LENIZA JANUÁRIO LUDWIG VARESCHI

CEVADA (*Hordeum vulgare*):

PROSPECÇÃO DE CULTIVARES, DESENVOLVIMENTO TECNOLÓGICO E AVALIAÇÃO DE CARACTERÍSTICAS FUNCIONAIS

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Tese apresentada ao Programa de Mestrado e Doutorado em Ciência e Tecnologia de Alimentos da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutor em Ciência de Alimentos.

Orientadora: Dra. Maria Victoria Eiras Grossmann
Coorientadora: Dra. Adelaide Del Pino Beléia

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Londrina, 02 de setembro de 2019

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“Sua tarefa é descobrir o seu trabalho e, então, com todo o coração, dedicar-se a ele.”

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LUDWIG, Leniza. **Cevada (*Hordeum vulgare*)**: prospecção de cultivares, desenvolvimento tecnológico e avaliação de características funcionais. 2019. 103 f. Tese (Pós-Graduação em Ciência e Tecnologia de Alimentos) – Universidade Estadual de Londrina, Londrina, 2019.

RESUMO

A cevada tem uma ampla aplicação. No entanto, as características tecnológicas exigidas apresentam padrões diferentes de acordo com o destino do cereal. O conteúdo de β-glucana dos grãos é o atributo mais importante para as variedades destinadas ao mercado de alimentos, devido às propriedades funcionais na redução da glicemia e colesterol. Alto teor de proteínas, peso hectolitro e taxa de descascamento também podem agregar valor a diferentes usos finais. No Brasil, o principal destino da cevada é a produção de malte; no entanto, nem todo lote atinge os padrões de maltagem. A farinha de cevada cervejeira é um ingrediente valioso na indústria de alimentos, mas seu uso é restrito devido a aspectos de qualidade, como variações de cor e presença de fragmentos de casca. Por outro lado, as variedades nuas apresentam qualidade superior, com melhores características sensoriais e composição nutricional para consumo humano. O sistema de visão computacional (CVS) pode fornecer uma classificação automática e precisa das amostras. Neste trabalho, foi proposto a CVS, combinado com a técnica do Conjunto de Partição de Espaço por Pirâmedes (SPPe), para distinguir os tipos de farinhas de cevada nua e cervejeira. Os resultados variaram de 75,00% a 100,00% de precisão, mostrando que a avaliação da amostra pelo CVS com SPPe foi altamente precisa, representando uma técnica potencial para a classificação automática da farinha de cevada. Paralelamente, para determinar a qualidade da cevada brasileira nas indústrias alimentícias, foram estudadas 9 cultivares de cevada coberta e 8 linhagens de cevada sem casca. Foram analisados os pesos de mil grãos (TKW), peso de hectolitro (HW), taxa de descascamento (HR), proteína e conteúdo de β-glucana. As linhagens nuas apresentaram médias maiores quando comparadas ao grupo com casca, exceto no teor de proteínas. As correlações entre "β-glucanas e HW", "β-glucanas e TKW" e "TKW e HW" foram positivas. Por outro lado, “HW e proteína” e “β-glucana e proteína” apresentaram correlação negativa. Os resultados foram utilizados para qualificar uma cultivar brasileira de cevada como a mais adequada para uso em alimentos: BRS Itanema. Ela foi utilisada em um estudo clínico para avaliar os efeitos da ingestão de 3 g / dia de β-glucana de cevada, por meio da ingestão de grãos integrais e biscoitos de cevada, na resposta glicêmica, colesterol e índices antropométricos por um estudo randomizado, duplo-cego e controlado realizado com 39 indivíduos brasileiros por 45 dias. A mesma intervenção clínica teve como objetivo comparar as respostas oxidativas na dieta baseada em trigo e em cevada, medindo indicadores de estresse oxidativo em amostras de sangue, uma vez que a cevada também contém fitoquímicos, incluindo ácidos fenólicos, flavonóides, lignanas, tocóis, fitoesteróis e folato. Ambos os grupos não apresentaram redução do colesterol, mas o grupo cevada ao final do estudo (T45) apresentou diminuição da glicemia. Os indicadores oxidativos de estresse apresentaram melhores resultados para malonaldeído (MDA), capacidade antioxidante total plasmática (TRAP), catalase (CAT), glutationa reduzida (GSH) e glutationa oxidada (GSSG) no grupo que consumiu trigo. Por outro lado, no grupo cevada, apenas MDA e TRAP melhoraram durante a intervenção dietética. A conclusão foi que uma ingestão regular de β-glucana de cevada levou a reduções significativas da glicemia, mas os produtos à base de trigo parecem ser mais efetivos nas respostas oxidativas quando adicionados regularmente a dieta humana.

Palavras-chave: β-glucana. Alimento funcional. Cultivar. Detecção por imagem. Estresse oxidativo.

LUDWIG, Leniza. **Barley (*Hordeum vulgare*):** cultivar prospection, technological development and evaluation of functional characteristics. 2019. 103 p. Thesis (Degree in Food Science and Technology) – State University of Londrina, Londrina, 2019.

