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Potencialidade do resíduo de cervejaria na terminação de bovinos e como fonte de compostos bioativos para incrementar a qualidade da carne

TESE DE DOUTORADO

Flávia Santi Stefanello

Santa Maria, RS, Brasil
2017

Potencialidade do resíduo de cervejaria na terminação de bovinos e como fonte de compostos bioativos para incrementar a qualidade da carne

Flávia Santi Stefanello

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia de Carnes e Derivados, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutor em Ciência e Tecnologia dos Alimentos.**

Orientador: Prof. Dr. José Laerte Nörnberg

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Elaborada por
Flávia Santi Stefanello

Como requisito parcial para a obtenção do grau de
Doutor em Ciência e Tecnologia dos Alimentos

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RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos
Universidade Federal de Santa Maria

Potencialidade do resíduo de cervejaria na terminação de bovinos e como fonte de compostos bioativos para incrementar a qualidade da carne

AUTORA: FLÁVIA SANTI STEFANELLO
ORIENTADOR: JOSÉ LAERTE NÖRNBERG
Local e Data de Defesa: Santa Maria-RS, 21 de agosto de 2017.

A qualidade dos alimentos é definida em termos da aceitabilidade do consumidor, por este motivo, os esforços para reduzir a oxidação têm aumentado. A incorporação dos antioxidantes naturais em carne pode ser através do contato direto na superfície ou pela suplementação. Na dieta animal com antioxidantes e deposição metabólica na carne para exercer sua atividade protetora. O resíduo úmido de cervejaria (RUC) é o mais abundante subproduto da indústria cervejeira, disponível a baixo custo como uma fonte valiosa de ácidos fenólicos. Neste sentido, objetivou-se avaliar diferentes condições de extração para a caracterização e identificação de polifenois de RUC e o seu efeito sobre o desempenho e atributos de qualidade da carne de bovinos sob sistema de confinamento. A fim de caracterizar o RUC e demais ingredientes utilizados nas dietas (silagem de milho, farelo de arroz, farelo de milho e farelo de trigo) foi efetuado a composição química, a determinação de fenólicos totais, flavonoides totais e atividade antioxidante *in vitro* dos extratos obtidos por extração convencional e assistida por micro-ondas; variando o solvente entre acetona 50%, metanol 50% ou NaOH 0,75% e amostras *in natura* ou desengorduradas. Utilizando HPLC-DAD foram identificados e quantificados os monômeros de compostos fenólicos de todas as amostras. Quatro formulações foram testadas variando o nível substituição de silagem de milho por RUC na terminação de bovinos de corte em delineamento inteiramente casualizado (DIC) com cinco repetições em cada tratamento. Durante o confinamento foram avaliados o consumo médio diário dos animais e o ganho de peso médio diário. A digestibilidade dos nutrientes da dieta e os níveis de compostos fenólicos e enzimas antioxidantes endógenas no sangue dos bovinos. O músculo *Longissimus thoracis* foi analisado 24 horas após o abate através da temperatura, pH, composição química, perfil de ácidos graxos, coloração, perda por cocção, perfil de textura, atividade de água, estabilidade oxidativa, proteica e microbiológica,

além da aceitabilidade dos produtos ao longo de sua vida útil por 75 dias de armazenamento embalado à vácuo sob refrigeração. A comparação entre o RUC e demais ingredientes utilizados nas dietas experimentais dos animais revelou que este resíduo possui a maior concentração de polifenóis identificados e biodisponíveis com consequente maior potencial antioxidante. O RUC em substituição a silagem de milho em até 35% na dieta de terminação de bovinos em confinamento possibilita excelente desempenho produtivo e redução significativa nos custos de produção, considerando os valores de mercado praticados atualmente. Quanto ao produto final, a carne bovina com a inclusão de RUC na dieta dos animais apresenta maior quantidade de ácidos graxos benéficos à saúde dos consumidores, com maior estabilidade oxidativa e aumento na maciez. Quanto a vida útil da carne bovina durante o armazenamento sob condições comerciais, a inclusão de até 35% de RUC nas dietas de terminação de bovinos como fonte forrageira não promoveu qualquer efeito indesejável. O uso do RUC em substituição a silagem de milho em dietas de bovinos de corte pode ser adotado como uma estratégia para reduzir o custo de alimentação e pelo uso de um material que apresenta a necessidade de ser reciclado. Além disso, o material é fonte de polifenois, proporcionando incremento em atributos importantes de qualidade da carne, tais como: maciez, perfil de ácidos graxos e estabilidade oxidativa.

Palavras-chave: Ácidos fenólicos. Atividade antioxidante. Carne bovina. Estabilidade oxidativa. Perfil de ácidos graxos. Resíduo agroindustrial.

ABSTRACT

Doctoral Thesis
Graduate Program on Food Science and Technology
Federal University of Santa Maria

Potentiality of brewer's spent grain in the finishing of cattle and as a source of bioactive compounds to increase meat quality

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ADVISER: JOSÉ LAERTE NÖRNBERG
Place and date of defense: Santa Maria-RS, august, 21, 2017.

Food quality is defined in terms of consumer acceptability, so efforts to reduce oxidation have increased. The incorporation of natural antioxidants into meat can be clone by direct contact on the surface or by supplementing. In the animal diet with antioxidants and metabolic deposition in the meat being to exert its protective activity. Brewer's spent grain (BSG) is the most abundant by-product of the brewing industry.It is available at low cost as a valuable source of phenolic acids. Thus, the objective was to evaluate different extraction conditions for the characterization and identification of BSG polyphenols and their effect on the performance and quality attributes of beef under feedlot. In order to characterize the BSG and other ingredients used in the diets (corn silage, rice bran, corn bran and wheat bran), chemical composition, determination of total phenolics, total flavonoids, and in vitro antioxidant activity of the extracts were performed. Extracts were obtained by conventional extraction and assisted by microwave. using solvent 50% acetone, 50% methanol, or 0.75% NaOH. Samples were studied in natura or deffated. Using HPLC-DAD, monomers of phenolic compounds from all samples were identified and quantified. Four formulations were tested by varying the replacement level of corn silage by BSG at the termination of beef cattle in a completely randomized design (DIC) with five replicates in each treatment. During feedlot, the average daily consumption of the animals and the average daily weight gain were evaluated. The digestibility of dietary nutrients and levels of phenolic compounds and endogenous antioxidant enzymes in bovine blood. Longissimus thoracis muscle was analyzed 24 hours after slaughter through temperature, pH, chemical composition, fatty acid profile, color, cooking loss, texture profile, water activity, oxidative, protein and microbiological stability, besides the acceptability of products throughout its shelf life for 75 days of vacuum-packed refrigerated storage. The comparison between BSG and other ingredients used in the experimental diets of the animals revealed that this residue has the highest concentration of

polyphenols identified and bioavailable with consequent higher antioxidant potential. The BSG in replacement of corn silage up to 35% in the finishing diet of cattle feedlot allows excellent production performance and a significant reduction in production costs, considering the market values currently practiced. Regarding the final product, beef with the inclusion of BSG in the animals' diet presents a higher amount of fatty acids that are beneficial to the health of the consumers. Moreover, meat has hight oxidative stability and increased tenderness. The inclusion of up to 35% BSG in the cattle finishing diets as a forage source did not promote any undesirable effect during the shelf life evaluation under commercial conditions. The use of BSG to replace corn silage in beef cattle diets can be adopted as a strategy to reduce feed costs. Furthemore, it is also a source of polyphenols from a material that needs to be recycled. Those substances have show that are able to improve important attributes related to meat quality, as: tenderness, fatty acid profile, and oxidative stability.

Keywords: Agroindustrial waste. Antioxidant activity. Beef. Oxidative stability. Phenolic acids. Profile of fatty acids.

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LISTA DE ABREVIATURAS

4-HBA: 4-hydroxybenzoic acid
ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ADIN: acid detergent insoluble nitrogen
ADL: acid detergent lignin
AE: ethyl acetate
BSG: brewer's spent grain
BW: body weight
CA: clorogenic acid
Caf: caffeic acid
CAT: catalase
Cat: catechin
CFU: colony forming units
CLA: conjugated linoleic acid
CP: crude protein
Cr_2O_3: chromium oxide III
CRD: completely randomized design
CS: corn silage
CV: coefficient of variation
DE: diethyl ether
DM: dry matter
DMD: dry matter digestible
DMI: dry matter intake
DPPH: radical livre 2,2-difenil-1-picrilhidrazil
EC: efficient concentration
ED: digestible energy
EE: ether extract
Epic: epicatechin
FA: factor A – two extraction methods
FB: factor B – two sample pre-treatments
FC: factor C – three extraction solvents
FCR: Folin-Ciocalteu reagent
FD: factor D – five evaluated foods
FEA: ferulic acid
FRAP: ferric ion reducing antioxidant power
GA: gallic acid
GAE: gallic acid equivalent
GPx: glutathione peroxidase
HBD: hydroxybenzoate derivatives
HCD: hydroxycinnamate derivatives
HPLC-DAD: cromatografia líquida de alta eficiência acoplada a detector de arranjo de iodo
HPLC: cromatografia líquida de alta eficiência
Kae-3DGlp: kaempferol-3DGlp
Kae: kaempferol
LDL: lipoproteínas de baixa densidade, também chamado de "mau colesterol"
LOD: limits of detection
LOQ: limits of quantification
MDA: malonaldehyde

MM: mineral matter
MS: matéria seca
MUFA: mono-unsaturated fatty acids
Myr: myricetin
N: newton
N.A.: not applicable
NaOH: hidróxido de sódio
NDFap: neutral detergent fiber corrected for ash and protein
NDIN: neutral detergent insoluble nitrogen
NFC: non-fiber carbohydrates
OM: organic matter
p-Cou: p-coumaric acid
PB: proteína bruta
PC: protein carbonyl
PCA: p-coumaric acid
Pro: protocatechuic acid
PTFE-TFM: polytetrafluorethylene
PUFA: polyunsaturated fatty acids
QE: quercetin equivalent
Quer: quercetin
RC: resíduo de cervejaria
Resv: resveratrol
RSD: residual standard deviation
RUC: resíduo úmido de cervejaria
SEM: standard error of mean
SFA: saturated fatty acids
Sin: sinapic acid
SOD: superoxide dismutase
Syr: syringic acid
TBARS: substâncias reativas ao ácido tiobarbitúrico
TDN: total digestible nutrients
TEAC: trolox equivalent antioxidant capacity
TFP: total fecal production
trans-cin: trans-cinnamic acid
trans-fer: trans-ferulic acid
Van: vanillic acid

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SUMÁRIO

1. INTRODUÇÃO

O cultivo comercial de cevada no Brasil destina-se a fabricação de malte, principal matéria-prima da indústria cervejeira, que fomenta a cultura no país (DE MORI, 2007). A cevada caracteriza-se pelas grandes quantidades de compostos fenólicos que podem servir como uma excelente fonte alimentar de antioxidantes naturais. Durante a produção de cerveja a partir do malte cervejeiro gera-se um resíduo considerado um subproduto industrial com baixo valor agregado, denominado de resíduo úmido de cervejaria (RUC).

O RUC é o mais abundante subproduto da indústria cervejeira. Indubitavelmente, isto é visto de forma negativa dentro das perspectivas de sustentabilidade, já que demanda custo com a remoção e tratamento. Atualmente, há uma crescente pressão para reduzir os subprodutos industriais, reutilizando-os em processos secundários para a redução da poluição ambiental (WATERS et al., 2012).

As composições típicas de RUC variam, mas sempre incluem altos níveis de fibra e proteína e particularmente, uma fonte potencialmente valiosa de ácidos fenólicos. Há evidências que sugerem que o ácido ferúlico, ácido p-cumárico, sinápico, caféico e ácido siríngico estão presentes em concentrações relativamente elevadas em RUC (BARTOLOMÉ et al., 2002; SZWAJGIER et al., 2010). Por conseguinte, há cada vez mais provas que sugerem que os ácidos fenólicos, incluindo aqueles encontrados em concentrações elevadas em RUC, podem conferir efeitos benéficos para a saúde humana e animal, incluindo potencial antioxidante, anti-inflamatório, anticancerígeno e anti-aterogênico (KANG et al., 2009; DAI; MUMPER, 2010; MAURYA; DEVASAGAYAM, 2010; YOSHIDA; KISUGI, 2010). No entanto, a incorporação de RUC em alimentos apresenta algumas limitações, principalmente sobre a aparência, pois, quando úmido o resíduo tem coloração marrom e pode alterar a coloração dos produtos, incluindo os produtos cárneos (MCCARTHY et al., 2013).

As evidências sugerem que o RUC, como parte de uma dieta animal, apresenta benefícios nutricionais para os ruminantes, quanto ao consumo e digestibilidade dos nutrientes da dieta (GERON et al., 2010; SILVA et al., 2010). Porém, pesquisas ainda precisam ser realizadas para avaliar o nível de suplementação ideal que resulte em melhoria das características qualitativas e, principalmente, da estabilidade da carne de bovinos ao longo da vida útil, sem comprometer o desempenho animal. Neste sentido, muitos estudos têm relatado a bioatividade de compostos fenólicos de diferentes fontes vegetais (NIETO et al.,

2010; RIVAROLI et al., 2016). No entanto, poucos avaliam os compostos bioativos de resíduos agroindustriais destinados ao consumo animal, como para ruminantes.

Os compostos fenólicos na dieta animal são capazes de melhorar a estabilidade oxidativa da carne, além de evitar alterações na sua coloração, ampliando a vida útil do produto final (CASTILLO et al., 2013). O efeito dose-resposta destas unidades, bem como o seu mecanismo de ação não estão completamente elucidados. No entanto, de acordo com Vasta e Luciano (2011), a utilização de plantas ricas em polifenois ou a suplementação de unidades purificadas destes compostos na dieta de pequenos ruminantes apresenta-se como uma estratégia promissora para a melhoria da qualidade da carne e do leite destes animais.

Dessa forma, estudos sobre a exploração do RUC na alimentação de bovinos de corte são relevantes, especialmente no sentido de avaliar se os compostos fenólicos são capazes de modificar os parâmetros de qualidade da carne nos níveis de concentração em que naturalmente ocorrem em seu estado original, paralelamente a viabilidade nutricional e econômica quando incluso como componente de dietas de terminação. Ainda, os produtos "naturalmente melhorados" poderiam ser comercializados a preços diferenciados, já que os consumidores parecem estar disponíveis à pagar mais por produtos naturalmente enriquecidos com compostos bioativos. Por conseguinte, o retorno financeiro aos produtores é um incentivo para adotar o sistema de alimentação enriquecida de compostos fenólicos na dieta de bovinos.

1.1 Objetivo geral

- Avaliar diferentes condições de extração para a caracterização e identificação de polifenois do resíduo de cervejaria e o seu efeito sobre atributos de qualidade da carne quando utilizado em substituição a silagem de milho em dietas de terminação de bovinos sob sistema de confinamento.

1.2 Objetivos específicos

- Estudar diferentes condições para a extração de polifenois do resíduo de cervejaria e dos demais ingredientes da dieta (silagem de milho, farelo de arroz, farelo de milho, farelo de trigo) para avaliar o efeito sob o teor de fenólicos e flavonoides totais nos extratos e a sua atividade antioxidante *in vitro*;

- Desenvolver um método de separação, identificação e quantificação dos monômeros de compostos fenólicos por HPLC-DAD para avaliação do resíduo de cervejaria e dos demais ingredientes da dieta (silagem de milho, farelo de arroz, farelo de milho, farelo de trigo);

- Determinar a composição centesimal e o perfil de ácidos graxos do resíduo de cervejaria e dos demais ingredientes da dieta (silagem de milho, farelo de arroz, farelo de milho, farelo de trigo);

- Substituir a silagem de milho por diferentes níveis de resíduo de cervejaria na dieta de bovinos de corte em sistema de confinamento para avaliar o desempenho produtivo dos animais e custos deste sistema;

- Quantificar os compostos fenólicos totais e enzimas antioxidantes na corrente sanguínea dos bovinos no ínicio e ao final do confinamento dos animais para avaliar a atividade antioxidante das dietas;

- Avaliar o efeito de resíduo de cervejaria na dieta de bovinos de corte sobre os atributos de qualidade nutricional, sensorial e estabilidade do músculo *Longissimus thoracis* no *post-mortem*;

- Acompanhar a vida útil do músculo *Longissimus thoracis* embalado à vácuo sob referigeração de bovinos de corte submetidos a diferentes níveis de resíduo de cervejaria em substituição a silagem de milho.

2. REVISÃO DE LITERATURA

A revisão de literatura está apresentada na forma de artigo científico, publicado na Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental – REGET – disponível em http://dx.doi.org/10.5902/2236117012979.

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RESÍDUO DE CERVEJARIA: BIOATIVIDADE DOS COMPOSTOS FENÓLICOS; APLICABILIDADE NA NUTRIÇÃO ANIMAL E EM ALIMENTOS FUNCIONAIS

Brewers' spent grain: bioactivity of phenolic compounds; applicability in animal nutrition and functional foods

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Resumo

A grande disponibilidade de resíduo de cervejaria (RC) a baixo custo, associado com o atual interesse nos benefícios à saúde dos ácidos fenólicos, abre novas possibilidades para o seu uso. Esta revisão detalha as potenciais bioatividades de compostos fenólicos presentes no RC e a sua incorporação na nutrição animal e em alimentos funcionais. A literatura demonstra que os compostos fenólicos, incluindo o ácido ferúlico, cafeico e p-cumárico podem ter efeitos antioxidantes, anticancerígenos, anti-inflamatório e anti-aterogênicos. Dado que estes ácidos fenólicos são alguns dos principais fenólicos em RC, espera-se que possam exibir propriedades semelhantes. O RC funciona como uma alternativa na nutrição animal, tendo muitos benefícios nutricionais. Embora tenham sido feitas tentativas para incorporar os componentes bioativos de RC em alimentos, é necessária mais investigação nesta área.

Palavras-chave: resíduo de cervejaria, ácidos fenólicos, bioatividade, nutrição animal, alimentos funcionais.

Abstract

The wide availability of brewers' spent grain (BG) low-cost, associated with the current interest in the health benefits of phenolic acids, opens new possibilities for the use. This review details the potential bioactivities of phenolic compounds present in the BG and its incorporation in animal nutrition and functional foods. The literature shows that the phenolic compound including ferulic, p-coumaric and caffeic acid can have antioxidant, anti-carcinogenic, anti-inflammatory and anti-atherogenic activities. Since these phenolic acids are some of the major phenolic in BG, it is expected that may exhibit similar properties. The BG works as an alternative for animal nutrition, with many nutritional benefits. Although some attempts have been made to incorporate the bioactive components in foods BG, more research is needed in this area.

Keywords: brewers' spent grain, phenolic acids, bioactivity, animal nutrition, functional foods.

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1. INTRODUÇÃO

A cevada destaca-se como o quarto colocado do ranking de produção de cereais no mundo (FAO, 2012), devido a sua ampla adaptabilidade ecológica, por ser matéria-prima na fabricação de cerveja e pela sua utilização na alimentação animal, além de ser reconhecida pelo elevado potencial produtivo e pela baixa incidência de doenças (SILVA et al., 2000; ZHANG et al., 2002; KURTZ, 2012).

A cevada tem inúmeras utilidades, esta pode apresentar aplicações alimentares e não alimentares (ZHAO et al., 2011). Diferentemente do mercado da cevada no mundo, onde 66% da produção é utilizada como forrageira e ração para as criações, o cultivo comercial de cevada no Brasil destina-se a fabricação de malte, principal matéria-prima da indústria cervejeira, sendo estas empresas responsáveis por fomentar a cultura no país (DE MORI, 2007). Durante a produção de cerveja gera-se um resíduo que é considerado um subproduto industrial com baixo valor agregado (STOJCESKA; AINSWORTH, 2008; WATERS et al., 2012), resultante da fase inicial do processo como uma fração sólida de malte de cevada remanescente após a produção de mosto, denominado de bagaço de cevada, definido nesta revisão como resíduo de cervejaria (RC).

O resíduo de cervejaria (RC) é o mais abundante subproduto da indústria cervejeira, representando cerca de 85% dos co-produtos gerados em todo o processo (MUSSATTO et al., 2006). Consequentemente, o RC apresenta uma produção anual em torno de 30 milhões de toneladas pelas cervejarias do mundo, das quais cerca de 3,4 milhões de toneladas são produzidas na Europa (STOJCESKA et al., 2008; NIEMI et al., 2012) e no Brasil a estimativa de disponibilidade ultrapassa 2 milhões de toneladas/ano (FARIA, 2003).

Indubitavelmente, isto é visto negativamente pela indústria dentro das perspectivas de sustentabilidade, já que demanda a remoção deste resíduo ou o custo do tratamento. O RC é geralmente incorporado na alimentação de animais, dentre outras aplicações comuns, a eliminação direta ao solo ou em aterro sanitário, as quais não são suficientes para drenar a grande quantidade produzida por ano (FILLAUDEAU et al., 2006; MUSSATTO et al., 2006). Atualmente, há uma crescente pressão para reduzir os subprodutos de processos industriais, reutilizando-os em processos secundários (WATERS et al., 2012). Neste contexto, as oportunidades econômicas para o RC, associadas a redução de poluição ambiental, podem ser realizadas através da expansão de sua funcionalidade.

As composições típicas de RC variam, mas sempre incluem altos níveis de fibra dietética, proteína e particularmente, aminoácidos essenciais, bem como níveis apreciáveis de minerais, polifenóis e lipídios (MUSSATTO; ROBERTO, 2005; MUSSATTO et al., 2006), o que respresenta características nutricionais altamente desejáveis para o consumo animal e também do ponto de vista dietético humano.

Além de suas características de composição, o baixo custo e o elevado nível de disponibilidade, tornam o RC adequado como ingrediente alimentar, de forma que, trabalhos experimentais buscam reaproveitar tal resíduo para o consumo humano (ÖZVURAL et al., 2009; WATERS et al., 2012; KIM et al., 2013; KTENIOUDAKI et al., 2013).

Nos últimos anos, vários estudos têm associado o consumo de alimentos ricos em compostos bioativos com a capacidade de promover uma série de benefícios para a saúde humana. Os compostos bioativos mais comuns incluem metabólitos secundários, tais como antibióticos, micotoxinas, alcalóides, pigmentos de grau alimentar, fatores de crescimento de plantas e compostos fenólicos (MARTINS et al., 2011; ROUTRAY; ORSAT, 2012; MENESES et al., 2013). Particularmente, compostos fenólicos são de grande interesse para cientistas, fabricantes e consumidores, devido a sua importância na qualidade dos alimentos e aos papéis de proteção e prevenção em certos tipos de câncer e outras doenças crônicas (BARBOSA-PEREIRA et al., 2013; KRISHNASWAMY et al., 2013).

Ácidos hidroxicinâmicos, que são os fenóis predominantes no RC (BARTOLOMÉ et al., 2002; MUSSATTO et al., 2007), têm demonstrado propriedade antioxidante e este efeito in vitro foi reportado ser semelhante àquele exibido pelos antioxidantes bem conhecidos como α-tocoferol e ácido ascórbico (MCCARTHY et al., 2012). Esta revisão detalha as potenciais bioatividades de compostos fenólicos presentes no RC e a sua incorporação na nutrição animal e em alimentos funcionais.

2. COMPONENTES FENÓLICOS DE RESÍDUO DE CERVEJARIA

Muitos estudos têm relatado a composição

centesimal de RC, que contém proteínas, gorduras, celulose, hemicelulose e lignina (Tabela 1).

Como está representado, há uma boa consistência no que diz respeito à composição de RC. No entanto, variações podem ocorrer devido a diferenças na variedade de cevada, o tempo de colheita, as características do lúpulo adicionado e a tecnologia da cervejaria (SANTOS et al., 2003).

Tabela 1. Composição química de resíduo de cervejaria

Componentes (expresso em % de matéria seca)							
Pesquisas	Proteína	Lipídios	Umidade	Celulose	Hemicelulose	Lignina	Amido
Santos et al. (2003)	24,2	3,9	3,4	-	-	-	-
El-Shafey (2004)	26,7	8,9	3,9	-	-	5,3	-
Mussatto e Roberto (2005)	15,3	-	4,6	16,8	28,4	7,8	-
Xiros (2008)	14,2	13,3	3,3	12,0	40,2	11,5	-
Treimo (2009)	23,4	-	-	-	-	12,6	7,8
Ktenioudaki et al. (2013); Reis e Abu-Ghannam (2014)	20,8	4,5	5,6	-	-	-	3,3

*valores expressos em % de matéria seca, documentada em 20% (EL-SHAFEY et al., 2004).

O RC é constituído predominantemente das camadas de pericarpo (casca) da semente, que são ricas em celulose, polissacarídeos não-celulósicos, lignina, proteína e gordura. Isso se reflete na composição do RC (Tabela 1), e, portanto, este resíduo pode ser considerado como um material lignocelulósico (MUSSATTO et al., 2006).

Além dos componentes descritos na Tabela 1, tem sido mostrado que RC também constitui uma fonte valiosa de vitaminas, minerais e aminoácidos, em particular para a alimentação animal. As vitaminas presentes em RC são biotina, ácido fólico, niacina, colina, riboflavina e tiamina, ácido pantotênico e piroxidina (HUIGE, 1994). O RC também é relatado por conter minerais tais como Ca, Cu, Fe, Mn, K e Na e ambos aminoácidos, essenciais (incluindo a lisina, histidina, metionina, fenilalanina, triptofano) e não-essenciais (incluindo alanina, serina, glicina, prolina) (HUIGE, 1994).

Os ácidos fenólicos, particularmente ácidos hidroxicinâmicos e hidroxibenzóicos são metabólitos secundários de plantas encontrados extensivamente em alimentos vegetais. Estes ácidos fenólicos são atualmente o foco de atenção, devido ao seu potencial para atuar como antioxidante, anti-inflamatório e compostos anticancerígenos (NAGASAKA et al., 2007).

Como mencionado anteriormente, RC é constituído predominantemente de camadas de pericarpo (casca) da semente e é em grande parte composta de paredes celulares. Uma vez que a maior parte dos compostos fenólicos dos grãos de cevada estão contidos na casca (MUSSATTO et al., 2006) e os ácidos hidroxicinâmicos acumulam-se nas paredes das células, o RC é uma fonte potencialmente valiosa de ácidos fenólicos.

Há evidências que sugerem que o ácido ferúlico e ácido p-cumárico estão presentes em concentrações relativamente elevadas em RC (BARTOLOMÉ et al., 2002).Tem sido relatado que, na sequência de ácidos ferúlico e p-cumárico, os próximos ácidos fenólicos mais abundantes em RC foram o sinápico, caféico e ácido siríngico (SZWAJGIER et al., 2010), apresentados na Tabela 2.

Tabela 2. Ácidos fenólicos mais abundantes presentes no resíduo de cervejaria conforme Szwajgier et al. (2010)

	Concentração de ácidos livres (mg/100 g matéria seca)
Ácidos fenólicos	Média
Ácido ferúlico	336,3
Ácido p-cumárico	64,4
Ácido sináptico	42,0
Ácido cafeico	9,9
Ácido siríngico	6,5
Ácido 4-OH-benzóico	1,2
Ácido clorogênico	0,6
Ácido protocatecuico	0,5

Evidências mais recentes mostram que a grande maioria dos ácidos fenólicos em RC é encontrada na forma ligada (FORSSELL et al., 2008), obtendo-se valores em torno de 0,7 a 0,8 % da matéria seca de RC correspondente a ácidos fenólicos ligados (TREIMO et al., 2009; ROBERTSON et al., 2010). Numerosos estudos têm sido realizados para extrair ácidos fenólicos de RC. Novas técnicas de extração, por exemplo, processo de derivatização rápida assistida por micro-ondas, têm sido investigado (ATHANASIOS et al., 2007).

Uma revisão dos métodos de extração, separação e detecção dos ácidos fenólicos em alimentos vegetais naturais demonstrou que os métodos mais utilizados envolvem a hidrólise ácida e saponificação (STALIKAS, 2007). Entretanto, a saponificação (envolvendo o tratamento de amostras com uma solução de NaOH 1-4 M) tem sido amplamente utilizada para extrair os ácidos hidroxicinâmicos de RC (BARTOLOMÉ et al., 2002; FAULDS et al., 2004).

Muitas pesquisas foram conduzidas voltadas para a atividade antioxidante dos ácidos hidroxicinâmicos, particularmente os ácidos ferúlico e p-cumárico. O potencial antioxidante do ácido ferúlico utilizando o método de DPPH foi demonstrado, entretanto o ácido ferúlico apresentou-se como um antioxidante menos potente do que o ácido caféico e α–tocoferol (BRAND-WILLIAMS et al., 1995). O ácido caféico foi demonstrado atuante como um antioxidante *in vitro* e sequestrador de radicais incluindo o DPPH e o superóxido ânion (GULCIN, 2006). Também tem sido demonstrado, utilizando o ensaio de DPPH, que uma série de ácidos hidroxicinâmicos atuam como antioxidantes, sequestradores de DPPH conforme a ordem: ácido cafeico> ácido sinápico = ácido ferúlico> ácido p-cumárico (KIKUZAKI et al., 2002).

Da mesma forma, mas utilizando um método alternativo, um estudo para investigar os compostos fenólicos em extrato de farelo de trigo e a sua atividade antioxidante descobriu que o ácido ferúlico foi um dos antioxidantes mais fortes, quando utilizado o sistema modelo de ácido linoleico β-caroteno. O ensaio do sistema de ácido linoléico β-caroteno, baseia-se no princípio de que a temperatura elevada da oxidação do ácido linoleico, produz peróxidos para descolorir o β-caroteno. O extrato de farelo de trigo com maiores concentrações de ácido ferúlico (após hidrólise alcalina) apresentou maior atividade antioxidante (KIM et al., 2006).

O ácido ferúlico e ácido cafeico têm sido relatados por terem um excelente potencial antioxidante em baixas concentrações, com a capacidade para sequestrar uma série de radicais livres testados (MAURYA; DEVASAGAYAM, 2010). Em um estudo recente sobre cervejas, foi encontrado uma correlação direta entre o poder antioxidante de redução do ferro e uma série de ácidos fenólicos incluindo os ácidos ferúlico, p-cumárico, cafeico, sinápico e vanílico (PIAZZON

et al., 2010). Dado que o ácido ferúlico é muito reconhecido como um antioxidante, e por isso está aprovado para utilização como um aditivo alimentar em alguns países para evitar a oxidação (ITAGAKI et al., 2009).

Além disso, é importante notar que, enquanto os compostos fenólicos podem ter um efeito antioxidante, tem demonstrado atuar como pró-oxidantes, sob certas condições, induzindo o stress oxidativo. Para o ácido cafeico, ácido ferúlico e p-cumárico atuarem como pró-oxidantes são necessárias concentrações elevadas (FERGUSON et al., 2005; MAURYA; DEVASAGAYAM, 2010).

Tem sido sugerido que o efeito pró-oxidante está relacionado com a presença de íons metálicos do organismo (por exemplo, devido à lesão do tecido libertando Fe e Cu) e é de relevância para a bioatividade dos compostos fenólicos *in vivo* (MORTON et al., 2000). Entretanto, a partir de estudos *in vivo* os ácidos hidroxicinâmicos apresentam propriedades antioxidantes. Tais estudos são essenciais para entender o papel biológico desses ácidos fenólicos (SHAHIDI; CHANDRA- SEKARA, 2010).

Em adição ao seu potencial antioxidante, há cada vez mais provas que sugerem que os ácidos fenólicos podem ter um efeito anticancerígeno. O ácido cafeico exibe um efeito antiproliferativo em várias células cancerígenas, incluindo da glândula mamária, o adenocarcinoma, leucemia linfoblástica e linhas celulares de câncer do colo do útero (CHANG et al., 2010). Os ácidos fenólicos incluindo o ácido caféico (KANG et al., 2009) e ácido vanílico (KIM et al., 2011), os polifenóis e quercetina (GARCÍA-MEDIAVILLA et al., 2007) foram capazes de inibir a expressão de COX-2, possivelmente reduzindo o risco de câncer.

