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Metabolite profile and consumer sensory acceptability of meat from lean Nellore and Angus × Nellore crossbreed cattle fed soybean oil

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ABSTRACT

Thirty each Nellore (NEL) and crossbred Angus × Nellore (AxN) were used to evaluate the effect of feeding soybean oil (SBO) and breed on meat sensory acceptability and its relation to muscle metabolite profiles. Cattle were fed for 133 d on two different diets: 1) basal feedlot diet (CON) and 2) CON diet with 3.5% added SBO. No interactions between diet and genetic group were detected for any traits measured. Meat from animals fed SBO diet had lower overall liking, flavor, tenderness and juiciness scores compared to meat from animals fed CON diet. The four most important compounds differing between animals fed CON and SBO diets were betaine, glycerol, fumarate, and carnosine, suggesting that metabolic pathways such as glycerolipid metabolism; glycine, serine and threonine metabolism; glutamine and glutamate metabolism; valine, leucine and isoleucine biosynthesis; and alanine, aspartate and glutamate metabolism were affected by diets. Nellore beef had a higher overall liking and meat flavor scores than AxN beef. The four most important compounds differing between breeds were glycine, glucose, alanine, and carnosine, which may indicate that metabolic pathways such as glutathione metabolism; primary bile acid biosynthesis; alanine, aspartate and glutamate metabolism; and valine, leucine and isoleucine biosynthesis were affected by genetic groups. Meat carnosine, inosine monophosphate, glutamate, betaine, glycerol and creatinine levels were correlated with sensory acceptability scores. Meat metabolite profiles and sensory acceptability were differentially impacted by diet and breed.

1. Introduction

Beef is a primary source of protein in human diets. Meat is also highly coveted by consumers for its impact on higher satiety centers and its gratifying sensory attributes, which include flavor, tenderness, and juiciness (Toldrá, 2017). Sensory and meat quality attributes are subject to several pre and post-slaughter factors, such as breed, age, feeding, sex, pre-slaughter management, the amount and composition of intramuscular fat (Arshad et al., 2018; Muchenje et al., 2009), and maturation (aging) time (Graham et al., 2010; Muroya, Oe, Nakajima, Ojima, & Chikuni, 2014).

Animal feeding regimes affect meat composition as well as sensory attributes (Oliveira et al., 2012; Partida, Olleta, Sañudo, Albertí, & Campo, 2007; Vatansever et al., 2000). Adding fat to cattle diets increases the energy density of the diet and also changes the fatty acid profile of meat (Silva et al., 2018; Wood et al., 2008). In Brazil, soybeans are one of the most popular means of adding energy to ruminant diets, mostly because the feedstuff is readily available, inexpensive and energy rich (Barletta et al., 2012), especially regarding oleic, linoleic and linolenic fatty acids (Silva et al., 2018). In general, meat from cattle fed vegetable oils has a lower n-6:n-3 polyunsaturated fatty acids ratio (Castro et al., 2016; Gonzalez, Moreno, Bispo, Dugan, & Franco, 2014; Wood et al., 2008), which can also negatively impact consumer acceptability (Okumura et al., 2007; Vatansever et al., 2000).

Breed also impacts sensory and meat quality attributes primarily due to differences in the amount and composition of intramuscular fat deposited in the meat (Muchenje et al., 2009; Nassu et al., 2017), tenderness (Wheeler, Shackelford, & Koohmaraie, 1997) and flavor

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compounds (Koutsidis et al., 2008a, 2008b). Development of the characteristic species-specific flavors and aromas are mostly located in the lipid fraction of meat, while the water-soluble fraction contains components that contribute to the development of 'meaty' flavor.

A number of attempts have been made to understand the role of muscle metabolite profiles on beef quality. Kim, Kemp, and Samuelsson (2016) reported that sensory differences in flavor and overall acceptability of dry-aged and wet-aged beef were due to differing amounts of specific amino acids. In addition, (King et al., 2019) used a metabolomic approach to identify candidate metabolic biomarkers of beef tenderness whereas Ma et al. (2017) used metabolomic profiling to explore the effect of postmortem aging on color and lipid oxidative stabilities across different bovine muscles. However, most of the aforementioned studies were performed using European beef breeds, which are normally slaughtered at higher levels of carcass fatness and therefore complicated by added lipid content in the muscle. Little is known, however, about how metabolites contribute to sensory properties in meat from extremely lean cattle, such as Nellore cattle, which form the basis for meat production in Brazil. In addition, few studies have explored breed effects on metabolic pathways that may affect the sensory attributes and acceptability of beef. Understanding the basis for how these metabolites affect meat quality in different breeds may help facilitate the creation of novel management schemes for mitigating limitation in beef quality.

One-dimensional (1D) proton nuclear magnetic resonance (^{1}H NMR) spectroscopy has been widely used to characterize and quantify low molecular weight metabolites in small samples (Emwas et al., 2019). ^{1}H NMR has been successfully used to obtain metabolic profiles in many meat products such as dry-cured hams and marinated meats (Zhang et al., 2018; Zhang, Yi, et al., 2019), which have been related to the effects of aging (Kim et al., 2016), breeds (Straadt, Aaslyng, & Bertram, 2014) and diets (Osorio, Moloney, Brennan, & Monahan, 2012; Zawadzki et al., 2017) on the sensory properties of meat. Therefore, the objective of this study was to evaluate the effects of soybean oil feeding on the metabolite profile and sensory properties of meat from Nellore (NEL) and Angus × Nellore (AxN) crossbreed cattle.

2. Material and methods

All procedures used in this study were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines (protocol 7294130616) and Institutional Committee Guidelines in Research with Human Subjects (protocol 58704616.7.0000.5422). Both procedures were approved by the committees of the Faculdade de Zootecnia e Engenharia de Alimentos – Universidade de São Paulo.