ABSTRACT

Barley has a wide range of end uses. However, the technological characteristics expected from barley present different standards according to the destination of the cereal. Grain β-glucan content is the most important attribute for varieties destined for the food market due to blood glucose and cholesterol-reducing properties. High protein content, test weight, and huller rate may also add value to different end uses. In Brazil, the main destination for barley is malt production; however, not every lot achieves malting standards. Flour from malting barley varieties is a valuable ingredient in the food industry, but its use is restricted due to quality aspects such as color variations and the presence of husk fragments. On the other hand, naked varieties present superior quality with better visual appearance and nutritional composition for human consumption. Computer Vision Systems (CVS) can provide an automatic and precise classification of samples, but identification of grain and flour characteristics require more specialized methods. It was proposed CVS combined with the Spatial Pyramid Partition ensemble (SPPe) technique to distinguish between naked and malting types of twenty-two flour varieties using image features and machine learning. The results ranged from 75.00% (k-NN) to 100.00% (J48) accuracy, showing that sample assessment by CVS with SPPe was highly accurate, representing a potential technique for automatic barley flour classification. In parallel, to determine the quality of Brazilian barley for food industries, 9 covered barley cultivars and 8 hull-less barley breeding lines were studied. Thousand kernel weight (TKW), hectoliter weight (HW), huller rate (HR), protein, and β-glucan contents were analyzed. The hull-less breeding lines presented higher averages when compared to the covered group, except in protein content. Correlations between "β-glucan and HW", "β-glucan and TKW", and "TKW and HW" were positive. On the other hand, "HW and protein content" and "β-glucan and protein content" presented a negative correlation. There are bromatological quality differences between Brazilian hull-less breeding lines and covered varieties. Results were used to qualify one Brazilian barley cultivar as the best barley for food uses: BRS Itanema. It was used in a clinical trial study to evaluate the effects of 3 g/day barley β-glucan intake (by eating whole grains and barley cookies) on glycemic response, cholesterol and anthropometric indexes by a randomized, double-blind, wheat-controlled intervention study conducted with 39 Brazilian individuals for 45 days. The same clinical intervention aimed to compare oxidative responses of wheat-based diet and barley-based diet by measuring stress oxidative indicators in blood samples, since whole grain barley also contains phytochemicals including phenolic acids, flavonoids, lignans, tocols, phytosterols and folate. Both groups showed no cholesterol reductions but barley group at the end of intervention time (T45) showed decrease in glycemia when compared to T0. Stress oxidative indicators showed better results for malonaldehyde (MDA), plasma total antioxidant capacity (TRAP), catalase (CAT), reduced glutathione (GSH) and oxidized glutathione (GSSG) in wheat group. On the other hand, in barley group, only MDA and TRAP improved during diet intervention. The conclusion was that a regular intake of barley β-glucan led to significant and safe reductions in glycaemia but wheat-based products seems to improve better the oxidative responses when added regularly in human diet.

Key words: β-glucan. Functional food. Cultivar. Image detection. Oxidative stress.

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LISTA DE ABREVIATURAS E SIGLAS

AMBEV	Companhia de Bebidas das Américas
ANVISA	Agência Nacional de Vigilância Sanitária
AOPP	Produtos Avançados de Oxidação Protéica
CAT	Catalase
CL-LOOH	Compostos Tert-butil Hidroperóxidos
CVS	Sistema de Visão Computacional
DNA	Ácido Desoxirribonucleico
FFT	Fast Fourier Transform
FN	False Negative
Fox-LOOH	Hidroperóxidos
FP	False Positive
GHS	Glutationa Reduzida
GLCM	Grey Level Co-occurrence Matrix
GSSG	Glutationa Oxidada
GT	Glutationa Total
HDL	Lipoproteína de Alta Densidade
HW	Peso Hectolitro
HR	Taxa de Descascamento
IBGE	Instituto Brasileiro de Geografia e Estatística
k-NN	k-Nearest Neighbors
LBP	Local Binary Patterns
LDL	Lipoproteína de Baixa Densidade
LOSO	Leave One Subject Out
MDA	Malonaldeído
ML	Aprendizado de Máquina
MLP	Multilayer Perceotron
NIRS	Near Infrared Spectroscopy
NOx	Metabólitos de Óxido Nítrico
PON1	Paraoxanase 1
RF	Random Forest
RNA	Ácido Ribonucleico
ROI	Região de Interesse

ROS	Espécies Reativas de Oxigênio
SM	Síndrome Metabólica
SOD	Superóxido Dismutase
SPPe	Conjunto de Partição de Espaço por Pirâmedes
SVM	Support Vector Machine
TKW	Peso de Mil Grãos
TN	True Negative
TP	True Positive
TRAP	Capacidade Antioxidante Total Plasmática
URL	Unidade Relativa de Luz

SUMÁRIO

1 INTRODUÇÃO

Historicamente, a cevada (*Hordeum vulgare* L.) está entre as culturas de cereais mais antigas no mundo. Evidências arqueológicas sugerem a existência de cevada no Egito, ao longo do rio Nilo, há cerca de 17.000 anos (Idehen et al., 2017). Por ser um cereal versátil e de bom aporte energético, sua aplicação consiste em uma ampla gama de usos finais. Para o consumo humano, aplica-se a cevada, majoritariamente, na produção de malte e cerveja. Os grãos também são empregados na alimentação animal, o que ajuda a explicar o porquê da cevada ser uma das culturas de cereais mais importantes do mundo, como o quarto cereal mais produzido depois do milho, trigo e arroz (Ferreira et al., 2016).

Geralmente, a cevada é classificada de acordo com seu uso. A classificação mais comum é a de cevada coberta e cevada sem casca. Esta última também é conhecida como nua é usada, principalmente, para consumo humano. Enquanto em alguns países, como o Japão, a cevada nua apresenta várias vantagens sobre as cultivares cobertas, incluindo prêmios mais altos para os produtores e demanda mais estável dos fabricantes de alimentos de cevada (Nakamine et al., 2012), no Brasil, variedades sem casca são apenas cultivadas em escalas experimentais e cevada com casca, para malte, é o principal destino do grão. Sayd et al. (2018) relataram o bom desempenho de cevada nua cultivada sob irrigação no cerrado brasileiro. No entanto, desde 1990, a área de cultivo tem diminuído porque, quando a cevada não é apropriada para o malte, o cereal é geralmente destinado, para alimentação animal, o que não é rentável para os agricultores. Além disso, problemas climáticos podem elevar os níveis de desoxinivalenol acima dos níveis aceitados por indústrias (De Mori & Minella, 2015).

Para usos alimentícios, o grão de cevada é primeiro brunido, para produzir a cevada perolada, e pode ser posteriormente processado na forma de grão, floco ou farinha. Em países ocidentais, a cevada perolada, em flocos ou moída, é usada em cereais matinais, sopas, mingau, misturas de farinha de panificação e alimentos para bebês. Nos países do Oriente Médio e da África, a cevada é perolada e moída para uso em sopa, pão achatado e mingau (Tamm et al., 2015).

O interesse pela cevada como cultura alimentar foi renovado, principalmente pelos efeitos das β-glucanas, que são os principais polissacáridos não-amiláceos presentes em vários tecidos da cevada. O consumo de β-glucana de cevada tem sido associado à diminuição do colesterol plasmático, redução do índice glicêmico e redução também do risco de câncer de cólon (Idehen et al., 2017). O conteúdo de β-glucana na cevada representa cerca de 2 a 10%, dependendo do genótipo e das condições do ambiente de cultivo (Baik & Ullrich,

2008). A cevada nua contém concentrações geralmente mais altas de β-glucana (Sterna et al., 2017), quando comparada às da cevada coberta, considerando que variedades nuas foram desenvolvidas para consumo humano em alimentos (Takeda et al., 2004).