Os estudos em animais também foram realizados para determinar o potencial anticancerígeno de ácidos fenólicos, e ambos os ácidos ferúlico e cafeico, foram capazes de previnir tumores induzidos em ratos (KAWABATA et al., 2000). Uma revisão publicada recentemente com compostos fenólicos de plantas informou que, desempenham um papel antagônico em todas as fases de desenvolvimento de câncer e que um estudo mais aprofundado sobre estes compostos vão fornecer informações sobre o seu possível uso farmacêutico futuro (DAÍ; MUMPER, 2010).

Em medicamentos orientais japoneses, *Cimicifuga heracleifolia* é muitas vezes utilizada como uma droga anti-inflamatória. O ácido ferúlico foi demonstrado estar entre os principais ácidos fenólicos em *C. Heracleifolia* (HE et al., 2006). Sakai et al. (1999) e Kim et al. (2012) demonstraram que o ácido ferúlico é capaz de reduzir a produção de moléculas de sinalização inflamatória de uma forma dose-dependente. Foi sugerido que o ácido ferúlico e ácido isoferúlico são responsáveis, pelo menos em parte, pelas propriedades anti-inflamatórias da droga *C. heracleifolia* (SAKAI et al., 1999).

O LDL oxidado é um marcador de risco bem conhecido de doença cardiovascular, que é causada principalmente pela aterosclerose (YOSHIDA; KISUGI, 2010). Existe evidência para o efeito de ácidos hidroxicinâmicos, incluindo ácido ferúlico, p-cumárico e sinápico sobre a inibição da oxidação de LDL humano i*n vitro* numa relação dose-dependente (ANDREASEN et al., 2001). Além disso, há a capacidade de extratos fenólicos de RC proteger contra danos ao DNA induzidos pela oxidação (MCCARTHY et al., 2012).

Em resumo, há cada vez mais provas que sugerem que os ácidos fenólicos, incluindo aqueles encontrados em concentrações mais elevadas em RC, podem conferir benefícios potenciais para a saúde incluindo potencial antioxidante, anti-inflamatório, anticancerígeno e anti-aterogênico.

3. INCORPORAÇÃO DE RESÍDUO DE CERVEJARIA EM NUTRIÇÃO ANIMAL

Como mencionado anteriormente, o RC contem cerca de 20 % de proteína e 70% de fibra, e é devido a esta composição química favorável que tem um grande potencial para o uso como um ingrediente bruto (MUSSATTO et al., 2006). Além disso, ao determinar aspectos produtivos e a economicidade do uso desse resíduo em substituição ao alimento concentrado na alimentação de cordeiros confinados em fase de terminação ficou comprovado haver redução linear no custo da alimentação dos animais com o aumento da quantidade de RC nas dietas (BROCHIER; CARVALHO, 2009).

O RC fermentado pode ser obtido pelo processo de fermentação microbiana de RC úmido. Em estudo de Geron et al. (2007) foram avaliadas as frações da proteína e dos carboidratos, a degradabilidade ruminal efetiva da matéria seca (MS) e proteína bruta (PB), a digestibilidade ruminal in vitro da MS e PB, a digestibilidade intestinal in vitro da proteína

não-degradada no rúmen e os perfis de aminoácidos e de ácidos graxos de RC úmido e RC fermentado. De maneira geral, o processo de fermentação anaeróbico não alterou as características nutricionais de RC.

A inclusão de até 24% do RC fermentado na MS em rações para bovinos não altera os processos de fermentação ruminal e digestão dos nutrientes (GERON et al., 2008), concordando com estudo de Silva et al. (2010), onde observou-se que RC úmido pode ser utilizado em níveis de até 25 % como substituto ao concentrado em dietas para cabras, sem alterar o consumo e a digestibilidade dos nutrientes.

O efeito de RC na composição e produção de leite de gado leiteiro também foi estudado. A inclusão de 0, 5, 10 e 15% de RC fermentado as rações de vacas leiteiras não altera o consumo dos nutrientes, os coeficientes de digestibilidade da matéria orgânica, carboidratos totais, fibra em detergente neutro e fibra em detergente ácido, contudo, verificou-se efeito linear positivo com relação aos coeficientes de digestibilidade da matéria seca, proteína bruta e extrato etéreo em função da inclusão do RC fermentado nas rações. A produção e a qualidade do leite não foram influenciadas, definindo-se que o RC fermentado pode ser incluído até 15% nas rações de vacas leiteiras sem alterar o consumo de nutrientes e a produção e qualidade do leite (GERON et al., 2010).

Embora a principal alternativa para o RC é, atualmente, como ração para gado leiteiro, pode-se relatar os benefícios do RC para uso como ração para aves e peixes. O efeito da substituição do farelo de arroz na dieta de peixe com 10-40 % de RC tem sido investigado (KAUR; SAXENA, 2004). O RC utilizado continha 19 % de proteína bruta, 18-20 % de fibra bruta e apresentava um bom perfil de aminoácidos. Verificou-se que a carpa (peixe de água doce) obteve melhor desempenho de crescimento em dietas contendo RC do que o grupo controle, em função da proteína de alta qualidade deste resíduo.

Um estudo mais recente mostrou que o RC biodegradado contém cisteína, lisina e metionina, além de outros catorze aminoácidos (ESSIEN; UDOTONG, 2008). Dependendo do microrganismo utilizado para degradar o RC, diferentes concentrações de aminoácidos foram encontradas, de forma consistente, a alanina na concentração mais elevada. Esta composição foi observada ser de particular importância para as aves, já que a cisteína, lisina e metionina são os principais aminoácidos necessários à alimentação destes animais.

Em resumo, as evidências sugerem que todo o RC, como parte de uma ração completa, tem benefícios nutricionais para uma variedade de animais, principalmente para bovinos, o que implica em uma utilização de rotina de RC como ração para esta categoria animal.

4. INCORPORAÇÃO DE RESÍDUO DE CERVEJARIA EM ALIMENTOS FUNCIONAIS

Para além da sua utilização como ração para animais, o RC pode ser incorporado em gêneros alimentícios destinados ao consumo humano. Dado o seu baixo custo e elevado valor nutricional, o RC representa um ingrediente ideal para a alimentação humana, particularmente onde existe necessidade de aumentar o teor de fibras.

Vários estudos têm avaliado o efeito da incorporação de RC como substituto de farinha de trigo, onde observa-se que a qualidade física de biscoitos é mantida, além de obter-se um aumento no teor de N, de fibra bruta e fibra dietética (ÖZTÜRK et al., 2002). Ktenioudaki et al. (2013) observaram que biscoito com 10% deste resíduo demonstrou elevado índice de crocância e foi altamente aceitável, destacando a possibilidade de utilização de RC para a formulação de lanches. No estudo de Stojceska e Ainsworth (2008), o RC foi incorporado em diferentes níveis (0-30 %) em pães de farinha de trigo tratada com quatro enzimas diferentes, de forma que o teor de fibra dos pães foi significativamente aumentado pela adição de RC e a sua utilização combinada com enzimas adequadas é capaz de melhorar a vida de prateleira, textura e volume dos pães.

Da mesma forma, quando a farinha de milho de lanche extrudido foi substituída por RC a níveis de 10, 20, 25 e 30 %, o percentual protéico, o teor de gordura e fibra aumentaram, enquanto que o amido diminuiu, de maneira que, foi sugerido que os alimentos fortificados com RC podem ser considerados como alimentos funcionais (AINSWORTH et al., 2007).

Inicialmente, acreditava-se que o RC era muito granular para adição direta à alimentação e que teria de ser primeiramente convertido a farinha antes da sua utilização. No entanto, Özvural et al. (2009) demonstraram que o RC de vários tamanhos de partículas, pode ser efetivamente utilizado na produção de salsichas, de maneira que a aceitabilidade é diminuída com o aumento do tamanho das partículas e a redução dos níveis de gordura.

Os autores sugeriram que RC pode ser utilizado para produzir produtos à base de carne de baixo teor de gordura e ricos em fibras, com a adição limitada entre 3% a 5% (ÖZVURAL et al., 2009). Kim et al. (2013) ao extraírem fibra alimentar de RC e adicionar em hambúrgueres de frango observaram que formulações contendo 3 % do extrato de fibra dietética garantiu maior aceitabilidade ao produto.

Em adição ao tamanho de partícula, há preocupações sobre a aparência do produto quanto a incorporação de RC em gêneros alimentícios, já que quando úmido, o RC é de cor marrom, o que poderá alterar a coloração característica do produto adicionado. Contudo, é imperativo que as propriedades organolépticas permaneçam aceitáveis para os consumidores e diversos trabalhos têm estabelecido o limite superior de adição de RC para que as características sensoriais dos produtos sejam mantidas (ÖZVURAL et al., 2009; KIM et al., 2013).

5. CONCLUSÕES

A literatura demonstra que os compostos fenólicos, incluindo o ácido ferúlico, p-cumárico e cafeico podem ter efeitos antioxidantes, anti-cancerígenos, anti-inflamatório e anti-aterogênicos. Dado que estes ácidos fenólicos são alguns dos principais fenólicos em RC, espera-se que este resíduo também possa exibir propriedades semelhantes com potencial de ser desenvolvido para uma série de bioatividades.

O RC atualmente funciona como uma alternativa na alimentação animal, tendo muitos benefícios nutricionais. Embora tenham sido feitas algumas tentativas para incorporar os componentes bioativos de RC em alimentos, é necessária mais investigação nesta área. Dada a natureza bioativa potencial dos extratos fenólicos de RC, e as grandes quantidades de RC produzidos anualmente a um baixo custo, é imperativo que seu uso alternativo seja explorado.

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3. DESENVOLVIMENTO

O desenvolvimento desta Tese foi dividido em quatro manuscritos, apresentados na forma de artigos científicos.

3.1 MANUSCRITO 1

ANALYSIS OF POLYPHENOLS IN BREWER'S SPENT GRAIN AND ITS COMPARISON WITH CORN SILAGE AND CEREAL BRANS COMMONLY USED FOR ANIMAL NUTRITION

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Analysis of polyphenols in brewer's spent grain and its comparison with corn silage and cereal brans commonly used for animal nutrition

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abstract

Brewer's spent grain (BSG) could be tested as an alternative source of polyphenols in animal nutrition. Proper extraction and analytical methods are critical for quantification. Thus, extraction for BSG, corn silage, and brans of rice, corn, and wheat were studied for the highest yield of polyphenols. A method for 18 phenolic monomers by HPLC-DAD was developed, validated, and applied to samples. An aqueous solution of NaOH (0.75% w/v) using integral samples for extraction resulted in the highest values for colorimetric measurements in all analyzed sources. Method by maceration showed the highest phenolic yield when applied in corn silage and BSG. However, for brans the best method was microwave assisted. Results from HPLC-DAD analysis clearly showed that native structures of phenolic compounds were simplified to its monomers allowing quantification and sample discrimination. BSG had the highest concentration of polyphenols and could be a promising and innovative source for animal feed studies.

1. Introduction

Brewer's spent grain (BSG) is a beer industry residue that represents more than 85% of the total produced by-products (Mussatto, Dragone, & Roberto, 2006). In 2014, Brazil has produced a total of 14 billion liters of beer reaching the third place in the global production ranking, just behind China and the United States of America (Isozaki, 2015). Worldwide breweries are capable to produced more than 30 million tons of brewer's spent grain per year and at a least 2 million of tons are produced just in Brazil (Niemiet al., 2012). This scenario emphasizes the relevance in the inclusion of this residue in the food production chain, as has already occurred with other wastes produced in large amounts by agroindustry.

Among the agricultural wastes that are annually produced in large amounts and used for animal consumption there is the corn silage. It is rich in cellulose, hemicellulose and lignin and widely used to feed ruminants (Kuzmanovic´ et al., 2015). Cereal brans are also important by-products obtained from corn, wheat, and rice flour production (Brewer, Kubola, Siriamornpun, Herald, & Shi, 2014; Zilic et al., 2013).

All those cereals grains were produced worldwide in 2015's recorded values of 1,336.6 billion tons, 729.1 million tons, and 494.7 million tons, respectively (FAO.Cereal Supply, Agriculture Organization of the United Nations(FAO), & Italy, 2016). Cereal brans are common ingredients in animal feed and some previous works in literature have already reported polyphenol profile and quantification results (Setyaningsih, Saputro, Palma, & Barroso, 2015; Vaher, Matso,Levandi, Helmja, & Kaljurand, 2010; Wang et al., 2014). Thus, comparisons of these materials with new ingredients in animal diet are important to evaluate the relevance of it as source of polyphenols.

It is noteworthy that corn silage is one of the main items of forage in the diet of cattle in many parts of the world and brewer's spent grain could be tested as an alternative source of polyphenols in animal nutrition because its chemical composition contains high levels of fiber and protein (Mussatto et al., 2006), which could suggest putative benefits for ruminants. The advantages of such substitution would entail the use of an agro-industrial waste of low commercial value to replace a dietary ingredient with a higher market value. Furthermore, this waste could be a promising source of polyphenols with greater antioxidant activity than corn silage.

As already reported by Masisi, Beta, and Moghadasian (2016) cereal by-products obtained from agricultural and agroindustry activities are promising sources of bioactive compounds. Most phenolic compounds can act as strong

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antioxidants (Jun, Shin,Song, & Kim, 2015) being helpful to promote human health against chronic diseases (Del Rio et al., 2013). Moreover, it could improve food quality either by direct application as food stabilizers (McCarthy et al., 2013) or indirectly by the animal diet increasing deposition in tissues (Castillo, Pereira, Abuelo, & Hernández,2013; Fruet et al., 2016). All future applications of these plantmatrices require previous studies on the evaluation of extraction conditions and polyphenols characterization.

Due to the polyphenol's chemical diversity and its interaction with other matrix constituents, extraction from vegetable sources are complex and the initial steps in samples preparation requires the evaluations of some important conditions (Masisi et al., 2016). Solvent composition and the energy supplied during extraction are some factors that greatly affect analyte recovery (Acosta-Estrada, Gutiérez-Uribe, & Serna-Saldívar, 2014; Wanyo, Meeso, & Siriamornpun, 2014). Since antioxidant capacity is mainly linked to the concentration of these compounds, it could also be affected as a property of the final extract mixture.

As a final remarkable consideration to this work, the use of phe-nolic compounds in ruminant diet was already reported as a promising strategy to improve animal well being for dairy cattle or as a supplementation strategy for antioxidant fortified milk and meat (Castillo et al., 2013; Fruet et al., 2016; Paraskevakis, 2015). The dose-response effect of these compounds, as well as their mechanism of action in ruminant animals has still not been fully elucidated. Therefore, there is a growing interest in the chemical elucidation and quantification of these compounds in food destined for animal con-sumption, which has a polyphenol profile that is still unclear.

The main purpose of this works is the evaluation of differ extraction conditions for characterization of polyphenols content or the identification of major monomers that could be present in brewer's spent grain. Moreover, corn silage and brans of corn, wheat, and rice were also evaluated for comparison purposes. Since this vegetable sources are the major ingredients used for animal feed. Extraction was carefully studied to determine the contri-bution of different solvents and the effect of microwave energy in the polyphenol's yield and profile. The method used for separation and quantification of phenolic compounds in extracts of cereal wastes by HPLC-DAD is detailed described.

2. Materials and methods

2.1. Chemicals and standards

4-Hydroxybenzoic acid (99%); caffeic acid (98%); catechin(98%); chlorogenic acid (95%); epicatechin (98%); gallic acid(98%); kaempferol (90%); kaempferol-3DGlp (97%); myricetin(96%); p-coumaric acid (98%); protocatechuic acid (97%); quercetin(95%); resveratrol (99%); sinapic acid (99%); syringic acid (95%); trans-cinnamic acid (99%); trans-ferulic acid (99%); vanillic acid (97%) were purchased from Sigma–Aldrich (St. Louis, Missouri, United States). HPLC-grade methanol used for mobile phase was obtained from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and formic acid used for mobile phase was obtained from Sigma–Aldrich (St. Louis, MO, U.S.A.). HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, Massachusetts, U.S.A.). Polytetrafluoroethylene syringe filter and membrane (PTFE) was from Allcrom (São Paulo, Brazil).

2.2. Samples

The corn silage was obtained from a commercial hybrid corn variety. The silage was ensiled using double plastic bag in micro-silos with a volume of forty liters. It was compressed and sealedimmediately after harvest. Silos were opened after 60 days. At this date, the dry matter content was 34.61%.

Rice, corn, and wheat bran were purchased directly from a specialized company (Cooperativa Agrícola Mista Ltda – CAMNPAL, Nova Palma, Brazil) in feed production for ruminants. It had a dry matter content of 88.94%, 88.12%, and 87.23% for rice, corn, and wheat, respectively.

The brewer's spent grain was obtained from a medium-sized brewery. All amount used in this work was collected from the same production lot and with a dry matter content of 18.97%.

Samples of corn silage and brewer's spent grain were pre-dried in a forced ventilation oven (55 °C; 72 h) with a final dry matter content of 94.18% and 95.84%, respectively. All samples were finely milled in a refrigerated analytical mill (Marconi, São Paulo, Brazil) during 1 min at 27,000 rpm, standardized to 0.5 mm of particle diameter using a sieve system, and stored at 20 °C until the extraction experiments. Samples were studied in two forms: one with its initial fat content and named as integral samples; and another after fat removal with ethyl ether using exhaustive extraction (Soxhlet apparatus), 4 h, lipid content in grams per 100 g of dry matter corresponding to the 3.2% corn silage; 19.9% rice bran; 3.9% corn bran; 4.2% wheat bran; 8.2% brewer's spent grain and named as defatted samples.

2.3. Extraction of phenolic compounds by maceration and microwave-assisted extraction

Maceration and microwave-assisted extractions were tested using the following five raw materials: corn silage; rice bran; corn bran; wheat bran, and brewer's spent grain. All of them were evaluated as integral and as defatted samples. The following solvents were tested: 50% methanol; 50% acetone, and 0.75% NaOH aqueous solution.

The microwave equipment (Synthos 3000, Anton-Paar, São Paulo, Brazil) equipped 16-carrousel containers (Rotor 16) was set up as detailed by Moreira et al. (2013) with some adaptations. For all solvents being tested, one gram of each sample was transferred to polytetrafluorethylene (PTFE-TFM) based tubes in a solid to liquid ratio of 1:20 w/v. Temperature was maintained and monitored at 100 °C under stirring (magnetic stirring bar, 200 rpm) in all the containers during the whole extraction time (15 min).

For maceration, powdered samples were mixed with each solvent (50% methanol; 50% acetone, and 0.75% NaOH aqueous solution) at a solid to liquid ratio of 1:10 and kept at room temperature (around 20 °C) for 24 h under constant stirring (magnetic stirring bar, 200 rpm) at dark (Brewer et al., 2014; Jun et al., 2015).

All the extracts obtained by both methods were centrifuged (15 min, 4000 rpm, MTD III Plus, Logen Scientific). For the extracts obtained with 0.75% of sodium hydroxide aqueous solution, the pH value in supernatant was adjusted to 6.5 with a 6 M hydrochloric acid solution. Supernatants were filtered (cellulose, 0.45 μm) and stored at 20 °C for further analysis.

2.4. Total phenolics and flavonoids

Total phenolic content was determined by the Folin-Ciocalteu reagent (FCR) colorimetric method (Singleton, Orthofer, &Ramuela-Raventos, 1999). Quantification was performed by a cali-bration curve using Gallic acid as an authentic phenolic standard (0–70 mg L^{1}; $Y = 0.013x + 0.013$; $R^2 = 0.999$). Results were expressed by equivalence as milligrams of gallic acid equivalent (GAE) per gram of sample.

Total flavonoid content was determined by a colorimetric method (Bao, Cai, Sun, Wang, & Corke, 2005). Quantification was done using quercetin as the authentic standard for flavonoids (0–80 mg L^{1}; $Y = 0.002x + 0.0223$; $R^2 = 0.999$). Results were expressed as milligrams of quercetin equivalents (QE) per gram of sample.

2.5. Antioxidant capacity

Scavenging capacity of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was used to determine antioxidant activity of samples as detailed by Brand-Williams, Cuvelier, and Berset (1995). Results were expressed as the effective dose for 50% of reduction in the initial levels of DPPH free radical. It was named as the ‘‘Efficient Concentration” (EC50) and determined by sequential sample dilutions versus the antioxidant activity scavenger percentage as previously described (Brand-Williams et al., 1995).

The ferric ion reducing antioxidant power (FRAP) was determined using the method described by Benzie and Strain (1996). Regression equation for quantification (Y = 0.060 x 0.068, R^2 = 0.994) was determined by a calibration curve using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 0–25 μM) as standard. Results were expressed as lM of mol TEAC (Trolox equivalent antioxidant capacity) per gram of sample.

Antioxidant activity was also measured using the ABTS cation radical (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) discoloration assay as described by Re et al. (1999). Regression equation (Y = 0.401x + 4.56, R^2 = 0.991) for quantification was determined by a calibration curves obtained with Trolox (10– 250 μM) as the standard antioxidant. Results were expressed as μM of TEAC per gram of sample.

2.6. Sample treatment for HPLC analysis

Just samples obtained in the best extraction conditions (integral or defatted samples; maceration versus microwave; solvent type) determined by the content of phenolic compounds, flavonoids, and antioxidant capacity were selected for polyphenols characterization by HPLC.

Thus, based in this first analytical approach only integral samples were used for HPLC extraction. For corn silage and brewer’s spent grain, the extraction by maceration was chosen. However, for rice, corn, and wheat brans, the method by microwave was the best procedure for extraction. For all samples, extraction using aqueous solution of sodium hydroxide was the solvent type with the higher yields as total polyphenols by colorimetric assay. However, 50% of acetone in water (v/v) was also analyzed to evaluate changes due to alkaline hydrolysis.

Extracts with 0.75% NaOH aqueous solution were purified to deplete possible salts as previously described by Ross, Beta, and Arntfield (2009) withsome modifications. Thus, a liquid-to-liquid extraction was performed using a mixture of diethyl ether/ethyl acetate (DE/EA 1:1, v/v). The DE/EA (supernatant) organic layer containing the analytes was collected and evaporated under rotary vacuum (10 min, 38 °C, Rotavapor R-300, Buchi Brasil Ltda). The residue was re-dissolved in methanol: formic acid (99.9: 0.1 v/v) to a known volume (2 mL). Previously to injection, samples were filtered (PTFE, 0.22 μm, 25 mm, simple pure syringe filters).

Extracts with 50% acetone solvent were evaporated under rotary vacuum (10 min, 38 °C, Rotavapor R-300, Buchi Brasil Ltda). The residue was re-dissolved in methanol: formic acid (99.9: 0.1 v/v) to a known volume (5 mL). Previously to injection, samples were filtered (PTFE, 0.22 μm, 25 mm, simple pure syringe filters).

2.7. Analysis of phenolic compounds by HPLC-DAD analysis

HPLC equipment (LC-20A Prominence, Shimadzu, Japan) was equipped with a quaternary pump (LC-20AD), manual injector, oven column (CTA-20A) and diode array detector (SPD-M20A). The LCsolutions Software (Version 3, Shimadzu, Columbia, U.S.A.) was used for data processing.

Samples and all standards solutions were injected at a volume of 20 mL in a reversed-phase column (C-18, 150 mm 4.6 mm, particle size 5 μm, Kinetix Core-Shell Technology) at 38 °C. Separation was optimized by gradient elution of three mobile phases at a flow rate of 1.0 mL $min^{-1.}$ Method suitability was evaluated by peak resolutions and by asymmetry factor of all 18 plant phenolic standards (see Section 2.1). Mobile phase A was ultrapure water (Milli-Q Gra-dient System, Millipore Corporation, Massachusetts, EUA) acidified with formic acid (99.9: 0.1 v/v); B was acetonitrile and formic acid (99.9: 0.1 v/v); and C was methanol and formic acid (99.9: 0.1 v/v). The standardized best gradient elution conditions was set as follows: 95% A, 0% B and 5% C from 0 to 4 min; 83.9% A, 4.1% B and 12% C from 4.1 to 8 min; 96% A, 0% B and 4% C from 8.1 to 11 min; 88% A, 0% B and 12% C from 11 to 11.5 min; 98% A, 0% B and 2% C from 11.5 to 13.6 min; 73.5% A, 14.5% B and 12% C from 13.6 to 14.5 min; 75.3% A, 16.7% B and 8% C from 14.5 to 16.7 min; 51.9% A, 40.1% B and 8% C from 16.7 to 40.1 min; 0% A, 100% B and 0% C from 40.1 to 47.1 min; 100% A, 0% B and 0% C from 47.1 to 60.01 min.

Absorption spectra was recorded from 200 to 800 nm and chromatograms for quantification purposes at 280 nm, 320 nm, and 360 nm for hydroxybenzoates and proanthocyanidins (Class A), hydroxycinnamates (Class B), and flavonols (Class C), respectively. Identification was performed by comparison with standard’s retention times and the characteristic bands from UV–visible absorption spectra.

Validation was performed to determine the reliability of the analytical method by linearity, limits of detection and quantifica-tion, and accuracy only with those standard compounds that were also detected in samples. Experiments were conducted by selecting the appropriate recommendation and concepts available in the International Conference on Harmonization (ICH, 2006).

Calibration curves were constructed using stock solutions of nine phenolic compounds (4-hydroxybenzoic acid; catechin; cafeic acid; chlorogenic acid; p-coumaric acid; trans-ferulic acid; sinapic acid; quercetin and kaempferol) in methanol. These solutions were diluted in the initial mobile phase in seven equidistant points within the concentration range of 0.1–80 mg L^{-1}. Linear regression residual standard deviation (r) and the slope (m) of calibration curves were used to calculate limits of detection (LOD) and quantification (LOQ).

Method precision was evaluated by using repeatability (intra-day) and intermediate precision (inter-day) studies. It was expressed as the coefficient of variation (CV) from retention time and peaks area values (n = 10).

2.8. Statistical analysis

The completely randomized design (CRD) was analyzed by a factorial of four independent variables (two extraction methods – factor A two sample pre-treatments – factor B three extraction solvents – factor C five evaluated foods – factor D). Experiments in three sample replications were analyzed (n = 3). Each sample replication was collected from the silo (20 tons) in different ran-domized sections. Qualitative data were analyzed using SAS (Statistical Analysis System) software, version 9.1. Analysis of variance (ANOVA) was performed and results were significant with $p < 0.05$.

3. Results and discussion

3.1. Evaluation of different extraction conditions for phenolic compounds analysis and the effect on the antioxidant capacity

This study has evaluated some of the most commonly used solvents in polyphenol’s extraction from various matrices. Moreover, it was done in

comparison with an aqueous extraction process under an alkaline condition. Thus, concentrations of 50% methanol and acetone in water were the solvents mixtures used for comparison with an extraction method that combine alkaline hydrolysis and an aqueous medium. Furthermore, the use of microwave energy during extraction was evaluated to increase the permeability of the solvent in samples.

Tables 1 and 2 shows the average results and statistical data treatment for total concentration of polyphenols, flavonols, and antioxidant activity measured by colorimetric assays. ANOVA revealed a significant effect for the interaction between all the factors (FA⁄FB⁄FC⁄FD) for all variables. Thus, for the total measurement of polyphenols and flavonoids, the most effective solvent was the aqueous solution of NaOH (0.75% v/v) and the least was

Table 1
Total concentration of phenolic compounds (PC) and flavonoids (FVN) in different cereal agricultural and agroindustry wastes submitted to different extraction conditions.

Sample type (Factor D)	Solvent (Factor C)	Sample pre-treatment (Factor B)	PC*		FVN*	
			Extraction methods (Factor A)			
			Maceration	Microwave	Maceration	Microwave
BSG	Acetone	Integral	3.06 ± 0.08Aijk	1.00 ± 0.06Bijklm	1.34 ± 0.03Adefg	0.18 ± 0.02Bfghi
		Defatted	3.43 ± 0.34Ahij	1.08 ± 0.20Bijkl	1.24 ± 0.08Adefgh	0.12 ± 0.07Bghi
	Methanol	Integral	0.98 ± 0.15Aop	0.97 ± 0.03Aijklmn	0.65 ± 0.02Ahijk	0.48 ± 0.04Aefghi
		Defatted	1.36 ± 0.09Anop	0.89 ± 0.09Ajklmno	0.64 ± 0.11Ahijk	0.15 ± 0.05Afghi
	NaOH	Integral	17.46 ± 0.38Aa	13.77 ± 0.34Ba	4.54 ± 0.23Aa	2.65 ± 0.51Bc
		Defatted	15.41 ± 0.08Abc	12.04 ± 0.02Bc	2.93 ± 0.22Ab	1.24 ± 0.07Bd
Corn silage	Acetone	Integral	4.72 ± 0.11Ae	2.49 ± 0.14Bh	1.43 ± 0.11Acdef	0.65 ± 0.08Bdefgh
		Defatted	4.54 ± 0.22Aef	2.57 ± 0.23Bh	1.45 ± 0.07Acd	0.81 ± 0.03Bde
	Methanol	Integral	3.41 ± 0.06Ahij	2.53 ± 0.09Bh	0.81 ± 0.05Afghijk	0.61 ± 0.07Aefghi
		Defatted	3.67 ± 0.25Aghi	2.62 ± 0.41Bh	0.82 ± 0.09Aefghij	0.54 ± 0.08Aefghi
	NaOH	Integral	15.65 ± 0.10Ab	13.82 ± 0.11Ba	2.69 ± 0.18Bb	3.32 ± 0.47Aab
		Defatted	14.80 ± 0.91Ac	12.77 ± 0.04Bb	0.92 ± 0.13Bdefghij	2.92 ± 0.17Abc
Corn bran	Acetone	Integral	1.35 ± 0.06Anop	0.28 ± 0.03Bno	0.35 ± 0.07Ajk	0.08 ± 0.02Ahi
		Defatted	1.25 ± 0.13Anop	0.32 ± 0.06Bmno	0.21 ± 0.02Ak	0.01 ± 0.00Ai
	Methanol	Integral	0.68 ± 0.04Ap	0.24 ± 0.15Ao	0.20 ± 0.01Ak	0.02 ± 0.00Ai
		Defatted	0.81 ± 0.02Ap	0.23 ± 0.07Ao	0.36 ± 0.04Ajk	0.01 ± 0.00Ai
	NaOH	Integral	3.19 ± 0.10Bij	5.23 ± 0.02Afg	0.93 ± 0.18Adefghij	0.92 ± 0.51Ade
		Defatted	4.88 ± 0.05Ae	4.65 ± 0.02Ag	1.11 ± 0.20Adefgh	0.73 ± 0.11Adefg
Rice bran	Acetone	Integral	4.67 ± 0.49Ae	1.38 ± 0.09Bijk	1.44 ± 0.27Acde	0.62 ± 0.12Bdefghi
		Defatted	4.59 ± 0.09Aef	1.62 ± 0.05Bi	0.87 ± 0.19Adefghij	0.52 ± 0.07Aefghi
	Methanol	Integral	3.66 ± 0.22Aghij	1.57 ± 0.07Bif	0.42 ± 0.25Ajk	0.77 ± 0.01Adef
		Defatted	3.97 ± 0.14Afgh	1.63 ± 0.08Bi	0.60 ± 0.20Aijk	0.50 ± 0.05Aefghi
	NaOH	Integral	4.24 ± 0.06Befg	7.83 ± 0.49Ad	4.38 ± 0.17Aa	3.55 ± 0.40Ba
		Defatted	2.96 ± 0.04Bjk	6.64 ± 0.12Ae	2.01 ± 0.30Ac	1.24 ± 0.07Bd
Wheat bran	Acetone	Integral	2.38 ± 0.03Akl	0.58 ± 0.02Blmno	0.71 ± 0.05Aghijk	0.10 ± 0.01Ahi
		Defatted	2.15 ± 0.02Alm	0.66 ± 0.13Blmno	0.68 ± 0.01Aghijk	0.12 ± 0.03Aghi
	Methanol	Integral	1.84 ± 0.02Almn	0.76 ± 0.02Bklmno	0.33 ± 0.04Ajk	0.06 ± 0.01Ahi
		Defatted	1.52 ± 0.03Amno	0.79 ± 0.11Bklmno	0.30 ± 0.03Ajk	0.03 ± 0.00Ai
	NaOH	Integral	6.62 ± 0.12Ad	7.07 ± 0.02Ae	2.84 ± 0.07Ab	0.45 ± 0.09Befghi
		Defatted	6.59 ± 0.19Ad	5.81 ± 0.15Bf	1.13 ± 0.15Adefghi	0.10 ± 0.02Bhi

Probability		ANOVA results Independent variables	
		PC	FVN
	FA	<0.001	<0.001
	FB	<0.001	<0.001
	FA*FB	0.0006	<0.001
	FC	<0.001	<0.001
	FA*FC	<0.001	<0.001
	FB*FC	<0.001	<0.001
	FA*FB*FC	<0.001	<0.001
	FD	<0.001	<0.001
	FA*FD	<0.001	<0.001
	FB*FD	<0.001	<0.001
	FA*FB*FD	<0.001	<0.001
	FC*FD	<0.001	<0.001
	FA*FC*FD	<0.001	<0.001
	FB*FC*FD	<0.001	<0.001
	FA*FB*FC*FD	<0.001	<0.001
CV (%)		4.73	7.61
Error Mean Square		0.04	0.03

Different uppercase letters in line indicate significant differences between means. Different lowercase letters in column indicate significant differences between means. The values were represented as mean plus standard deviation of analyzes in triplicate. Significant effect for the factors when $p < 0.05$.