2.1. Feedlot trial

Sixty 24-month-old non-castrated male cattle, 30 each NEL and AxN, with an average initial body weight (BW) of 363 ± 28 kg, were fed two different diets: (1) a normal feedlot diet containing no soybean oil (CON) and (2) a normal diet containing 3.5% soybean oil (SBO), in a randomized complete block (initial BW) design with a 2 × 2 factorial arrangement.

Animals were housed in individual (6 × 3 m) concrete pens with ad libitum access to water. Cattle were submitted to a 10-d adaptation period, during which time concentrate levels were gradually increased. At the end of the adaptation period, animals were fed twice a day, at 8 am and 4 pm, over a 133-d period. Every two days, refusals were collected and weighed, and feed offerings were adjusted to ensure 5% to 10% refusal. Experimental diets were formulated using RLM (Esalq/USP, Piracicaba, São Paulo, Brazil) software, and nutritional demands were estimated by the CNCPS system (Fox, Sniffen, O'Connor, Russell, & Van Soest, 1992) to meet animal nutrient requirements of intact crossbred AxN animals with the aim of maximum weight gain (Table 1).

Table 1
Dietary ingredients and chemical composition (DM basis) of finishing diets.

Item	Diet	
	Control	Soybean oil
Ingredients (%)		
Sugarcane bagasse	5.0	5.0
Corn silage	10.0	10.0
Ground corn grain	58.0	54.5
Ground citrus pulp	16.0	16.0
Soybean oil	0.0	3.5
Soybean meal	9.0	9.0
Urea	1.2	1.2
Mineral mixture[1]	0.8	0.8
Chemical composition[2] (%)		
Dry matter (% as fed)	73.1	73.3
Neutral detergent fiber	21.1	20.6
Crude protein	15.1	14.7
Ether extract	3.11	6.45
Total digestible nutrients	78.9	82.3
Calcium	0.48	0.48
Phosphorus	0.37	0.36
Fatty acid composition (% of total fatty acid)		
14:0	0.09	0.08
16:0	13.89	12.28
18:0	2.68	3.28
9c-18:1	34.39	28.82
18:2 n-6	43.77	47.56
18:2 n-3	1.62	3.30

[1] The trace mineral mixture contained (per kg) the following: calcium (min/max), 200–250 g; phosphorus, 20 g; magnesium, 15 g; sulphur, 32 g; sodium, 80 g; zinc, 2000 mg; copper, 450 mg; manganese, 800 mg; iodine, 45 mg; cobalt. 27 mg; selenium, 18 mg; monensin sodium, 1500 mg; vitamin A, 60,000 UI; vitamin D3, 45,000 UI; vitamin E, 400 UI.

[2] The diets were formulated to meet or exceed all nutrient requirements of finishing non-castrated crossbred Angus × Nellore males according to nutritional demands estimated by the CNCPS system (Fox et al., 1992) by using the RLM (Esalq/USP, Piracicaba, São Paulo, Brazil) software.

2.2. Meat sampling

After 133 d on feed, animals were transported to a Federal inspected commercial slaughterhouse (Frigorifico Dom Glutao, Ibitinga, SP, Brazil) located 184 km from the experimental site. Upon arrival, animals were lariaged for 10 h with free access to water and then slaughtered according to Humanitarian slaughter procedures as required by Brazilian law. Briefly, animals were restrained in a stunning box and stunned using a penetrative captive bolt, followed by bleeding through the jugular vein and carotid artery.

After a 48 h chilling (0 to 2 °C), the left side of each carcass was cut between the 12th and 13th rib, and a 5 cm cross-section of the *longissimus thoracis* (LT) muscle was sampled, vacuum packaged and aged (0 to 4 °C) for 7 d (9 d post-slaughter). After aging, two 2.5-cm thick LT samples were collected for sensory and metabolomic analyses.

2.3. Consumer acceptance sensory analysis

Steaks were roasted in an oven at 170 °C (Model F130/L – Electric Furnaces Golden Arrow Industry and Commerce Ltda., São Paulo, SP, Brazil). Internal temperature of each steak was monitored using individual thermometers until an internal temperature of 40 °C was attained, at which point steaks were flipped and cooked to an internal temperature of 71 °C, as recommended by the American Meat Science Association (AMSA, 2015).

Cooked samples were cut into 1.27 × 1.27 × 2.54 cm pieces, avoiding fat and any visible connective tissues, and wrapped in aluminum foil and placed in a water bath (70 °C) prior to serving them to the panelists. Samples were placed in a plastic cup with a three digit

random number for each treatment and served in random order to the panelists with filtered water and unsalted crackers to cleanse the palate between samples. Sensory analyses were performed in individual booths under controlled conditions of red light and temperature (22 ± 2 °C).

A total of 12 sensory sessions (nine panelists per session; a total of 108 consumer panelists) were performed to evaluate the four treatments (one sample per treatment) for overall liking, juiciness, tenderness, and flavor using a nine-point hedonic scale (extremely dislike – 1; extremely like – 9) (AMSA, 2015). In addition, overall liking data of each panelist were used to build clusters so that consumer groups were identified and a percentage acceptance of each treatment was established.

2.4. Extraction of polar metabolites from meat

A total of 0.5 g of meat sample (n = 15/treatment) aged for 7 d was macerated and homogenized using a ultra-turrax®. Metabolites were extracted with 3.5 mL of a cold methanol/water solution (4:3 v/v) while vortexing for 1 min, as previously described by Beckonert et al. (2007). Samples were stored on ice for 15 min and then centrifuged for 15 min at 10,000*g* and 4 °C to remove the protein precipitate and connective tissue. Supernatants were transferred to 1.5 mL Eppendorf tubes and freeze-dried. Remaining residues were reconstituted in 600 μL of 100 mM phosphate buffer (containing 10% D_2O and 90% H_2O, pH 7.0) and 60 μL internal standard solution (containing 5 mM 3-(trimethylsilyl)-1-propanesulfonic acid sodiumsalt (DSS)) as a quantitation standard and chemical shift reference and 100 mM imidazole as a pH indicator). Samples were centrifuged at 10,000*g* for 3 min at 4 °C to remove any precipitate. Supernatants (600 μL) were transferred to standard 5 × 178 mm thin-walled NMR tubes (VWR International).