Devido aos hábitos alimentares e estilo de vida, a Síndrome Metabólica (SM) tem se tornado uma doença de grande prevalência em diversos países, inclusive em países orientais. Em 2007, foi estimado que 50% dos homens e 20% das mulheres no Japão sofriam com esta síndrome. A Síndrome Metabólica é caracterizada por três ou mais alterações, incluindo obesidade abdominal, hipertensão arterial sistêmica, triglicerídeos séricos elevados, glicemia alta e baixos níveis de colesterol HDL (high-density lipoprotein). No Brasil, a SM já é uma realidade mesmo em crianças, variando sua prevalência entre 1,6 e 12,8% (De Moraes et al., 2009). É consenso, no entanto, que mudanças precoces nos componentes da SM estão associadas a um alto risco de desenvolver essa condição na idade adulta. A SM aumenta o fator de risco de doenças cardiovasculares associado com o aumento dos níveis de colesterol (Aoe et al., 2017).

O consumo de certos alimentos pode auxiliar na diminuição de riscos de algumas doenças crônicas, como a Síndrome Metabólica. Duas metanálises já foram publicadas reafirmando os efeitos benéficos do consumo regular de cevada, com porção mínima diária de 3 g de β-glucanas, na redução da glicemia (Ames et al., 2016) e redução do colesterol (Tiwari & Cummins, 2011). Várias agências reguladoras de alimentos aprovaram alegações de saúde para β-glucanas em produtos de cevada ou aveia para redução de colesterol (FDA, 1997; EFSA, 2011). Porém, atualmente, acredita-se que, além das β-glucanas, estes benefícios também estão associados a compostos antioxidantes presentes nestes alimentos, como carotenoides e flavonoides, que podem proteger os principais biomoléculas contra danos oxidativos. Entre os principais cereais, a cevada contém a maior quantidade de vitamina E (tocóis), que representa um importante antioxidante nos alimentos. Porém, para desenvolver novos produtos e otimizar o consumo de cevada como alimento funcional, é necessário ampliar o conhecimento sobre as variações no teor de fibra e componentes bioativos em cevada e suas relações mútuas (Sterna et al., 2017).

Nos últimos anos, o Brasil importou cerca de 350 mil toneladas anuais para suprir as necessidades domésticas e 75% da cevada destinou-se à produção de malte para a fabricação de cerveja (Ferreira et al., 2016). As cultivares brasileiras foram, ao longo dos anos, selecionadas para a indústria de malte e cervejaria, o que contribuiu para baixas concentrações de β-glucana na cevada nacional, uma vez que a fibra interfere negativamente no processo de maltagem (Lizarazo, 2003). A cevada pode ser rejeitada para malte, devido a altos níveis de

proteína, alta concentração de β-glucana ou variações de tamanho de grão; e não está claro se, após este descarte, a cevada brasileira coberta, ou cervejeira, pode ou não ser destinada à indústria alimentícia como um potencial ingrediente funcional.

Ademais, os métodos para a caracterização da cevada como matéria-prima e destinação final dentro da própria indústria de alimentos são, muitas vezes, dispendiosos e subjetivos. Necessita-se desenvolver tecnologias rápidas, não invasivas e de baixo custo para aplicação na indústria, a fim de otimizar os processos e melhor destinar os ingredientes que sofrem variações no campo para sua melhor aplicação final, potencializando suas vantagens e minimizando seus defeitos. Sykorova et al. (2009) analisaram seis variedades de grãos de cevada a fim de viabilizar análises de imagens digitais para determinação de características de formato dos grãos e obtiveram uma correlação estatisticamente aceitável para uso em escala industrial.

Pelo que sabemos, nada foi relatado na literatura aberta sobre as diferenças entre as cultivares brasileiros de cevada coberta e as linhagens brasileiras de cevada nua, seu conteúdo de β-glucana e desempenho industrial, ao considerar a aplicação de grãos para consumo humano em alimentos. Além dos aspectos tecnológicos e nutricionais, não há também estudos de intervenção em humanos que avalie a cevada brasileira como um ingrediente ou alimento funcional no Brasil, apesar da cevada ser reconhecida em muitos países como ingrediente ou alimento capaz de auxiliar na redução da glicemia e colesterolemia se consumida diariamente em porção adequada.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliação/comprovação do efeito funcional relacionado ao consumo de cevada brasileira.

2.1 OBJETIVOS ESPECÍFICOS

- Caracterizar as cultivares e linhagens de cevada quanto à performance industrial e aos teores de β-glucanas e proteínas;

- Analisar o possível efeito do genótipo e do local de cultivo na composição química e nas características físicas dos grãos;

- Desenvolver um método de análise de imagem para discriminar farinhas de cevada com casca e de cevada nua;

- Selecionar a cultivar com maior teor de β-glucanas, para ser cultivado em nova safra;

- Empregar produtos derivados do cultivar selecionado em estudo de intervenção em humanos, para avaliar o efeito destes produtos na glicemia e nos perfis lipídico e oxidativo.

3 CAPÍTULO I _ REVISÃO BIBLIOGRÁFICA

3.1 CEVADA

Taxonomicamente, a cevada (*Hordeum vulgare* L.) é um cereal, pertencente à família das gramíneas e ao gênero *Hordeum*. A sua principal característica morfológica é a inflorescência, em forma de espiga (Ullrich, 2014). A cevada foi um dos primeiros grãos cultivados juntamente com trigo, ervilha, lentilha, caprinos, ovinos e bovinos datando de cerca de 17.000 anos atrás no Crescente Fértil de Oriente Médio (Idehen et al., 2017).

A cevada foi, presumivelmente, usada pela primeira vez como alimento humano, mas evoluiu, principalmente, para a aplicação em malte e cerveja, em parte devido ao aumento da importância do trigo e arroz. Contudo, ao longo de sua história, permaneceu como uma importante fonte de energia para algumas culturas, principalmente na Ásia e norte da África (Shewry & Ullrich, 2011). A cevada é, indiscutivelmente, o cereal mais adaptado, dentre as espécies de grãos com produção em latitudes e altitudes mais elevadas (Baik & Ullrich, 2008).