* Total concentration of phenolic compounds is expressed as mg Gallic acid equivalent/g of sample; total concentration of flavonoids is expressed as mg of Quercetin equivalents/g of sample. BSG = brewer's spent grain; FA = factor A – two extraction methods; FB = factor B – two sample pre-treatments; FC = factor C – three extraction solvents; FD = factor D – five evaluated foods; CV = coefficient of variation.

Table 2
Antioxidant capacity indifferent cereal agricultural and agroindustry wastes submitted to different extraction conditions.

Sample type (Factor D)	Solvent (Factor C)	Sample pre-treatment (Factor B)	FRAP*		ABTS*		DPPH	
			Extraction methods (Factor A)					
			Maceration	Microwave	Maceration	Microwave	Maceration	Microwave
BSG	Acetone	Integral	0.89 ± 0.04Akl	0.48 ± 0.02Bij	0.93 ± 0.04Aefg	0.06 ± 0.01Af	29.27 ± 0.69Bij	60.06 ± 0.70Ae
		Defatted	1.31 ± 0.04Aj	0.52 ± 0.03Bij	0.63 ± 0.05Afg	0.02 ± 0.00Af	34.50 ± 0.67Bgh	89.20 ± 0.99Aa
	Methanol	Integral	0.67 ± 0.02Almn	0.68 ± 0.01Ahi	0.56 ± 0.08Afg	0.49 ± 0.09Af	40.33 ± 0.91Bf	65.66 ± 0.82Ad
		Defatted	0.79 ± 0.02Aklmn	0.59 ± 0.02Aij	0.35 ± 0.04Ag	0.23 ± 0.04Af	42.98 ± 0.50Bf	73.60 ± 1.01Ac
	NaOH	Integral	5.99 ± 0.06Aa	4.88 ± 0.02Ba	4.61 ± 0.41Ba	7.17 ± 0.87Aa	7.03 ± 0.10Ap	10.49 ± 0.21Aq
		Defatted	5.33 ± 0.03Ab	4.35 ± 0.01Bb	4.80 ± 0.57Aa	5.59 ± 0.57Aabc	8.08 ± 0.14 Bp	13.76 ± 0.08Aq
Corn silage	Acetone	Integral	2.17 ± 0.14Ag	1.05 ± 0.07Bfg	1.79 ± 0.02Acdefg	0.44 ± 0.06Af	27.04 ± 0.86Bjkl	60.63 ± 0.91Ae
		Defatted	1.66 ± 0.11Ai	1.26 ± 0.04Bf	1.75 ± 0.10Acdefg	0.52 ± 0.04Af	30.67 ± 0.81Bhij	62.34 ± 0.82Ade
	Methanol	Integral	1.41 ± 0.02Aij	0.61 ± 0.04B	1.64 ± 0.30Adefg	1.43 ± 0.09Aef	28.00 ± 0.43Bjk	79.80 ± 0.60Ab
		Defatted	1.65 ± 0.03Ai	1.10 ± 0.02Bf	2.08 ± 0.21Acdef	0.95 ± 0.10Aef	39.67 ± 0.49Bfg	78.72 ± 0.88Ab
	NaOH	Integral	3.41 ± 0.08Bc	5.09 ± 0.31Aa	3.87 ± 0.69Bab	6.02 ± 0.19Aab	13.41 ± 0.09Bo	20.61 ± 0.21Ano
		Defatted	2.79 ± 0.06Bd	4.48 ± 0.09Ab	3.47 ± 0.40Babc	5.45 ± 0.33Aabc	16.55 ± 0.16Bno	23.96 ± 0.19An
Corn bran	Acetone	Integral	0.64 ± 0.01Amn	0.36 ± 0.01Bj	0.23 ± 0.05Ag	0.02 ± 0.00Af	47.35 ± 0.30Ae	35.22 ± 0.40Bkl
		Defatted	0.59 ± 0.02An	0.35 ± 0.03Bj	0.34 ± 0.07Ag	0.01 ± 0.00Af	50.57 ± 0.82Bc	55.25 ± 0.39Afg
	Methanol	Integral	0.15 ± 0.01Bo	0.42 ± 0.02Aj	0.36 ± 0.08Ag	0.27 ± 0.01Af	28.26 ± 0.31Bijk	36.69 ± 0.31Ajk
		Defatted	0.14 ± 0.00Bo	0.41 ± 0.06Aj	0.35 ± 0.09Ag	0.17 ± 0.07Af	36.46 ± 0.83Bg	43.09 ± 0.74Ai
	NaOH	Integral	1.19 ± 0.01Bj	2.30 ± 0.03Ae	3.14 ± 0.20Aabcd	3.32 ± 0.21Ad	42.69 ± 0.23Af	28.74 ± 0.27Bm
		Defatted	1.66 ± 0.02Bi	2.17 ± 0.03Ae	3.25 ± 0.19Aabcd	2.55 ± 0.18Ade	55.13 ± 0.51Aa	36.90 ± 0.42Bjk
Rice bran	Acetone	Integral	2.24 ± 0.04Afg	0.93 ± 0.04Bgh	2.23 ± 0.21Acdef	0.37 ± 0.00Bf	17.72 ± 0.83Bmn	49.52 ± 0.68Ah
		Defatted	2.03 ± 0.08Agh	1.03 ± 0.02Bfg	2.50 ± 0.26Abcde	0.94 ± 0.11Aef	20.59 ± 0.89Bm	58.84 ± 0.55Aef
	Methanol	Integral	1.45 ± 0.04Aij	1.00 ± 0.01Bg	1.99 ± 0.17Acdefg	0.93 ± 0.04Aef	18.45 ± 0.41Bmn	39.91 ± 0.95Aij
		Defatted	1.85 ± 0.02Ah	1.10 ± 0.04Bfg	1.80 ± 0.35Acdefg	1.04 ± 0.21Aef	24.62 ± 0.41Bkl	88.29 ± 1.02Aa
	NaOH	Integral	3.24 ± 0.05Bc	3.53 ± 0.27Ac	4.61 ± 0.58Aa	5.29 ± 0.52Abc	32.18 ± 0.58Ahi	19.11 ± 0.16Bo
		Defatted	2.71 ± 0.08Bde	3.01 ± 0.02Ad	4.27 ± 0.31Aab	4.97 ± 0.46Abcd	49.74 ± 0.74Ac	24.34 ± 0.14Bn
Wheat bran	Acetone	Integral	0.86 ± 0.01Aklm	0.54 ± 0.01Bij	0.71 ± 0.01Aefg	0.04 ± 0.00Af	49.13 ± 0.67Ac	23.28 ± 0.12Bn
		Defatted	0.94 ± 0.03Ak	0.51 ± 0.02Bij	0.69 ± 0.02Afg	0.07 ± 0.00Af	51.69 ± 0.43Aa	53.87 ± 0.96Ag
	Methanol	Integral	0.71 ± 0.01Aklmn	0.49 ± 0.00Aij	1.12 ± 0.11Aefg	0.53 ± 0.09Af	23.64 ± 0.55Bl	32.73 ± 0.79Al
		Defatted	0.72 ± 0.00Aklmn	0.51 ± 0.05Aij	0.81 ± 0.03Aefg	0.33 ± 0.09Af	45.16 ± 0.79Ae	41.94 ± 0.53Ai
	NaOH	Integral	2.65 ± 0.04Bde	2.95 ± 0.05Ad	4.07 ± 0.35Aab	4.95 ± 0.26Abcd	48.10 ± 0.37Ad	16.97 ± 0.21 Bp
		Defatted	2.48 ± 0.16Bef	2.81 ± 0.06Ad	4.33 ± 0.49Aa	4.17 ± 0.41Acd	51.16 ± 0.77Ab	17.82 ± 0.37 Bp

Probability		ANOVA results Independent variables		
		FRAP	ABTS	DPPH
	FA	<0.001	0.031	<0.001
	FB	<0.001	0.042	<0.001
	FA*FB	0.823	0.093	<0.001
	FC	<0.001	<0.001	<0.001
	FA*FC	<0.001	<0.001	<0.001
	FB*FC	<0.001	0.042	<0.001
	FA*FB*FC	<0.001	0.059	<0.001
	FD	<0.001	<0.001	<0.001
	FA*FD	<0.001	<0.001	<0.001
	FB*FD	<0.001	0.635	<0.001
	FA*FB*FD	<0.001	0.643	<0.001
	FC*FD	<0.001	<0.001	<0.001
	FA*FC*FD	<0.001	<0.001	<0.001
	FB*FC*FD	<0.001	0.992	<0.001
	FA*FB*FC*FD	<0.001	<0.001	<0.001
	CV (%)	4.23	5.69	2.88
Error Mean Square		0.05	0.27	1.27

Different uppercase letters in line indicate significant differences between means. Different lowercase letters in column indicate significant differences between means. The values were represented as mean plus standard deviation of analyzes in triplicate. Significant effect for the factors when $p < 0.05$.

* Antioxidant activity was expressed as mmol of Trolox antioxidant capacity/g of sample for FRAP and ABTS assays. The effective concentration for 50% of DPPH free radial scavenger capacity values was shown mg/ml of extract. BSG = brewer's spent grain; FA = factor A – two extraction methods; FB = factor B – two sample pre-treatments; FC = factor C – three extraction solvents; FD = factor D – five evaluated foods; CV = coefficient of variation.

50% methanol. Only rice bran has showed higher results for total polyphenols with acetone than with the alkaline aqueous solvent for extraction by maceration. But, extraction by maceration still showed higher levels of flavonoids when sample was extracted under the alkaline aqueous solution than with organic solvents. Nonetheless, maceration has not resulted in the highest quantification results for polyphenols in rice bran but, microwave has. Thus, in microwave assisted extraction the use of aqueous NaOH (7.5% v/v) solution has resulted in the highest values for quantification in this sample as this solvent was also effective in all other samples.

All these results did not allow us to have a conclusive answer about the best set of conditions for characterization or quantification of monomers since measurements by colorimetric assays do not provide enough information about structural changes in target compounds. Results only showed that final extract is more reactive when alkaline condition is applied during extraction. Thus, analysis by chromatographic tools should provide specific information

about extract composition and future application for each extraction procedure.

In general, statistical treatment of data has showed that lipid removal prior to extraction has negatively influenced the efficiency of extraction process for almost all samples ($p < 0.05$). Some exception has occurred for corn bran in which fat removal has resulted in higher values of total polyphenols for extraction with aqueous NaOH. However, for the total flavonoid content the answer is not the same, and similar results were obtained for integral and defatted samples. The increment in total polyphenols concentration could be due to concentration after the fat removal. Furthermore, pre-treatment of samples by exhaustive extraction with ethyl ether is a time consuming analytical step with hazardous chemicals. Hence, it should only be adopted as a standard extractive procedure if concentration of all target compounds is greater increased.

The effect of extraction methodology by maceration or by microwave was significant ($p < 0.05$) for polyphenols recovery and it has showed sample-specific behavior due to the interaction effects among factors (FA⁄FB⁄FC⁄FD, $p < 0.001$, Table 1). Thus, the extraction by maceration was more effective in obtaining total polyphenols and flavonoids from corn silage and for brewer's spent grain samples. However, for rice, corn, and wheat brans extraction by microwave showed the best results for total polyphenols content (Table 1). This behavior could be due to structural characteristics of each group of samples in which brans are reported as having similar structure with a lower content of lignin and a greater number of phenolic compounds linked by ester-bound than ether forms (Cao, Wang, Yang, & Jiang, 2015). Nonetheless, brewer's spent grain is predominantly composed of seed coatings, which is majorly composed of cell wall structures with high lignin content (McCarthy et al. 2013). Corn silage also has a high lignified cell wall structure with phenolic compounds majorly linked by ether-bounds than by ester linkages after processing in silo storage period (Cao, Jin, Yang, Li, & Jiang, 2016).

Thus, it could be assumed that microwave method was not able to promote sufficient molecular movement and rotation to over-come the barrier imposed by the phenolic compounds dimerization with the cell wall (Moreira et al., 2013; Ostrander et al.,1999). Extraction by maceration was performed for a longer time (24 h) than by microwave (15 min) with could have favored alkaline hydrolyses and matrix linked polyphenols release.

When phenolic compounds were extracted from brewer's spent grain using alkaline solvent, values of 19.5 and 16.2 mg GAE/g for clear type and dark type of malt were observed, respectively (Moreira et al., 2013). These values agree to the results found in our study for brewer's spent grain also under alkaline conditions (Table 1).

In a study with corn silage by Besle et al. (2010), the total phenolic content of samples subjected to extraction with ethanol was 6.5 mg GAE/g silage. These results were lower than ours considering the best conditions for extraction (for integral sample, alkaline solvent for extraction, and maceration extraction method, Table 1).

Results for rice bran obtained by maceration with 50% methanol, polyphenols and flavonoid concentration were in accordance with those previously recovered for water extraction condition at 70 °C and 2 h of stirring (3.52 mg GAE/g and 3.88 mg QE/g dry matter for total phenolics and flavonoids) (Wanyo et al., 2014). However, when using the microwave and alkaline aqueous (0.75% NaOH) condition, the total phenolic content was higher than previous reports (Table 1).

Results for wheat bran in alkaline extraction of integral samples (Table 1) recovered more than three times the levels achieved with hydro alcoholic solvents (methanol and acetone). However, Brewer et al. (2014) usinga protocol with 80% methanol as solvent followed by extract hydrolysis had obtained values of 7.36 mg GAE/ g and 0.21 mg QE/g for total polyphenols and flavonoids in wheat bran, respectively. These results are similar with those found in the present study for integral wheat bran samples that were extracted in alkaline aqueous solvent (0.75%) under microwave (Table 1). This could indicate that extract hydrolysis is occurring and increased levels determined by FCR method is majorly due to it. Thus, determination of extract composition by liquid chromatography could clearly indicate differences between acetone and aqueous sodium hydroxide as extraction solvents.

In a study with corn bran, extraction by alkaline hydrolysis followed by partition with ethyl acetate and diethyl ether (1:1, v/v), values of 5.8 mg GAE/g bran and 0.3 mg QE/g bran were found for total phenolics and flavonoids, respectively (Zilic et al., 2013). These results corroborate with results obtained for corn bran when using microwaved assisted alkaline extraction (Table 1).

Because of multiple reaction characteristics and mechanisms, a single antioxidant assay is not accurate to reflect the antioxidant capacity of a mixed or complex system (Denardin et al., 2015; Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2009). For thisreason, three different antioxidant assays were conducted to clarify different aspects of the antioxidant capacity of agricultural and agroindustry wastes and its extracts. The antioxidant capacity of all wastes was directly proportional to the total phenolic and flavonoid content, as reported in most studies of natural antioxidants (Huang, Ou, & Prior, 2005; Zhen et al., 2016).

The in vitro antioxidant activity evaluated by the FRAP and ABTS technique presented different interaction effects between the extraction factors. Overall, the ABTS values were higher than the FRAP values for all samples, both by the maceration and microwave extraction method. Whereas some of the interaction effects were not significant for results obtained by ABTS (FA⁄FB⁄FC, FB⁄FD, FA⁄FB⁄FD, FB⁄FC⁄FD; Table 2) and were significant for FRAP it could be assumed that this second method was more sensitive to identify differences in antioxidant capacity between samples.

In some possible future applications of these residues, direct use of the matrix could not be done and natural antioxidants in liquid extracts could be required. Thus, the measurement of antioxidant activity by DPPH was expressed in EC 50 to determine the characteristics of the obtained extract. As expected, EC 50 values showed inversely proportional to total phenolic and flavonoid concentrations. Since extracts obtained under alkaline conditions were more efficient as antioxidants by DPPH method, the study of this method of extraction is encouraged for the development of a final preparation as food additive.

3.2. Characterization of polyphenols by high-performance liquid chromatography coupled with diode array detection (HPLC-DAD)

Fig. 1 and Table 3 showsthe separation and the system suitability parameters obtained in the chromatographic method. Thus, the standardized conditions used in this work have resulted in a separation method for 18 monomeric polyphenols with the recommended selective quality (AOAC, 2012) for quantification. Regression analysis have resulted in calibration curves that are significant models without lack of fitness and with correlation coefficients greater than 0.99. These results demonstrated that the working range was linear and models had ability for predictions (ICH, 2006). Quantification limits obtained for each of the analytes had predicted RSD values that are higher than those experimentally obtained (HorRat value, Horwitz & Albert, 1995) which means that all compounds have acceptable repeatability and accuracy in parameters used for quantification (peak areas values) and also identification (retention times values).

Based on the results obtained for the determination of total phenolic compounds and antioxidant activity by colorimetry, only extracts obtained using 50% acetone and alkaline conditions with the highest recovery were selected for analysis by HPLC (Fig. 2).

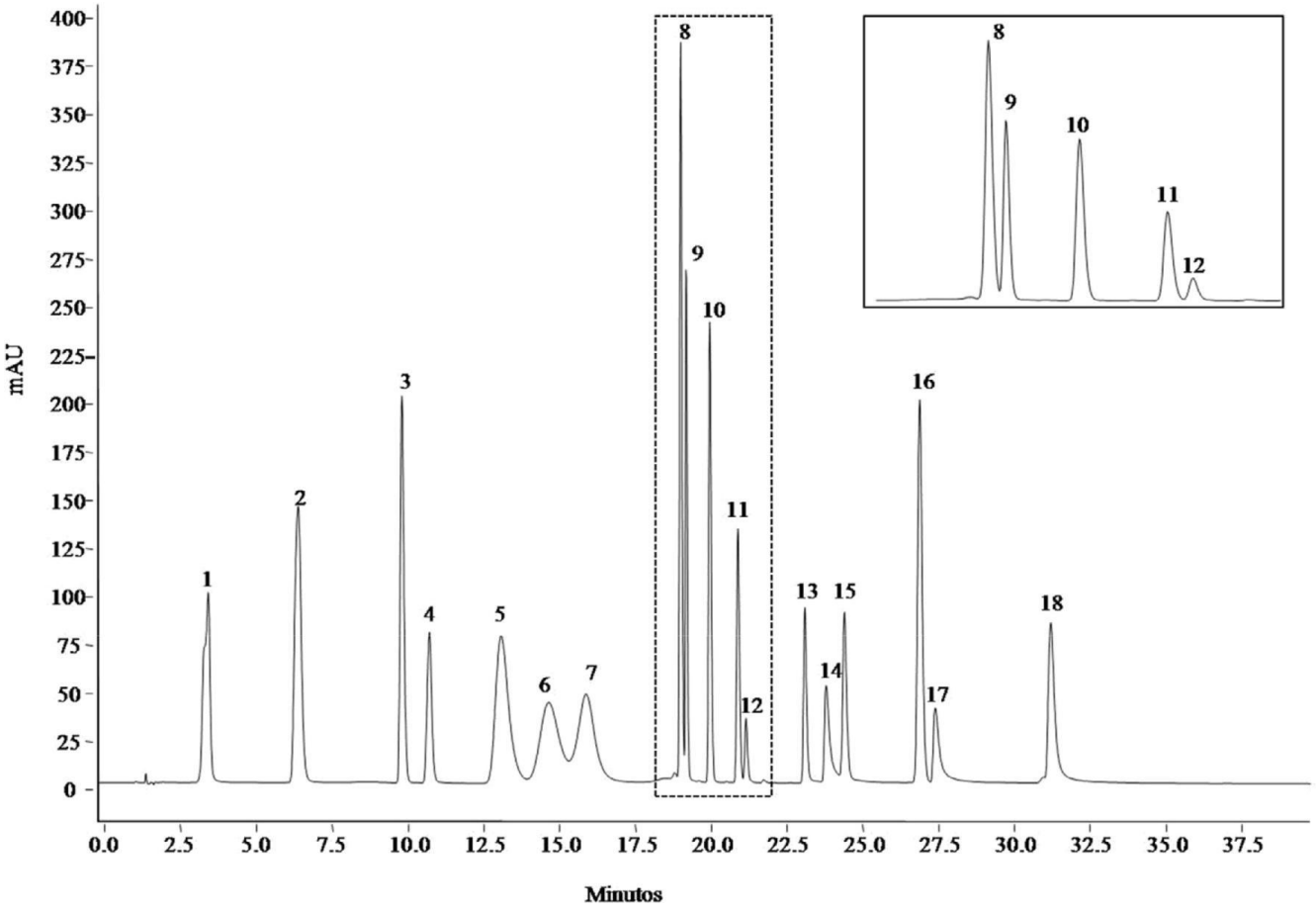

Fig. 1. HPLC-DAD chromatogram of calibration standards of phenolic compounds at 280 nm. (1) gallic acid, (2) protocatechuic acid, (3) 4-hydroxybenzoic acid, (4) catechin, (5) vanillic acid, (6) caffeic acid, (7) clorogenic acid, (8) syringic acid, (9) epicatechin, (10) p-coumaric acid, (11) trans-ferulic acid, (12) sinapic acid, (13) kaempferol-3DGlp, (14) myricetin, (15) resveratrol, (16) trans-cinnamic acid, (17) quercetin, (18) kaempferol.

Acetone is one of the two extraction procedures with organic solvents that had the highest results in relation to total measurements. Thus, it was chosen to determine the extension of chemical changes in polyphenols native forms due to hydrolyses when the alkaline aqueous solvent was used.

HPLC analysis revealed that the extraction method assisted by microwave using 50% acetone resulted in chromatograms with the separation of a complex mixture of phenolic compounds (Fig. 2). This was consistent with numerous studies in which plant phenolic compounds generally had occurred esterified with other phenolic acids, bonded to glycosides, or forming polymers in their native forms (Zhen et al., 2016). These results are a strong evidence that alkaline conditions have resulted in structural simplification and it could not be used for characterization purposes of native forms of polyphenols. In these kinds of studies, extractions with mixtures of organic solvent and water should be adopted and mass spectrometry may be necessary for correct identification.

However, the development of extraction methods and quantification by HPLC-DAD is a simple alternative to determine the total content of monomers that could be delivered from samples. This information does not provide a complete characterization, but it is also relevant in relation to nutritional intervention studies. Therefore, the present study also assessed the composition of the polyphenols after alkaline extraction to determine whether this condition could simplify the composition of the polyphenols for separation and quantification of monomers by HPLC.

The chromatograms in Fig. 2 shows the separation of the phenolic compounds obtained with 0.75% NaOH solvent after microwave-assisted extraction for rice bran, corn bran and wheat bran, and after maceration extraction for brewer's spent grain and corn silage. There was a decrease in the number of compounds present in the extract resulting in the expected simplification of the structures. Furthermore, there was only possible to detect and quantify monomers when using the extraction with alkali (Table 4). Thus, it is an evidence that extraction under alkaline conditions had hydrolyzed polyphenols during extraction providing a final extract that is directly able to determine monomers that could be delivered from sample.

Nevertheless, extraction using acetone represents a quantification of the native polymerized forms of polyphenols that could not have the same reactivity than monomers commonly used for quantification, as the galic acid. Thus, for the extraction using sodium hydroxide quantification by FCR assay is a measurement of total monomers that could be release from matrix and be quantified by equivalence with a compound that has similar chemical structure.

In all samples extracted by alkaline hydrolyses three phenolic acids derived from hydroxycinnamates (p-coumaric acid, trans-ferulic acid, sinapic acid) were detected and quantified (Table 4).

In the brewer's spent grain there was the following descending order in concentration: trans-ferulic acid, p-coumaric acid, and sinapic acid (Table 4). Barbosa-Pereira, Angulo, Paseiro-Losada, and Cruz (2013) determinedthe phenolic profile from a crudeextract of brewer's spent grain (on a pilot scale)

Table 3
System suitability and validation figures of the HPLC-DAD method for determination of plant phenolic compounds.

Phenolic compounds*	Channel	Retention time (min) (n = 10)	Peak Resolution (n = 10)	Asymmetry factor (n = 10)	Linear range (mg L^{-1})	Regression equation*	p-Value	R^2	LoD (mg L^{-1})	LoQ (mg L^{-1})	Repeatability Intra-day precision, CV (%) n = 10		Intermediate Inter-day precision, CV (%) n = 10	
											Rt	Peak area	Rt	Peak area
GA	280	4.27	N.A.	0.37	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Pro	280	7.23	7.99	0.37	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
4-HBA	280	10.26	9.88	0.23	0.1–80	$Y = 3\ 10^{7}.X\ 3430$	<0.001	0.9998	0.0016	0.0048	0.18	2.64	1.38	4.01
Cat	280	11.98	3.29	0.29	0.1–50	$Y = 2\ 10\ .X\ 2675.4$	<0.001	0.9996	0.0014	0.0042	0.15	2.59	1.01	4.64
Van	280	14.03	5.05	0.98	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Caf	320	15.86	2.02	0.16	0.1–80	$Y = 1\ 10^{8}.X\ 44141$	<0.001	0.9996	0.0021	0.0065	0.25	2.70	0.24	4.82
CA	320	16.61	1.13	0.17	0.1–80	$Y = 8\ 10\ .X\ 44259$	<0.001	0.9971	0.0056	0.0170	0.26	2.95	1.67	4.26
Syr	280	19.27	5.34	0.71	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Epic	280	19.42	1.07	0.85	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
p-Cou	320	20.25	5.58	0.16	0.1–30	$Y = 2\ 10^{8}.X +9424.3$	<0.001	0.9997	0.0006	0.0017	0.03	2.68	0.11	4.17
Trans-fer	320	21.16	5.27	0.20	0.5–50	$Y = 1\ 10^{7}.X +55619$	<0.001	0.9969	0.0033	0.0099	0.03	2.67	0.07	4.24
Sin	320	21.45	1.45	0.38	0.1–10	$Y = 6\ 10\ 1686.3$	<0.001	0.9998	0.0002	0.0006	0.03	2.70	0.05	4.19
Kae-3DGlp	360	23.30	10.46	0.24	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Myr	360	23.97	2.87	0.29	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Resv	305	24.61	2.40	0.00	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	NA	N.A.	N.A.
Trans-cin	280	27.08	9.58	0.14	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Quer	360	27.58	1.63	0.41	0.6–80	$Y = 9\ 10^{/}.X\ 212239$	<0.001	0.9953	0.0213	0.0645	0.02	3.05	0.08	3.14
Kae	360	31.39	11.02	0.42	0.6–80	$Y = 1\ 10^{8}.X\ 405191$	<0.001	0.9942	0.0155	0.0469	0.02	2.54	0.05	4.49

* (GA) gallic acid, (Pro) protocatechuic acid, (4-HBA) 4-hydroxybenzoic acid, (Cat) catechin, (Van) vanillic acid, (Caf) caffeic acid, (CA) clorogenic acid, (Syr) syringic acid, (Epic) epicatechin, (p-Cou) p-coumaric acid, (trans-fer) trans-ferulic acid, (Sin) sinapic acid, (Kae-3DGlp) kaempferol-3DGlp, (Myr) myricetin, (Resv) resveratrol, (trans-cin) trans-cinnamic acid, (Quer) quercetin, (Kae) kaempferol. N.A.: not applicable.