2.5. NMR spectroscopy

One dimensional proton nuclear magnetic resonance (1D ^{1}H NMR) spectroscopy was used for metabolite profiling (EMBRAPA Instrumentation, São Carlos, SP, Brazil). The ^{1}H NMR spectra were acquired at 300 K on a Bruker Avance 14.1 T spectrometer (Bruker Corporation, Karlsruhe, Baden-Württemberg, Germany) at 600.13 MHz for ^{1}H, using a 5 mm Broadband Observer (BBO) probe. Deuterium oxide was used as a lock solvent and DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) was used as the chemical shift reference for ^{1}H. Standard one-dimensional (1D) proton NMR spectra were acquired using a single 90° pulse experiment. Water suppression was performed using the BRUKER "zgesgp" pulse sequence (excitation sculpting with gradients) and the following acquisition parameters were used: 13.05 μs for the 90 degree pulse, 0.5 s relaxation delay, 64 K data points, 64 scans, 3.89 s acquisition time, and 14.03 ppm spectral width.

2.6. Spectral processing and metabolite quantitation

The 1D ^{1}H NMR spectra were processed using Chenomx NMR Suite Professional 7.7 software (Chenomx Inc., Edmonton, Canada). Phasing and baseline correction were performed and the pH was calibrated using the resonances from imidazole. The spectra were referenced to the DSS methyl peak at 0.00 ppm. The same peak was also used as a chemical shape indicator, to serve as an internal standard for quantification.

Thirty-one metabolites were quantified in the 1D ^{1}H NMR spectra of meat extracts using the Profiler module on the Chenomx NMR Suite Professional software with an in-built 1D spectral library. Quantitation was based on comparing the area of selected metabolite peaks with the area under the DSS methyl peak, which corresponds to a known concentration of 0.5 mM in each sample. The resulting metabolite concentration table (31 metabolites × 15 samples each treatment) was exported to Excel where sample identifiers were added.

2.7. Statistical analysis

Data were evaluated as a randomized complete block design in a 2 × 2 factorial arrangement (genetic group and diet) using 15 replications per treatment (animal was the experimental unit). Metabolomic data were analyzed considering the fixed effects of diet, genetic group and the diet*genetic group interaction and block (initial BW) was used as random effect. For sensory traits, the previous model was used and the panelist was included as a random effect. Analyzes were carried out using the Mixed procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The least squares means (LSMEANS) statement was used to calculate the adjusted means for treatment and the means were compared by Student's *t* test. Differences were considered statistically significant when $P \leq 0.05$.

Metabolomic data also were processed statistically using MetaboAnalyst 4.0 (Chong et al., 2018). The metabolite concentration table was uploaded to MetaboAnalyst, and data were log-transformed and Pareto-scaled prior to analysis. Partial least square discriminant analysis (PLS-DA) was performed using a 10-fold cross validation method, and the values for R^2 (cumulative interpretation ability of model) and Q^2 (predictive ability of model) were employed as initial indicators for evaluating the goodness of fit. In the PLS-DA model, a variable importance in the projection (VIP) plot was used to rank the metabolites based on their importance in discriminating groups. Correlation analysis were performed between sensory properties and metabolites and a heatmap was generated to denote the enrichment of the corresponding correlation. The PatternHunter method was used, and Pearson correlation was applied as distance measure. In addition, pathway analysis was performed with metabolite quantification data sets according to group using the *Bos taurus* library.

3. Results

3.1. Consumer sensory acceptability

No interactions were detected between diet and genetic group for any of the traits evaluated.

Meat from animals fed CON diets received greater overall liking ($P < 0.001$), tenderness ($P < 0.001$) and juiciness ($P < 0.001$) scores and tended to have higher flavor ($P = 0.053$) scores when compared to meat from animals fed the SBO diet (Table 2). In addition, meat from NEL cattle had greater overall liking ($P = 0.044$) and flavor ($P = 0.044$) scores than AxN beef. No differences were observed between genetic group for tenderness and juiciness.

3.2. Meat metabolites and their pathways

Based on 1D ^{1}H NMR analyses, 31 compounds were identified in

Table 2
Effect of diet (DT) and genetic group (GG) on the consumer acceptability of *Longissimus thoracis*.

Traits[1]	DT[2]		GG[3]		SEM	*P* value		
	CON	SBO	NEL	AxN		DT	GG	DT*GG
Overall liking	6.9	6.3	6.7	6.4	0.12	< 0.001	0.044	0.634
Flavor	6.7	6.5	6.7	6.5	0.12	0.053	0.044	0.843
Tenderness	6.8	5.8	6.4	6.2	0.16	< 0.001	0.298	0.379
Juiciness	6.7	6.2	6.6	6.3	0.13	< 0.001	0.114	0.843

[1] For the consumer acceptance test a structured hedonic scale of nine points was used, ranging from "dislike extremely" (note 1) to "like extremely" (note 9).

[2] CON = basal diet without soybean oil inclusion; SBO = basal diet containing 3.5% soybean oil inclusion in replacing of ground corn grain.

[3] NEL = Nellore; AxN = crossbred Angus × Nellore.