Vários estudos mostraram que genótipo, fatores ambientais e a oferta de fertilizantes afetam, tanto o rendimento, como a composição de grãos de cevada (Tamm et al., 2015). Temperaturas altas aceleram o desenvolvimento das plantas, com um efeito negativo nos grãos (Ullrich, 2014). Na fase de maturação, o clima seco e quente aumenta o teor de proteína e, consequentemente, a dureza do endosperma, o que é indesejável, tanto para a produção de malte, quanto para qualidade e processamento da cerveja (Lewis & Young, 1995).

Apesar de vários cereais (sorgo, trigo e milho) poderem ser maltados, a cevada, tradicionalmente, é a mais utilizada na produção de malte para cerveja, pois ela produz enzimas de forma equilibrada e sua casca é utilizada no processo de filtração do mosto (Lewis; Young & Brewing, 1995). A composição desejável da cevada cervejeira é alto conteúdo de amido (61%) e baixo teor de proteínas (Reinold, 1997).

Paralelamente a estes parâmetros desejáveis à maltagem, há a utilização da cevada na alimentação humana, visando, entre outros benefícios nutricionais, o efeito funcional de redução do colesterol. Para estes efeitos, é desejável um alto teor de β-glucanas e proteínas, o que não condiz com a cevada cervejeira. A cevada nua é uma variedade melhorada geneticamente, que perde facilmente a casca durante a colheita. Esta variedade é rejeitada pelas cervejarias, devido ao fato de possuir alto teor de fibras alimentares solúveis (5,2-5,7%) e proteínas (15,1-16,5%), que seriam prejudiciais para o processamento da bebida (Lizarazo,

2003). Em virtude de o foco da produção de cevada nacional ser amplamente voltado à indústria cervejeira, estas variedades nuas ainda estão em pesquisa no Brasil, não sendo utilizadas por indústrias nacionais para a produção de alimentos.

3.1.1 Produção

No mundo, a produção de cevada, em 2.016, totalizou 291.577.988 toneladas em uma área de 47.661.470 ha. Porém, a produção mundial anual de 2017 caiu para 147.404.260 toneladas em uma área de 47.009.175 ha. Na União Europeia, a produção de cevada foi equivalente a 59.815.725 toneladas em 2016 e 57.261.785 toneladas em 2017. Nos Estados Unidos e Canadá foram contabilizadas 4.338.850 e 8.839.400 toneladas em 2016; e 3.090.010 e 7.891.300 em 2017, respectivamente (FAOSTAT, 2019).

Mundialmente, a principal utilização da cevada é a alimentação animal, representando 65% de toda a produção. A indústria cervejeira consome aproximadamente 33% dos grãos e, por fim, a utilização direta na alimentação humana totaliza 2% (Idehen et al., 2017).

Em 2015, 2016 e 2017 o Brasil produziu respectivamente o equivalente a 186.285, 379.687 e 300.947 toneladas de cevada em uma área de 86.409, 91.055 e 122.019 hectares (IBGE, 2019). Em 2018 e 2019, o estado do Paraná teve a maior área plantada de cevada, sendo 55.675 e 58.075 ha, e produção de 215.957 e 263.997 toneladas, respectivamente. No Rio Grande do Sul, no mesmo período, as áreas plantadas foram de 41.811 e 43.510, sendo suas respectivas produções de 93.362 e 125.921 toneladas. São Paulo teve plantados 2.100 e 2.200 ha com produção de 12.432 e 12.740 toneladas. Em Santa Catarina foram cultivados 860 e 1.350 ha e colhidos 3.330 e 4.586 toneladas (IBGE, 2019).

No Brasil, há diversas variedades de cevada disponíveis, cada uma delas com características específicas e indicadas para cultivo em determinadas regiões. No presente trabalho, as variedades estudadas estão listadas a seguir, juntamente com as características das plantas descritas pela EMBRAPA (s.d., 2019):

BRS Brau: cultivar que apresenta um porte anão, sob condições normais de desenvolvimento, chegando a 76 cm de altura. Seu rendimento médio chega a 6.000 kg/ha. Possui ciclo precoce, sendo 88 dias até o espigamento e 132 dias até a maturação. É moderadamente resistente à mancha reticular e suscetível ao oídio, mancha marrom e giberela. Seu grão é classificado como classe 1, para o malte, e atende às principais especificações da indústria cervejeira. As regiões indicadas para seu cultivo são: RS, SC e PR.

BRS Elis: cultivar que apresenta porte anão, não ultrapassando 80 cm de altura. Seu potencial produtivo é superior a 6.000 kg/ha. É uma cultivar de ciclo precoce, de aproximadamente 88 dias até o espigamento e até 130 dias para a maturação. É moderadamente resistente ao oídio, moderadamente suscetível à giberela e suscetível à ferrugem da folha, mancha reticular e mancha marrom. Responde positivamente à adubação nitrogenada e ao plantio no espaçamento de 17 cm x 34 cm (semeadura pareada). O baixo porte confere bom nível de resistência ao acamamento. O seu potencial de classificação comercial é classe 1 e média superior a 85%. Apresentando melhor performance agronômica nas zonas de primaveras mais frescas das regiões produtoras do RS, SC e PR. Foi desenvolvida pela Embrapa, em parceria com outras instituições.

BRS Korbel: foi desenvolvida pelo programa de melhoramento da Embrapa Trigo em parceria com a FAPA (Fundação Agrária de Pesquisa Agropecuária) e Ambev. É uma cultivar de cevada cervejeira de duas fileiras de grãos, porte baixo, ciclo de 125 a 135 dias, combina vantagens de alto potencial produtivo, excelente qualidade de malte e principalmente a resistência ao oídio. Tem apresentado melhor desempenho agronômico nas regiões mais frescas do sul do Brasil.

BRS Sampa: cultivar que apresenta porte anão, em média 77 cm, com produtividade média de 6.500 kg/ha. Seu ciclo é precoce, levando até 75 dias para o espigamento e 135 dias para a maturação. É moderadamente suscetível à mancha reticular e suscetível ao oídio, ferrugem da folha, mancha marrom, giberela e brusone. Sua classificação comercial é de classe 1, superior a 75%. O malte obtido dos grãos atende a todas as especificações da indústria cervejeira. Os melhores resultados em rendimento foram obtidos em semeaduras realizadas na primeira quinzena de maio. Apresenta uma boa resistência ao acamamento, devido ao seu porte baixo. As áreas de adaptação são SP e GO.