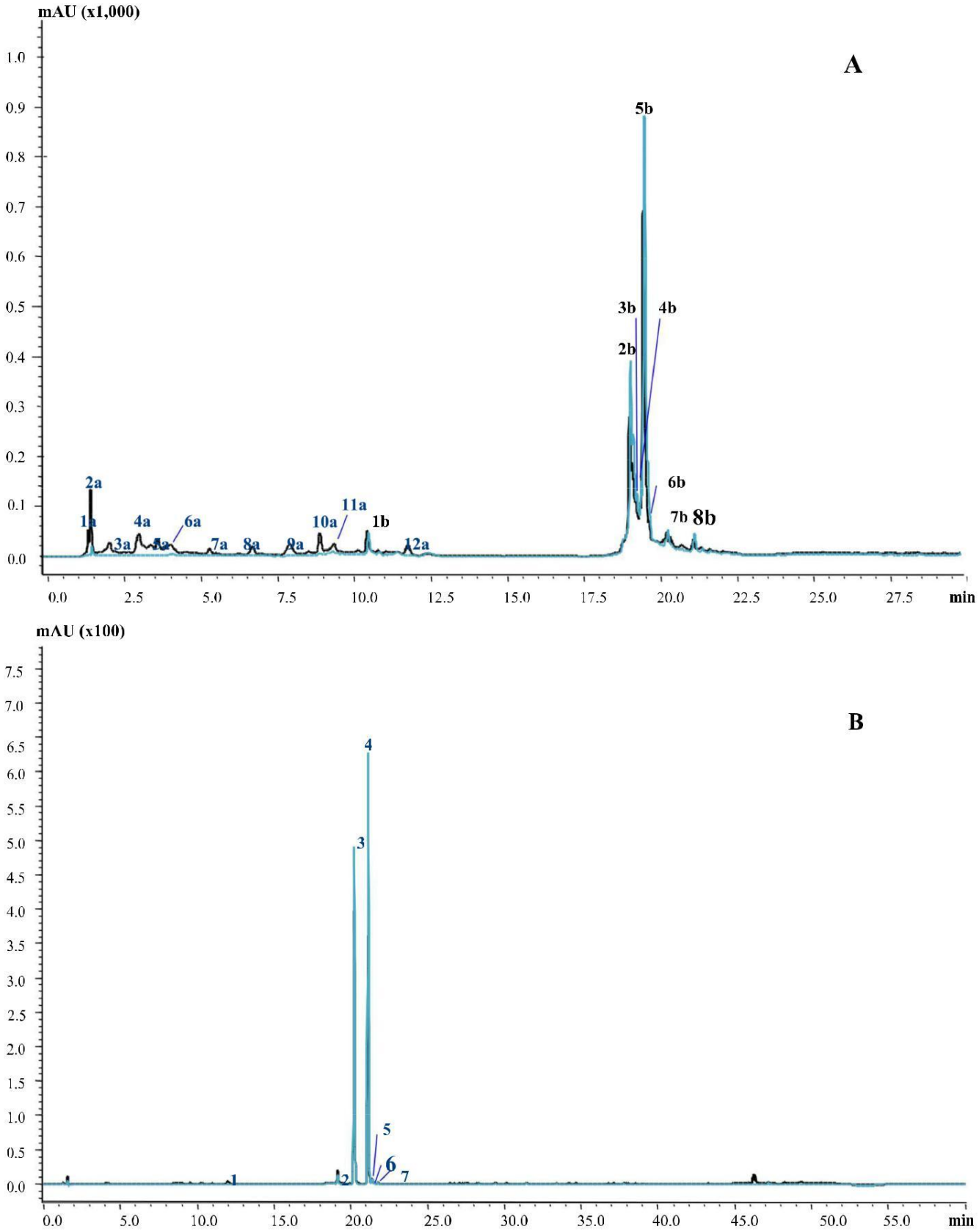

Fig. 2. HPLC-DAD chromatograms of phenolic compounds extracted using acetone and alkaline solvent from brewer's spent grain (A, B), corn silage (C, D), corn bran (E, F), rice bran (G, H), and wheat bran (I, J), respectively. Peak assignment is in agreement with Table 4. Black, blue, and red were used for chromatograms at 280, 320, and 360 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

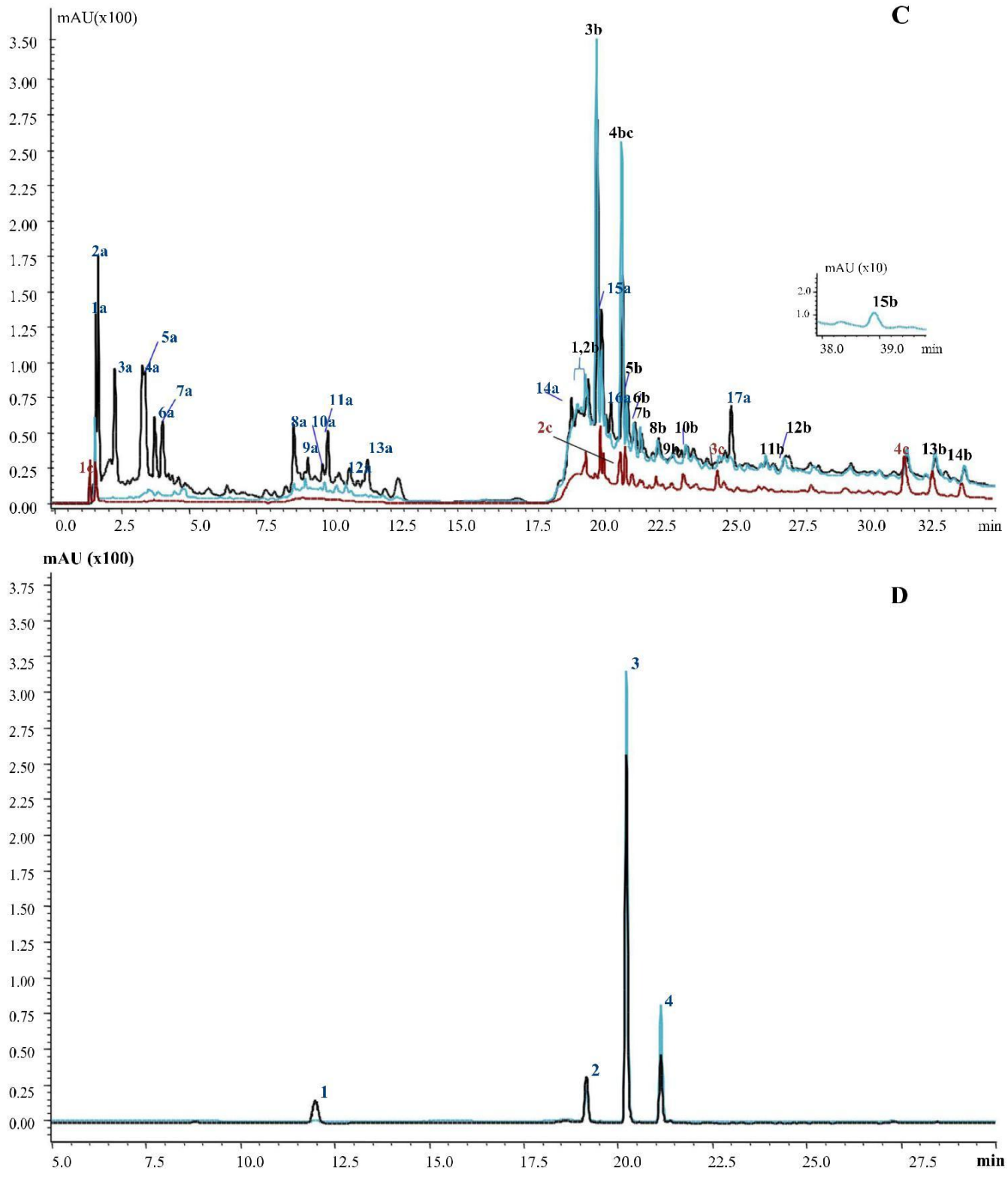

Fig. 2 (continued)

and obtained ferulic acid as the highest concentration (39.52 mg/g), followed by p-coumaric acid (11.41 mg/g), which is in agreement with our findings.

Corn silage is rich in phenolic compounds and 4-hydroxybenzoic acid, p-coumaric acid and ferulic acid are usually found (Besle et al., 2010) which was also found when acetone solvent was used for extraction (Table 4).

Cao et al. (2016) quantified ferulic and p-coumaric acid in corn silage samples in values of 9.0 and 11.1 g kg ^{1}of dry sample respectively. The p-coumaric acid content reported by these authors was also higher than the concentration of trans-ferulic acid in corn silage. Our results are in agreement with these findings and we also showed that the total content of p-coumaric

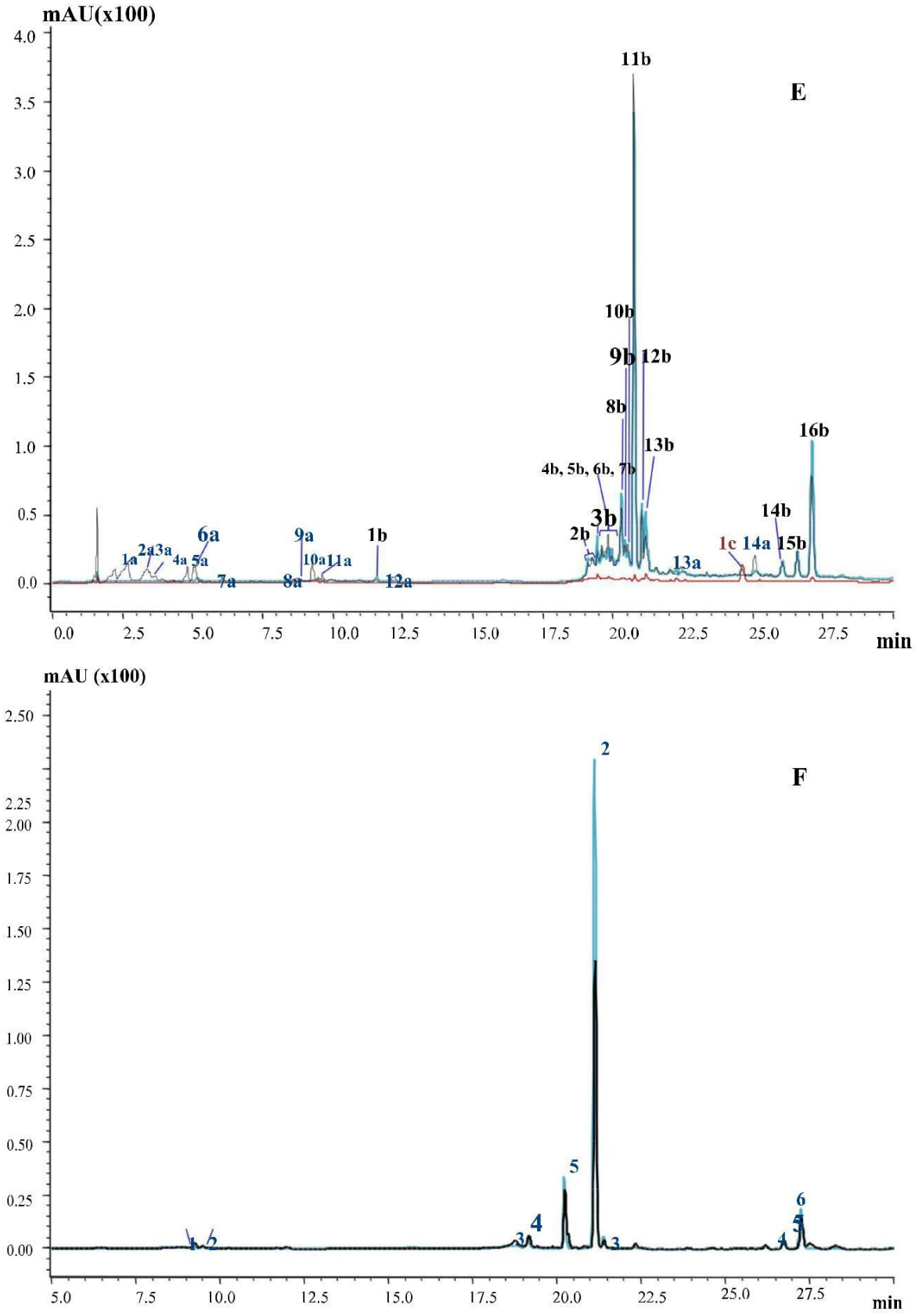

Fig. 2 (continued)

that could be realese from corn silage (extraction with alkaline solvent, Table 4) is ate a least four times greater the initial content in sample (extraction using acetone as solvent, Table 4).

Phenolic acids majorly found are the trans-ferulic acid followed by the p-coumaric acid (Buranov & Mazza, 2009). It agrees with results obtained for corn bran using the aqueous alkaline condition during extraction, which has

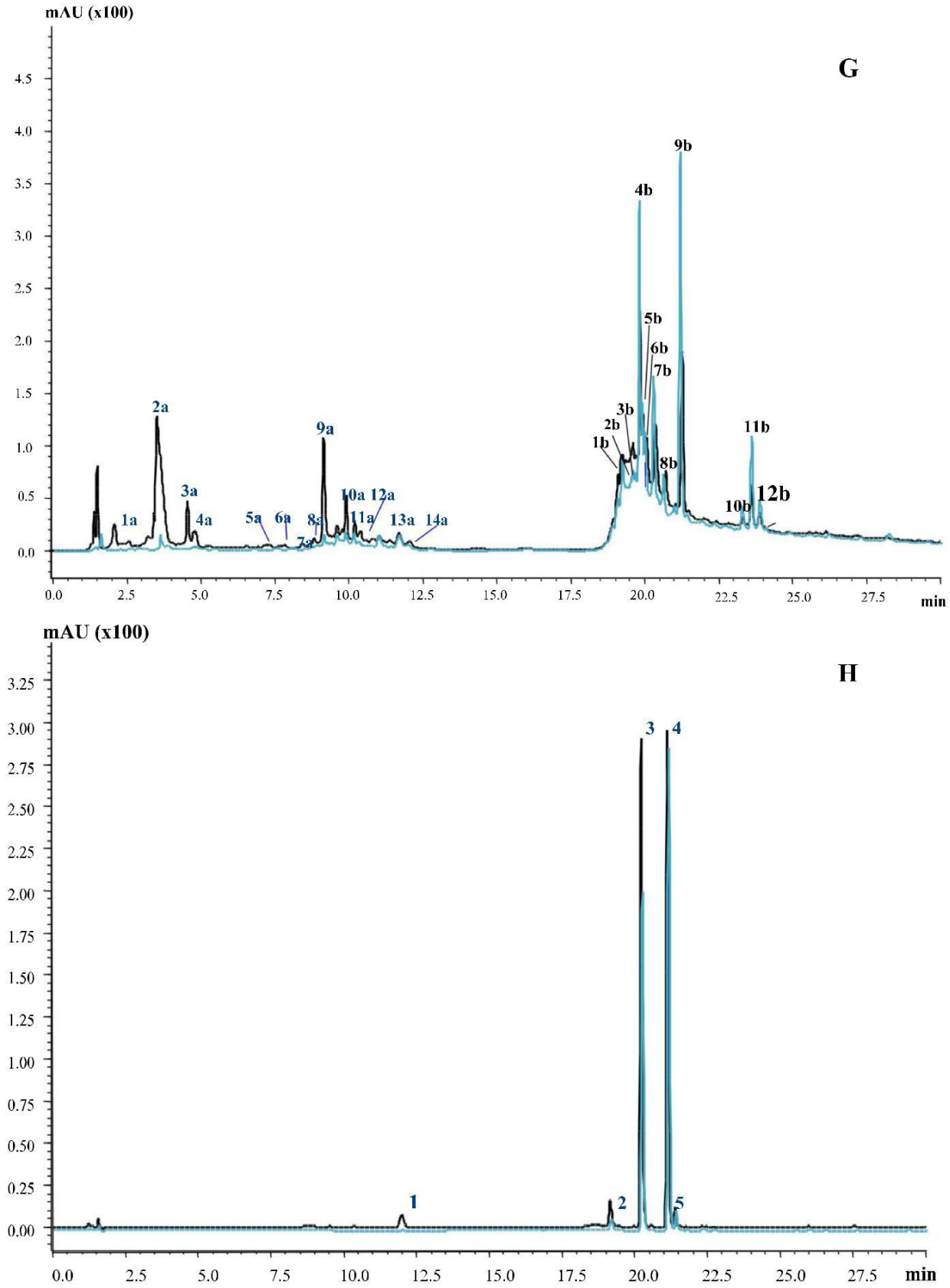

Fig. 2 (continued)

also resulted in higher quantification of ferulic acid than p-coumaric acid in this cereal waste (Table 4).

Rice bran as showed as grater content trans-ferulic acid p-coumaric acid in both extraction methods (Table 4). Jun, Song,Yang, Youn, and Kim (2012) evaluated the composition of phenolic acids in pigmented rice bran and also

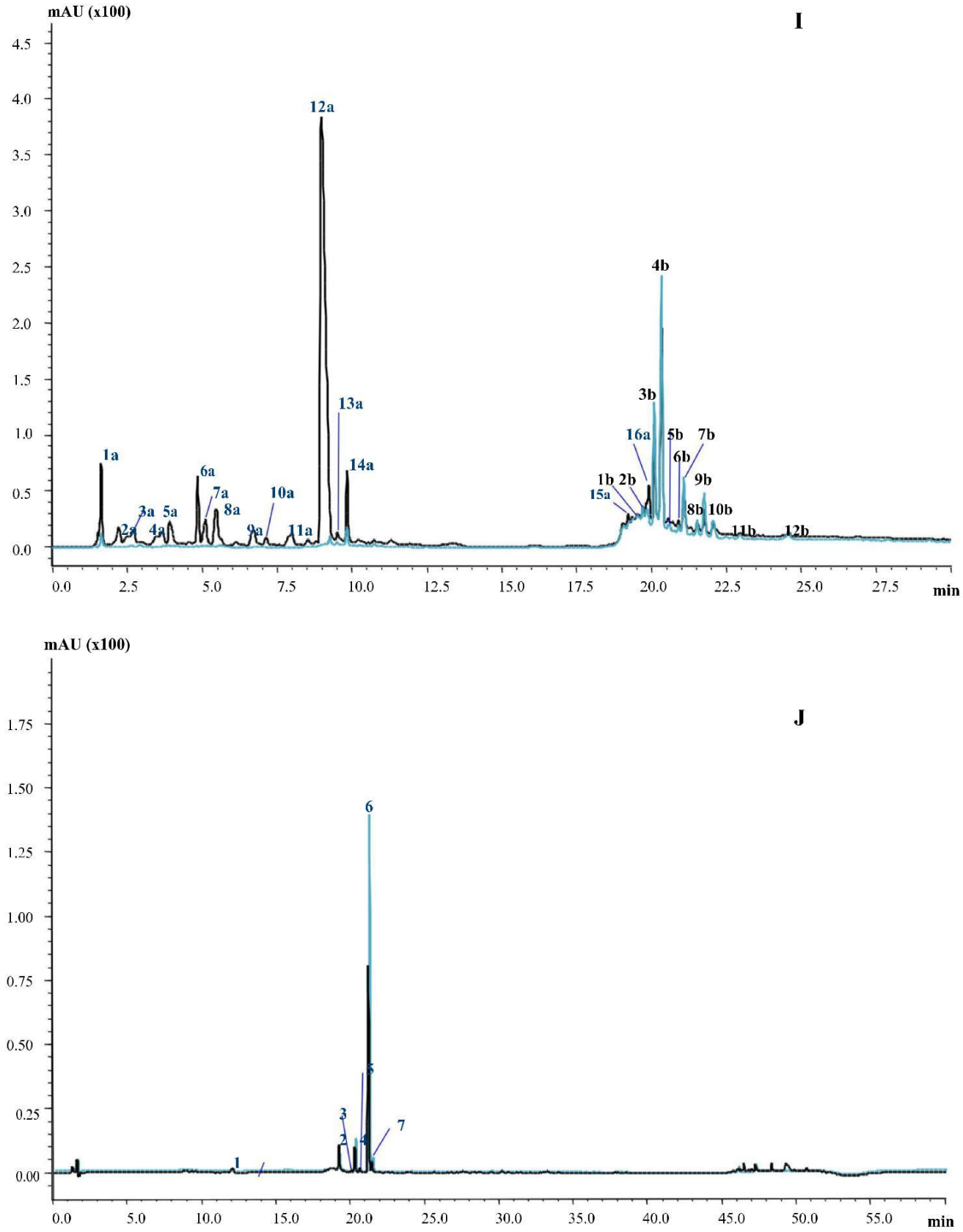

Fig. 2 (continued)

found that the most abundant acid was ferulic acid (57% of total phenolic acids). It seems interesting to highlight that the two major contributors for the antioxidant activity of wheat (UC66049 Triticum aestivum L.) was trans-ferulic acid, followed by p-coumaric acid (Tyl & Bunzel, 2012) which corroborates our results (Table 4).

Table 4
Spectral information. tentative identification. and quantification of plant phenolic compounds from cereal wastes obtained from agricultural and agroindustry practices.

Peak#	Channel	Rt	$k_{(max)}$	Tentative classification and identification	Relative percentage**	Concentration (mg/100 g)
Brewer's spent grain						
Acetone solvent for extraction						
1a	280 nm	1.5 ± 0.06	274	Hydroxybenzoate	5.9 ± 0.0	n.q.
2a	280 nm	1.6 ± 0.05	269	derivatives (HBD) or	17.5 ± 0.0	n.q.
3a	280 nm	2.3 ± 0.01	255	Tannins	4.1 ± 0.0	n.q.
4a	280 nm	3.3 ± 0.13	270		9.4 ± 1.2	n.q.
5a	280 nm	4.0 ± 0.31	265		4.2 ± 0.4	n.q.
6a	280 nm	4.3 ± 0.28	260 (280 sh)		4.7 ± 1.7	n.q.
7a	280 nm	5.5 ± 0.35	283		2.8 ± 0.2	n.q.
8a	280 nm	6.9 ± 0.15	257, 277		5.5 ± 0.1	n.q.
9a	280 nm	8.1 ± 0.09	274		10.3 ± 0.4	n.q.
10a	280 nm	9.1 ± 0.05	278		9.3 ± 1.3	n.q.
11a	280 nm	9.5 ± 0.06	277		4.3 ± 0.0	n.q.
12a	280 nm	11.7 ± 0.32	280	Catechin	8.0 ± 1.9	6.84 ± 1.40
13a	280 nm	46.2 ± 0.02	275	HBD or Tannins	14.0 ± 0.5	n.q.
1b	320 nm	10.4 ± 0.18	297	Hydroxycinnamate	2.1 ± 0.2	n.q.
2b	320 nm	19.1 ± 0.10	299	derivatives (HCD)	30.6 ± 0.9	n.q.
3b	320 nm	19.4 ± 0.08	301, 315		2.9 ± 1.7	n.q.
4b	320 nm	19.6 ± 0.05	319		2.6 ± 0.5	n.q.
5b	320 nm	19.7 ± 0.01	300		55.5 ± 2.5	n.q.
6b	320 nm	19.9 ± 0.05	299, 309*		1.4 ± 0.3	n.q.
7b	320 nm	20.4 ± 0.18	309	p-Coumaric Acid	2.8 ± 0.0	0.84 ± 0.21
8b	320 nm	21.3 ± 0.17	322 (290 sh)	t-Ferulic Acid	2.1 ± 0.0	0.56 ± 0.11
Alkaline solvent for extraction						
1	280 nm	12.1 ± 0.09	282	HBD or Tannins	32.9 ± 2.7	n.q.
2	280 nm	19.3 ± 0.10	279*, 308		67.1 ± 2.7	n.q.
3	320 nm	20.4 ± 0.10	309	p-Coumaric Acid	39.0 ± 1.6	103.17 ± 12.28
4	320 nm	21.3 ± 0.11	322 (290 sh)	t-Ferulic Acid	59.4 ± 1.4	207.68 ± 11.74
5	320 nm	21.6 ± 0.12	313	Sinapic Acid	0.9 ± 0.2	8.66 ± 1.02
6	320 nm	21.7 ± 0.12	321 (288 sh)	HCD	0.2 ± 0.1	n.q.
7	320 nm	22.0 ± 0.12	322 (295 sh)		0.5 ± 0.1	n.q.
Corn silage						
Acetone solvent for extraction						
1a	280 nm	1.5 ± 0.01	274	HBD or Tannins	7.6 ± 0.2	n.q.
2a	280 nm	1.6 ± 0.01	271		9.5 ± 0.5	n.q.
3a	280 nm	2.2 ± 0.01	260		10.1 ± 0.7	n.q.
4a	280 nm	3.3 ± 0.02	269		11.2 ± 1.1	n.q.
5a	280 nm	3.4 ± 0.02	267		8.6 ± 0.8	n.q.
6a	280 nm	3.8 ± 0.10	261		2.1 ± 2.2	n.q.
7a	280 nm	4.2 ± 0.15	264		7.0 ± 0.8	n.q.
8a	280 nm	9.1 ± 0.16	278		6.0 ± 0.3	n.q.
9a	280 nm	9.5 ± 0.09	280		1.5 ± 0.3	n.q.
10a	280 nm	10.1 ± 0.11	282		1.5 ± 0.1	n.q.
11a	280 nm	10.3 ± 0.13	255	4-Hydroxybenzoic Acid	5.2 ± 0.3	1.74 ± 0.06
12a	280 nm	11.3 ± 0.22	275	HBD or Tannins	2.0 ± 0.2	n.q.
13a	280 nm	12.0 ± 0.24	282	Catechin	5.0 ± 1.3	4.09 ± 0.15
14a	280 nm	19.2 ± 0.02	276	Epicatechin	4.8 ± 0.6	10.80 ± 0.003
15a	280 nm	20.3 ± 0.01	278	HBD or Tannins	9.8 ± 0.9	n.q.
16a	280 nm	20.7 ± 0.03	280		2.1 ± 0.2	n.q.
17a	280 nm	25.2 ± 0.01	278		6.0 ± 0.2	n.q.
1b	320 nm	19.7 ± 0.02	327	HCD	3.9 ± 1.1	n.q.
2b	320 nm	19.8 ± 0.01	269, 335		1.2 ± 0.9	n.q.
3b	320 nm	20.2 ± 0.02	309	p-Coumaric Acid	38.3 ± 3.3	6.38 ± 0.88
4b	320 nm	21.1 ± 0.00	322 (292 sh)	t-Ferulic Acid	29.2 ± 1.7	5.46 ± 0.53
5b	320 nm	21.3 ± 0.16	271, 337	HCD	3.4 ± 0.5	n.q.
6b	320 nm	21.6 ± 0.12	326		3.3 ± 0.2	n.q.
7b	320 nm	21.8 ± 0.05	323 (283 sh)		2.4 ± 1.1	n.q.
8b	320 nm	22.4 ± 0.02	272, 325		1.9 ± 0.5	n.q.
9b	320 nm	23.0 ± 0.02	272, 330		1.4 ± 0.1	n.q.
10b	320 nm	23.5 ± 0.00	313		2.8 ± 0.3	n.q.
11b	320 nm	26.5 ± 0.01	288, 328*		1.3 ± 0.1	n.q.
12b	320 nm	27.1 ± 0.04	298		2.3 ± 1.4	n.q.
13b	320 nm	32.9 ± 0.10	269, 340*		4.6 ± 0.6	n.q.
14b	320 nm	34.1 ± 0.11	270, 338*		2.6 ± 0.3	n.q.
15b	320 nm	39.1 ± 0.13	314		1.4 ± 0.1	n.q.
1c	360 nm	1.4 ± 0.01	363	Flavonol derivatives	10.1 ± 1.8	n.q.
2c	360 nm	20.4 ± 0.07	269, 351*		11.6 ± 0.5	n.q.
3c	360 nm	24.7 ± 0.02	358		20.5 ± 1.2	n.q.
4c	360 nm	31.8 ± 0.05	351		57.8 ± 1.1	n.q.

Table 4 (continued)

Peak#	Channel	Rt	$k_{(max)}$	Tentative classification and identification	Relative percentage**	Concentration (mg/100 g)
Alkaline solvent for extraction						
1	280 nm	12.0 ± 0.06	280	HBD or Tannins	45.4 ± 0.0	n.q.
2	280 nm	19.2 ± 0.02	270*, 309		54.6 ± 0.0	n.q.
3	320 nm	20.2 ± 0.01	309	p-Coumaric Acid	77.4 ± 0.0	43.0 ± 5.17
4	320 nm	21.1 ± 0.00	322 (290 sh)	t-Ferulic Acid	22.2 ± 0.0	14.9 ± 2.00
5	320 nm	21.4 ± 0.00	289	HCD	0.4 ± 0.0	n.q.
Corn bran						
Acetone solvent for extraction						
1a	280 nm	2.2 ± 0.01	262	HBD or Tannins	6.4 ± 1.4	n.q.
2a	280 nm	2.7 ± 0.01	284		12.8 ± 3.3	n.q.
3a	280 nm	3.3 ± 0.03	269		8.6 ± 2.7	n.q.
4a	280 nm	3.6 ± 0.06	261		2.4 ± 0.3	n.q.
5a	280 nm	3.7 ± 0.04	0		34.7 ± 1.2	n.q.
6a	280 nm	4.7 ± 0.13	257		5.1 ± 1.5	n.q.
7a	280 nm	5.0 ± 0.13	253		7.1 ± 1.8	n.q.
8a	280 nm	6.0 ± 0.11	284		1.6 ± 0.7	n.q.
9a	280 nm	8.5 ± 0.10	277		1.2 ± 0.2	n.q.
10a	280 nm	8.8 ± 0.08	271		0.7 ± 0.1	n.q.
11a	280 nm	9.2 ± 0.08	278		6.5 ± 1.8	n.q.
12a	280 nm	9.5 ± 0.07	268*, 318, 378		0.9 ± 0.3	n.q.
13a	280 nm	12.0 ± 0.13	248, 291		3.0 ± 0.9	n.q.
14a	280 nm	22.5 ± 0.04	274		1.3 ± 2.5	n.q.
15a	280 nm	25.1 ± 0.03	288		7.7 ± 6.0	n.q.
1b	320 nm	11.4 ± 0.12	310 (295 sh)	HCD	0.4 ± 0.0	n.q.
2b	320 nm	19.2 ± 0.06	285 (309 sh)		2.1 ± 0.4	n.q.
3b	320 nm	19.4 ± 0.05	325 (295 sh)		1.8 ± 0.1	n.q.
4b	320 nm	19.5 ± 0.04	290		1.2 ± 0.1	n.q.
5b	320 nm	19.6 ± 0.05	282, 311		1.1 ± 0.0	n.q.
6b	320 nm	19.8 ± 0.04	269, 307		1.6 ± 0.0	n.q.
7b	320 nm	19.9 ± 0.04	316		1.4 ± 0.1	n.q.
8b	320 nm	20.2 ± 0.04	309	p-Coumaric Acid	6.5 ± 0.1	0.88 ± 0.03
9b	320 nm	20.4 ± 0.03	311	HCD	1.6 ± 0.0	n.q.
10b	320 nm	20.5 ± 0.01	291		1.3 ± 0.2	n.q.
11b	320 nm	20.8 ± 0.02	297		45.6 ± 0.3	n.q.
12b	320 nm	21.1 ± 0.05	309		11.1 ± 0.2	n.q.
13b	320 nm	21.3 ± 0.11	321 (295 sh)	t-Ferulic Acid	1.2 ± 0.3	n.q.
14b	320 nm	26.2 ± 0.08	293, 309	HCD	2.1 ± 0.2	n.q.
15b	320 nm	26.7 ± 0.09	292, 308		3.2 ± 0.1	n.q.
16b	320 nm	27.3 ± 0.09	316 (293 sh)		17.8 ± 0.5	n.q.
1c	360 nm	24.7 ± 0.07	359	Flavonol derivatives	100.0 ± 48.2	n.q.
Alkaline solvent for extraction						
1	280 nm	9.3 ± 0.02	256	HBD or Tannins	13.4 ± 3.2	n.q.
2	280 nm	9.6 ± 0.05	280 (310 sh)		3.9 ± 1.2	n.q.
3	280 nm	18.8 ± 0.01	258		20.1 ± 7.0	n.q.
4	280 nm	19.3 ± 0.17	278*, 309		19.8 ± 3.9	n.q.
11	280 nm	27.5 ± 0.00	257		16.5 ± 3.7	n.q.
12	280 nm	28.3 ± 0.02	252		26.3 ± 7.9	n.q.
5	320 nm	20.2 ± 0.00	309	p-Coumaric Acid	9.6 ± 0.5	0.33 ± 0.16
6	320 nm	21.1 ± 0.00	322 (290)	t-Ferulic Acid	64.4 ± 7.3	3.0 ± 1.42
7	320 nm	21.4 ± 0.00	316	Sinapic Acid	1.9 ± 0.3	0.31 ± 0.05
8	320 nm	26.2 ± 0.00	310 (290 sh)	HCD	2.2 ± 0.8	n.q.
9	320 nm	26.7 ± 0.00	293, 308		2.3 ± 1.0	n.q.
10	320 nm	27.3 ± 0.00	316 (294 sh)		19.6 ± 6.9	n.q.
Rice bran						
Acetone solvent for extraction						
1a	280 nm	2.2 ± 0.01	263	HBD or Tannins	5.2 ± 0.3	n.q.
2a	280 nm	3.6 ± 0.01	265		47.4 ± 3.0	n.q.
3a	280 nm	4.6 ± 0.10	257		7.0 ± 0.4	n.q.
4a	280 nm	4.9 ± 0.10	252		4.2 ± 0.2	n.q.
5a	280 nm	7.4 ± 0.11	261*, 313		1.5 ± 0.2	n.q.
6a	280 nm	7.9 ± 0.13	264*, 308		1.3 ± 0.1	n.q.
7a	280 nm	8.5 ± 0.06	281		0.6 ± 0.1	n.q.
8a	280 nm	8.8 ± 0.06	292 (263 sh)		0.7 ± 0.0	n.q.
9a	280 nm	9.1 ± 0.07	268		16.9 ± 1.5	n.q.
10a	280 nm	9.8 ± 0.08	269* (297)		6.5 ± 0.4	n.q.
11a	280 nm	10.1 ± 0.09	263*, 310		2.7 ± 0.2	n.q.
12a	280 nm	10.3 ± 0.10	257 (300 sh)		1.9 ± 0.2	n.q.
13a	280 nm	11.6 ± 0.10	280*, 327		3.0 ± 0.2	n.q.
14a	280 nm	12.0 ± 0.09	278 (319 sh)		1.1 ± 0.2	n.q.
1b	320 nm	19.2 ± 0.07	318	HCD	3.1 ± 0.3	n.q.