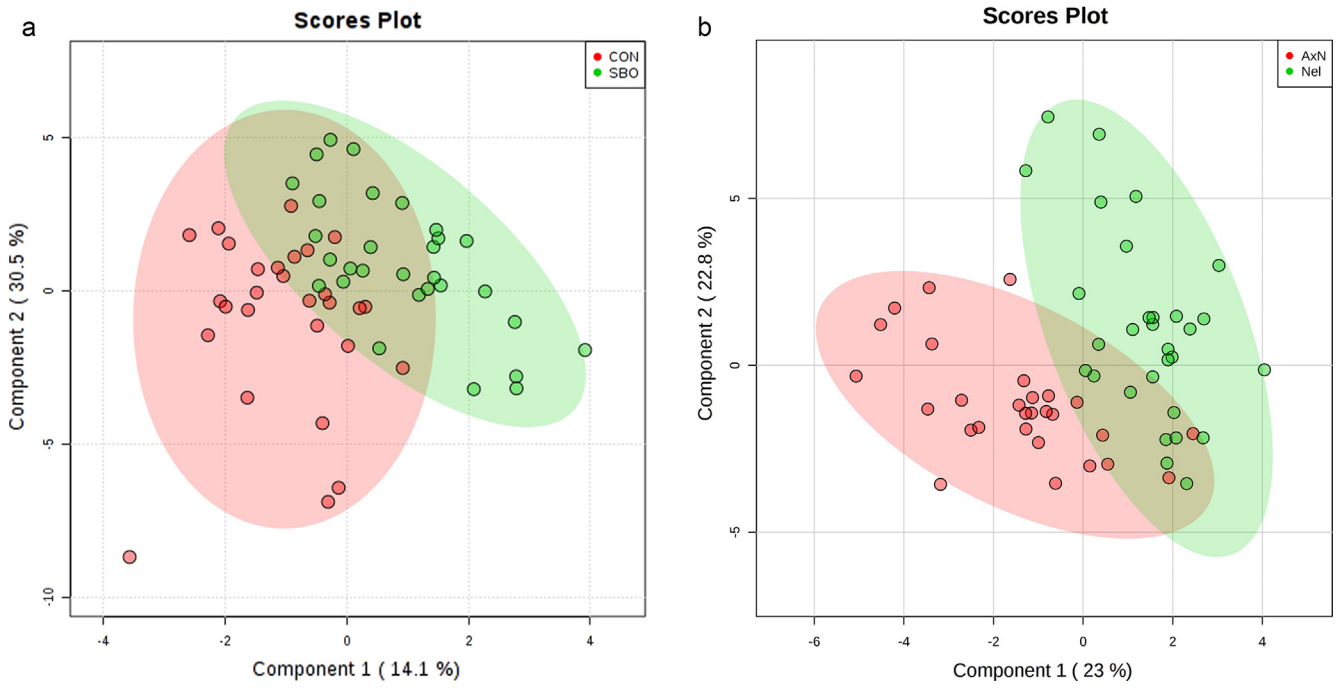

Fig. 1. Partial least square discriminant analysis (PLS-DA) scores plot of metabolome distribution according to the diet (A; CON = control diet; SBO = diet with 3.5% soybean oil; R2 = 0.68 and Q2 = 0.41) and breed (B; NEL = Nellore; AxN = crossbred Angus × Nellore; R2 = 0.77 and Q2 = 0.55).

meat (Supplementary file 1). Based on PLS-DA score sample clustering patterns, compounds segregated according to diet (Fig. 1A) and breed (Fig. 1B), which suggests differences in metabolite concentrations between treatments.

Based on diets and VIP analysis, the most important compounds differing between animals fed CON and SBO diets included (in order of importance) betaine, glycerol, fumarate, carnosine, creatinine, glutamate, inosine monophosphate (IMP), isoleucine, valine, alanine, methionine, anserine, threonine, carninitine, and glutamine (Fig. 2A). Betaine ($P < 0.001$) and glycerol ($P < 0.001$) were higher in meat from animals fed SBO diets, whereas carnosine ($P = 0.005$) was higher in meat from animals fed CON diets (Table 3). The main metabolic

Table 3
Metabolites that differ significantly ($P < 0.05$) in the beef samples according to the diets.

Metabolite, μmol/g meat	Diets[1]		SEM	Fold change	*P* value
	CON	SBO			
Betaine	0.73	1.28	0.086	1.75	< 0.001
Carnosine	12.01	3.24	0.962	3.71	0.005
Glycerol	1.65	2.81	0.229	1.70	< 0.001

[1] CON = basal diet; SBO = basal diet containing 3.5% soybean oil.