BRS Itanema: apresenta porte médio, chegando a 90cm de altura e potencial produtivo de até 7.000 kg/ha. Seu ciclo é precoce, com até 65 dias para o espigamento e 125 dias para a maturação. É moderadamente resistente à mancha reticular e é suscetível ao oídio, mancha marrom e brusone. É uma cevada cervejeira, de duas fileiras de grãos. Sua classificação comercial é classe 1 e média de 85%. Seu malte atende satisfatoriamente às principais especificações da indústria cervejeira. Apresenta ampla adaptação nas principais regiões irrigadas de SP, em altitudes superiores a 600 m e tem demonstrado desempenho agronômico competitivo também nos estados de GO, MG e DF. Foi desenvolvida pela Embrapa, em parceria com outras instituições.

BRS Manduri: cultivar que apresenta porte anão, não ultrapassando 80cm de altura sob condições normais de desenvolvimento. Seu rendimento potencial é de 6.500 kg/ha. Seu ciclo é precoce, levando cerca de 70 dias até o espigamento e 130 para a maturação. É moderadamente resistente à mancha reticular e moderadamente suscetível ao oídio e ferrugem da folha. É suscetível à mancha marrom, giberela e brusone. O baixo porte da planta confere resistência ao acamamento, e os melhores resultados em rendimento e em tamanho de grão foram em semeaduras realizadas na primeira quinzena de maio. Apresenta potencial de classificação comercial de classe 1 superior a 80%. As áreas de adaptação são: SP (irrigado), MG, Go e DF. Foi desenvolvida pela Embrapa, em parceria com outras instituições.

BRS Aliensa: cultivar cevada superprecoce desenvolvida pela EMBRAPA para a alimentação animal, cultivada majoritariamente na região do estado de São Paulo na entressafra verão/inverno por ser uma alternativa de manejo para a otimização de áreas agrícolas, produzindo grãos e mantendo cobertura no solo.

MN6021: cultivar desenvolvida pela Cia de Bebidas das Américas (AmBev) para a indústria cervejeira, cultivada majoritariamente na região do estado do Rio Grande do Sul[1].

BRS Cauê: cultivar de porte anão, com altura média de 75 cm. As melhorias na genética resultaram em cultivares com melhor padrão de qualidade para malte cervejeiro**,** maior resistência a doenças (como o oídio) e aumento de produtividade em até 15%**.** A pesquisa também gerou cultivares com ciclo ajustado às características regionais, com ampliação do período vegetativo para escape da geada. O processo de desenvolvimento da cultivar que foi conduzido com foco regional e sob encomenda por meio de parceria direta com usuário industrial. O trabalho resultou no conjunto de cinco cultivares de cevada lançadas a partir de 2008, dentre elas, BRS Cauê, com indicação para cultivo nos estados do Rio Grande do Sul, Santa Catarina e Paraná.

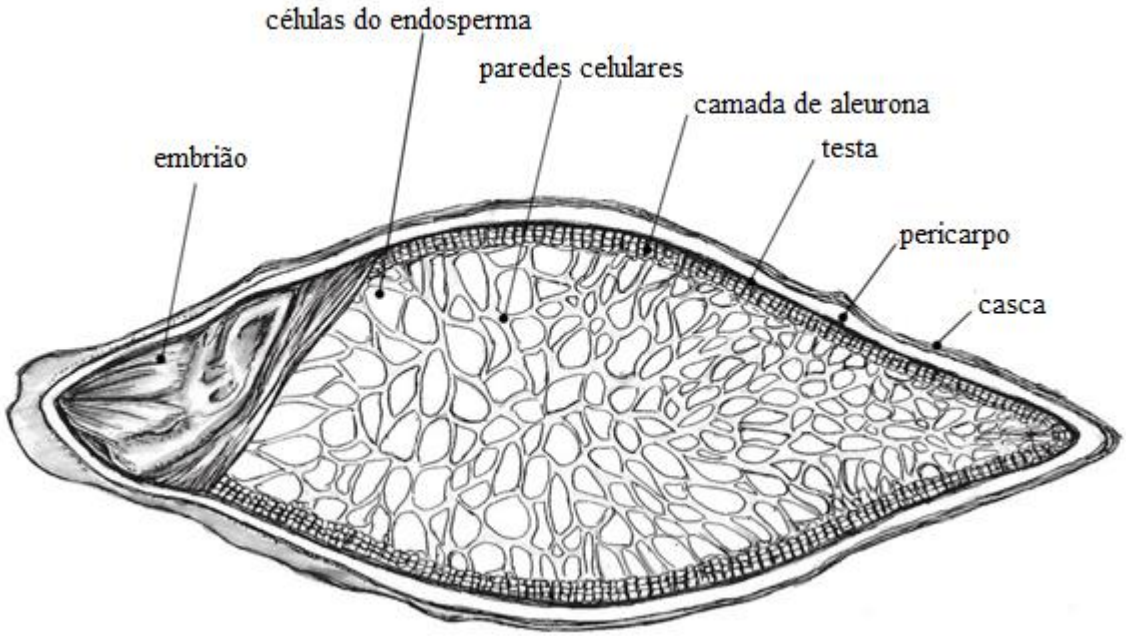
Além dos cultivares comerciais, foram estudadas oito linhagens experimentais de cevada nua. Estas linhagens foram cultivadas em Passo Fundo-RS e foram originadas por cruzamentos de materiais internacionais e nacionais.

[1] Comunicação pessoal de Vitor Antunes Monteiro, colaborador da Ambev, em 06 de outubro de 2015, recebida por correio eletrônico.

3.1.2 Morfologia do Grão

Cevada cervejeira, como arroz e aveia, é colhida com casca. A cariopse é composta pelo pericarpo, testa, aleurona, gérmen ou embrião, e endosperma (Figura 1). As células da aleurona compõem uma das camadas mais externas do grão, que é formada por duas ou três camadas celulares. É na parede das células da aleurona que a β-glucana está presente, em maior concentração, nos grãos de cevada. As paredes celulares são o terceiro maior componente do endosperma e estão constituídas principalmente por 22,2% de arabinoxilanas (pentosanas), 72,3% de (1→3)(1→4)-β-glucanas e 5,5% de proteínas (Gubatz & Weschke, 2014).

