C18:1
273 (Daza, Ruiz & Lopez-Bote, 2005). C18:1 is the main MUFA in plant and animal
274 tissues and in the human diet, with strong associations with health indicators in
275 humans such as improved lipid profile, decreased blood pressure and modulation
276 of insulin resistance, endothelial function, and glycemic control (Guasch-Ferré et
277 al., 2015).
278 Previous research in our group examined the aromatic compounds responsible
279 for the Iberico cooked ham pork flavor by studying fat and volatile profiles
280 compared to a common commercial crossbreed (Large White x Landrace) and
281 found that the main flavor differences were related to the C18:1 content and the
282 MUFA/PUFA ratio (Benet et al., 2015). The high C18:1 content in the Iberico pork
283 resulted in higher values of odor-active aroma compounds from the Maillard
284 reaction, which are related to roast flavors and a higher overall flavor liking (Benet
285 et al., 2016). The current project aimed at producing pork meat with high C18:1
286 content and assessing the effect on pork sensory attributes based on consumer
287 preferences with an emphasis on characterizing flavor perception in volunteers
288 with a Chinese background.
289 The three experimental feeds were formulated to model diets used by the pork
290 industry worldwide (corn-soybean and wheat-canola) or specifically in Australia
291 (sorghum/lupins) where the study was developed. The main interest was to study

how different formulations (Diet) would interact with the macadamia (high MUFA/PUFA ratio) or corn (low MUFA/PUFA ratio) oil supplements (Oil).

3.1 Pig performance parameters

No significant (P>0.05) main effects on performance indicators (final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR)) were observed related to Diet (CS, SL or WC) or Oil (macadamia or corn) (additional details on feed intake and growth performance have been provided in supplementary material). However, there was a significant (P=0.04) interaction between the two main effects on ADG indicating that macadamia or corn oils affected performance depending on Diet type. The macadamia oil supplement favored the WC diet but impacted negatively the final BW when supplemented to CS or SL diets showing reductions of 1.8 and 3.7% respectively compared with the corn oil supplement.

3.2 Fatty acid composition in feeds, pork loins, and juice losses after cooking

The initial objective was to increase the C18:1 content in pork fat by supplementing a standard pig feeding program with a C18:1-rich oil. Macadamia oil was selected due to the high C18:1 content (57%), low C18:2 content (2.58%), and the high MUFA/PUFA ratio of 30.63. In contrast, corn oil was selected based on a high C18:2 and low C18:1 contents (44% and 36.0%, respectively) resulting in a low MUFA/PUFA ratio of 0.832.

The analysis of the fatty acid composition of the experimental feeds is presented in Table 1. The FA profiles obtained were consistent with the high or low MUFA and PUFA profiles of the added Oil. Quantitatively, macadamia compared to corn oil supplemented feeds had a 67% higher MUFA/PUFA ratio across the three Diets. The FA content in the lipid fraction of the cereal and pulses also played a