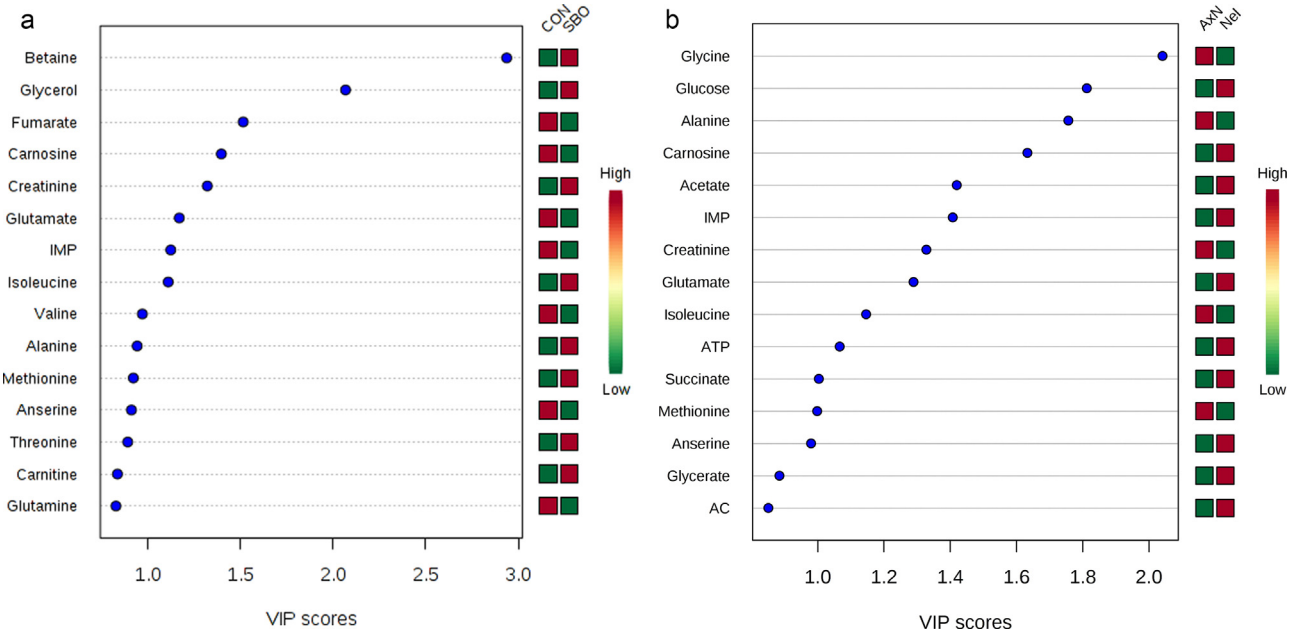

Fig. 2. Variable importance in projection (VIP) plot according to the diet (A; CON = control; SBO = diet with 3.5% soybean oil) and breed (B; NEL = Nellore; AxN = crossbred Angus × Nellore). *IMP = inosine monophosphate; ATP = adenosine triphosphate; AC = acetyl carnitine.

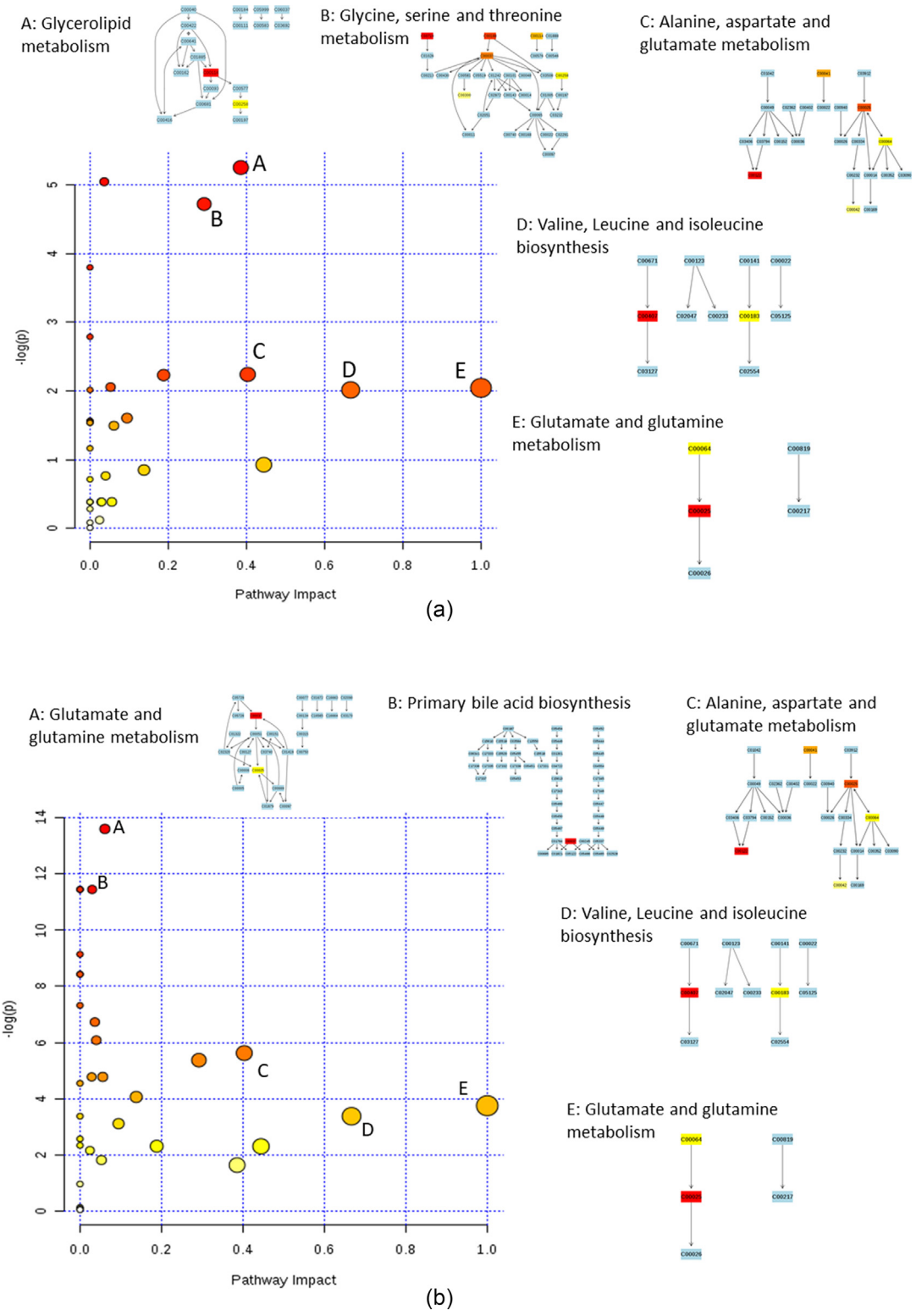

Fig. 3. Pathway analysis using metabolites according to the diet (A; CON = control; SBO = diet with 3.5% soybean oil) and breed (B; NEL = Nellore; AxN = crossbred Angus × Nellore).

pathways affected by dietary treatments were glycerolipid metabolism; glycine, serine and threonine metabolism; glutamine and glutamate metabolism; valine, leucine and isoleucine biosynthesis; and alanine, aspartate and glutamate metabolism (Fig. 3A).