(continued on next page)

Table 4 (continued)

Peak#	Channel	Rt	$k_{(max)}$	Tentative classification and identification	Relative percentage**	Concentration (mg/100 g)
2b	320 nm	19.5 ± 0.07	305		1.9 ± 0.2	n.q.
3b	320 nm	19.6 ± 0.07	318		1.3 ± 0.0	n.q.
4b	320 nm	19.8 ± 0.07	325		21.2 ± 1.6	n.q.
5b	320 nm	19.8 ± 0.08	325		6.5 ± 1.1	n.q.
6b	320 nm	19.9 ± 0.09	326		4.8 ± 0.1	n.q.
7b	320 nm	20.2 ± 0.10	309	p-Coumaric Acid	9.8 ± 1.3	1.40 ± 0.95
8b	320 nm	20.6 ± 0.10	337	HCD	3.3 ± 0.2	n.q.
9b	320 nm	21.14 ± 0.10	322	t-Ferulic Acid	31.2 ± 3.2	9.0 ± 5.8
10b	320 nm	23.2 ± 0.06	331	HCD	3.0 ± 0.4	n.q.
11b	320 nm	23.6 ± 0.05	329		10.5 ± 0.6	n.q.
12b	320 nm	23.8 ± 0.04	327		3.4 ± 0.2	n.q.
Acetone solvent for extraction						
1	280 nm	12.0 ± 0.019	280	HBD or Tannins	39.4 ± 0.9	n.q.
2	280 nm	19.2 ± 0.005	279, 308		60.6 ± 2.6	n.q.
3	320 nm	20.2 ± 0.001	309	p-Coumaric Acid	7.9 ± 1.7	17.9 ± 5.83
4	320 nm	21.1 ± 0.002	322 (290 sh)	t-Ferulic Acid	43.4 ± 6.1	34.5 ± 6.44
5	320 nm	21.4 ± 0.020	318	Sinapic acid	48.7 ± 0.5	3.1 ± 0.59
Wheat bran						
Acetone solvent for extraction						
1a	280 nm	1.6 ± 0.01	271	HBD or Tannins	3.7 ± 0.0	n.q.
2a	280 nm	2.2 ± 0.02	263		1.7 ± 0.0	n.q.
3a	280 nm	2.7 ± 0.01	283		2.3 ± 0.0	n.q.
4a	280 nm	3.6 ± 0.03	261		2.5 ± 0.1	n.q.
5a	280 nm	3.8 ± 0.04	265		2.7 ± 0.2	n.q.
6a	280 nm	4.7 ± 0.07	257		3.5 ± 0.0	n.q.
7a	280 nm	5.0 ± 0.07	254		2.4 ± 0.0	n.q.
8a	280 nm	5.3 ± 0.07	283		3.2 ± 0.0	n.q.
9a	280 nm	6.7 ± 0.03	282		2.0 ± 0.0	n.q.
10a	280 nm	7.1 ± 0.02	263		0.7 ± 0.0	n.q.
11a	280 nm	8.2 ± 0.13	283		1.9 ± 0.0	n.q.
12a	280 nm	9.0 ± 0.01	278		67.4 ± 0.4	n.q.
13a	280 nm	9.5 ± 0.00	278		0.5 ± 0.0	n.q.
14a	280 nm	9.9 ± 0.02	280		4.1 ± 0.0	n.q.
15a	280 nm	18.9 ± 0.09	263*, 291		0.3 ± 0.3	n.q.
16a	280 nm	19.2 ± 0.02	278*, 309		1.1 ± 0.4	n.q.
1b	320 nm	19.7 ± 0.03	270, 339*	HCD	2.2 ± 0.1	n.q.
2b	320 nm	19.8 ± 0.02	345		3.4 ± 0.1	n.q.
3b	320 nm	20.1 ± 0.02	271, 337*		19.8 ± 0.1	n.q.
4b	320 nm	20.3 ± 0.00	271, 337*		43.9 ± 0.3	0.99 ± 0.05
5b	320 nm	20.6 ± 0.01	271, 348*		1.2 ± 0.0	n.q.
6b	320 nm	21.0 ± 0.03	282, 327*		1.2 ± 0.0	n.q.
7b	320 nm	21.1 ± 0.04	322 (293 sh)	t-Ferulic acid	11.2 ± 0.1	n.q.
8b	320 nm	21.7 ± 0.09	272, 332*	HCD	2.4 ± 0.1	n.q.
9b	320 nm	21.9 ± 0.10	271, 330*		7.5 ± 0.6	n.q.
10b	320 nm	22.3 ± 0.12	271, 330*		3.8 ± 0.4	n.q.
11b	320 nm	23.2 ± 0.15	349		0.6 ± 0.0	n.q.
12b	320 nm	24.8 ± 0.13	326 (297 sh)		1.7 ± 0.5	n.q.
13b	320 nm	28.2 ± 0.00	320 (294 sh)		1.1 ± 0.1	n.q.
Alkaline solvent for extraction						
1	280 nm	12.0 ± 0.00	280	HBD or Tannins	18.3 ± 1.1	n.q.
2	280 nm	19.2 ± 0.00	279 (290 sh)		65.4 ± 8.2	n.q.
3	280 nm	20.0 ± 0.00	262		4.7 ± 0.6	n.q.
4	280 nm	20.6 ± 0.00	275*, 289		11.6 ± 2.5	n.q.
5	320 nm	20.2 ± 0.00	308	p-Coumaric Acid	8.0 ± 1.4	0.30 ± 0.24
6	320 nm	21.1 ± 0.00	322 (290 sh)	t-Ferulic Acid	89.3 ± 1.6	7.06 ± 4.82
7	320 nm	21.4 ± 0.00	318	Sinapic acid	2.7 ± 0.8	0.43 ± 0.46

Results are mean ± standard deviation (n = 3) and expressed in mg/100 g sample. Rt: retention time; n.q.: non quantified. sh: shoulder.

* Indicates absorption band with the greatest intensity.

** The relative percentage was determined for each chromatogram in separate for 280 nm, 320 nm, and 360 nm.

4. Conclusion

Microwave was not the best extraction procedure for all samples being efficient only for bran samples. Thus, for brewers' spent grain and the corn silage, maceration for long times have resulted in higher quantification results. It emphasizes, the need to study carefully the answer of each sample matrix to extraction conditions.

HPLC analysis has clearly showed that the native forms of these compounds were changed when extraction is performed in alkaline conditions. Thus, this method is recommended only for quantification of total monomers that could be obtained from matrix. It is a valuable information for nutritional intervention studies. Moreover, extracts with high amount of these monomer showed increased antioxidant activity, which could make this procedure interesting for pharmaceutical purposes and in the development of future applications. Extraction using the mixture of acetone and water at 50% (v/v) was the best solvent for characterization studies aiming to determine the native phenolic compounds profile.

Finally, the comparison between brewers' spent grain and all other analyzed samples that are commonly used for ruminant's animal feed had re-

vealed that it has the highest concentration of identified polyphenols. Thus, it is a promising source of antioxidant for nutritional intervention studies. Future scientific researches should be realized for a complete characterization and to determine how diet management by brewers' spent grain inclusion could affect animal performance and the meat and milk quality.

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3.2 MANUSCRITO 2

BREWER'S SPENT GRAIN IN REPLACEMENT OF CORN SILAGE AT THE CATTLE FINISHING: NUTRITIONAL, PRODUCTIVE, ECONOMIC EFFECTS AND MEAT QUALITY

Artigo em fase final de revisão pelos autores para ser submetido à revista

Animal Feed Science and Technology

(configurado conforme as normas da revista)

Brewer's spent grain in replacement of corn silage at the cattle finishing: nutritional, productive, economic effects and meat quality

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Abstract

Brewer's spent grain (BSG) is the most abundant by-product of the brewing industry, with a nutrient composition that is compatible with ruminant feeding. Moreover, it presents high values of polyunsaturated fatty acids and it is a valuable source of phenolic compounds with high antioxidant potential. Thus, the objective was to evaluate the effect of the usage of BSG in the finishing of feedlot steers as a partial replacement of corn silage (CS) on intake, digestibility, performance, carcass characteristics, production costs and in meat quality. Twenty male Angus cattle from the same herd and born at the same time of calving, with a mean age of 16 months old and 280 ± 20 kg of body weight, were housed in individual pens with 25 m^2 of area, equipped with individual feeders and drinking troughs. After 20 days, the animals were submitted to levels of BSG (0, 15, 25 and 35% on dry basis) to replace CS, in diets with corn grain, wheat bran and rice bran concentrate and mineral-vitamin premix in a 50:50 ratio, balanced for similar levels of protein and energy, for 90 days (three first periods of 21 days and the last period of 27 days) in a completely randomized design with five repetitions per treatment. The nutrient intake was calculated by the difference between the supply and the leftovers, while fecal production was estimated using chromium III (Cr_2O_3) as an external indicator. The digestibility of the diets was determined by the difference between the consumption and the fecal excretion of nutrients. The cattle were weighed during the experimental and slaughter periods, when the body weight, finishing and conformation of the carcasses were evaluated. After cooling, the carcasses were again weighed for yield evaluation and pH and temperature were measured. In the *Longissimus thoracis* muscle, analyzes of the centesimal composition, cholesterol, oxidative stability and meat fatty acid profile were performed. The results showed that the BSG in substitution of CS up to 35% in the finishing diet of beef cattle in feedlot allows excellent productive performance, without affecting the weight gain and carcass yield of the animals, but with better feeding conversion and reduction in the production costs, together with a final product with a higher amount of fatty acids beneficial to the health of the consumers in the intramuscular lipids, without altering the levels of fat and cholesterol and with greater oxidative stability.

Key-words: agroindustrial byproduct; consumption; digestibility; oxidative stability; phenolic acids; production costs

1. Introduction

The whole plant corn silage is often used as a source of energy and forage in diets of beef cattle in feedlot system (Klopfenstein et al., 2013; Vasconcelos and Galyean, 2007). This system is numerically low in Brazil. Since the great majority of cattle are raised and finished on pastures. Thus, this extensive Brazilian breeding system is typical. However, the exponential growth of feedlots in the country in the last decade it has become apparent (Kamali et al., 2016; Millen et al., 2009).

Nowadays, corn silage is one of the main forage sources used globally. It produces excellent gains in the growth and finishing of beef cattle in a feedlot system (Klopfenstein et al., 2013; Freitas et al., 2014; Weber et al., 2011). However, this system is subject to variations in feed sources due to the high price of grains, protein sources and forage availability. In this way, new unconventional alternatives tend to be exploited (Campos et al., 2014; Correia et al., 2016; Eliyahu et al., 2015). In addition, there is currently increasing pressure to reuse industrial by-products in order to reduce environmental pollution (Waters et al., 2012) and global policies have encouraged the use of low-cost resources in animal feeding (Anandan et al., 2012; Santana Filho et al., 2016). According to its composition, there are several industrial by-products that can be used as alternative sources of feeding and are able to maintain or improve the nutrition and performance of ruminants (Eliyahu et al., 2015). Also resulting in final products with high quality, meat (Castro et al., 2016) and milk (Neto et al., 2015).

The brewing industry generates an industrial by-product with low commercial value. Brewer’s spent grain (BSG) is characterized by solid fraction of barley malt that remains after the production of wort (Steiner et al., 2015; Waters et al., 2012). This by-product is the most abundant, representing about 85% of the waste generated in the whole process (Tang et al., 2009), with an annual production of around 38.6 million tons by the world's breweries (Mussatto, 2014). Considering that about 20 kg of BSG per 100 L of beer are produced

(McCarthy et al, 2013), the estimated availability of this residue in Brazil exceeds 2.8 million tons per year, since it is produced around 14 billion liters of beer annually in the country (CERVBRASIL, 2015).

Typical BSG compositions change but always include high levels of fiber (39.9 to 63.7% dry matter - DM) and protein (14.2 to 26.7% DM), as well as appreciable levels of lipids (3.9 to 13.3% DM) and minerals (11.6 to 15.5 g/kg DM) (McCarthy et al., 2013; Mussatto, 2014). Among lipids, a remarkable amount of fatty acids with beneficial properties is identified, such as linoleic acid. In addition to the mentioned nutrients, it is important to note that BSG contains a considerable amount of phenolic compounds (284.20 to 291.47 mg of gallic acid equivalent per 100 g DM) with high antioxidant potential (Fãrcas et al., 2015; Mussatto, 2014). Among these compounds, phenolic acids, particularly hydroxycinnamic acids, as ferulic acid and p-coumaric acid are present in high concentrations in this residue (McCarthy et al., 2013; Stefanello et al., 2018). In cattle production systems in wich high-quality products (milk or meat) are required, the use of diets rich in certain fatty acids and antioxidants that are capable not only of improving the health of animals, but also adding value to the final product and consumer health benefits are desirable (Castillo et al., 2013).

On the other hand, phenolic acids in the diet of ruminants, especially ferulic acid and p-coumaric acid, are receiving increasing attention because of their association with lignification of the plant cell wall (Cao et al., 2015). Although the negative effects of the relation between lignin and forage digestibility have been well characterized over the last few years (Wang et al., 2013), the available knowledge on the digestion and microbial metabolism of these phenolic acids in the digestive tract of ruminants is limited (Cao et al., 2016a).

In this context, the objective was to evaluate the potential use of BSG as a source of nutrients and bioactive compounds in the finishing of feedlot steers, in partial replacement of corn silage, through consumption, digestibility, productive performance, carcass

characteristics, production costs and meat quality produced.

2. Material and Methods

2.1 Animals and diets

The work was carried out in accordance with the Ethics Committee of Federal University of Santa Maria, Brazil, registered under protocol 096/2014. Twenty Angus breed castrated males, with a mean age of 16 months old and 280 ± 20 kg of live weight were used. Animals were from the same herd, born at the same time of calving, and randomly distributed in four homogeneous groups housed in individual pens with total area of 25 m^2. They had free access to water and feeding.

The cattle were fed twice a day (8h00 and 16h00) with rice bran, wheat bran, ground corn grain meal and mineral-vitamin supplement and. The BSG was used instead of CS as forage, in different levels: zero, 15, 25, and 35% on the dry matter basis of the diets. CS was stored in nylon bags, while BSG was stored in a surface silo. All BSG used in this study was obtained from pilsen malt. This type of malt is the most produced in Brazil and originates from germinated barley grains and later dried at a maximum temperature of 85 ºC, in a characteristic process for the acquisition of color and aroma of the pilsen type.

The ingredients of the diets were chemically analyzed for the centesimal composition and characterized as alternative sources of bioactive compounds through fatty acid profile and phenolic compounds (Table 1). Table 2 shows the participation of the ingredients and the estimated chemical composition of the experimental diets. The methodologies used in the chemical analyzes are described in item 2.2. The diets were balanced to be similar in protein and energy as evaluated by the Cornell Net Carbohydrate and Protein System software (CNCPS, 2005).

2.2 Intake and digestibility of experimental diets

The experimental period was 110 days, with 20 days of adaptation of the animals to

the management, experimental facilities and diets. During the experimental period, food was supplied daily in the form of a complete mixture, and offered twice a day (morning and afternoon), with refuse of approximately 15% of the quantity offered. In this sense, the foods and refusal samples were weighed daily for each animal.

The diets offered and the refusal samples were separated daily by animal and experimental period, packed in plastic bags, identified and stored in a freezer at -10° C. Subsequently, these samples were partially dried in an oven with forced air circulation (55° C) to constant weight (about 72 hours), ground and stored in sealed containers for further chemical analysis. In this way, the nutrients intake was calculated by the difference between the offered and the refusal.

Fecal production was estimated using chromium oxide III (Cr_2O_3) as an external indicator. During 15 days, during the last experimental period, hard gelatin capsules containing 1000 mg Cr_2O_3 each were given daily to each animal orally (five capsules in the morning and another five in the afternoon, totaling 10 g/ animal/ day of Cr_2O_3). In the last five days of the period, fecal samples were collected, approximately 400 g per animal, twice a day, after each feeding.

Fecal samples were stored frozen (-20° C), then subjected to partial drying in a forced air oven at 55° C until constant weight (about 72 hours), ground in a sieve with holes of 1mm diameter (Wiley mill) and stored for further analysis. For determination of chromium concentration, the fecal samples were composed by animal/ day. The chromium (Cr) content of feces was determined by atomic absorption spectrophotometry, after heating in muffle (550° C) and acid-perchloric solubilization (Czarnocki et al., 1961).

In the fecal, foods and refusal samples, dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) were analyzed according to AOAC (1995) (methods 967.03, 942.05, 954.05 and 920.39, respectively). Determinations of acid detergent fiber

(ADFa), in neutral detergent fiber corrected for ash and protein (NDFap) and acid detergent lignin (ADL) were performed according to Van Soest et al. (1991). The non-fibrous carbohydrate (NFC) contents were calculated by the equation proposed by Hall (2003). Dry matter digestibility (DMD) and its main components (organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber corrected for ash and protein (NDFap) and non-fibrous carbohydrates NFC)) of the experimental diets were determined by the difference between intake and fecal excretion. The fecal production was estimated, in the different treatments, through the fecal chromium (Cr) concentration, using the formula: total fecal production (TFP) = (g Cr consumed/ day) / (g Cr/ g fecal DM).

The digestibility (D) of DM and other constituents of the diets were calculated by the equation: D = (g of DM or nutrient intake - g of DM or nutrient excreted) / g of DM or nutrient consumed. From the digestibility data the total digestible nutrients concentration (TDN) of the diets was calculated using the equation proposed by Weiss (1999): TDN (%) = NDFapd + NFCd + CPd + EEd x 2.25. Total digestible nutrients were converted to digestible energy (ED) by multiplication by 4.4 (NRC, 2016).

2.3 Performance and carcass characteristics

The cattle were weighed at the beginning, after the adaptation period (20 days), every 21 days and at the end of the experimental period, before shipment to the slaughterhouse, after 12-hour solids fasting. The cattle were slaughtered in a commercial slaughterhouse, after resting and a water diet, following the legislation used by the Brazilian slaughter industries.

The animal carcasses were classified in the following items: carcass weight, animal age, finishing and conformation. The half-carcasses were then identified, weighed, washed and brought to cooling for 24 h at 2^{o} C. After the cooling period, the carcasses were again weighed and the pH was verified (PH-2600 digital potentiometer; Icel Manaus, Manaus, Amazônia, Brazil), equipped with a penetration electrode and temperature probe in each

experimental half-carcass.

2.4 Analyzes of meat chemical composition

In the deboning line the *Longissimus thoracis* muscle was removed from both sides of the carcasses. Subsequently, they were divided into sections of 2.5 cm thick, obtained perpendicularly to the length of the muscle. The portions were then identified and packed in a vacuum machine (Cryovac®, São Paulo, Brazil) in polyamide packages of low gas permeability and stored at 4° C ± 2° C until the time of analysis.

Protein and ash were determined according to protocols 960.52 and 923.03, respectively, from AOAC (1995) and moisture was determined by oven weight loss at 105° C. The fat was extracted with hexane and isopropyl alcohol, as described by Hara and Radin (1978), and used for the quantification of the fat and cholesterol content by enzymatic method (Saldanha et al., 2004), as well as for analysis of fatty acid profile.

Lipid oxidation was evaluated by determination of thiobarbituric acid reactive substances (TBARS) as described by Raharjo et al. (1992). Oxidation values were calculated from a standard curve of 1,1,3,3-tetraethoxypropane (TEP) (T9889, Sigma-Aldrich, St. Louis, USA) and expressed in milligrams of malonaldehyde per kg of meat (mg MDA / kg of meat).

The extracted fat, after being weighed in test tubes, was transesterified and methylated according to the procedures described by Christie (1982). The fatty acid profile was determined using Agilent 6890N (Agilent Technologies, St. Clara, USA) gas chromatograph equipped with a flame ionization detector (FID), automatic injector and Supelco 2560 capillary column (Sigma-Aldrich, St. Louis, USA) with 100 m length x 0.25 mm thickness and 0.20 μm film. The identification of the fatty acids was performed by comparison with the known retention times of methyl esters (Sigma-Aldrich, St. Louis, USA): FAME Mix-37 (code 47885-U), methyl ester of the vaccenic acid (code 46905-U), mixture of C18:2 CLA isomers (code 47792). Fatty acid quantification in mg per g of lipids was performed using

C23:0 and the theoretical correction factor, as well as the conversion factor for fatty acid methyl ester, according to the methodology proposed by Tonial et al. (2014).

2.5. Economic analysis

Economic analysis was performed for all diets and value of food purchased in August 2014 was used. The price was calculated in Brazilian money converted to dollar (US $) based on the 2014 average value (one dollar is equivalent to R$ 2.35 reais). The price used for concentrated was $ 0.28/ kg DM for all treatments and a cost of $ 0.37/ kg DM was used for corn silage and BSG's price was $ 0.10/ kg DM. Commercial's value of bovine cold carcass was $ 4.50/ kg carcass.

2.6. Statistical analysis

A completely randomized design with four treatments and five replications was used, making a total of 20 animals. Data were submitted to variance analysis, regression and test for the lack of adjustment of the equations (lack-of-fit) to 5% of significance, using the PROC REG of the Statistical Analysis System (SAS, 2008). When the data did not fit any of the regression models, the averages were compared by the Tukey test, at 5% significance. The mathematical model used in the regression study was: $\hat{y}ij = b0 + b1Xi + b2\ Xi2 + b3\ Xi3 + aj + \varepsilon ij$, where: $\hat{y}ij$ = dependent variables; b's = regression coefficients; Xi = independent variables; aj = regression deviations; and εij = residual random error.

3. Results

The mean values of DM intake in grams for kg of body weight (BW) has a linear behavior reducing as animals are fed with increasing levels of BSG ($P < 0.05$; Table 3). The intake of organic matter (OM), neutral detergent fiber (NDFap) and non-fibrous carbohydrates (NFC), expressed in g/kg of BW, also presented lower linear response ($P < 0.05$) in the treatments with BSG. While the ether extract (EE) intake showed an increasing linear effect with the inclusion of BSG and crude protein (CP) intake had no any effect (P

<0.05; Table 3).

The digestibility coefficients of DM, OM, CP and NDFap were explained by linear regression and values were decreased as the inclusion levels of BSG in diet were increased (P <0.05). For EE, NFC and digestible energy (ED) none of the regression models were capable to explain results variation (P >0.05) for the different diets (Table 3).

No differences in linear and quadratic responses were observed for body weight gain, carcass weight, carcass dressing percentage, fat's typification, temperature and pH among cattle confined to different levels of brewer's spent grain (BSG), replacing corn silage (CS) (P >0.05). However, feeding conversion (kg DM / kg BW gain) showed a linear effect with the reduction of this value when BSG were used on to feed animals (P <0.05; Table 4).

Regarding the economic viability of this system of steers finishing, there was a linear and quadratic effect of the net revenue with the inclusion of the BSG, indicating that the increase of the level of this residue in the cattle diet promoted reduction in the cost of the animals feeding, mainly due to the acquisition value of this residue, since the values practiced at the time of the research were much lower than the other ingredients, because it is an agroindustrial byproduct, still unexplored (Table 5).

The chemical composition of the beef produced, represented by the values of moisture, protein, lipids, ash and cholesterol were not affected by the inclusion of BSG in substitution to CS in the animals diets (P >0.05; Table 6). The meat lipid oxidation was linearly lower with the inclusion of 25 and 35% BSG in the cattle diet (P >0.05; Table 6).

In general, the fatty acid profile of the *Longissimus thoracis* muscle was similar (P >0.05) among animals fed with levels of inclusion of BSG (Table 6). However, it should be noted that polyunsaturated fatty acids had higher linear values (P <0.05) in those animals with inclusion of BSG in the diet; among these, the concentrations of linoleic acid and conjugated linoleic acid (P <0.05) are particularly noteworthy.

4. Discussion

Regarding the average daily intake, although the BSG represents an important option as a source of fiber in the ruminant diet, having presented 49.92% of NDFap, a value similar than corn silage (53.14%), from the physical point of view (particle size) characteristics are distinct. As the mean particle size of the BSG is smaller than the mean particle size of corn silage, intakes of equivalent amounts of NDF stimulate the chewing activity and saliva flow with different intensities (Allen, 2000; Firkins, 1997). In addition to the smaller particle size, BSG has a higher specific gravity than corn silage. The combination of these factors contributed to reduce the retention time of rumen BSG diets, which increases the probability of passage of potentially digestible NDF to the lower digestive tract and, consequently, may decrease the digestibility of NDF in the total digestive tract (Firkins, 1997), with proportional effects on dry matter and organic matter intake.

In addition, it should be considered that the inclusion of unsaturated fat in ruminant feeding entails reduced digestion of structural carbohydrates in the rumen, and that this reduction can only be attenuated when protected fats are used (Castro et al., 2016; Doreau and Chilliard, 1997). A lower intake of dry matter and lower organic matter in the added BSG treatments in the diets may also have been influenced by the increase in the unsaturated lipids content, which increased according to the inclusion of this residue in the diets (Table 1).

In this context, another aspect that deserves attention in the digestion of food by ruminants is the presence of phenolic compounds such as ferulic acid (FEA) and p-coumaric acid (PCA), the main phenolics present in the cell wall of the BSG and CS used in the experimental diets (Table 1). The ester-like bonds of the phenolic acids to the plant cell wall, together with other cellular components, can be disrupted by esterases activities produced by rumen microorganisms capable of releasing the free form of FEA and PCA into the rumen (Cao et al., 2016b).

However, different forms of physical association of these phenolic acids to the cell wall of feed may impose restriction on the digestibility of fiber in ruminants. For instance, the lignin content of the cell wall and the ether-type bonds of the phenolic acids have been confirmed as limiting factors for rumen degradation, since they restrict the accessibility of polysaccharide hydrolysis enzymes to their substrates in forages (Cao et al., 2015; Cao et al., 2016a).

The ruminal microorganisms present greater capacity to degrade FEA in the ester-like bound form than PCA in this form (Cao et al., 2016b). Thus, even if BSG has higher levels of FEA than PCA, when we compare the PCA levels of the residue with CS, the values are much lower in CS (Table 1). Thus, there may be a limitation of DM and OM intake in g kg-1 BW of the animals submitted to BSG-inclusion diets (Table 4) by the high concentration of PCA.

This limitation is due to the slow food passage rate, because of the delayed digestion of the fiber by ruminal microorganisms, and because the PCA hinders the accessibility of enzymes to their substrates (Cao et al., 2016b).

In this context, the voluntary intake and the digestibility of NDFap of the animals submitted to the diets with inclusion of BSG were linearly lower. From five moist by-products of the food industry (Aspergillus residue, soybean pulp (okara), pomegranate pulp, grape pulp and avocado pulp) tested by Eliyahu et al. (2015) in sheep feeding, there were many discrepancies between in vitro and in vivo NDF digestibility values of the residues. This leads to the condition that in addition to lignin content, phenolic contents should be considered to explain NDF intake and digestibility in ruminants, since these compounds positively or negatively influence the accessibility and degradability of the fiber by ruminal bacteria in agreement with the results of this study.

The non-fibrous carbohydrate (NFC) intake in g/ kg BW was also linearly lower in the added diets of BSG (Table 3). If we consider the chemical composition characteristics of this

294 ingredient of the diet, an insignificant starch content can be observed, because it is the
295 remaining solid fraction of barley malt, which has endosperm starch as the main substrate for
296 fermentation (McCarthy et al., 2013).

297 Meanwhile, crude protein intake had no effect for diets with inclusion of BSG (Table
298 3), since diets were balanced to similar levels in protein. The excess protein in the cattle diet
299 may lead to a decrease in the animals weight gain, because of the energy cost of excess
300 nitrogen clearance (Nuñez et al., 2015). However, diets did not promote excessive values of
301 nitrogen to the point of influencing the animal performance.

302 Often, feed intake by ruminants is directly influenced by odor and palatability, so it
303 requires long periods of adaptation to introduce different ingredients to feeding (Pilajun et al.,
304 2016). However, there was an excellent acceptability of the BSG in feedlot cattle, since the
305 animals started the residue intake and only after consumed the CS. Such ingestive behavior of
306 the animals did not affect values of voluntary intake of CP with the inclusion of BSG in the
307 diet.

308 Likewise, voluntary intake of EE in g/ kg of BW was linearly higher in animals
309 submitted to diets with inclusion of BSG, although diets were balanced for energy levels,
310 energy source in diets with BSG was higher from lipids, as the BSG had 8.16 g $100g^{-1}$ DM
311 from EE while the CS 3.21g $100g^{-1}$ DM (Table 1). Thus, the higher EE intake (a fraction
312 metabolically 2.25 times more energetic than protein and carbohydrates) satisfactorily
313 compensated for the lower OM intake and digestibility of BSG diets, confirmed by the similar
314 energy value between experimental diets (Table 3) and performance and carcass
315 characteristics.

316 The productive indices represented by the body weight and cold carcass parameters of
317 the animals were not affected by the substitution of CS for BSG in the animals' diet (Table 4).
318 However, feeding conversion of BSG-fed animals was linearly more efficient, since daily dry

matter intake was lower with the addition of this by-product in the diet and weight gain was not influenced by the different diets offered. In the current scenario, this becomes an important positive point for the inclusion of BSG in ruminant feeding, since feeding conversion is a strong ally for the choice of ingredients that promote the reduction of costs of beef cattle diets.

The average weight gain of the animals was 1.22 to 1.38 kg/ day for the different diets offered (Table 4). Coleman et al. (2016) when evaluating the performance of 20-month-old Angus steers, compatible with the cattle used in this study, observed lower values of daily average weight gain (0.72 kg/ day) for steers fed exclusively with ryegrass (*Lolium multiflorum*). Even comparing two distinct finishing systems, it can be inferred that the cattle feedlot subjected to 50:50 diets (forage:concentrated) with the inclusion of up to 35% BSG in diets as a source of forage meets market expectations for performance of animals and makes this alternative economically viable (Table 5).

Still, corroborating the results obtained, Ban-Tokuda et al. (2007) evaluated 10 crossbred Brahman cattle with a mean age of 22 months, fed an experimental diet composed of corn silage, brewer's spent grain and concentrate (50:30:20) during a 6-month finishing period, obtained average daily gain of 0.93 kg, reaching values around 1.2 kg in the first month and 0.80 kg at the end of the sixth month.

Thus, considering all the productive parameters evaluated the indication of the BSG use as a source of forage in the diet of beef cattle is confirmed. In addition, the economic aspects are highlighted, among them the linearly lower feedlot feeding cost for the diets added to this residue (Table 5). Compared to the reduced production cost, we can still observe higher partial net revenue for the diets with the inclusion of BSG.

Another important aspect in relation to the production costs of formulated diets is the use of other ingredients of lower commercial value, besides the BSG, such as wheat bran and

rice bran (agro-industrial by-products), allowing the achievement of adequate protein levels without the need to use soybean meal, the main protein source used in the formulations and which is generally expensive.

The availability of this ingredient directly impacts its commercial cost. Due to the high production of BSG by the brewing industry and demands still little explored. Nowaday it is possible to acquire this residue at a low cost; because this waste is commonly discarded in landfills, that if not adequately controlled, can lead to an environmental problem. Potential uses in food, in the chemical, pharmaceutical and biofuel industry (Xiros and Christakopoulos, 2012) are suggested for this purpose. However, it is important to highlight that the viability of BSG in ruminant feeding is a strong ally for the correct destination of this residue.