317 role in the final profile of each feed group. Linoleic acid is predominant in corn
318 grains (56% C18:2 versus 27% C18:1), soybean (54% C18:2 versus 22% C18:1),
319 wheat (57% C18:2 and 15% C18:1) and lupins (31% C18:1 and 48% C18:2).
320 However, oleic acid is the main fatty acid in the lipid fraction of sorghum grains
321 (31% C18:1 and 45% C18:2), and canola meal (56% C18:1 and 22% C18:2) (de
322 Blas, Mateos & Garcia-Rebollar, 2010). Thus, the CS feed contained a lower
323 amount of oleic acid (31.3 and 38.0 %) compared to the SL (35.0 and 42.1 %) or
324 the WC (33.0 and 43.1 %) Diets for corn or macadamia oils, respectively. These
325 results confirmed the findings by Benz and co-workers who reported higher levels
326 of MUFA in fat deposits of sorghum-fed compared with corn-fed pigs (Benz et al.,
327 2011). In addition, CS presented the highest level of linoleic acid (in both oils -
328 46.2 and 31.1% respectively-) compared with the other two diets (SL -39.2 and
329 27.0%- and WC -42.0 and 25.0%-). Overall, the lowest and highest MUFA/PUFA
330 ratios were recorded for the corn oil supplemented CS diet (ratio of 0.67) and the
331 WC diet supplemented with macadamia oil (ratio of 2.01), respectively.
332 The fatty acid composition of feeds was partially reflected in the composition of
333 the LD as shown in Table 2. Macadamia oil-fed pigs resulted in pork with FA
334 levels higher in C14:0, C16:1, and C18:1 and decreased levels of C18:2
335 compared to the corn oil-fed group ($p=0.02$, $p<0.0001$, $p=0.001$, and $p=0.0005$,
336 respectively). Across Diets, the ratios of total MUFA/PUFA were significantly
337 ($P<0.01$) higher in macadamia compared to corn oil groups. Scheeder and co-
338 workers had previously reported that increasing dietary C18:1 levels had a
339 significant impact on the fatty acid profiles in pork LD (Scheeder, Glaser,
340 Elchenberger & Wenk,2000). In monogastric animals such as the pig, dietary fatty
341 acids are absorbed from the intestine and incorporated into tissue lipids. In

342 particular, PUFA linoleic and α-linolenic cannot be synthesized *de novo* in animal
343 tissues resulting in that their concentrations respond rapidly to dietary changes.
344 In contrast, saturated and MUFA can be synthesized *de novo* what minimizes the
345 impact of dietary manipulations (Wood and Enser 1997).
346 The difference in the C18:1 and total MUFA was also diet-dependent as shown
347 by a significant ($p \leq 0.05$) interaction Diet by Oil, indicating that the CS diet had a
348 significantly higher impact on C18:1 levels in LD than the WC or SL diets (Table
349 2, Figure 1). In addition, the SL diet with macadamia did not show a significant
350 increase in loin MUFA suggesting a limited transfer of C18:1 from the feed to
351 muscle fat. The CS and the SL control diets (without Oil) contained the lowest
352 (31%) and the highest (35%) levels of C18:1, respectively. Another important
353 aspect of how cereals and pulses may affect the fatty acid composition of pork
354 came because of the fatty acid composition of the juice losses after cooking.
355 Firstly, because the level of C18:1 and MUFA in the fat of the juice of macadamia
356 fed pigs (47.60% and 50.62% respectively) was significantly ($p<0.001$) higher
357 than in the corn oil-fed group (44.15% and 44.57% respectively). Secondly,
358 because the MUFA/PUFA ratio in the juice was significantly ($p=0.01$) higher in
359 the WC (3.4) than in the other two diets (3.1 in CS and 2.9 on SL). In summary,
360 the WC compared to the CS seems to have a negative impact on the absorption
361 and deposition of C18:1 and MUFA and a second negative impact related to
362 higher loss of MUFA during cooking. To the best of our knowledge, this is the first
363 time that clear evidence has been reported indicating that some cereals and
364 pulses may affect the transfer of dietary fats to body fat deposits.