Figura 1 – Corte transversal da cariopse de cevada



Fonte: Reich, 2019

3.1.3 Composição Química

3.1.3.1 Proteínas

As proteínas têm uma grande influência na qualidade do grão de cevada. As enzimas determinam a grande maioria das reações químicas que ocorrem no grão durante todo o seu desenvolvimento, e o resultado dessas reações determina a composição final e as

propriedades do grão. Outras proteínas desempenham importantes papéis estruturais ou fornecem defesa contra ataque de patógenos, enquanto as proteínas de armazenamento servem como garantia de nutrição durante a germinação e crescimento das plântulas e influenciam o processamento e propriedades do grão. A quantidade e a composição das proteínas da cevada tem sido, portanto, objeto de intensos estudos para mais de um século. Como as proteínas são mais heterogêneas em sua estrutura, propriedades biofísicas e funções do que outras macromoléculas, como carboidratos, por exemplo, uma ampla gama de abordagens é necessária para elucidar suas estruturas, funções e interações (Svensson, 2014).

Alto teor de proteína é um fator indesejável para as indústrias de malte, que esperam apenas um teor de proteína entre 10 a 12% (Baik et al., 2011). Geralmente, o teor de proteína da cevada é altamente dependente da cultivar e difere nas condições de crescimento, particularmente na taxa e no momento da fertilização com nitrogênio. O maior teor de proteína bruta na cevada foi geralmente acompanhado por menores teores de amido e fibra alimentar. Algumas investigações mostraram que um aumento no teor de proteína foi acompanhado por uma diminuição nos aminoácidos essenciais, principalmente lisina. Entretanto, estudos científicos poloneses demonstraram que os valores nutricionais (escore químico, índice de aminoácidos essenciais e valor biológico das proteínas) em variedades de cevada nua mostraram boa qualidade protéica para animais monogástricos (Sterna et al., 2017).

A concentração de proteínas da cevada é entre 10-20% (Karboune et al., 2018), e se encontra, em sua maioria, no endosperma, na forma de uma matriz proteica, cuja quantidade e consistência variam, dependendo das condições ambientais e de manejo no campo. As proteínas da cevada podem ser classificadas, de acordo com a solubilidade, em: albuminas (hidrossolúveis) e globulinas (solúveis em soluções salinas diluídas), que são proteínas fisiologicamente ativas e estão localizadas na camada de aleurona e no gérmen. As proteínas de reserva são constituídas pelas glutelinas (solúveis em ácidos e bases diluídas) e as prolaminas ou hordeínas (solúveis em álcool 70%). Este último grupo encontra-se formando a matriz proteica do endosperma (Macgregor & Fincher, 1993). As prolaminas da cevada, assim como as procedentes do trigo e do centeio, são responsáveis pelas reações de intolerância que ocorrem em indivíduos com doença celíaca. Essas prolaminas compreendem uma mistura de peptídeos que podem ser divididos em quatro frações: B, C, γ e D. A fração D (elevado peso molecular), por formar géis, exerce maior influência sobre a qualidade do malte, apesar de corresponder a menos de 5% do total da fração proteica do grão (Macgregor & Fincher, 1993). Como todos os cereais, a cevada apresenta baixos níveis dos aminoácidos lisina, treonina e metionina (Hoseney, 1986). Porém, as proteínas de cevada podem contribuir nutricionalmente em

suplementos alimentares por suas propriedades funcionais, incluindo capacidade de emulsificação e estabilidade, formação de espuma, elasticidade, coesão e capacidade de retenção de água que melhoram as propriedades alimentares reológicas. Além disso, as proteínas da cevada constituem uma boa fonte de aminoácidos essenciais e não essenciais, como a treonina, valina, fenilalanina e arginina (Karboune et al., 2018).

3.1.3.2 Lipídeos

A concentração de lipídeos na cevada é de, aproximadamente, 3,3%. Um terço desta concentração está presente no gérmen dos grãos. Considerando que o gérmen representa apenas 3% do peso total do grão, esta proporção sugere que 30% do gérmen são constituídos por lipídeos. Os lipídeos presentes nos grãos de cevada podem ser divididos em lipídeos não polares (72%), glicolipídeos (10%) e fosfolipídeos (21%). A cevada também contém tocoferóis, na ordem de 5,0 mg/100g. Os lipídeos na cevada são similares aos encontrados nos demais cereais e os ácidos graxos são um pouco mais saturados do que os encontrados no grão de trigo (Hoseney, 1986).

3.1.3.3 Carboidratos

Os carboidratos são a maior fonte de energia do grão de cevada. Representam cerca de 70% do peso seco do grão e estão localizados, principalmente, no endosperma. O maior componente da cevada é o amido, seguido pelas fibras alimentares e por proteínas (Zhu, 2017).

O amido está armazenado no endosperma em forma de duas populações de grânulos, embebidos numa matriz de proteína. Os grânulos grandes, entre 15 e 25 µm, constituem 90% do peso do amido no grão, enquanto os pequenos, entre 2 e 5 µm, constituem os 10% restantes. O tamanho, distribuição e proporção dos grânulos grandes e pequenos dentro do endosperma parecem estar relacionados com a origem genética (Tang et al., 2000).

O amido é constituído, basicamente, por dois tipos de polímeros de α-D-glucose: a amilose, de cadeia linear e ligações α-(1→4), e a amilopectina, de cadeia ramificada com ligações α-(1→4) e α-(1→6). Estes polímeros são degradados, principalmente, por duas enzimas amilolíticas: α-amilase e β-amilase. A α-amilase cliva as ligações α-(1→4) dos dois polímeros, em posições aleatórias, enquanto que a β-amilase faz a clivagem a partir dos terminais redutores, liberando maltose. Estas enzimas têm grande importância na fermentação para produção da cerveja, pois hidrolisam o amido formando açúcares passíveis de fermentação (Lewis & Young, 1995).