Regarding the meat characteristics, there was no statistical difference in the composition of moisture, protein, total lipids and minerals among treatments (Table 6). It should be noted that even though the results of EE consumption with BSG inclusion in the diets were linearly higher, and this tends to increase fat deposition intramuscularly in ruminants, the total lipid content did not show any difference with the addition of this residue in the diet (Hocquette et al., 2010). Likewise, the cholesterol values in the muscle did not present differences with the substitution in the levels of the CS by BSG in the cattle diets. In agreement, Freitas et al. (2014) also found no difference in muscle cholesterol in cattle fed on pasture or with a diet rich in concentrate.

TBARS technique is widely used to evaluate lipid oxidation in meat (Campo et al., 2006). The lowest values of TBARS were found in treatments 25 and 35% BSG and this can be attributed to the higher content of phenolic acids in this residue, such as PCA and FEA. Those compounds have high antioxidant power (Fãrcas et al., 2006; Stefanello et al., 2018) and could be responsible to a protective effect. However, Campo et al. (2006) evaluated the

relation between TBARS and rancidity perceptions by consumers and found that the sensory threshold in meat is 2 mg of malonaldehyde per kg. Consequently, in the present study all groups showed acceptable values of lipid oxidation.

The most abundant fatty acids found in meat of feedlot cattle in diets containing different levels of BSG to replace CS were palmitic acid (16:0), stearic acid (18:0) and oleic acid (C18: 1n9c) (Table 6). The different diets of the finishing animals showed no linear or quadratic effect on these fatty acids. In general terms, this composition is characteristic of beef and is in agreement with the majority of meat quality researches currently developed (Coleman et al., 2016; Correia et al., 2016; Łozicki et al., 2012).

Thus, considering the lipid profile of the dietary ingredients, low but similar amounts of stearic acid (C18:0) were given to the animals submitted to the different experimental diets (Table 1). The level of C18:0 in the animals diet does not justify the high concentration of this acid in beef, since when C18:0 reaches adipose tissue it is partially converted to oleic fatty acid (C18:1n9c) through the enzyme delta -9 desaturase (Castro et al., 2016). On the other hand, the high concentration of this fatty acid (C18:0) in beef may be due to the ruminal biohydrogenation process, which converts unsaturated fatty acids with 18 carbons of the diet into C18:0 (Correia et al., 2016).

In turn, the oleic acid abundance (C18:1n9c) in beef can be attributed to the high amount of this acid present in the ingredients used in diets (Table 1), when it is identified that the rice bran, ground corn and wheat bran presented respectively 43.02%, 30.86% and 20.21%, while CS presented 28.31% and BSG 13.62% oleic acid. Despite these different values in the diet ingredients, there was no difference in the composition of oleic acid in the beef.

It was verified that the diets offered did not influence in a linear or quadratic manner the values of C18: 3n3c in beef, since the linolenic acid contents did not present significant

394 difference between the CS and BSG ingredients (Table 1). However, the values of C18: 2n6c
395 of beef were linearly affected by the diets, obtaining high values in those animals that had the
396 inclusion of BSG in their diet. Possibly due to the lipid profile of this residue, with 54.34% of
397 linoleic acid in its composition, while CS showed 44.99%.
398 Similarly, conjugated linoleic acid (CLA) C18:2n9c11t and linolelaic acid
399 (C18:2n6t9t12), as well as the vaccenic acid (C18:1n11t) presented linearly higher values in
400 the beef submitted to the addition of BSG in diets. Modification of rumen unsaturated fatty
401 acids (biohydrogenation) tends to convert oleic (C18: 1n9c), linoleic (C18: 2n6c) and
402 linolenic (C18: 3n3c) unsaturated fatty acids to stearic acid (C18:0) (Correia et al., 2016).
403 Nevertheless, in some occasions changes occur in this route and the final product will be
404 some trans fatty acids as a consequence of incomplete biohydrogenation (Jenkins et al., 2008).
405 In this context, trans fatty acids found in higher amounts in the meat of animals submitted to
406 diets with the inclusion of BSG are mainly a consequence of higher values of linoleic acid in
407 this diet ingredient. The linear effect of the inclusion of BSG in the diet on docosahexaenoic
408 acid (C22:6n3) is also due to the conversion of linoleic acid to rumen (Baba et al., 2016).
409 Corroborating the results obtained in this study, Castro et al. (2016) observed that the
410 addition of 4% of different vegetable oils (palm oil, olive oil and soybean oil) in cattle
411 finishing diets did not affect the animals performance and the carcass quality, but
412 considerably modified the fatty acid profile in meat. When grouped the fatty acids, the
413 different diets did not promote linear and quadratic modifications in the levels of SFA
414 (saturated fatty acids), MUFA (monounsaturated fatty acids) and n-3 (omega 3) of beef
415 among treatments. On the other hand, the levels of polyunsaturated fatty acids - PUFA, n-6
416 (omega 6) and n-6 / n-3 ratio were linearly higher in those animals submitted to the added
417 BSG diets.
418 Modifications that occurred with C18:2n6c fatty acid in beef were sufficient to alter

the sum of PUFA in those added diets of BSG, most likely being the main unsaturated fatty acid of this category. In addition, the values of CLA, also belonging to the PUFA group, together with C18:2n6c had a direct effect on the total omega 6 fatty acids, promoting a linear effect in the beef of BSG added diets. Values for vaccenic acid (CLA precursor in humans), CLA and total polyunsaturated fatty acids were higher, factors that are strongly correlated with reduced risk for cardiovascular disease, type-2 diabetes, and some types of cancer in humans (Gladyshev et al., 2015; Kratz et al., 2013).

5. Conclusions

Brewer's spent grain replacing corn silage up to 35% in the finishing diet of feedlot cattle does not affect the weight gain and carcass yield of the animals and results in higher feed conversion and reduction in production costs. Regarding the final product, beef from feedlot animals with a diet containing a brewer's spent grain instead of corn silage has a higher amount of fatty acids beneficial to consumers' health, such as vaccenic, linoleic and conjugated linoleic acid (CLA) in intramuscular lipids, with no change in fat and cholesterol content and with greater oxidative stability.

The ruminal digestion of phenolic acids as ferulic and p-coumaric acid from corn silage and brewer's spent grain (BSG) when used in a proportion of up to 15:35 of the finishing diet of beef cattle does not present a negative effect on the productive and economic indexes, as well as on the nutritional quality of the meat produced. In addition, phenolic compounds of BSG have high potential to be explored as bioactive compounds in ruminant feeding, since they have reduced the meat lipid oxidation.

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Weiss, W.P., 1999. Energy prediction equations for ruminant feeds. In: CORNELL NUTRITION CONFERENCE FOR FEED MANUFACTURERS, 61, 1999, Ithaca. Proceedings. Ithaca: Cornell University, 176-185.

640 Table 1. Proximate composition, fatty acid profile and phenolic compounds of the

641 ingredients used in experimental diets

Ingredients	Concentrated			Forage	
	Rice bran	Ground corn grain	Wheat bran	Brewer's spent grain	Corn silage
Proximate composition (g/ kg DM - %)					
DM	889.4	881.3	872.3	213.2	329.7
MM	101.7	15.0	46.7	39.7	100.5
CP	161.2	97.5	176.6	201.2	72.2
EE	199.1	39.8	42.1	81.6	32.1
NDFap	267.6	154.6	417.9	499.2	531.4
NFC	270.4	693.1	316.7	178.3	263.8
ADIN	1.6	0.9	0.9	3.6	3.8
NDIN	4.3	8.1	6.4	16.5	15.5
ADL	61.7	15.0	46.7	39.7	40.5
Fatty acid profile (g/ kg methyl esters of fatty acid - %)					
14:0	2.5	2.4	2.9	3.9	5.9
16:0	169.5	130.4	154.2	209.6	143.2
16:1n-7	1.5	1.9	1.8	1.8	3.5
18:0	17.4	24.9	13.2	19.9	28.0
18:1n-9	430.2	308.6	202.1	136.2	283.1
18:2n-6	339.7	499.9	557.9	543.4	449.9
18:3n-3	16.1	12.0	44.1	52.9	57.4
20:0	8.7	5.4	1.5	3.3	5.0
20:1n9c11	5.6	2.7	8.0	9.6	2.6
22:0	3.3	1.7	1.2	3.2	2.5
24:0	5.3	2.9	1.6	2.6	4.0
SFA	206.7	170.6	179.5	248.8	198.3
MUFA	437.4	316.6	217.4	153.8	292.3
PUFA	355.9	512.7	603.2	598.4	510.4
Phenolic compounds (in kg DM)					
Total phenolics (g GAE)[1]	7830.0	5230.0	7070.0	17460.0	15650.0
Phenolic acids (g)[2]					
p-coumaric acid	0.18	0.003	0.003	1.03	0.43
trans-ferulic acid	0.34	0.03	0.07	2.08	0.15
Sinapic acid	0.03	0.003	0.004	0.09	-
Total flavonoids (g QE)[3]	3.5	0.9	0.4	4.5	2.7

642 DM = dry matter; MM = mineral matter; CP = crude protein; EE = ether extract; NDFap =
643 neutral detergent fiber corrected for ash and protein; NFC = non-fiber carbohydrates; ADIN =
644 acid detergent insoluble nitrogen; NDIN = neutral detergent insoluble nitrogen; ADL = acid
645 detergent lignin; C14:0 = myristic acid; C16:0 = palmitic acid; C16:1n7 = palmitolenic acid;
646 C18:0= stearic acid; C18:1n9 = oleic acid; C18:2n6 = linoleic acid; C18:3n3 = alpha linolenic
647 acid; C20:0 = arachidic acid; C 20:1n9c11 = gondoic acid; C22:0 = behenic acid; C24:0 =
648 lignoceric acid; SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA =
649 polyunsaturated fatty acids; GAE = Gallic acid equivalent; QE = quercetin equivalent.
650 [1]Obtained by colorimetric method with the Folin-Ciocalteu reagent according to Singleton et

al. (1999).

[2]Obtained by high performance liquid chromatography with methodology developed and validated by the authors in a previous study (Stefanello et al., 2018)

[3]Obtained by colorimetric method described by Bao et al. (2005)

Table 2. Participation of ingredients in experimental diets and proximate composition

Ingredients (%)		BSG 0%	BSG 15%	BSG 25%	BSG 35%
Concentrated	Rice bran	5	10	14	18
	Ground corn grain	32	18	18	18
	Wheat bran	9	18	14	10
Forage	Brewer's spent grain	-	15	25	35
	Corn silage	50	35	25	15
Mineral and vitamin supplement[1]		4	4	4	4
Proximate composition[2] (g/ kg DM*, except for DM)*					
	DM	570.3	562.5	560.1	555.4
	OM	928.4	932.0	936.4	940.8
	CP	121.2	125.3	133.5	147.1
	EE	50.5	56.7	69.3	82.6
	NDFap	376.7	374.0	361.3	348.0
	NFC	380.0	376.0	372.3	363.1

[1]Mineral and vitamin supplement corresponding to vitamin A, D and E, microminerals (Mn, Zn, Fe, Cu, I, Se and Co) and ionophore.

[2]DM = dry matter expressed as natural base; OM = organic matter; CP = crude protein; EE = ether extract; NDFap = neutral detergent fiber corrected for ash and protein; NFC = non-fiber carbohydrates; 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the diet; 25% BSG = addition of 25% of brewer's spent grain in the diet; 35% BSG = addition of 35% of brewer's spent grain in the diet.

735 Table 3. Voluntary intake, digestibility and energetic value of experimental diets

Components	Treatments				L[1]	Q[1]
	0% BSG	15% BSG	25% BSG	35% BSG		
Voluntary intake						
DM (g/kg BW)	28.31	26.37	26.06	25.08	0.014	0.725
OM (g/kg BW)	26.32	24.63	24.43	23.62	0.028	0.719
CP (g/kg BW)	3.46	3.31	3.46	3.66	0.239	0.098
EE (g/kg BW)	1.46	1.53	1.83	2.09	0.0001	0.031
NDFap (g/kg BW)	10.46	9.59	9.23	8.59	0.0001	0.993
NFC (g/kg BW)	10.95	10.22	9.91	9.28	0.003	0.892
Digestibility						
DM	0.69	0.69	0.63	0.64	0.0005	0.979
OM	0.74	0,74	0.68	0.69	0.002	0.963
CP	0.72	0.69	0.66	0.68	0.013	0.174
EE	0.92	0.91	0.90	0.91	0.244	0.354
NDFap	0.56	0.52	0.42	0.43	0.0006	0.645
NFC	0.89	0.93	0.90	0.90	0.957	0.052
ED (Mcal/kg DM)	3.27	3.29	3.14	3.28	0.585	0.376

736 [1]Probabilistic value for linear (L) or quadratic (Q) effect of the diets on each variable. DM =
737 dry matter; OM = organic matter; BW = body weight; CP = crude protein; EE = ether extract;
738 NDFap = neutral detergent fiber corrected for ash and protein; NFC = non-fibrous
739 carbohydrates; ED = digestible energy; 0% BSG = no addition of brewer's spent grain in the
740 diet; 15% BSG = addition of 15% of brewer's spent grain in the diet; 25% BSG = addition of
741 25% of brewer's spent grain in the diet; 35% BSG = addition of 35% of brewer's spent grain
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Table 4. Effects of different levels of brewer's spent grain (BSG) in the finishing cattle diet on productive and carcass performance

Characteristics	Treatments				L[1]	Q[1]
	0% BSG	15% BSG	25% BSG	35% BSG		
Body weight, BW						
Initial (kg)	322.0	321.0	340.0	338.0	0.390	0.903
Final (kg)	432.0	432.0	453.0	462.0	0.180	0.661
Gain (kg/ day)	1.22	1.23	1.25	1.38	0.110	0.319
Total gain (kg)	110.0	111.0	113.0	124.0	0.110	0.319
Feeding conversion (kg DM/ kg BW gain)	9.97	9.22	9.39	8.28	0.029	0.611
Cold carcass, CC						
Weight (kg)	205.29	206.90	212.43	223.87	0.208	0.524
Dressing (%)	47.0	47.6	46.6	47.8	0.475	0.227
Fat's typification	3.0	3.0	3.2	3.2	0.234	0.890
Temperature (^{o}C)	4.5	4.4	4.5	4.3	0.378	0.877
pH	5.65	5.42	5.46	5.41	0.146	0.379

[1]Probabilistic value for linear (L) or quadratic (Q) effect of the diets on the studied variables. 0% BSG= no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the diet; 25% BSG = addition of 25% of brewer's spent grain in the diet; 35% BSG = addition of 35% of brewer's spent grain in the diet.

798 Table 5. Economic aspects related to the levels of substitution of corn silage by

799 brewer's spent grain in the diet of feedlot cattle

Characteristics	Treatments				L[1]	Q[1]
	0% BSG	15% BSG	25% BSG	35% BSG		
Available concentrate (kg DM day^{-1})	5.3	5.3	5.5	5.5	-	-
Available CS (kg DM day^{-1})	7.3	5.1	3.8	2.3	-	-
Available BSG (kg DM day^{-1})	0.0	1.7	2.9	4.0	-	-
Feeding total cost ($ kg DM day^{-1})	4.2	3.4	3.3	2.8	<0.0001	<0.0001
Gross revenue ($ kg BW day^{-1})	5.5	5.5	5.6	6.2	0.081	0.168
Net revenue ($ kg BW day^{-1})	1.3	2.1	2.3	3.4	<0.0001	<0.0001

800 [1]Probabilistic value for linear (L) or quadratic (Q) effect of the diets on each variable. DM =
801 dry matter; CS = corn silage; BSG = brewer's spent grain; BW = body weight. 0% BSG = no .
802 addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent
803 grain in the diet; 25% BSG = addition of 25% of brewer's spent grain in the diet; 35% BSG =
804 addition of 35% of brewer's spent grain in the diet.

837 Table 6. Proximate composition in g $100g^{-1}$, cholesterol in mg $100g^{-1}$ of meat, lipid
838 oxidation in mg of MDA kg^{-1} of meat and meat fatty acid profile (mg g^{-1} of lipids) of feedlot
839 steers in diets containing different levels of brewer's spent grain to replace corn silage

Characteristics	Treatments				L[1]	Q[1]
	0% BSG	15% BSG	25% BSG	35% BSG		
Moisture	73.05	72.69	72.75	72.65	0.463	0.792
Crude protein	22.02	22.30	22.18	21.84	0.070	0.811
Total lipids	3.58	4.15	4.39	4.36	0.062	0.411
Ash	1.63	1.61	1.48	1.62	0.763	0.583
Cholesterol	69.80	67.67	72.93	73.12	0.094	0.389
Lipid oxidation	0.079	0.074	0.023	0.025	0.035	0.115
Fatty acid profile (mg/g of lipids)						
14:0	20.20	18.67	25.23	21.69	0.145	0.707
15:0	2.57	2.79	3.45	3.02	0.017	0.150
16:0	214.47	188.62	225.97	206.99	0.898	0.639
17:0	24.19	19.89	22.86	22.17	0.572	0.349
18:0	141.44	144.82	165.28	152.91	0.202	0.533
18:1n-9t	2.72	3.77	3.18	3.25	0.330	0.136
18:1trans-11	10.24	16.51	22.97	24.48	0.0001	0.567
18:1n-9c	296.16	261.40	278.51	289.08	0.732	0.071
18:1n-7c	9.93	8.19	9.08	9.29	0.441	0.054
18:2n6 trans-9 trans-12	2.15	3.17	3.36	3.89	0.003	0.739
18:2n-6	20.90	28.40	28.99	30.99	0.001	0.285
18:3n-6	0.15	0.19	0.32	0.44	0.395	0.418
18:3n-3	3.09	3.45	2.98	2.92	0.628	0.530
18:2cis-9 trans-11 (CLA)	2.72	3.34	4.11	3.94	0.008	0.422
20:0	1.12	1.27	1.17	1.18	0.747	0.457
20:4n-6	6.18	6.67	5.57	6.63	0.906	0.721
22:5n-3	3.82	3.15	2.86	3.31	0.172	0.132
22:6n-3	0.39	0.53	0.75	0.70	0.009	0.494
SFA	397.22	374.72	440.37	404.96	0.332	0.878
MUFA	354.37	321.04	348.72	359.80	0.651	0.086
PUFA	44.67	55.29	53.08	57.96	0.005	0.465
n-6	32.59	42.81	40.91	45.31	0.004	0.419
n-3	9.35	9.15	8.06	8.71	0.321	0.637
n-6/n-3	3.56	4.74	5.24	5.25	0.004	0.262

840 [1]Probabilistic value for linear (L) or quadratic (Q) effect of the diet on each variable. C14: 0,
841 myristic acid; C15: 0, pentadecanoic acid; C16: 0, palmitic acid; C16: 1, palmitolenic acid;
842 C18: 0, stearic acid; C18: 1n9t, elaidic acid; C18: 1 trans11, vaccenic acid; C18: 1n9c, oleic
843 acid; C18: 1n7c, 7-octadecenoic acid; C18: 2n6t9t12, linolelaic acid; C18: 2n6c, linoleic acid;
844 C18: 3n6c, gamma-linolenic acid; C18: 3n3c, alpha-linolenic acid; C18: 2n9c11t, conjugated
845 linoleic acid (CLA-rumenic acid); C20: 0, arachidic acid; C20: 4n6, arachidonic acid; C22:
846 5n3, docosapentaenoic acid; C22: 6n3, docosahexaenoic acid. SFA-saturated fatty acids;
847 MUFA-monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; UFA - unsaturated
848 fatty acids; n-6-omega-6; n-3-omega-3.

3.3 MANUSCRITO 3

STABILITY OF CATTLE'S MEAT FINISHED IN FEEDLOT WITH LEVELS OF BREWER'S SPENT GRAIN IN THE DIET

Artigo em fase final de revisão pelos autores para ser submetido à revista

Meat Science

(configurado conforme as normas da revista)

Stability of cattle's meat in feedlot with different levels of brewer's spent grain in the diet

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Abstract

It was evaluated the potential use of brewer's spent grain (BSG) in partial substitution of corn silage (CS) in the finishing of feedlot steers on stability of lipid, protein, color and microbiological of vacuum packed beef for 75 days under refrigerated storage. Twenty male castrated cattle were distributed in four treatments in a completely randomized design (CRD), with five replicates each: 50% concentrate + 50% CS; + 35% CS + 15% BSG; + 25% CS + 25% BSG; 15% CS + 35% BSG for 90 days. After the animals were slaughtered and the carcasses cooled, the Longissimus thoracis muscle was collected for analyzes. The lipid and protein oxidation, color parameters and microbiological stability of beef were not affected by diets (P>0.05). However, it oscillated throughout the storage time (P<0.05). BSG can be included in finishing diets of beef cattle by up to 35% (dry basis) as a forage source without adverse effects on the beef shelf life.

Key-words: beef color, lipid oxidation, microbial counts, phenolic compounds, protein oxidation, shelf life

1. Introduction

The beef shelf life is conditioned by oxidative processes that are influenced by temperature, exposure to oxygen, light and microbial growth (Guerra-Rivas et al., 2016). Due to changes in purchasing and consumption habits, the industry has attempted to extend the shelf life of the meat by storage practices such as vacuum packaging and modified atmosphere packaging (Guerra-Rivas et al., 2016; Wyrwisz, Moczkowska, Kurek, Stelmasiak, Półtorak, & Wierzbicka, 2016).

However, the joint use of new technologies is essential to increase and improve the production of beef quality in order to meet the demands and consolidate the existing markets

and access the new ones (Correia et al., 2016). The consumer's increasingly demanding preference for natural and health-enhancing products has intensified the search for alternative methods to delay lipid oxidation in foods, such as the use of natural antioxidants in animal feeding, thus avoiding any further meat manipulation (Castillo, Pereira, Abuelo, Hernández, 2013, Guerra-Rivas et al., 2016).

In this context, it is known that meat quality can be affected by animal nutrition, and several agroindustrial by-products can be used as viable alternatives in diets (Oliveira et al., 2015, Guerra-Rivas et al., 2016). This directly implies the sustainability of the systems, taking into account global policies that have encouraged the use of by-products in order to compile two major objectives, the decrease of environmental pollution by industries and the use of low-cost animal feeding resources (Anandan, Zoltan , Khan, Ravi, Blümmel, 2012; Santana Filho et al., 2016).

The brewing industry generates a low value-added industrial by-product called brewer's spent grain - BSG (Steiner, Procopio, Becker, 2015). This by-product accounts for about 85% of the waste generated in the whole process with an annual output of around 38.6 million tons by the world's breweries (Mussatto, 2014). Typical compositions of BSG vary, but always include high levels of fiber and protein, as well as appreciable levels of lipids and minerals (McCarthy, O'Callaghan, Piggott, Fitzgerald, O'Brien, 2013, Mussatto, 2014).

In addition to these nutrients, BSG contains a high amount of phenolic compounds with high antioxidant potential (Mussatto, 2014; Farcãs, Socaci, Dulf, Tofanã, Mudura, Diaconeasa, 2015), among them, phenolic acids, particularly hydroxycinnamic acids, such as ferulic acid and p-coumaric acid (McCarthy et al., 2013, Stefanello et al., 2018). In addition, BSG was compared with other ingredients (corn silage, rice bran, corn bran and wheat bran), which were used in ruminant feeding, and showed the highest concentration of polyphenols among the samples evaluated (Stefanello et al., 2018).

Thus, the objective of this study was to evaluate the potential of the use of BSG in partial replacement of corn silage in the finishing of feedlot steers on stability of lipid, protein, color and microbiological of vacuum packed beef for 75 days under refrigerated storage.

2. Material and Methods

2.1 Animals and diets

The work was carried out in accordance with the Ethics Committee of Federal University of Santa Maria, Brazil, registered under protocol 096/2014.Twenty Angus breed castrated males, with a mean age of 16 months and 280 ± 20 kg of live weight, from the same herd, born at the same time of calving, randomly distributed in four homogeneous groups housed in individual pens with total area of 25 m^2.They had free access to drinking water and feeding.

The cattle were fed twice daily (8h00 and 16h00) with rice bran, wheat bran, and ground corn grain meal and mineral-vitamin supplement and, as forage, the BSG was used instead of CS, in levels: zero, 15, 25 and 35% on the dry basis of the diets. The diets were provided in a way to provide a forage:concentrated ratio of 50:50 balanced to be similar in protein and energy as evaluated by the Cornell Net Carbohydrate and Protein System software (CNCPS, 2005).

2.2. *Longisimus thoracis* muscle sampling

The experimental period was 110 days, with 20 days of adaptation of the animals to the management, experimental facilities and diets. After, the cattle were slaughtered in a commercial slaughterhouse, after resting and a water diet, following the legislation used by the Brazilian slaughter industries. In the deboning line the *Longissimus thoracis* muscle was removed from both sides of the carcasses. Subsequently, they were divided into sections of 2.5 cm thick, obtained perpendicularly to the length of the muscle. The portions were then

identified and packed in a vacuum machine (Cryovac®, São Paulo, Brazil) in polyamide packages of low gas permeability and stored at 4° C ± 2° C throughout the experimental period (0, 15, 30, 45, 60 and 75 days of storage).

2.3 Lipid oxidation

The lipid oxidation of meat was assessed by monitoring the levels of thiobarbituric acid reactive substances (TBARS) using the method of Raharjo, Sofos, and Schmidt (1992) and the results were expressed in mg of malonaldehyde (MDA) per 1000 g of meat throughout the experimental period (0, 15, 30, 45, 60 and 75 days of storage).

2.4. Protein oxidation

Protein oxidation of meat was assessed by determining protein carbonyl (PC) content throughout the experimental period (0, 15, 30, 45, 60 and 75 days of storage). The meat samples were homogenized with phosphate buffered saline (1:4, w/v). PC content was determined at 370 nm using 2,4-dinitrophenyl hydrazine (Levine et al., 1990) and normalized to the protein content. Total protein was determined at 625 nm after reaction with Folin Ciocalteu and bovine serum albumin was used as standard (Lowry, Rosebrough, Farr, & Randall, 1951).

2.5. pH measurements

The pH of the meat samples was determined by blending 10 g of meat with 100 ml of distilled water for 1.5 min in a homogenizer. The pH values were measured using a standardized electrode attached to a digital pH meter (Model DM-22–DIGIMED, São Paulo, Brasil) throughout the experimental period (0, 15, 30, 45, 60 and 75 days of storage).

2.6. Meat color

2.6.1 Instrumental color

Flesh color was assessed in the meat against a white surface using a CR-700 Chromameter (Minolta, Osaka, Japan; CIE1976 L* a* b* and CIE L* C* h*), with a standard

illuminant D65, 28 supplementary standard observers and a standard calibration plate (number15233011). L* coordinate indicates lightness, while a* and b* are the chromaticity coordinates, where 1a* is the red direction, 2a* is the green direction, 1b* is the yellow direction, and 2b* is the blue direction.

Thirty minutes prior to the assessment, the samples were removed from their vacuum packages, and surface samples were exposed to air for the oxygenation of myoglobin. Six color measurements were performed directly on meat samples at randomly selected points per sample throughout the experimental period (0, 15, 30, 45, 60 and 75 days of storage).

2.6.2 Metmyoglobin measurements

Metmyoglobin concentrations in the total heme pigments were evaluated using a modification of the procedures described by Krzywicki (1979).Samples were blended with five volumes of cold 0.04 M phosphate buffer at pH 6.8 for 10 s in a homogenizer. After standing at 4 °C for 1 h, the mixture was centrifuged at 5.000 g for 30 min at 4 °C. The supernatants were then removed and filtered using filter paper. The filtrate was measured at absorbance of 525, 572, and 700 nm using a spectrophotometer (SP-220 Biospectro brand, São Paulo, Brazil). The percent of metmyoglobin was calculated using the following formula: Metmyoglobin % = 1.395 − (A572 − A700)/ (A525 − A700) × 100 where *A* = absorbance at the assigned nm.

2.7 Microbiological analyzes

For microbiological assays, after opening the pack 10 g was taken a septically from each tray and homogenized with 90 ml of buffered peptone water for 2 min in a sterile plastic bag. Serial decimal dilutions were made in sterile buffered peptone water and, in duplicate, 1ml samples of appropriate dilutions were spread onto selective agar plates throughout the experimental period (0, 15, 30, 45, 60 and 75 days of storage) for each sample.

The microbiological analyses of the samples that were performed were: mesophilic

aerobic plate count determined on plate count agar (PCA, MERCK) incubated at 36 ± 1°C for 48 h and psychrotrophic bacteria counts determined on plate count agar (PCA, MERCK) incubated at 7 a 10 °C for 7 days (APHA, 2001; BRASIL, 2003). The detection limit of the above techniques was 1 log CFU g−1.

2.8 Statistical analysis

The experimental design was completely randomized with four treatments and five replications. The data were subjected to analysis of variance using the SAS MIXED procedure (version 9.2, SAS Institute Inc., Cary, NC, USA). Data was analyzed as repeated measures by comparing 12 covariance structures for each variable and the covariance structure that yielded the smallest Bayesian information criterion was used for the results presented. The adjusted means for each treatment were calculated using the least squares means (LSMEANS) statement. Data were submitted to regression analysis and test for the lack of adjustment of the equations (lack-of-fit) to 5% of significance, using the PROC REG of the Statistical Analysis System (SAS, 2008). The mathematical model used in the regression study was: $\hat{y}ij = b0 + b1Xi + b2\ Xi2 + b3\ Xi3 + aj + \varepsilon ij$, where: $\hat{y}ij$ = dependent variables; b's = regression coefficients; Xi = independent variables; aj = regression deviations; and εij = residual random error.

3. Results and Discussion

3.1 Lipid and protein oxidation

Lipid and protein oxidation are considered the main non-microbial factors that affect the deterioration of meat quality (Falowo, Fayemi, &Muchenje, 2014). However, the susceptibility to oxidation may be influenced by the breed and animal species, muscle type evaluated, as well as the diet provided to the animals (Falowo et al., 2014; Min, Nam, Cordray, & Ahn, 2008).

Beef lipid oxidation as measured by MDA production was not affected by diets with different levels of brewer's spent grain (BSG) in substitution of corn silage (CS) ($P>0.05$), however, it increased over the refrigerated storage time ($P<0.05$) (0, 15, 30, 45, 60 and 75 days) (Table 1). When comparing the antioxidant potential of CS with that of the BSG used in the animal diets, higher total phenolic and flavonoid values were observed in the residue, respectively 15.6 mg of gallic acid equivalent (GAE)/g silage and 2.7 mg of quercitin equivalent (QE) / g silage and 17.5 mg GAE / g residue and 4.5 mg QE / g residue (Stefanello et al., 2018). Nevertheless, this in vitro value difference was not reproduced in vivo, which may be justified by the CS high antioxidant potential (Kuzmanovic et al., 2015).

With the beef aging, all MDA values were increased regardless of the evaluated treatment ($P<0.05$), however, low lipid oxidation was observed in the meats (Table 1), considering that the sensory perception threshold of rancidity is 2 mg MDA / kg beef (Campo, Nute, Hughes, Enser, Wood & Richardson, 2006).

In addition to the high antioxidant potential of forage sources used in experimental diets, the observed results may also be related to the animal feeding history. The steers used in the experiment came from matrices kept in pastures grown throughout the gestational period and postpartum, as well as during weaning until the movement to the confinement system, which occurred at about 16 months of age. During this period, feeding exclusively on pasture, allowed β-carotene reserves that presents protective effect of the beef against the lipid oxidation, after a short period of confinement as implemented in this study (Realini, Duckett, Brito, DallaRizza& De Mattos, 2004; Nieto, Díaz, Bañón, Garrido, 2010).

The beef cattle carbonyl content submitted to the different levels of substitution of CS for BSG in the diets is presented in Figure 1. The beef protein oxidation was not affected by the different diets ($P>0.05$), but increased throughout the storage time ($P <0.05$).

These results are in agreement with the lipid oxidation data, suggesting a correlation between lipid and protein oxidation already reported in other meat surveys (Estevez, 2011; Lund, Heinonen, Baron, & Estevez, 2011; Canto et al., 2016). In general, the oxidation of lipids and myoglobin leads to the formation of by-products, such as peroxyl radicals and oxidized iron, which in turn accelerate protein oxidation (Estevez, 2011).