365 *3.3 Pork physicochemical measures*

366 Based on previous literature, altering the lipid composition in pig meat (e.g. by
367 increasing C18:1) had no impact on objective meat physicochemical indicators
368 (Nuernberg et al., 2004; Yang & Lien 2016). Our data was consistent with the
369 previous reports in that there was no impact of Diet or Oil as the main effects on
370 pork physicochemical parameters except for color at 24 h (Table 3). Macadamia
371 oil was associated with a higher "blueness" (or b*) index at 24h which was not
372 confirmed at the 72-hours post-mortem time point. In addition, macadamia
373 decreased the L* value (where 0 = black and 100 = white) of pork making it darker
374 in the CS diet compared to the SL or WC groups. The opposite occurred with the
375 corn oil supplement which is consistent with the findings of Larick and co-workers
376 (1992), who reported a lighter, green note in pork from corn-fed pigs. The lighter
377 color was associated with an increase in the oxidative status particularly fat
378 oxidation (Larick et al., 1992). Thus, the data obtained seems to support the
379 observation that C18:1-rich pork is less prone to oxidation relative to PUFA.
380 However, neither Diet nor Oil had a significant impact on the lipid oxidation.
381 Finally, a significant interaction between Diet and Oil was observed affecting pH.
382 In brief, supplementing macadamia oil to the CS diet increased while the SL and
383 WC diets decreased the pH of the pork loin samples. In line with our results, pork
384 high in pH is generally darker in color (Miller 2020) and has higher water-holding
385 and may result in greater sensory tenderness and juiciness scores (Lonergan et
386 al., 2007). The latter is consistent with the results on sensory perception
387 presented in the next section where the CS with macadamia loin samples were
388 ranked with superior pleasantness by consumers.

3.4 Sensory analysis (trained panelist and consumer)

A second principal aim of the research project was to deliver pork with a distinctive pleasant bouquet for the consumer. To secure the feasibility of the test using non-trained panelists, the number of samples was reduced by focusing on three of the treatments. The treatments were selected to evaluate the hypotheses that: 1) a high C18:1 content and MUFA/PUFA ratio would result in improved cooked pork flavor perceived by volunteer panelists, and 2) a feed formulation based on locally produced cereal and pulses would further improve pork flavor compared to a global feed standard the CS diet. To assess hypothesis 1, the samples selected were WC with macadamia oil (WCM) and the CS with corn oil (CSC) for high and low C18:1, respectively. To assess hypothesis 2, the WCM was compared to the CS with macadamia (CSM) treatments. In other words, WCM samples were used as a control and were pair-tested to CSC and CSM. The selected sensory descriptors were partially selected to be suitable for non-trained consumers to evaluate the sensory quality of pork flavor. The group of volunteers included a subgroup of Chinese panelists to identify potential ethnic biases in the evaluation. The results showed that the Chinese cohort consistently rated lower than the non-Chinese cohort in all the selected attributes (Table 4). A lower rating associated with a Chinese cohort compared to Western panelists has also been reported in other studies involving meat sensory tests (O'Reilly et al., 2020). Differences in flavor liking are related with familiar sensory cues associated to the cultural background (Feng & O'Mahony, 2017). Importantly, the most relevant findings on the consumer sensory test were independent of ethnicity while showing a significant impact due to Diet. The panelists consistently rated the hedonic attributes of the CSM higher than the other two treatment

414 samples tested (Table 4). The increase of C18:1 in the profile of pork loins
415 improved liking values in the CS diet associated with the high MUFA/PUFA ratio
416 of the juice released during cooking. The increased ratio of MUFAs over PUFAs
417 cooked pork has been previously associated with a profile of volatile compounds
418 resulting in a desirable roasted meaty flavor (Zhao et al., 2017, Benet et al., 2016;
419 Nuernberg et al., 2004) confirming that using feed ingredients rich in C18:1 has
420 the potential to create a distinctive pork signature flavor resulting in an improved
421 liking by consumers regardless of their ethnicity background (Chinese vs non-
422 Chinese in this study).
423 The last section of the questionnaire for the pair-wise comparisons referred to the
424 ability of the panelist to sense a difference between the samples and "if yes" to
425 indicate the preferred one. The two questions were: 1- "Is there a difference?";
426 and 2- "Which sample do you prefer?". When the WCM (highest C18:1 and
427 MUFA/PUFA ratio) was compared to the CSC (lowest C18:1 and MUFA/PUFA
428 ratio), 87.65% of the responses detected a difference but no significant ($p>0.05$)
429 preference for one or the other were recorded. In contrast, when the sample
430 WCM was compared with the CSM, 84.08% of the panelists were able to detect
431 differences of which a significant ($p=0.027$) 65% of the panelists preferred the
432 CSM sample over the WCM (data not shown). Consistently, when the sensory
433 attributes from WCM and CSM where compared, the improvement associated
434 with the CSM diet affected "pleasantness" and "tenderness" ($p<0.05$), "juiciness"
435 ($p<0.01$), and "intensity", "caramelized", "fatty flavor" and "fattiness" ($p<0.001$)
436 (Figure 2, a and b). Finally, the unpaired analysis of the two CS groups differing
437 in Oil (macadamia (CSM) vs corn (CSC)) confirmed an improvement in attributes
438 associated with the macadamia which positively affected "pleasantness"