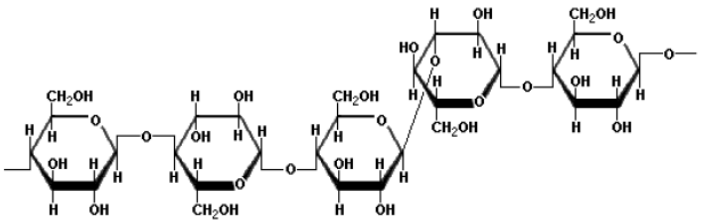
A proporção de amilose/amilopectina, em amido de diferentes genótipos de cevada, é variável. O amido ceroso (com aproximadamente 100% de amilopectina) e o de alta amilose (acima de 35%) são encontrados em diferentes cultivares. Genótipos com maior concentração de amilopectina em sua constituição são também os que apresentam maior concentração de β-glucanas (Baik & Ullrich, 2008).

3.1.3.4 Fibras

A cevada é fonte de fibras alimentares solúveis e insolúveis. As fibras insolúveis estão presentes, majoritariamente, na casca. Esta, consiste em 10% do peso seco dos grãos e é formada por celulose, hemicelulose, lignina e pequenas quantidades de proteína. Para o consumo humano, as cascas da cevada são removidas (Anderson & Aman, 2008). Já, nos grãos sem casca, arabinoxilanos, β-glucanas e celulose são os principais constituintes dos polissacarídeos não-amiláceos. Enquanto a estrutura molecular e propriedades físico-químicas e funcionais das β-glucanas têm sido extensivamente pesquisadas durante os últimos 60 anos, o papel e o potencial dos arabinoxilanos da cevada na nutrição humana e nos processos industriais ainda estão sendo investigados. Estes polissacáridos são constituídos, predominantemente, das pentoses: arabinose e xilose. Estudos recentes, revelaram o potencial, dos arabinoxilanos para a saúde (Izydorczyk, 2014).

As β-glucanas ((1,3) (1,4)-β-glucanas), fibras solúveis, são polímeros de glicose de estrutura linear, unidos por ligações glicosídicas β-(1,4) e β-(1,3) (Figura 2). A distribuição de β-glucanas nos grãos de cereais varia dependendo da variedade e, portanto, as frações de moagem correspondentes, ricas em β-glucanas, podem diferir. Frações de cevada perolada, sem casca, mostraram que o teor de β-glucanas era muito baixo nas regiões 20% mais externas do grão. Para cevada sem casca, as cultivares apresentaram β-glucanas predominantemente na região do núcleo subaleurônico. Porém, mais de 80% das β-glucanas estão distribuídos, uniformemente, no endosperma, para cultivares com alto teor de β-glucanas (Stevenson, 2009). Ludwig et al., 2018 estudaram 27 genótipos de cevada de dois locais de origem (República Checa e Espanha). As amostras incluíram cultivares de cevada nua e com casca e a variação de β-glucana no grupo foi de 2,40 até 7,42 g/100 g.

Figura 2 - Estrutura química das β-1,3 e β-1,4 -D-glucanas



Fonte: Zamora, 2011

As variações na concentração de β-glucana encontradas entre cultivares são o resultado das diferenças genéticas e ambientais. Estas variações podem ser aproveitadas por malteadores e indústrias de alimentos, na escolha de cultivares de cevadas. Um dos principais fatores ambientais que influenciam os níveis de β-glucanas parece ser a disponibilidade de água durante a maturação dos grãos. Foi relatado que condições de alta umidade podem causar uma diminuição nos níveis deste componente, de modo que altos níveis de irrigação podem reduzir seu conteúdo no grão. Condições de crescimento, localização e fatores ambientais tiveram efeitos significativos sobre o teor de β-glucanas nos cultivares de cevada sem casca (Stevenson, 2009).

Rey et al. (2009) relataram que as cevadas cerosas são as que apresentam maior concentração de β-glucanas. Porém, estas cultivares apresentam problemas significativos durante o plantio, gerando perdas de produtividade, devido à ausência de casca em algumas variedades (*hull-less barley*). Os mesmos autores relataram ainda que, pelos níveis de β-glucana serem insignificantes na casca do grão, o processo de descascamento na indústria não ocasionaria perdas nutricionais para humanos, mas a presença da casca seria um facilitador do bom desenvolvimento dos grãos no campo.

3.1.3.5 Cinzas

As cinzas são compostos inorgânicos. Os minerais encontrados na cevada são nitrogênio, fósforo, potássio, cálcio, magnésio e sódio. Eles vão se concentrando gradativamente nos grãos conforme ocorre a maturação do cereal. O acúmulo de ferro e zinco também ocorre no decorrer do ciclo (aproximadamente 1 µg por grão), assim como o acúmulo de magnésio e cobre, chegando a 0,5 e 0,1 µg por grão, respectivamente (Duffus & Cochrane, 1993).

3.1.3.6 Compostos antioxidantes

A cevada contém fitoquímicos importantes que exercem potencial efeito antioxidante em organismos vivos, quando consumida regularmente. Os principais fitoquímicos da cevada incluem compostos fenólicos (que podem ser encontrados livres ou associados a moléculas de fibras), flavonóides, lignanas, vitamina E (tocóis), esteróis e folatos. Compostos fenólicos fornecem funções essenciais na reprodução e crescimento, atuam como mecanismos de defesa contra patógenos, parasitas e predadores, bem como contribuem para a cor das plantas. Especialmente as catequinas, procianidinas e prodelfinidinas são os principais compostos na fração fenólica livre do grão de cevada, enquanto os ácidos fenólicos, como os ácidos ferúlico, cumárico e vanílico, são os principais constituintes da fração fenólica ligada (Ludwig et al., 2018). Esteróis e tocóis são os principais componentes em óleos vegetais da cevada (Idehen et al., 2017).

A cevada compete bem com outros grãos de cereais importantes, como trigo, aveia, centeio e arroz, em termos de conteúdo e diversidade de fitoquímicos (Idehen et al., 2017). Além disso, a cevada tem algumas propriedades fitoquímicas únicas, é a que possui a maior quantidade de vitamina E, que representa um importante antioxidante nos alimentos. Os tocóis, no gérmen de cevada, são predominantemente tocoferóis (cerca de 97%), enquanto os do endosperma são predominantemente tocotrienóis (80-90%). Por outro lado, o maior nível de atividade da vitamina E é produzido pelo α-tocoferol, seguido pelo β-tocoferol e α-tocotrienol, que têm 40% e 30% da atividade do α-tocoferol, respectivamente, em uma base de peso igual (Sterna et al., 2017).