In regards to breed and VIP analysis, the most important compounds differing between breeds were (in order of importance) glycine, glucose, alanine, carnosine, acetate, IMP, creatinine, glutamate, isoleucine, ATP, succinate, methionine, anserine, glycerate, and acetyl carnitine (Fig. 2B). Concentrations of acetate ($P = 0.002$), carnosine ($P < 0.001$), glucose ($P < 0.001$), glutamate ($P = 0.002$), inosine monophosphate ($P = 0.005$) and succinate ($P = 0.030$) were greater in NEL beef, whereas alanine ($P < 0.001$), creatinine ($P = 0.005$), glycine ($P < 0.001$) and methionine ($P = 0.012$) were higher in AxN group (Table 4). The main metabolic pathways affected by breed were

Table 4
Metabolites that differ significantly ($P < 0.05$) in the beef samples according to the genetic groups.

Metabolite, μmol/g meat	Genetic group[1]		SEM	Fold change	*P* value
	NEL	AxN			
Acetate	0.18	0.10	0.017	1.80	0.002
Alanine	1.09	1.94	0.193	1.78	< 0.001
Carnosine	12.97	8.37	0.962	1.55	< 0.001
Creatinine	0.50	0.72	0.052	1.44	0.005
Glucose	4.36	2.54	0.353	1.72	< 0.001
Glutamate	0.44	0.29	0.071	1.52	0.002
Glycine	0.76	1.23	0.071	1.62	< 0.001
IMP[2]	0.94	0.56	0.093	1.68	0.005
Methionine	0.80	1.14	0.092	1.43	0.012
Succinate	0.06	0.04	0.008	1.50	0.030

[1] NEL = Nellore breed; AxN = crossbred Angus × Nellore.
[2] IMP = inosine monophosphate.

glutathione metabolism; primary bile acid biosynthesis; alanine, aspartate and glutamate metabolism; and valine, leucine and isoleucine biosynthesis (Fig. 3B).

3.3. Consumer sensory acceptability × meat metabolites correlations

As expected, overall liking, tenderness, flavor, and juiciness scores were highly correlated (Fig. 4). Similarly, acetyl carnitine, choline, adenine, succinate, anserine, valine, glutamate, IMP, fumarate, NADH, carnitine, lactate and beta-alanine, which have several common pathways were moderately to highly correlated with each other. Most of metabolites were lowly correlated with consumer sensory acceptability scores. However, some moderate correlations ($r > 0.3$ or $r < -0.3$) were observed for overall liking with carnosine, betaine, glycerol, creatinine, IMP, and glutamate (Fig. 5A); flavor with carnosine, alanine, glycine, IMP, glutamate, and creatinine (Fig. 5B); tenderness with betaine, glycerol, carnosine, creatinine, glutamate, and fumarate (Fig. 5C); and juiciness with carnosine, betaine, glutamate, glycerol, IMP, and creatinine (Fig. 5D).

4. Discussion

Kim et al. (2016) reported some meat metabolites are related to meat taste and flavor, either directly or indirectly since some substrates in chemical reactions form flavor compounds during cooking (Zhang, Pan, et al., 2019). Similarly, Straadt et al. (2014) showed some metabolites are associated with changes in tenderness. Therefore, variations in meat metabolite concentration may impact sensory acceptability of meat (Kim et al., 2016; Kodani, Miyakawa, Komatsu, & Tanokura, 2017; Nishimura, Rhue, Okitani, & Kato, 1988).

In the present study, flavor, tenderness and overall liking scores were greater for meat from CON cattle when compared to their SBO counterparts. In addition, diet altered important pathways, such as glutamate and glutamine metabolism and valine, leucine and isoleucine biosynthesis, and beef with higher overall liking scores had smaller concentrations of betaine, carnitine and glycerol.

According to Mottram (1998), the flavor of cooked meat is derived from the Maillard reaction, due to the interaction between reducing sugars and amino acids, and the thermal degradation of lipids, which produce desirable flavor characteristics in the meat. Further, carnosine positively impact sensory sensations because it is associated with the umami flavor (Lana et al., 2015; Nishimura et al., 1988). Carnosine facilitates the generation of several important nitrogen-containing volatiles, which are known to elicit roasted and nutty flavor sensations (Chen & Ho, 2002). Carnosine is also an antioxidant that scavenges radicals and binds metals (Wu, Shiau, Chen, & Chiou, 2003), which contributes to greater overall oxidative stability in beef and may positively affect the sensory perception of flavor.

In the present study, betaine was the most important metabolite that differed with diet, as indicated by the highest correlation with sensory scores. Betaine was negatively correlated with overall liking, tenderness and juiciness scores. In addition, carnosine was the fourth most important metabolite differing with diet and was moderate-highly correlated with overall liking, flavor, tenderness and juiciness scores. Straadt et al. (2014) reported that betaine concentration was negatively associated with beef tenderness, while carnosine concentration was positively associated with meat tenderness. Betaine has been reported to protect cells against hypertonic stress (Alfieri et al., 2006), preventing muscle cells from apoptosis and, thus, increasing their survival. Therefore, it is possible that during the postmortem period, a decrease in muscle cell apoptosis may inhibit activation of caspases-3, which may then lead to decreased myofilament degradation and consequently an impact on meat tenderization (Picard & Gagaoua, 2017). Even though postmortem tenderization is due to proteolysis of larger protein structures within the meat (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010), the amount of amino acids in the meat are likely indicative of proteolysis (Graham et al., 2010, 2012) and can be used to evaluate how meat metabolites reflect postmortem tenderization. Regardless, these data suggest that meat metabolite profiles may prove useful in predicting diet-mediated changes in sensory attributes for consumers.

Similar to dietary effects, genetic group also differed in sensory acceptability and meat metabolite profile. Consumers rated NEL beef as more flavorful and more acceptable than AxN. Indeed, this result may be also associated to the meat metabolites, mainly reducing sugars and amino acids, which are involved in Maillard reactions and the modification of flavor profiles (Koutsidis et al., 2008a, 2008b). This is in good agreement with the observation that the most important metabolites differentiating genetic groups were reducing sugars, such as glycine and glucose. According to the metabolic pathway analyses, amino acid metabolism, such as glutamate and glutamine metabolism, alanine, aspartate and glutamate metabolism, and valine, leucine and isoleucine biosynthesis were most affected by breed.