(p=0.014), “fatty flavor” (p=0.035) and “caramelized notes” (p<0.001), with a trend in fattiness (p=0.056) and intensity (p=0.093) (Figure 2c). This comparison proved the main hypothesis that the taste of pork samples high in C18:1 and MUFA/PUFA ratio associated with macadamia oil, resulted in the highest hedonic values.

Our results agreed with previous literature showing that “caramelized/roasted” and “fatty flavor” notes were two of the main components in meat flavor (Mottram, 1998). High MUFA levels in pork have been associated with higher values of odor-active aroma compounds from Maillard reactions, associated with pleasant roast flavor, and a higher sensory liking (Zhao et al., 2017; Benet et al., 2015 and 2016, Nuernberg et al., 2004). PUFA are easily oxidized which increases the risk of rancid odors in pork potentially affecting consumer choices (Nuernberg et al., 2004). However, oxidation biomarkers were not influenced by Diet or Oil in our experiments (as TBARS, Table 3). Thus, the lack of oxidative events may have benefited the outcome of sensory acceptance of the corn oil supplemented feeds relative to anticipated results in favor of other feed formulations such as WC which did not occur.

Conclusions

Our study provides evidence that dietary C18:1-rich oils have the potential to manipulate fat profiles by increasing C18:1 in pork LD and produce a flavor signature of a high pleasing standard. The results provided valuable insight regarding the complexity of producing a high C18:1 meat profile in pigs. In particular, the interaction between oil sources (e.g. macadamia or corn oil in this project) and the main dietary ingredients (cereals and pulses) are key aspects to take into account that affects the efficiency of transferring C18:1 and other fatty

acids from feeds to meats. The consumer sensory study confirmed that the flavor of pork samples high in C18:1 and MUFA/PUFA ratio associated with macadamia oil had the highest hedonic values and acceptance independent of the ethnic group.

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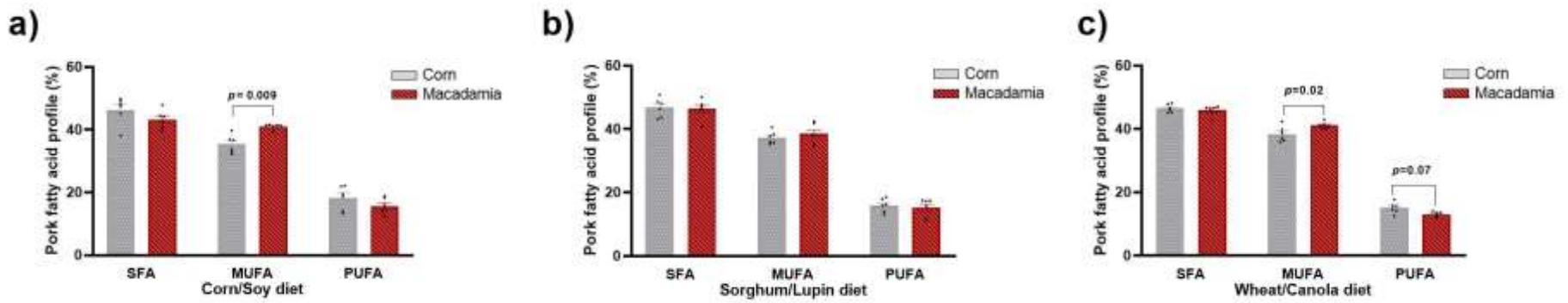