Canto et al. (2016) when evaluating the protein oxidation of Nellore bulls packed aerobically stored steaks at 4 °C for nine days obtained increased values with the progression of storage time. In agreement, Cho, Kang, Seong, Park and Kang (2015) observed an increase in the protein oxidation in cattle *Longissimus lumborum* Bos taurus steaks during 12 days of storage under refrigeration in air permeable packaging.

The beef pH values submitted to the different levels of substitution of CS for BSG in the diet during refrigerated storage and vacuum packaging of the meat are presented in Figure 2. The pH values were also not affected by the diets ($P>0.05$), however, they decreased over the storage time ($P<0.05$).

All samples showed the reduction of pH over time, presumably due to the growth of lactic acid bacteria, which due to their metabolism, consume cellular glycogen and then lactic acid is released, causing acidification (Cayré, Vignolo, &Garro, 2003). In Brazil, slaughterhouse only export meat with a pH below 5.8, measured directly on the *Longissimus dorsi* muscle 24 h post-mortem (Santana et al, 2014), and in this respect, pH values from day 1, for all treatments are in accordance with national legislation. Even though with aging pH decreased for all treatments, it remained within the desirable range for beef from 5.4 to 5.8 (Purchas & Aungsupakorn, 1993).

3.2 Meat color

The color measurements and percentage of the metmyoglobin pigment are shown in Fig. 3 and Fig. 4. The storage time significantly affected the evolution of beef color

parameters submitted to different levels of CS replacement by BSG in diets ($P<0.05$). In turn, the different diets did not result in a difference in the color parameters evaluated over the entire storage period ($P>0.05$) (Figure 3).

The luminosity values (L *) behavior increased during the 75 days of storage (Figure 3) ($P<0.05$). This can be explained by the protein degradation process during meat aging, which promotes the weakening of the protein structures, which results in a greater dispersion of light, thus increasing L* values (MacDougall & Taylor, 1975). Wyrwisz, Moczkowska, Kurek, Stelmasiak, Półtorak & Wierzbicka (2016) when evaluating the beef color changes under refrigerated storage during 21 days also obtained increasing values of L* throughout the aging period of the meat.

Some authors have observed that the dietary inclusion of phenolic compounds produces meat with higher L* values because they act as iron chelating agents promoting a lower hemoglobin concentration in the blood and probably lower concentration of myoglobin in the muscle before slaughter (Samman et al. , 2001). Our results support this suggestion, since the CS and BSG used in animal diets are characterized as polyphenol-rich ingredients (Stefanello et al, 2018), resulting in meats with high L* values.

The values of red (a *) and yellow (b *) behaved similarly, remained almost stable for the first 15 days, then increased up to 45 days and then decreased throughout storage (75 days)($P<0.05$) (Figure 3). In contrast, previous studies have reported the ability of some phenolic compounds added to the cattle diet to act as antioxidants and to extend the typical red meat color for a longer period of time (Scollan, Hocquette, Nuernberg, Dannenberger, Richardson & Moloney, 2006; González-Ríos et al., 2016). Faustman, Sun, Mancini & Suman (2010) indicated that the mechanism by which phenolic compounds improve meat color stability is due to the inhibition of lipid oxidation.

Simitzis, Deligeorgis, Bizelis, Dardamani, Theodosiou & Fegeros, (2008) reported that dietary natural antioxidants increased color parameters (a* and b*) in meat of lambs fed with oregano essential oil. Accordingly, the phenolic compounds of the diets used in this experiment maintained the values of a* and b* elevated up to day 45 of storage. Luciano et al. (2011) have shown that feeding ruminants with diets rich in polyphenols improves the antioxidant status of the muscle, decreases the oxidation of myoglobin and meat discoloration.

In turn, the hue parameter (h^o) remained stable for the first 30 days of storage and then increased ($P<0.05$) (Figure 3). The hue angle (h^o) provides a more realistic view of the meat cut than the individual color coordinates L*, a* and b* (Luciano, Monahan, Vasta, Biondi, Lanza, & Priolo, 2009). Therefore, an increase in h^o values over the storage time is considered a good descriptor of meat color as it correlates with the visual evaluation of meat discoloration and the accumulation of metmyoglobin on the meat surface (Luciano et al., 2011).

Metmyoglobin pigment values increased during the 75 days of storage ($P<0.05$) (Figure 4). The conversion of oximioglobin to metmyoglobin results in meat discoloration and interactions between lipid oxidation and discoloration are known (Faustman, Sun, Mancini and Suman, 2010). It is known that the meat that presents less oxidation will have greater color preservation (Monteschio et al., 2017). However, as in this experiment, Zouaghi & Cantalejo (2016) indicated that the loss of meat coloration throughout the refrigerated storage cannot be avoided, but delayed by the application of strategies that avoid oxidation.

3.3 Microbiological analyzes

The mesophilic and psychrotrophic content of beef submitted to different levels of substitution of CS for BSG in diets is presented in Figure 5. The microbiological stability of beef was not affected by the different diets ($P>0.05$), but oscillated over the storage time ($P<0.05$).

The initial bacterial load is essential to determine the meat shelf life. A high number of microorganisms in the meat before storage reduce shelf life, since the established limit for microorganisms will be reached faster (Berruga, Vergara and Gallego, 2005). Nevertheless, although the initial values of all treatments were low in concentration prior to storage, all microbial populations increased significantly during vacuum packed refrigerated storage.

For both evaluated microorganisms, the counts remained below 7 log CFU g-1, up to 60 days of storage, which limits the product as unfit for consumption according to the International Commission on Microbiological Specifications for Foods (ICMSF , 1986). However, the inclusion of polyphenols from the forage sources used in cattle diets was not able to extend the meat shelf life, that is, it had no significant effect on its microbial deterioration.

In contrast to our results, Rota, Herrera, Martínez, Sotomayor and Jordán (2008) provided evidence of the efficacy of polyphenols as antimicrobial agents capable of altering bacterial cell membranes and microbial enzymatic metabolism with high antibiotic activity.

4. Conclusions

Brewer’s spent grain may be included in cattle finishing diets by up to 35% (dry basis) as a forage source without adverse effects on the meat shelf life during storage under commercial conditions. Although the addition of increasing levels of brewer’s spent grain in the animals' diets did not provide significant differences in the oxidative, protein, color and microbiological stability of beef in relation to the control treatment, it is inferred that all the diets used were effective in maintenance of the meat quality throughout its shelf life. The use of brewer’s spent grain to replace corn silage in beef cattle diets can be adopted as a strategy to reduce feeding costs and also as an alternative source of polyphenols from a material that needs to be recycled.

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Table 1. Determination of TBARS (in mg MDA / kg of sample) during beef stocking from finished cattle with different levels of brewer's spent grain in the diet*

	t0	**t15**	**t30**	**t45**	**t60**	**t75**	Average treatment
T0%	0.079	0.133	0.237	0.171	0.278	0.038	0.156
T15%	0.073	0.181	0.180	0.223	0.318	0.091	0.178
T25%	0.023	0.157	0.443	0.245	0.304	0.194	0.228
T35%	0.025	0.198	0.442	0.267	0.250	0.194	0.219
Average time	0.050	0.167	0.325	0.227	0.288	0.114	**$y=0.044+0.012t - 0.0001t^2$

*Diets consist of corn silage replaced by brewer's spent grain levels with T0% (0.0% BSG), T15% (15% BSG), T25% (25% BSG), T35% (35% BSG) of the total diet; t0 = storage day 1; t15 = 15 days storage; t30 = 30 days of storage; t45 = 45 days of storage; t60 = 60 days of storage; t75 = 75 days of storage.

** Quadratic polynomial regression equation according to the mathematical model y = b0 + b1*X + b2*X2.where y = dependent variable TBARS; B's = regression coefficients; X = independent variable (storage time).

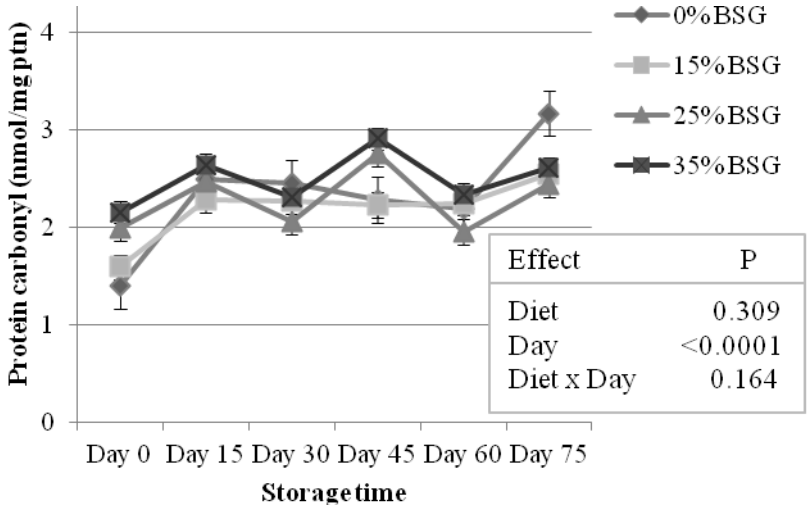

Figure 1. Least squares means ± SEM for protein carbonyl of samples from bovine meat submitted to different levels of corn silage replacement by brewer's spent grain in the diet. P = probability; 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

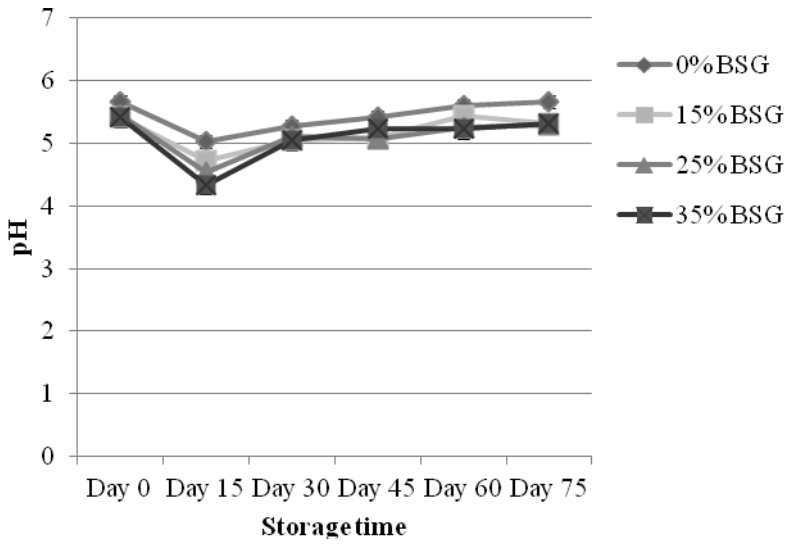

Figure 2. Least squares means ± SEM for pH samples from bovine meat submitted to different levels of corn silage replacement by brewer's spent grain in the diet. P = probability; 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

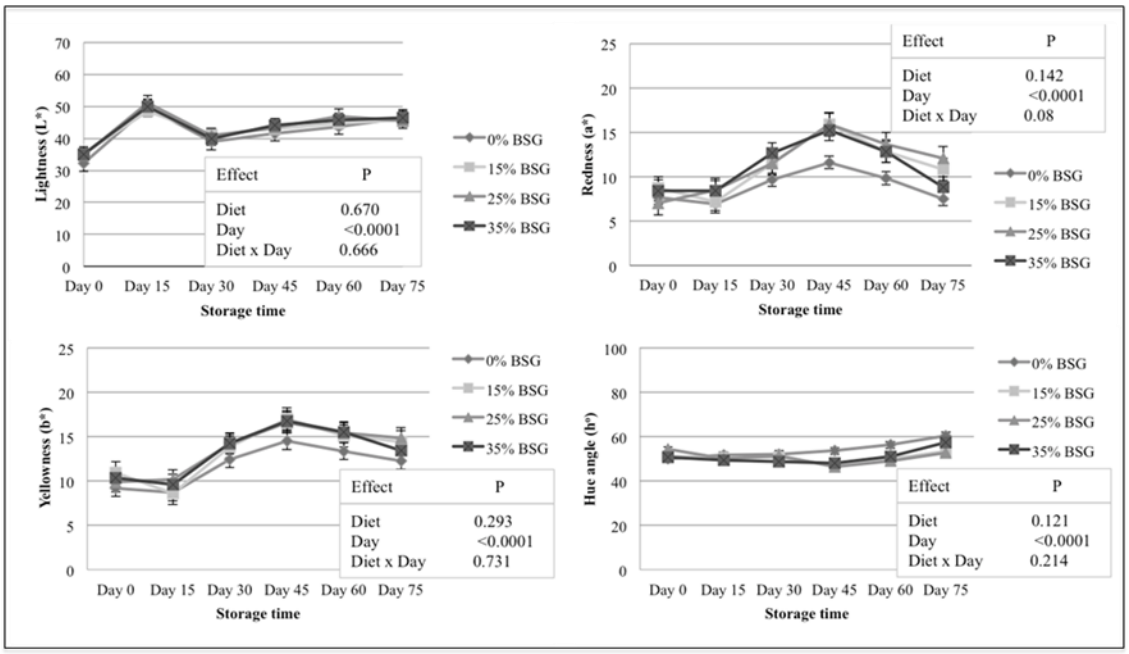

Figure 3. Least squares means ± SEM for lightness (L*), redness (a*), yellowness (b*) and hue angle (h^o) samples from bovine meat submitted to different levels of corn silage replacement by brewer's spent grain in the diet. P = probability; 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

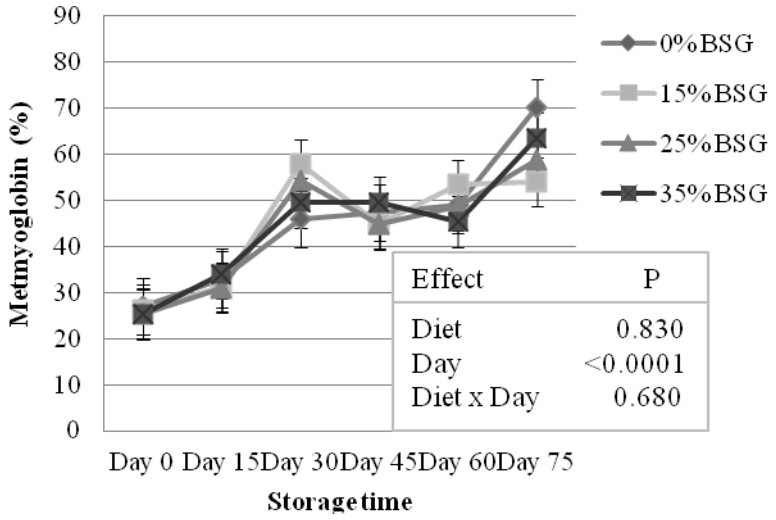

Figure 4. Least squares means ± SEM for metmyoglobin (%) samples from bovine meat submitted to different levels of corn silage replacement by brewer's spent grain in the diet. P = probability; 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

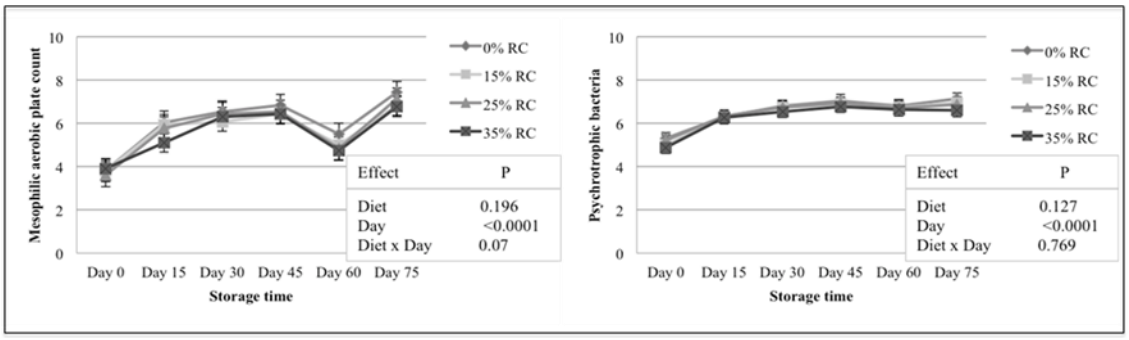

Figure 5. Least squares means ± SEM for mesophilic aerobic plate count and psychrotrophic samples from bovine meat submitted to different levels of corn silage replacement by brewer's spent grain in the diet. P = probability; 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

3.4 MANUSCRITO 4

BIOAVAILABILITY OF PHENOLIC COMPOUNDS AND MEAT QUALITY OF BEEF CATTLE SUBMITTED TO LEVELS OF BREWER'S SPENT GRAIN IN THE DIET

Artigo em fase final de revisão pelos autores para ser submetido à revista

Food Chemistry

(configurado conforme as normas da revista)

Bioavailability of phenolic compounds and meat quality of beef cattle submitted to levels of brewer's spent grain in the diet

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Abstract

The objective of this study was to evaluate the bioavailability of phenolic compounds in cattle submitted to diets with the inclusion of increasing levels of brewer's spent grain (BSG) in substitution of corn silage (CS). Additionally, effects of the inclusion of BSG on the quality of the meat produced were evaluated through analysis of chemical, physical, microbiological and sensory characteristics. Twenty steers were fed forage and concentrate (CS and BSG) in a 50:50 ratio for 110 days (20 days of adaptation period). The cattle were allocated into four treatments in a completely randomized design (CRD), with five replicates each: 50% concentrate + 50% CS (0%); + 35% CS + 15% BSG (15%); + 25% CS + 25% BSG (25%); 15% CS + 35% BSG (35%). Bovine blood was collected from the jugular vein in both heparinized and non-heparinized vials before the feedlot of animals and at the end of the 110-day period. Total blood and serum were kept refrigerated (2 to 8 °C) while being transported to the laboratory and frozen (-18 °C) until analysis of antioxidant enzymes and total phenolic content. After slaughter, the carcasses were chilled and the *Longissimus thoracis* muscle was collected for the analysis of centesimal composition, lipid and protein oxidation, pH measurement, color evaluation through the parameters L*, a*, b*, hue angle (h^o) and metmyoglobin content, cooking loss, texture profile, water activity, count of aerobic mesophilic microorganisms, psychrotrophic, total and fecal coliforms, *Staphylococcus aureus*, Salmonella SP and sensorial analysis. Total phenolic compounds in bovine serum were affected by the interaction of the different diets and the duration of feedlot ($P \leq 0.05$). The enzymes catalase (CAT) and superoxide dismutase (SOD) did not present significant difference between the control treatment and those with the addition of BSG to the treatment ($P \geq 0.05$), but reduced with feedlot time ($P \leq 0.05$). The GPx enzyme was affected by the treatments and feedlot ($P \leq 0.05$). The chemical composition of the beef did not present a significant difference with the inclusion of the BSG ($P \geq 0.05$). The lipid oxidation and the pH of the meat were linearly lower ($P \leq 0.05$). Protein oxidation was higher with the inclusion of BSG ($P \leq 0.05$). There was no difference in the evaluated color parameters, in the loss of cooking content or in the cohesiveness, fibrousness and shear force parameters of the meat ($P \geq 0.05$). The elasticity and hardness of the meat presented lower values with the inclusion of BSG to the treatments ($P \leq 0.05$). There was a difference in water activity and mesophilic and psychrotrophic meat counts ($P \leq 0.05$) but not in meat counts of total and fecal coliforms, *Staphylococcus aureus* and Salmonella sp with the inclusion of BSG in the diets ($P \geq 0.05$). There was no difference in sensorial attributes of the meat ($P \geq 0.05$), except for the tenderness which was higher in meat from animals whose diets contained BSG. In conclusion, BSG may be used in the finishing diets of cattle as a source of phenolic compounds with antioxidant activity, promoting improvement in meat quality through increased oxidative stability and greater tenderness.

Key-words: antioxidant enzymes, beef, blood's phenolic, feedlot, oxidative stability, sensory characteristics

1. Introduction

In biological systems, antioxidant defense mechanisms are agents that prevent damage from free radicals (Valko, Leibfritz, Moncol, Cronin, Mazur & Telser, 2007). These mechanisms include activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), among others. However, endogenous antioxidant defenses are not always sufficient to completely neutralize reactive oxygen species (Boaventura et al., 2015). Thus, antioxidants derived from the diet, including phenolic compounds, appear particularly important in improving the antioxidant status of the body (Yang & Liu, 2013).

Phenolic acids are some of the major phenolic compounds present in brewers' spent grain (BSG), including ferulic, p-coumaric and caffeic acid (McCarthy et al., 2013; Stefanello et al., 2018). These compounds have antioxidant, anticancer, anti-atherogenic and anti-inflammatory properties (McCarthy et al., 2013). Thus, BSG phenolics are expected to exhibit similar properties and have the potential to be used for a number of bioactivities, including antioxidant activity.

The use of natural antioxidants, especially those extracted from plants, has great potential for application and great consumer acceptance for use in meat and meat products (Nerín et al., 2006). The incorporation of the natural antioxidants can be done through direct contact at the surface of the meat, using active packaging or by supplementing the animal diet with antioxidants and metabolic deposition in the meat exerting protective activity (Luciano et al., 2011; Castillo, Pereira, Abuelo & Hernández, 2013).

Currently, BSG is already being used in animal feed, since it has nutritional benefits, such as high fiber and protein content (Wang, Luo, Myung & Liu, 2014). Given the potential bioactive nature of phenolic compounds present in BSG as well as the large amounts of waste produced annually as a low-value by-product (Tang et al., 2009; McCarthy et al., 2013), it is imperative that it be explored as a source of compounds.

The objective of this study was to evaluate the bioavailability of phenolic compounds diets with increasing levels of BSG as partial replacement of CS in finishing cattle by means of blood parameters and meat quality.

2. Material and Methods

2.1 Animals and diets

The work was carried out in accordance with the Ethics Committee of Federal University of Santa Maria, Brazil, registered under protocol 096/2014. Twenty Angus breed castrated males, with a mean age of 16 months and 280 ± 20 kg of live weight, from the same herd, randomly distributed in four homogeneous groups housed in individual pens with total area of 25 m^2. They had free access to water and feeding.

The cattle were fed twice daily (8h00 and 16h00) with rice bran, wheat bran, ground corn grain meal and mineral-vitamin supplement and, as forage, the BSG was used instead of CS, in levels: zero, 15, 25 and 35% on the dry basis of the diets. The diets were provided in a way to provide a forage:concentrated ratio of 50:50 balanced to be similar in protein and energy as evaluated by the Cornell Net Carbohydrate and Protein System software (CNCPS, 2005).

2.2. *Longisimus thoracis* muscle sampling

The experimental period was 110 days, with 20 days of adaptation of the animals to the management, experimental facilities and diets. After, the cattle were slaughtered in a commercial slaughterhouse, after resting and a water diet, following the legislation used by

the Brazilian slaughter industries. In the deboning line the *Longissimus thoracis* muscle was removed from both sides of the carcasses. Subsequently, they were divided into sections of 2.5 cm thick, obtained perpendicularly to the length of the muscle. The portions were then identified and packed in a vacuum machine (Cryovac®, São Paulo, Brazil) in polyamide packages of low gas permeability and stored at 4° C ± 2° C.

2.3 Antioxidant enzymes in cattle blood

Cattle blood was collected from the jugular vein into heparinized bottles. For jugular bleeding the animal was restrained in a crush with head bail and the employment of nose grips. This procedure was performed prior to commencement of feedlot of the animals referred to as the period before diet and at the end of the 110 days of feedlot called the period after diet.Whole bovine blood was kept refrigerated (2 a 8 °C) to transport the farm to the laboratory and frozen (-18 °C) until the analysis.

Catalase (CAT) activity was determined at 240 nm using hydrogen peroxide (H_2O_2) as substrate (Aebi, 1984).The pseudo-first-order reaction constant (k) of the decrease in H_2O_2 absorption at 37 °C was determined, andthe activity was expressed as k /mg protein.Protein was measured using bovine serum albumin as the standard (Lowry, Rosebrough, Farr, & Randall, 1951). Superoxide dismutase (SOD) activity was determined based on its ability to inhibit the auto-oxidation of epinephrine to adrenochrome at an alkaline pH; this activity was determined at 480 nm at 32°C (Misra & Fridovich, 1972). The method used to evaluate glutathione peroxidase (GPx) activity in erythrocytes is to add to the red cell lysate a mixture containing NADPH, glutathione - GSH, glutathione reductase - GR, EDTA (chelating agent which also has the function of preventing the oxidation of GSH) and phosphate buffer. An aliquot of the mixture is added to the hemolysate, kept in a water bath at 37° C for 1 min, then adding H_2O_2 to start the reaction, which is monitored in a spectrophotometer at 340 nm when NADPH is converted to NADP (Paglia & Valentine, 1967).

2.4 Total phenolic compounds in cattle blood serum

Cattle blood was collected from the jugular vein into non-heparinized bottles following the same procedure described in item 2.3. Total phenolic compounds in serum were determined after a procedure of extraction/hydrolysis, as described by Serafini, Maiani & Ferro-Luzzi (1998). To remove protein interferences, serum protein was precipitated with 0.75 mol/L meta-phosphoric acid. For hydrolyzing the conjugated forms of polyphenols, 200 μL of 1 mol/L HCl was added to 100 μLof the serum, vortexed for 1 minute, and incubated at 37 °C for 30 minutes. Later, 200 μL of 2 mol/L NaOH in 75%methanol was added, and the resulting mixture was vortexed for 2 minutes and incubated at 37°C for 30 minutes. This step breaks down the bonds of polyphenols with lipids and enables the extraction of lipid-bound phenolic compounds. Then, 200 μL of 0.75 mol/L meta-phosphoric acid was added after vortexing for 2 minutes to remove serumproteins, and the sample was centrifuged at 10.000 rpm for10 minutes. The supernatant was removed and diluted before the colorimetric analysis with Folin-Ciocalteu (Singleton, Orthofer & Ramuela-Raventos, 1999). The results were expressed as milligrams of gallic acid equivalentes (GAE) per liter.

2.5 Meat's quality

2.5.1 Chemical characteristics

Protein and ash were determined according to protocols 960.52 and 923.03, respectively, from AOAC (1995) and moisture was determined by oven weight loss at 105° C. The fat was extracted with hexane and isopropyl alcohol, as described by Hara and Radin (1978), and used for the quantification of the fat and cholesterol content by enzymatic method (Saldanha, Mazalli, Bragagnolo, 2004). The lipid oxidation of meat was assessed by monitoring the levels of thiobarbituric acid reactive substances (TBARS) using the method of Raharjo, Sofos, and Schmidt (1992) and the results were expressed in mg of malonaldehyde (MDA) per kg of meat.

Protein oxidation of meat was assessed by determining protein carbonyl (PC) content. The meat samples were homogenized with phosphate buffered saline (1:4, w/v). PC content was determined at 370 nm using 2,4-dinitrophenylhydrazine (Levine et al., 1990) and normalized to the protein content. Total protein was determined at 625 nm after reaction with Folin Ciocalteu and bovine serum albumin was used as standard (Lowry et al., 1951).

The pH of the meat samples was determined by blending 10 g of meat with 100 ml of distilled water for 1.5 min in a homogenizer.The pH values were measured using a standardized electrode attached to a digital pH meter (Model DM-22–DIGIMED, São Paulo, Brasil).

2.5.2 Physical characteristics

Flesh color was assessed in the meat against a white surface using a CR-700 Chromameter (Minolta, Osaka, Japan; CIE1976 L* a* b* and CIE L* C* h*), with a standard illuminant D65, 28 supplementary standard observer and a standard calibration plate (number15233011). L* coordinate indicates lightness, while a* and b* are the chromaticity coordinates, where 1a* is the red direction, 2a* is the green direction, 1b* is the yellow direction, and 2b* is the blue direction. Thirty minutes prior to the assessment, the samples wereremoved from their vacuum packages, and surface samples were exposed to air for the oxygenation of myoglobin. Six color measurements were performed directly on meat samples at randomly selected points per sample.

Metmyoglobin concentrations in the total heme pigments were evaluated using a modification of the procedures described by Krzywicki (1979).Samples were blended with five volumes of cold 0.04 M phosphate buffer at pH 6.8 for 10 s in a homogenizer. After standing at 4 °C for 1 h, the mixture was centrifuged at 5.000 g for 30 min at 4 °C. The supernatants were then removed and filtered using filter paper. The filtrate was measured at absorbance of 525, 572, and 700 nm using a spectrophotometer (SP-220 Biospectro brand,

São Paulo, Brazil). The percent of metmyoglobin was calculated using the following formula: Metmyoglobin % = 1.395 − (A572 − A700)/ (A525 − A700) × 100 where *A* = absorbance at the assigned nm.

To calculate the cooking loss, the steaks were weighed and baked in an industrial electric oven at a temperature of 175 °C until reaching an internal temperature of 70 °C (Abularach, Rocha & Felício, 1998). The weights after cooking were recorded, and the total loss was established from the difference.

After the baked samples had cooled, six cylindrical samples, cut in the longitudinal direction of the fibre (1 cm diameter) were removed from each sample using a steel cutter with a cylindrical mould to measure the shear force.The Warner–Bratzler shear force (WBSF) measurement was performed using a texture analyser (Stable Micro Systems, TA.XTplus TextureAnalyser, UK), and the test speed was 3.30 mm/s to measure the force necessary to transversally cut each cylinder. The average cutting force was calculated, representing the shear force of each sample, as described by Abularach et al. (1998). The texture profile analyzes were performed on the same texturometer. Baked samples with 2 cm diameter and 1 cm height were analyzed in double compression cycle using cylindrical probe with 45 mm diameter and compression of 50% of their original height. The parameters evaluated were: cohesiveness, elasticity, fibrousness and hardness (N) (Bourne, 1978).

The water activity was measured in specific equipment (Aqualab, model CX 2, São Paulo, Brazil) using approximately 10g of shredded sample, provided that the cylinder of the equipment was completed, applying the principle of dew point, where the water is condensed on a mirrored and cold surface and detected by infrared sensor.

2.5.3 Microbiological characteristics

For microbiological assays, after opening the pack 10 g was taken aseptically from each tray and homogenized with 90 ml of buffered peptone water for 2 min in a sterile plastic

bag. Serial decimal dilutions were made in sterile buffered peptone water and, in duplicate, 1ml samples of appropriate dilutions were spread onto selective agar plates.

The microbiological analyses of the samples that were performed were: mesophilic aerobic bacterias determined on plate count agar (PCA, MERCK) incubated at 36 ± 1°C for 48 h; psychrotrophic bacterias count determined on plate count agar (PCA, MERCK) incubated at 7 a 10 °C for 7 days; coliforms count determined on violet red bile agar (VRB, MERCK) at 36 ± 1 °C for 18 a 24 h; then for confirmation of the presence of total coliforms is made by inoculating the suspected colonies in brilliant-green bile lactose broth (BRILA, MERCK) and subsequent incubation at 36 ± 1°C for 24 a 48 h; and for the confirmation of the presence of thermotolerant coliforms is made by means of the inoculation of the suspicious colonies in EC (*Escherichia coli*) broth and subsequent incubation at a selective temperature of 45 ± 0,2ºC for 24 a 48 ha water bath with shaking or water circulation; *Staphylococcus aureus* count determined on Baird-Parker agar incubated at 36 ± 1°C for 24 h; pre-enrichment for Salmonela is based on the incubation, at 36 ± 1ºC for 16 to 20 h, of 25 ± 0,2 g of the sample, added of 225 mL of the specific diluent, and for selective enrichment was in the rappaport and tetrathionate broth for 41 ± 0.5 °C for 24 to 30 hours; followed by isolation and selection, biochemical identification and serum agglutination test (APHA, 2001; BRASIL, 2003).