Glutamate and IMP were also associated with the umami flavor (Chaudhari, Pereira, & Roper, 2009; Chaudhari & Roper, 2010). Further, these metabolites were also correlated with greater flavor acceptability in NEL group. IMP was the sixth most important metabolite to differ with genetics and was moderate-highly positively correlated with overall liking and flavor scores. Dang, Gao, Ma, and Wu (2015) reported that umami is a Japanese concept meaning savory or delicious and is elicited by two types of chemical compounds, such as monosodium glutamate and aspartate, and purine nucleotides, such as inosine monophosphate (IMP) and guanosine monophosphate. Glutamate (glutamine derivative) is the most abundant free amino acid in the brain and is the major excitatory neurotransmitter of the vertebrate central nervous systemic, which likely stimulates the brain and provides more desirable flavour sensations (Chaudhari et al., 2009; Chaudhari & Roper, 2010; Tapiero, Mathé, Couvreur, & Tew, 2002). This concept is consistent with the results of Lana et al. (2015) and Kim et al. (2016), who also reported that a higher glutamate concentration was associated with a greater flavor acceptability of beef.

Other non-volatile flavor precursor compounds including sugar, creatinine and carnosine also influence the flavor, tenderness and juiciness of meat (Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009; Ritota, Casciani, Failla, & Valentini, 2012). In this study, creatinine was the seventh most important metabolite to differ across genetic groups and was moderate-highly negatively correlated with overall liking, flavor, tenderness and juiciness scores. In this regard, lower amounts of this metabolite in NEL may be due to its conversion to a brothy taste modifier, N-(4-methyl-5-oxo-1-imidazolin-2-yl) sarcosine, through an aminocarbonyl reaction with methylglyoxal during heat treatment (Nissen & Young, 2006). Given creatinine plays a key role in normal muscle metabolism and function by being phosphorylated to phosphocreatine by the creatine kinase enzyme and creating a readily

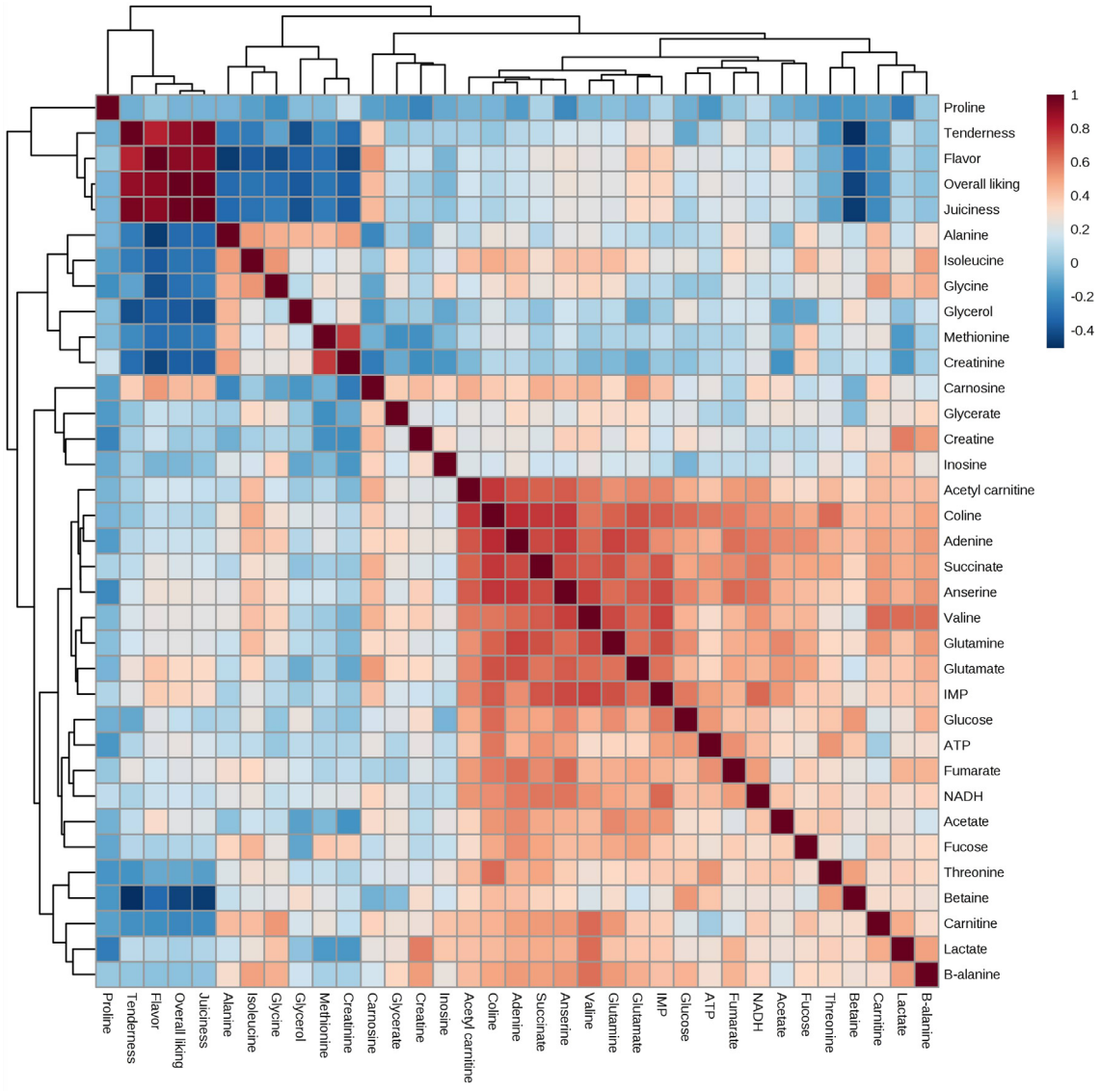

Fig. 4. Heatmap overview of the consumer sensory acceptability × meat metabolites correlations. *IMP = inosine monophosphate; ATP = adenosine triphosphate; NADH = Nicotinamide adenine dinucleotide.

available energy source (Demant & Rhodes, 1999; Wyss & Kaddurah-Daouk, 2000), it is possible that larger amounts of this metabolite may delay post-mortem lactate formation via glycolysis and retard the pH decline, which could improve the water-holding capacity (Nissen & Young, 2006) and impact meat tenderness and juiciness.