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Figure 1. Fatty acid profiles of pork (raw Longissimus dorsi, n=6) expressed within each Diet group (corn/soy –A-, sorghum/lupins –B- and wheat/canola –C-) enriched with corn or macadamia oils. Fatty acids have been categorised in: Saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA). P values in each graph indicate a significant difference (when $p<0.05$) or a trend (when $p<0.10$) using a student-t test to compare the Oil effect (macadamia vs corn) for each fatty acid group within each Diet type.

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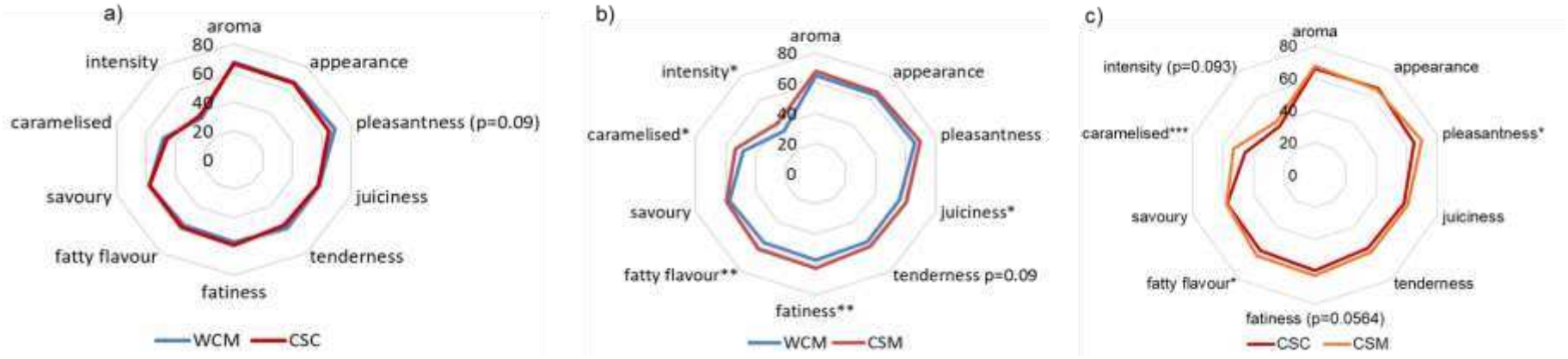

Figure 2. Cobweb-plot of the paired T-test analysis (WCM versus CSM and CSC) and unpaired T-test analysis (CSC versus CSM) comparing sensory attributes of pork loins based on consumer (non-trained) panelists assessment across ethnicity and gender (n=82). The values represent the Mean ± SE. Statistical significance represented as * = $p<0.05$, ** = $p<0.01$, and *** = $p<0.001$. A) Comparing pigs fed high vs low MUFA/PUFA samples (WCM vs CSC, respectively); where WCM describes the wheat-canola feed with macadamia oil while CSC describes the corn-soybean feed with corn oil. B) Comparing pigs fed wheat-canola (WC) or corn-soybean (CS) cereal formulations (WCM vs CSM); where WCM describes the wheat-canola feed with macadamia oil while CSM describes the corn-soybean feed with macadamia oil. C) Comparing pigs fed corn-soybean diets with macadamia (M) or corn oil (C) supplements (CSM vs CSC); where CSC describes the corn-soybean feed with corn oil while CSM describes the corn-soybean feed with macadamia oil.

Table 1 Fatty acid profile (%) of fat (% extracted from solid w/w) from experimental feeds fed to pigs from 70 kg of body weight until slaughter (corn/soy –CS-, wheat/canola –WC- and sorghum/lupins –SL-) supplemented with 2 oils (*corn* -C- or macadamia –M-).