2.5.4 Sensory characteristics

The development of the descriptive terminology of samples was carried out based on Nassu, Borba & Verruma-Bernardi (2011). Each descriptive term was consensually defined, together with reference materials, which were used in the training. For the final selection of the tasters after training three evaluations were carried out. Each taster received a sample of the three bovine cuts: hind muscle, hard cushion and fillet mignon. The tasters were instructed individually to evaluate the cuts, and to mark in scales of 10 cm, the point in which they

judged to be each attribute observed for the three beef cuts. The tasters were selected according to their ability to discriminate each attribute in the samples, in addition to the repeatability and agreement with the team.

For the sensory analyses, the meat samples were baked in an electric oven at a temperature of 175 °C until reaching an internal temperature of 75 °C. After cooling, they were cut and offered to 12 trained panelists in three repetitions for each sample. The tasters evaluated the intensity of each descriptor in the samples, using the quantitative descriptive analysis record consensually developed. This line scale was anchored on the left (0 cm) with a descriptive term representing the lowest degree and the right end (10.0 cm) of the scale was a descriptive term representing the highest sensorial degree for each sensory trait.

2.6 Statistical analysis

The experimental design was completely randomized with four treatments and five replications. The data were subjected to analysis of variance using the SAS MIXED procedure (version 9.2, SAS Institute Inc., Cary, NC, USA). The adjusted means for each treatment were calculated using the least squares means (LSMEANS) statement. Data were submitted to regression analysis and test for the lack of adjustment of the equations (lack-of-fit) to 5% of significance, using the PROC REG of the Statistical Analysis System (SAS, 2008). The mathematical model used in the regression study was: $\hat{y}ij = b0 + b1Xi + b2\ Xi2 + b3\ Xi3 + aj + \varepsilon ij$, where: $\hat{y}ij$ = dependent variables; b's = regression coefficients; Xi = independent variables; aj = regression deviations; and εij = residual random error.

3. Results and Discussion

For the phenolic compounds present in the BSG to exert their beneficial effects on the animals and to compose the beef, it is essential that they be absorbed in the gastrointestinal tract and circulates in the bloodstream. Results for total phenolic compounds in serum extracted before and after feedlot from bovine fed diets containing different amounts of BSG

as a replacement for corn silage (CS) are presented in Figure 1A. Total phenolic compounds in bovine serum were affected by the interaction of the different diets and the period of feedlot ($P \leq 0.05$).

Animals receiving 35% BSG in the diet had the highest total phenolic values in the bloodstream after a 90-day feedlot time. As such, it is possible to confirm the high antioxidant potential of BSG, a finding that is in agreement with previous *in vitro* studies (McCarthy et al., 2013; Stefanello et al., 2018). Moreover, it indicates that phenolics from BSG may be absorbed into the bloodstream of ruminants.

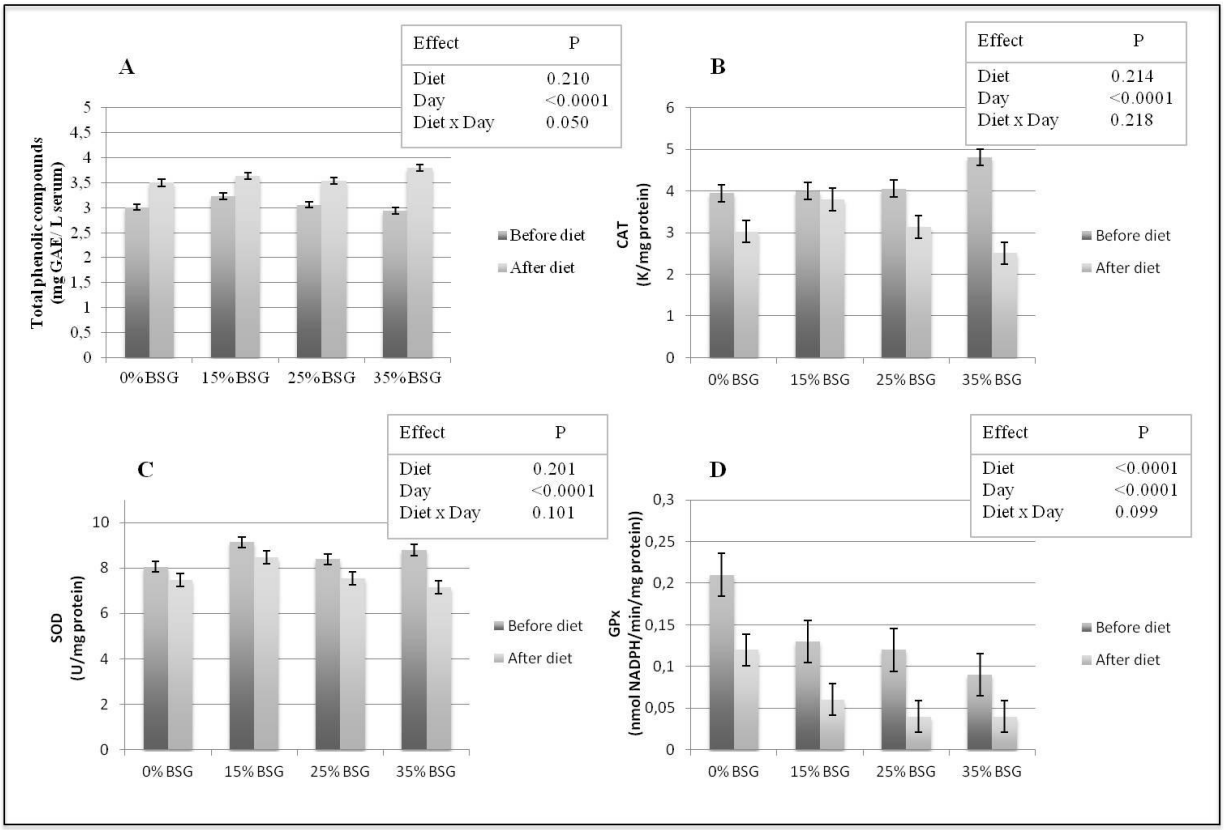

Figure 1. Total phenolic compounds (A), catalase (CAT) (B), superoxide dismutase (SOD) (C), glutathione peroxidase (GPx) (D)activities from blood of steers fed with diets containing different levels of brewer's spent grain to replace corn silage. Data are shown as the means ± SEM (n = 5). P = probability to 5% of significance to linear regression analysis; 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

Accordingly, Liu, Yang, Chen, Wang (2008) when working with isolated and associated supplementation of selenium and vitamin E in cattle, reported increased lipid

stability of blood when associated with dietary antioxidants. Likewise, Chandra, Aggarwal, Singh, Kumar & Upadhyay (2013) obtained similar results when evaluating the influence of some antioxidants (zinc and vitamin E, isolated and associated) on lipid peroxidation in bovine blood. Their findings indicate lower peroxidation indexes with the association of antioxidants.

The values of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) are presented, respectively, in Figures 1B and 1C. None of the tested enzymes showed significant difference between the control treatment and diets with inclusion of BSG ($P \geq 0.05$). However, a decrease was observed after feedlot time ($P \leq 0.05$). The antioxidant enzyme, GPx was affected by the diets ($P \leq 0.05$) with different levels of BSG as well as by the feedlot period ($P \leq 0.05$) as seen in Figure 1D.

The activity of the antioxidant enzymes CAT, SOD and GPx is commonly used to evaluate the body's defense capacity against the action of free radicals (Linke et al., 2005). The reduction in the activity of these antioxidant enzymes in the bovine serum after a period during which the animals were fed a diet rich in phenolic compounds probably occurred, due to a protective effect related to the antioxidant potential of these compounds.

The partial substitution of CS for BSG did not influence the values of CAT and SOD enzymes, confirming the high antioxidant potential of both sources (McCarthy et al., 2013; Kuzmanovic et al., 2015). On the other hand, the GPx enzyme was sensitive to the difference in the antioxidant potential of CS and BSG (Stefanello et al., 2018). A decrease in GPx values could be observed in treatments with the inclusion of BSG (15%, 25% and 35%) when compared to the control, which contained only CS as a forage source in the diet.

The high total phenolic content in the bovine blood after the period of feedlot with a diet rich in natural antioxidants from either the CS or BSG justifies the reduction in the levels of antioxidant enzymes, since the phenolics in the diet were able to promote improvement in

the intracellular redox balance, resulting in a decrease in the endogenous response adaptive to oxidative stress (Somacal et al., 2015). In assessing the influence of metabolic disturbances on the oxidative stress of dairy heifers, Dobbelaar, Bouwstra, Goselink, Jorritsma, Van Den Borne and Jansen (2010) found that the inclusion of vitamin E in animal diet did not lead to changes in either SOD or GPx.

The chemical composition of the beef, represented by the values of moisture, protein, lipids, ash and cholesterol, did not present linear, quadratic or cubic difference by inclusion of BSG in substitution to CS ($P \geq 0.05$; Table 1). Thus, it is inferred that the similar food energy level along with the feedlot period did not allow different values of intramuscular fat deposition and, consequently, did not alter the centesimal composition of the meat when analyzing the different treatments.

Accordingly, Freitas et al. (2014) also found no differences between the chemical composition of the *Longissimus dorsi* muscle of Hereford steers, finishing in feedlot or in improved Pampa biome pastures. The values found for moisture, protein, lipid, ash and cholesterol are in agreement with other studies and can be considered within characteristic limits on the chemical composition of beef (Leheska et al, 2008; Freitas et al, 2014).

The lipid oxidation of the meat was linearly lower with the inclusion of 25 and 35% of BSG in diet ($P \leq 0.05$; Table 1). This suggests that BSG has greater antioxidant potential than CS in agreement with results obtained by Stefanello et al. (2018) when evaluating the total phenolic and flavonoid content and *in vitro* antioxidant activity of BSG, CS and bran used in ruminant feed.

However, the TBARS values for all treatments did not exceed the oxidation value of 2.3 mg malonaldehyde/kg, established as the upper limit for meat oxidation, determined by consumers' rejection of the product (Nerín et al., 2006). A possible explanation for this

311 behavior may be the high content of phenolic compounds found in both forage sources used
312 in diets, BSG and CS (McCarthy et al., 2013, Kuzmanovic et al., 2015).

313 Table 1. Physicochemical characteristics of meat of feedlot steers in diets containing
314 different levels of brewer's spent grain to replace corn silage

Characteristics	Treatments				L[1]	Q[1]	C[1]
	0% BSG	15% BSG	25% BSG	35% BSG			
Moisture	73.05	72.69	72.75	72.65	0.463	0.792	0.813
Crude protein	22.02	22.30	22.18	21.84	0.070	0.811	0.795
Total lipids	3.58	4.15	4.39	4.36	0.062	0.411	0.872
Ash	1.63	1.61	1.48	1.62	0.763	0.583	0.532
Cholesterol	69.80	67.67	72.93	73.12	0.094	0.389	0.137
Lipid oxidation	0.079	0.074	0.023	0.025	0.035	0.115	0.149
Protein oxidation	1.41	1.59	1.99	2.15	0.013	0.049	0.107
pH	5.66	5.42	5.47	5.41	0.046	0.098	0.159
Lightmess (L*)	32.24	35.11	34.71	35.11	0.210	0.376	0.666
Redness (a*)	7.65	8.76	7.05	8.46	0.729	0.928	0.084
Yellowness (b*)	9.18	9.88	10.96	10.35	0.326	0.416	0.731
Hue angle (h°)	50.04	51.40	54.23	50.79	0.330	0.152	0.215
Metmyoglobin	26.97	26.28	25.44	25.20	0.561	0.849	0.955

315 [1]Probabilistic value for linear (L), quadratic (Q) or cubic (C) effect of the diet on each
316 variable.Moisture, crude protein, total lipids, ash are expressed in g $100g^{-1}$dry matter;
317 cholesterol in mg $100g^{-1}$ofmeat; lipid oxidation in mg of malonaldehyde kg^{-1} of meat; protein
318 oxidation in nmol mg $protein^{-1}$; metmyoglobin in percentage.0% BSG = no addition of
319 brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the
320 total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG =
321 addition of 35% of brewer's spent grain in the total diet.
322
323 The carbonyl content of bovine meat submitted to the different levels of substitution
324 of CS for BSG in the diets is presented in Table 1. The protein oxidation of beef presented a
325 linear and quadratic difference with higher values for the treatments with the inclusion of
326 BSG ($P \leq 0.05$). Increased levels of carbonylated proteins demonstrate that muscle protein has
327 been subjected to oxidative stress that leads to the degradation of some amino acids such as
328 lysine, proline, arginine and histidine residues (Stadtman & Levine, 2003). These results
329 indicate that the antioxidant sources evaluated were not efficient in preventing carbonyl
330 formation.

331 High carbonyl content was observed when TBARS values were compared to the
332 carbonyl content of the beef, indicating that protein oxidation occurred more rapidly than lipid

oxidation. These results are in agreement with other previous studies in biological systems (Srinivasan & Hultin, 1994; Turgut, Soyer, Işıkçı, 2016).

The pH value of the meat was linearly lower with the inclusion of BSG ($P \leq 0.05$; Table 1). Different diets are able to alter the pH values of the meat. MacKintosh, Richardson, Kim, Dannenberger, Coulmier & Scollan (2017) reported that the inclusion of different levels of lucerne (Medicago sativa L.) to bovine diets resulted in different meat pH values; however, if the pH of the meat is lower than 6.0 in all cases, it is unlikely to be of practical importance.

In this context, all meat pH values were within the normal range, from 5.41 to 5.66 (Hui, Guerrero & Rosmini, 2006). In agreement, Gonzalez-Ríos et al. (2016) inferred that pH values within the normal limit indicate that the animals were submitted to minimum stress conditions and that carcass cooling was adequate. This information is highly relevant for the evaluation of meat quality.

The results of the color and the percentage of metmyoglobin pigment measurements are shown in Table 1. The inclusion of BSG as a CS replacement did not result in linear, quadratic or cubic effects in the meat color parameters evaluated ($P \geq 0.05$). Accordingly, Rivaroli et al. (2016) observed the lack of effect on the color of beef with the addition of essential oils in the diet of the animals. Zawadzki et al. (2011) showed that the addition of another natural additive (propolis) in the bovine diet produced no difference in the color of meat when compared to the control treatment.

The cooking loss values for beef are shown in Table 2. The inclusion of BSG resulted in no linear, quadratic or cubic difference for this parameter ($P \geq 0.05$). Gonzalez-Ríos et al. (2016) reported higher values for beef cooking loss when animals were fed 6 mg of ferulic acid per kg body weight for the last 60 days of finishing or supplemented with 6 mg/kg of Zilpaterol chloride for the last 30 days of finishing when compared to control, which animals were fed only the basal diet. This change in the values of beef cooking loss was attributed to

an increase in the size of muscle fiber due to the growth promoting effect of the ferulic acid and Zilpaterol chloride present in the bovine diet (Gonzalez-Ríos et al., 2016).

In this sense, the inclusion of BSG promoted no changes in the size of muscle fiber. However, it did not promote variation in the water retention capacity of myofibrillar proteins and, subsequent variations in the values of meat cooking loss (Huff-Lonergan & Lonergan, 2005).

Table 2. Cooking loss and texture profile of meat of feedlot steers in diets containing different levels of brewer's spent grain to replace corn silage.

Characteristics	Treatments				L[1]	Q[1]	C[1]
	0% BSG	15% BSG	25% BSG	35% BSG			
Cooking loss	25.6	22.3	18.9	21.6	0.335	0.525	0.653
Cohesiveness	0.42	0.46	0.44	0.44	0.329	0.324	0.446
Elasticity	1.06	1.08	0.91	1.02	0.223	0.377	0.043
Fibrousness	1.85	1.74	1.68	1.80	0.457	0.305	0.451
Hardness	251.00	220.75	181.54	198.00	0.813	0.851	0.005
Shear force	2.42	3.48	2.07	2.58	0.830	0.805	0.269

[1]Probabilistic value for linear (L), quadratic (Q) or cubic (C) effect of the diet on each variable.Cooking loss is expressed in percentage; cohesiveness is dimensionless; elasticity and fibrousnes are expressed in cm;hardness in N; shear force in kg cm^{-1}. 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

The results of beef texture are shown in Table 2. The inclusion of BSG in substitution to CS in the bovine diets did not result in linear, quadratic or cubic differences in the cohesiveness, fibrousness and shear force parameters of the meat ($P \geq 0.05$). On the other hand, elasticity and hardness of the meat presented a cubic difference with lower values when BSG was included in the diets ($P \leq 0.05$). In this sense, it is known that the oxidation conditions in the postmortem muscle can inactivate or modify the calpain system (Harris, Huff-Lonergan, Lonergan, Jones and Rankins, 2001). Thus, it is suggested that the increase in the level of antioxidants in meat can improve its tenderness (Huff Lonergan, Zhang & Lonergan, 2010).

The tenderness is a characteristic of great importance to consumers; as such the objective measure tenderness corresponds to shear force (Santana et al., 2014). According to

Swan, Esguerra & Farouk (1998), beef is considered to be acceptably tenderness when its shear force values are lower than 8 kgf. Thus, the meat from the animals in our experiment is considered tenderness, regardless of the treatment.

The results of beef water activity and its microbiological evaluation are shown in Table 3. The inclusion of BSG resulted in a linear difference in water activity and linear, quadratic and cubic difference in mesophilic and psychrotrophic meat counts ($P \leq 0.05$). On the other hand, there was no difference in meat counts of total and fecal coliforms, *Staphylococcus aureus* and Salmonella sp ($P \geq 0.05$).

Table 3. Water activity and microbiological parameters of meat of feedlot steers in diets containing different levels of brewer's spent grain to replace corn silage.

Characteristics	Treatments				L[1]	Q[1]	C[1]
	0% BSG	15% BSG	25% BSG	35% BSG			
Water activity	0.9888	0.9885	0.9889	0.9903	<0.0001	0.078	0.843
Mesophilic aerobic	3.82	3.80	3.60	3.91	<0.0001	<0.0001	<0.0001
Psychrotrophic	5.29	5.01	5.21	4.88	<0.0001	<0.0001	<0.0001
Total coliforms	1.72	0.92	0.92	1.42	0.417	0.067	0.948
Thermotolerant coliforms	0.0	0.0	0.0	0.0	-	-	-
Staphylococcus aureus	1.23	1.15	0.62	1.28	0.732	0.270	0.192
Salmonella sp	0.0	0.0	0.0	0.0	-	-	-

[1]Probabilistic value for linear (L), quadratic (Q) or cubic (C) effect of the diet on each variable.Water activityis expressed in units within a scale of 0 to 1; counts of all microorganisms in log $CFUg^{-1}$, where CFU=colony forming units.0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

Salmonella sp. was not detected in any sample, which is in accordance with Brazilian legislation (Brasil, 2001). The limits for S. aureus, total and thermotolerant coliforms established by Brazilian legislation were also met, since the values should be lower than 3.70 Log CFU/g (Brasil, 2001). Therefore, the samples from all treatments demonstrate an excellent microbiological quality, since the counts were very low for bovine meat and the initial bacterial load is fundamental to determine its shelf-life (Guerra-Rívas et al., 2016).

As for the count values for mesophilic and psychrotrophic, both remained well below

7 log CFUg-1, the limit that determines the product as unfit for consumption according to the International Commission on Microbiological Specifications for Foods (ICMSF, 1986). The differences found suggest that the inclusion of BSG in the diets can promote antimicrobial action against psychrotrophic bacteria in beef, while it did not present this effect on the mesophilic bacteria count in meat. In this sense, in a study conducted by Kim, Cho and Han (2013) when evaluating meat with the inclusion of Chamnamul plant extract (typical East Asian plant) rich in phenolic compounds, the antioxidant and antimicrobial activity of the compound was observed in the meat.

However, differences in mesophilic and psychrotrophic counts as well as different values of water activity for beef between treatments may be attributed to operational procedures throughout the slaughtering process and carcass cooling conditions regardless of the diet (Panagou, Papadopoulou, Carstensen & Nychas, 2014).

The results for each descriptive sensory attribute of the beef from cattle finished with the substitution of CS at different levels by BSG in the diets are shown in Table 4 and Figure 2. The inclusion of BSG in the bovine diets did not result in changes in the attributes of appearance, aroma, flavor and texture of the meat ($P \geq 0.05$), except for tenderness that behaviour linear and quadratic was superior in diets containing BSG.

Importantly, the sensory analysis is an important tool to assess attributes that cannot always be measured objectively by means of readily available instrumental analysis, such as aroma, flavor and texture - tenderness and juiciness. In such cases, evaluation by human perception by means of panel of tasters is the best strategy (Nassu et al., 2011).

In turn, animal nutrition is able to directly influence the sensory characteristics of the beef perceived by the consumers (Campo, Nute, Hughes, Enser, Wood & Richardson, 2006; MacKintosh et al., 2017). For example, the panelists tended to express decreased preference towards meat from animals fed diets rich in unsaturated fatty acids (Campo et al., 2006) due

to the increase of the lipid oxidation products within the meat (Yang, Brewster, Beilken, Lanari, Taylor & Tume, 2002). Phenolic compounds such as α-tocopherol have been reported to increase lipid stability and reduce the development of off-flavor in beef (MacKintosh et al., 2017).

Table 4. Results for each sensory descriptive attribute of meat of feedlot steers in diets containing different levels of brewer's spent grain to replace corn silage

	Meat's attribute	Treatments				L[1]	Q[1]	C[1]
		0% BSG	15% BSG	25% BSG	35% BSG			
Appearance	Brown color	2.89	4.80	4.57	3.25	0.609	0.062	0.120
	Nerves' presence	2.25	2.43	2.92	1.50	0.556	0.348	0.378
	Hydration's degree	5.31	5.12	4.31	5.09	0.628	0.771	0.775
Odor	Beef	4.88	5.67	5.17	5.48	0.631	0.831	0.859
	Blood	2.1	2.02	0.73	1.52	0.263	0.485	0.362
Flavor	Beef	5.07	4.84	5.37	4.74	0.903	0.973	0.947
	Liver	1.35	1.77	1.50	0.62	0.358	0.275	0.465
	Fat	1.51	1.58	1.47	1.05	0.537	0.714	0.879
	Metal	1.78	1.47	2.29	1.81	0.733	0.944	0.774
Texture	Tenderness	6.29	6.66	7.13	8.29	0.015	0.035	0.083
	Juiciness	4.62	4.56	4.62	5.69	0.459	0.379	0.412
	Fibrousness	3.59	4.13	3.23	2.58	0.251	0.302	0.458

[1]Probabilistic value for linear (L), quadratic (Q) or cubic (C) effect of the diet on each variable.Sensory traits were evaluated by a trained 12-member panel. The preparation and presentation ofmeat samples to the panelist, was according to AMSA guidelines (AMSA, 1995).0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

In this regard, the phenolic compounds of both forage sources were able to maintain the sensory attributes of characteristic and desirable appearance, odor and taste of beef. When the inclusion of BSG did promote changes, it was towards increased tenderness, which is highly desirable (Figure 2).

Studies based on sensory evaluations attributed less tenderness to the meat of grass-

fed beef cattle fed than to the meat of animals fed a concentrate diet, probably because of the difference in the intramuscular fat content (Sitz, Calkins, Feuz, Umberger & Eskridge, 2005; Mackintosh et al., 2017). In contrast, the results of this study did not present a statistically significant difference in the fat contents of beef even though the inclusion of BSG in the diets was able to promote more meat tenderness according to the perception of the panelists.

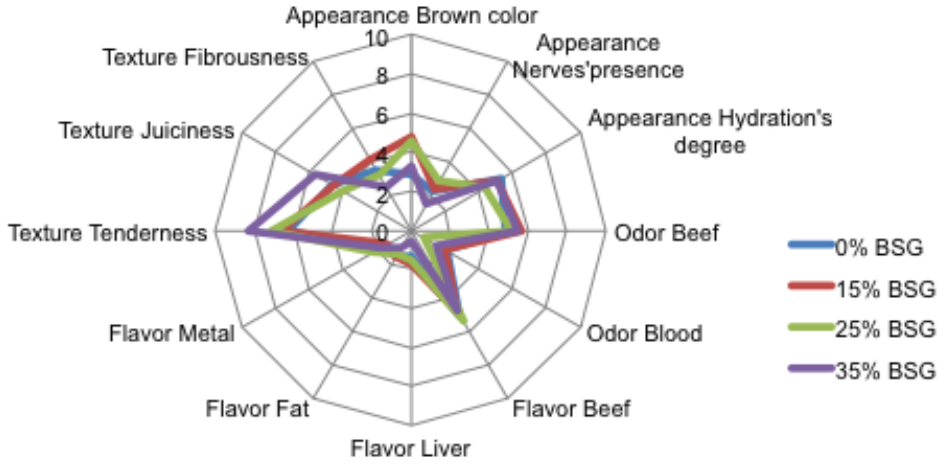

Figure 2. Spider graph with the averages of each attribute of meat of feedlot steers in diets containing different levels of brewer's spent grain to replace corn silage. 0% BSG = no addition of brewer's spent grain in the diet; 15% BSG = addition of 15% of brewer's spent grain in the total diet; 25% BSG = addition of 25% of brewer's spent grain in the total diet; 35% BSG = addition of 35% of brewer's spent grain in the total diet.

4. Conclusions

The brewers' spent grain can be used in the cattle finishing diets as a source of phenolic compounds bioavailable in the blood of animals with antioxidant activity, promoting improvement in meat quality through increased oxidative stability and greater tenderness.

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4. CONSIDERAÇÕES FINAIS

Inicialmente focamos nossa pesquisa para responder qual método deve ser adotado para determinar a composição e os níveis de monômeros de compostos fenólicos que poderiam ser liberados das amostras de resíduo úmido de cervejaria (RUC), silagem de milho, farelo de arroz, de milho e de trigo, bem como evidenciar o potencial antioxidante promissor deste resíduo em comparação aos demais ingredientes que são comumente utilizados na alimentação animal.

Com relação ao tipo de solvente, os resultados mostraram que a extração com 50% de acetona deve ser a forma preferencial para fins de caracterização dos fenólicos na sua forma natural. No entanto, se o interesse é apenas determinar qual amostra libera maior quantidade de monômeros e sua quantificação, o melhor método deve ser a extração com solvente alcalino.

Em relação à energia ou ao método de extração, cada ingrediente avaliado apresentou um comportamento específico, sendo o convencional o melhor método para a silagem de milho e resíduo de cervejaria, enquanto que a extração por micro-ondas foi o melhor método para os farelos de arroz, milho e trigo. Também concluímos sobre o pré-tratamento da amostra, sendo que a remoção de gordura não é um procedimento recomendado para os ingredientes avaliados.

Quanto ao tipo de amostra, a comparação entre o RUC e demais ingredientes comumente utilizados na alimentação de ruminantes revelou que este resíduo possui a maior concentração de polifenóis identificados e consequente maior potencial antioxidante.

O RUC em substituição a silagem de milho em até 35% na dieta de terminação de bovinos em confinamento possibilita excelente desempenho produtivo e redução significativa nos custos de produção. A degradação ruminal dos ácidos fenólicos, ferúlico (FEA) e p-cumárico (PCA), provenientes do RUC utilizado na dieta de terminação de bovinos de corte não apresentou efeito negativo sobre os índices produtivos e econômicos, bem como na qualidade nutricional da carne produzida. Além disso, comprovou-se por meio de parâmetros sanguíneos e de qualidade da carne, que os compostos fenólicos provenientes do RUC e da silagem de milho, assim como dos demais ingredientes usados nas dietas experimentais, são absorvidos e metabolizados por bovinos, contribuindo para melhora na estabilidade oxidativa do sangue e da carne produzida.

Quanto ao produto final, a carne bovina proveniente de animais confinados com dieta contendo RUC em substituição a silagem de milho apresentou maior quantidade de ácidos graxos benéficos à saúde dos consumidores, tais como vacênico, linoleico e rumênico (CLA - ácido linoleico conjugado) nos lipídios intramusculares, sem alteração nos teores de gordura e de colesterol e com maior estabilidade oxidativa e aumento na maciez.

Considerando a vida útil da carne bovina durante o armazenamento sob condições comerciais, a inclusão de até 35% de RUC nas dietas de terminação de bovinos como fonte forrageira não promoveu qualquer efeito indesejável, sendo que todas as dietas utilizadas foram eficazes na manutenção da qualidade da carne ao longo de sua vida útil.

O uso do RUC em substituição a silagem de milho em dietas de bovinos de corte pode ser adotado como uma estratégia para reduzir o custo de alimentação e também como uma fonte alternativa de polifenóis a partir de um material que apresenta a necessidade de ser reciclado.

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ANEXO 1

Registro do projeto no Comitê de Ética em Pesquisa Animal

UNIVERSIDADE FEDERAL DE SANTA MARIA
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS-UFSM

CARTA DE APROVAÇÃO

A Comissão de Ética no Uso de Animais-UFSM, analisou o protocolo de pesquisa:

Título do Projeto: "Potencialidade do resíduo de cervejaria na terminação de bovinos e como fonte de compostos bioativos para incrementar a qualidade da carne e produto cárneo."

Número do Parecer: 096/2014

Pesquisador Responsável: Prof. Dr. José Laerte Nörnberg

Este projeto foi **APROVADO** em seus aspectos éticos e metodológicos. Toda e qualquer alteração do Projeto, assim como os eventos adversos graves, deverão ser comunicados imediatamente a este Comitê.

OBS: Anualmente deve-se enviar à CEUA relatório parcial ou final deste projeto.

Os membros da CEUA-UFSM não participaram do processo de avaliação dos projetos onde constam como pesquisadores.

DATA DE APROVAÇÃO: 11/09/2014.

Santa Maria, 12 de setembro de 2014.

Prof. Dr. Alexandre Krause
Coordenador da Comissão de Ética no Uso de Animais- UFSM

VITA

Flávia Santi Stefanello, filha de Anacleto Luiz Moro Stefanello e Maria de Fátima Santi Stefanello, nascida em 10 de outubro de 1986, em Nova Palma – RS. Estudou no Escola Estadual Dom Érico Ferrari (São Francisco – Interior – Nova Palma – RS) até concluir o ensino fundamental e na Escola Estadual Tiradentes (Nova Palma – RS) onde concluiu o ensino médio em 2003. Em 2004 ingressou no curso de graduação em Medicina Veterinária da Universidade Federal de Santa Maria (UFSM- Santa Maria/RS). Formou-se médica veterinária em janeiro de 2009. Neste mesmo mês, iniciou atividade como responsável pelo Controle de Qualidade de um abatedouro de bovinos Frigorífico Silva Indústria e Comércio Ltda, Santa Maria – RS. Em março de 2011, concomitante com o trabalho nesta mesma empressa ingressou no Mestrado do Programa de Pós-graduação em Ciência e Tecnologia de Alimentos da Universidade Federal de Santa Maria sob orientação do Prof. Dr. Ernesto Hashime Kubota. Em dezembro de 2011 assumiu dedicação exclusiva ao mestrado como bolsista CAPES, obtendo o título de mestre em janeiro de 2013. Em janeiro de 2012 foi selecionada no Programa Especial de Graduação de Formação de Professores para Educação Profissional da Universidade Federal de Santa Maria, concluindo-o em agosto de 2013. Ainda no ano de 2013 ingressou no Doutorado do Programa de Pós-graduação em Ciência e Tecnologia de Alimentos da Universidade Federal de Santa Maria sob orientação do Prof. Dr. José Laerte Nörnberg, como bolsista CAPES até julho de 2015. Concomitante ao doutorado foi convocada em agosto de 2015 para atuar como médica veterinária responsável pelo Serviço de Inspeção Municipal de Nova Palma – RS, mediante aprovação em concurso público, onde permanece atualmente. Foi submetida à banca de defesa de tese em agosto de 2017.