5. Conclusions

Sensory attributes and some beef muscle metabolites are affected by diet and breed. Concentrations of various metabolites are also associated with sensory properties of beef. Therefore, the relative abundance of these metabolites may be useful to estimate changes in consumer acceptability of beef. Futher studies are needed to better understand the exact mechanisms by which various metabolites direct or indirectly drive beef quality.

CRediT authorship contribution statement

Daniel S. Antonelo: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Nara R.B. Cônsolo:** Methodology, Formal analysis, Writing - original draft. **Juan F.M. Gómez:** Conceptualization, Methodology. **Mariane Beline:** Methodology. **Rodrigo S. Goulart:** Writing - original draft. **R.R.P.S. Corte:** Writing - original draft. **Luiz A. Colnago:** Software, Resources. **M. Wes Schilling:** Formal analysis, Writing - review & editing. **David E. Gerrard:** Conceptualization, Writing - review & editing, Supervision. **Saulo L. Silva:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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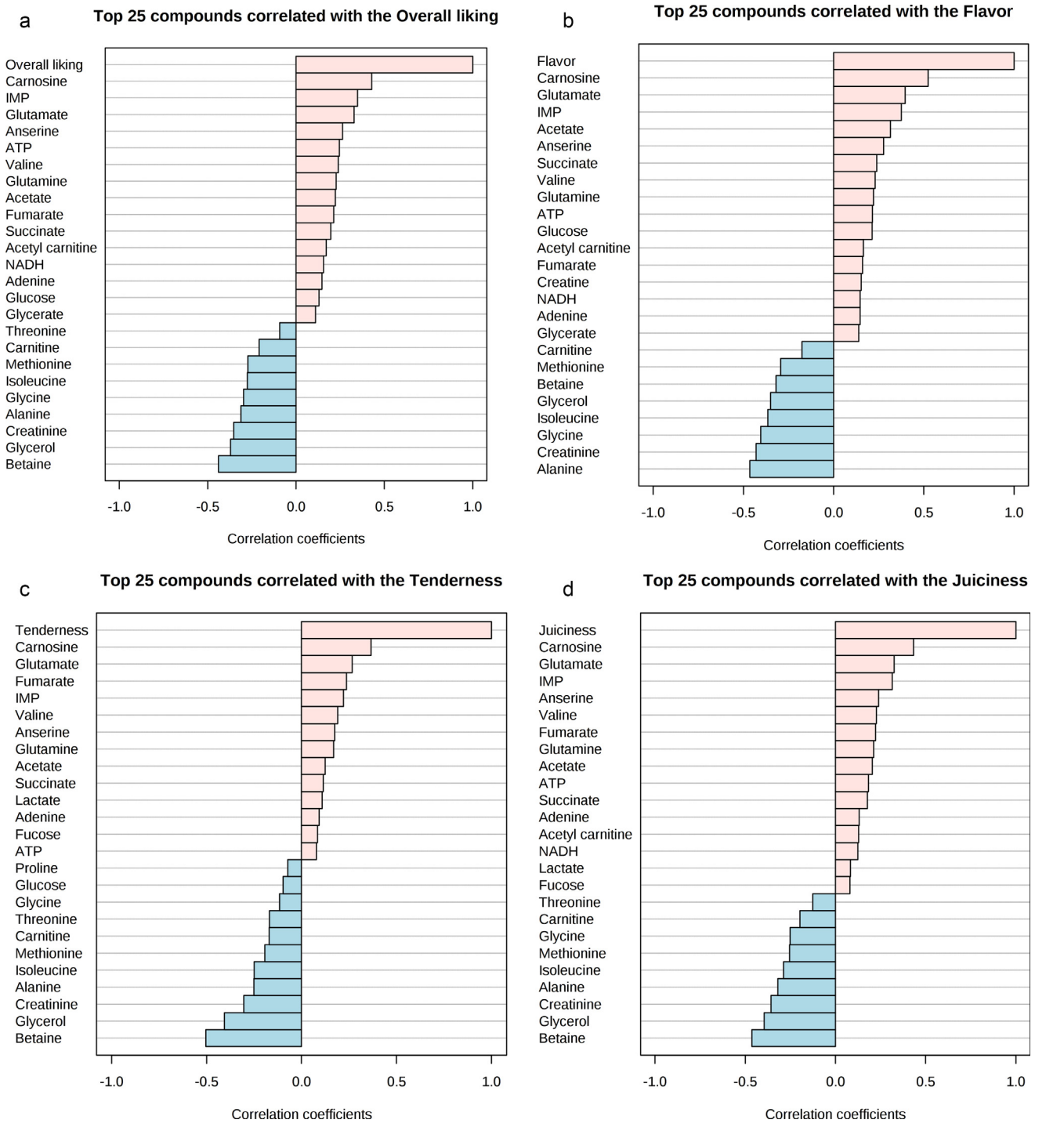

Fig. 5. Metabolites that were associated with overall liking (A), flavor (B), tenderness (C) and juiciness (D) in a consumer sensory acceptability test using Pearson correlation as a distance measure. *IMP = inosine monophosphate; ATP = adenosine triphosphate; NADH = Nicotinamide adenine dinucleotide.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109056.

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