Diet	CS		SL		WC	
Oil	C	M	C	M	C	M
% Fat in the feed	4.36	4.49	4.44	4.49	4.94	5.19
C14:0	0.00	0.36	0.31	0.33	0.33	0.36
C16:0	15.53	13.99	15.75	12.92	15.99	12.71
C16:1	0.35	7.41	0.43	7.69	0.77	9.02
C18:0	3.73	3.96	5.06	4.1	4.23	3.55
C18:1	31.28	37.94	34.84	42.14	32.86	43.08
C18:2	46.16	31.12	39.2	26.93	42.02	24.64
C18:3	1.92	1.69	2.85	2.01	2.70	2.19
C20:0	0.64	1.54	0.57	1.58	0.60	1.85
C20:1	0.39	1.35	0.46	1.32	0.50	1.82
C22:0	0.00	0.64	0.52	0.96	0.0	0.78
SFA	19.9	20.49	22.21	19.89	21.15	19.25
MUFA	32.02	46.7	35.73	51.15	34.13	53.92
PUFA	48.08	32.81	42.05	28.94	44.72	26.83
Ratio MUFA/PUFA	0.67	1.42	0.85	1.77	0.76	2.01

605 Table 2 Fatty acid profile (% of fat) of raw *longissimus dorsi* samples on saturated (SFA), monounsaturated (MUFA), polyunsaturated
606 (PUFA), and MUFA/PUFA ratio from pigs fed with 3 different cereal diets (corn/soy –CS-, wheat/canola –WC- and sorghum/lupins –
607 SL-) supplemented with 2 different oils (corn -C- or macadamia –M-) (mean ± SE, n=6).

Diet	CS		SL		WC		p-value		
Oil	C	M	C	M	C	M	Diet	Oil	D:O
C14:0	0.88±0.09^{abc}	0.93±0.12^{bc}	0.80±0.05^{ab}	0.97±0.09^{c}	0.79±0.06^{a}	0.84±0.09^{abc}	0.127	0.023	0.360
C16:0	24.34±1.14	23.36±0.98	23.77±1.06	24.05±0.99	23.61±0.64	23.61±0.64	0.980	0.253	0.311
C16:1	1.75±0.25^{a}	2.39±0.33^{b}	1.57±0.26^{a}	2.28±0.19^{b}	1.59±0.30^{a}	2.30±0.30^{b}	0.605	0.0001	0.962
C18:0	20.62±3.12	18.62±2.28	21.62±1.63	20.87±1.97	21.16±1.46	20.90±1.14	0.172	0.188	0.616
C18:1	33.63±2.18^{a}	38.70±0.63^{c}	35.71±0.33^{ab}	36.22±2.59^{b}	36.81±1.66^{bc}	38.75±0.78^{c}	0.071	0.001	0.030
C18:2	14.13±2.83^{c}	10.31±1.83^{ab}	12.00±1.59^{bc}	10.33±2.19^{ab}	10.78±1.71^{b}	7.91±0.60^{a}	0.008	0.0005	0.461
C18:3	0.22±0.25	0.40±0.14	0.39±0.11	0.42±0.16	0.40±0.09	0.40±0.05	0.302	0.222	0.397
C20:0	0.49±0.23	0.34±0.13	0.52±0.10	0.07±0.06	0.46±0.07	0.47±0.07	0.831	0.120	0.468
C22:0	0.01±0.03	0.03±0.03	0.07±0.06	0.08±0.08	0.04±0.06	0.05±0.03	0.113	0.443	0.975
SFA	46.34±3.57	43.28±2.45	46.78±2.43	46.33±2.57	46.51±1.07	45.87±1.28	0.198	0.098	0.355
MUFA	35.37±2.38^{a}	41.08±2.09^{d}	37.28±1.67^{ab}	38.50±2.57^{bc}	38.40±2.41^{b}	41.05±0.92^{cd}	0.104	0.0001	0.049
PUFA	18.28±3.14^{c}	15.68±2.09^{abc}	15.94±1.77^{bc}	15.17±2.36^{ab}	17.5±0.73^{ab}	13.08±0.73^{a}	0.016	0.024	0.590
Ratio MUFA/PUFA	2.02±0.44^{a}	2.68±0.33^{bc}	2.38±0.29^{ab}	2.64±0.58^{bc}	2.59±0.39^{b}	3.15±0.18^{c}	0.027	0.003	0.547

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610 Table 3 Carcass parameters (pH, temperature, hot carcass weight and backfat (P2) recorded 45 minutes post-slaughter) and
611 *longissimus dorsi* quality parameters (drip loss, muscle pH evolution (24, 48 and 72h), oxidation level (TBARS), muscle tenderness
612 (WBSF) raw and cooked, cooking loss (fresh and after freezing) and color evolution (L, a and b measured at 24 and 72 h post-
613 slaughter) of pigs fed with three different cereal diets (corn/soy –CS-, wheat/canola –WC- and sorghum/lupins –SL-) supplemented
614 with 2 different oils (corn -C- or macadamia –M-) (mean ± SE, n=6).

Diet	CS		SL		WC		p-value		
Oil	C	M	C	M	C	M	Diet	Oil	D:O
pH 45 min after slaughter	6.45±0.08	6.50±0.1	6.61±0.06	6.46±0.07	6.56±0.04	6.43±0.05	0.719	0.242	0.372
Temp 45 min after slaughter	36.95±0.21	36.38±0.36	35.90±0.21	35.55±0.15	36.55±0.13	35.85±0.33	0.253	0.243	0.950
Hot carcase BW (kg)	82.10±0.54	79.41±1.17	79.18±0.31	75.55±0.47	77.80±0.40	82.18±0.33	0.176	0.672	0.078
P2 fat	11.66±1.16	12.50±0.67	10.50±0.45	9.67±0.43	10.50±1.01	10.16±0.73	0.068	0.880	0.635
Drip loss at 48 h (%)	5.10±0.70	4.70±2.00	5.06±2.00	6.10±1.40	5.80±2.00	6.20±1.60	0.643	0.976	0.416
pH 24 h	5.37±0.03^{a}	5.50±0.05^{b}	5.46±0.04^{ab}	5.39±0.04^{a}	5.39±0.04^{ab}	5.36±0.02^{a}	0.378	0.725	*0.032*
pH 48 h	5.45±0.03^{ab}	5.60±0.08^{c}	5.52±0.06^{bc}	5.45±0.03^{ab}	5.56±0.07^{abc}	5.42±0.01^{a}	0.383	0.614	*0.021*
pH 72 h	5.50±0.03^{ab}	5.65±0.10^{b}	5.61±0.07^{b}	5.44±0.02^{a}	5.53±0.08^{ab}	5.43±0.03^{a}	0.258	0.472	*0.025*
pH after freezing	5.84±0.06	5.94±0.13	5.88±0.10	5.90±0.07	5.80±0.05	5.90±0.06	0.795	0.178	0.792
TBARS (µmol MDA/kg)	13.19±0.58	11.82±0.61	12.64±0.50	13.13±0.52	13.82±1.01	12.15±0.72	0.723	0.110	0.197
WBSF raw	35.48±2.20	28.10±1.12	36.20±3.10	33.73±5.04	30.61±1.51	26.32±3.24	0.113	0.064	0.710
WBSF cooked	52.09±3.35	44.12±5.22	54.84±4.93	48.26±7.90	53.00±4.35	41.91±5.00	0.725	0.066	0.915
Cooking loss fresh (% water)	49.85±1.06	51.19±2.79	47.74±1.71	48.14±0.90	49.41±1.08	49.43±0.51	0.259	0.124	0.289

Cooking loss after freezing	7.06±0.88	6.74±1.30	8.36±1.20	7.52±0.87	8.08±0.52	6.32±1.17	0.259	0.124	0.289
L*24	54.50±0.80	51.60±0.90	53.30±1.00	54.50±0.80	55.20±1.10	56.30±1.00	0.108	0.810	0.052
a*24	5.50±0.50	6.60±0.40	5.90±0.40	6.70±0.50	6.10±0.40	6.30±0.	0.875	0.108	0.532
b*24	4.20±0.60^{a}	4.40±0.80^{a}	4.50±0.40^{a}	5.00±0.20^{ab}	4.60±0.40^{a}	6.00±0.60^{b}	0.117	0.048	0.232
L*72	53.20±0.80^{ab}	52.80±0.90^{a}	55.20±1.00^{ab}	55.50±0.40^{ab}	54.70±1.40^{ab}	56.00±1.00^{b}	0.039	0.632	0.685
a*72	7.70±0.50	9.60±0.90	7.80±1.30	7.60±0.60	8.30±1.20	5.50±0.20	0.083	0.619	0.093
b*72	7.70±0.60	8.30±0.50	8.10±1.20	9.20±0.20	8.20±0.80	7.50±0.70	0.245	0.593	0.878

Different letters denote significant differences between groups at P< 0.05. TBARS refers to Thiobarbituric Acid Reactive Substances; WBSF refers to Warner-Bratzler Shear Force; L refers to lightness (0 = black, 100 = white). a* refers to redness/greenness color (positive value: red, negative values: green). b* refers to yellowness/blueness color (positive value: yellow, negative value: blue) using a Konica Minolta Chroma Meter Model CR-400. Different letters denote significant differences between groups at P< 0.05. L* refers to lightness (0 = black, 100 = white). a* refers to redness/greenness color (positive value: red, negative values: green). b* refers to yellowness/blueness color (positive value: yellow, negative value: blue) using a Konica Minolta Chroma Meter Model CR-400.*

620 Table 4 Effect of Diet (each of the three dietary groups of samples tested -WCM, CSC, or CSM-) on the sensory attributes of pork loin
621 based on the assessment of consumer (non-trained) panelists across ethnicity and gender (n= 82) and effect of ethnicity (Chinese
622 (n=39) versus Non-Chinese (n=43) origin on the evaluation of sensory attributes of pork loin based on the assessment of consumer
623 (non-trained) panelists across gender. Values represent the Mean ± SE.

	CSC	CSM	WCM	p-value	Chinese	Non-Chinese	p-value
Aroma	65.5±2.0	69.3±2.0	66.3±1.7	0.242	64.0±2.0	70±2.0	0.029
Appearance	64.6±1.9	67.1±1.9	65.4±1.6	0.538	64.7±1.9	66.8±1.9	0.400
Pleasantness	64.5±2.0	70.0±2.0	67.6±1.7	0.111	64.8±1.9	70.0±1.8	0.041
Juiciness	56.4±1.7	61.1±1.7	57.2±1.4	0.036	55.4±1.8	61.1±1.7	0.026
Tenderness	54.3±1.7	59.8±1.7	56.8±1.4	0.044	53.9±1.8	60.1±1.8	0.015
Fattiness	59.4±1.6	61.5±1.6	57.6±1.3	0.014	57.1±1.7	61.9±1.7	0.045
Fatty flavour	58.2±1.7	60.8±1.7	56.4±1.4	0.033	57.5±1.7	59.4±1.7	0.407
Savoury	58.2±1.6	59.0±1.6	57.8±1.4	0.591	57.3±1.9	59.4±1.8	0.406
Caramelised	47.5±1.9	52.1±1.9	48.4±1.7	0.079	48.3±2.1	50.4±2.1	0.462
Intensity	37.2±1.7	40.4±1.7	35.8±1.4	0.0006	38.7±1.9	87.0±1.9	0.519

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