Ludwig et al. (2018) estudaram 27 genótipos de cevada de dois locais de origem (República Checa e Espanha). As amostras incluíram cultivares de cevada nua e com casca e o conteúdo de tocol variou de 39,9 até 81,6 μg/g, evidenciando que a cevada deve sim ser considerada uma boa fonte de compostos bioativos, especialmente por causa do amplo espectro de fitoquímicos com potenciais benefícios para a saúde, além da fibra solúvel (β-glucana).

3.1.4 Características Tecnológicas

Além das características nutricionais e de composição química, as características geométricas dos grãos de cereais, incluindo a cevada, são muito importantes no planejamento dos processos industriais, tais como: transporte aéreo, secagem, moagem e maltagem. Em particular, o tamanho dos grãos e a sua uniformidade são importantes determinantes da qualidade da maltagem. Estes requisitos físicos interferem diretamente na eficiência da separação da cevada de eventuais materiais estranhos, bem como na classificação do cereal efetuada pelas máquinas. O formato, peso e densidade dos grãos também são significativos para o bom planejamento dos processos de secagem e benefiamento, como regulagem de temperatura, tempo de processamento, etc. Além do teste de peso, outro critério para malte de cevada é que pelo menos 85% dos grãos de cevada maltada (livre de matéria estranha) deve ser retido em uma peneira de 2,5 mm (Sykorova et al., 2009).

Os componentes presentes na cevada, tais como teores de proteína e β-glucana, além das características morfológicas dos grânulos de amido, são influenciados pelo ambiente de cultivo e podem influenciar no desempenho dos grãos na indústria. O estudo preliminar da composição, microestrutura e rendimento para processamentos específicos das matérias primas pode ser feito de forma eficiente e rentável para as diversas aplicações da cevada (Lizarazo, 2003).

O peso molecular e a solubilidade das β-glucanas são afetados não só pelo genótipo, ambiente e aporte agronômico, mas também por métodos de processamento de alimentos. O teor de β-glucanas em um produto acabado (por exemplo, pão, bolo, muffins) depende de vários fatores na cadeia de produção. As operações de processamento de alimentos afetam principalmente o peso molecular e a solubilidade das β-glucanas. Um estudo acerca dos efeitos do processamento nas características da β-glucana em pasta de semolina apontou que o processamento das massas não afetou significativamente a quantidade de β-glucana, mas afetou as propriedades físico-químicas de suas moléculas nos produtos finais. Em todas as massas, a extrusão e a secagem foram prejudiciais às propriedades da β-glucana, enquanto o cozimento aumentou significativamente a capacidade de extração destas β-glucanas e a diminuição do peso molecular da β-glucana e, por sua vez, sua viscosidade diminuiu, o que prejudica sua eficácia fisiológica (Marconi et al., 2017).

3.1.5 Novos Métodos Para Caracterização de Cereais e Derivados

Para aprovar um lote de cevada como matéria-prima e melhor destiná-lo industrialmente é necessário realizar algumas análises físicas e químicas, que geralmente são caras, consomem tempo e / ou requerem analistas e equipamentos especializados (Szczypinski, 2015). Na área agrícola e de produção industrial de alimentos, muitas vezes, são necessárias tecnologias rápidas e precisas para aumentar o desempenho de processamento, melhorando a qualidade do produto. Sensores de imagem e sistemas de visão computacional foram desenvolvidos para classificar a qualidade do produto, discriminar entre variedades e detectar contaminantes ou substâncias inerentes do próprio produto (Patrício & Rieder, 2018).

A avaliação da qualidade pode ser realizada por um Sistema de Visão Computacional (CVS) baseado em um dispositivo de aquisição (câmera digital, barato e amplamente disponível) e modelos de previsão, usando algoritmos de aprendizagem de máquina. Este tipo de abordagem apresenta várias vantagens, incluindo rapidez, baixo custo e precisão. Pode ser aplicado a grãos / sementes (Sabanci et al., 2016; Aslan et al., 2017), farinhas (Kurtulmu et al., 2014), ou outros subprodutos agrícolas. Sykorova et al. (2009) avaliaram variedades de cevada para testar o potencial de uso da análise digital de imagens para determinar as características do tamanho do grão, obtendo resultados satisfatórios. Para atender à demanda por produtos de alta qualidade, os grãos são classificados de acordo com suas características, antes de serem enviados para processamento. Estudos visam determinar a geometria e a cor do grão, com a finalidade de identificar as espécies, variedades e tipos de contaminação microbiológica, bem como a extensão dos danos mecânicos e / ou térmicos (Lopes et al., 2019).

Sendo não invasivos e não empregando reagentes químicos, esses métodos podem ser considerados como tecnologias ecológicas. Inspeção humana por exame visual exige muito tempo, é entediante e ineficiente. Além disso, a inspeção manual de produtos em processo é difícil, considerando a amostragem em linhas de processamento. A visão de máquina é adequada para essa tarefa, sendo uma alternativa econômica e rápida para a inspeção de processamento de alimentos (Mittal, 1996). Adulteração, contaminação ou, simplesmente, classificação de produtos, de acordo com suas características visuais são uma necessidade comum no processamento de alimentos. É essencial investigar métodos objetivos que possam quantificar os aspectos visuais dos produtos alimentares (Foca et al., 2011).

Em relação a todas as técnicas livres de produtos químicos disponíveis, ainda existem alguns desafios comuns, antes da implementação de resultados de pesquisas de laboratório recentes para aplicações industriais, como a construção de algoritmos inovadores

de análise de dados que possam filtrar completamente as informações redundantes e a exploração de técnicas estatísticas apropriadas para melhorar a robustez do modelo de operações em tempo real (Su & Sun, 2018).

3.1.6 Benefícios a Saúde

3.1.6.1 Ação biológica das β-glucanas

As β-glucanas são fibras solúveis. Estas fibras solúveis se dissolvem em água, formando géis viscosos. Não são digeridas no intestino delgado e são facilmente fermentadas pela microflora do intestino grosso (Bernaud & Rodrigues, 2013). A característica já conhecida do consumo regular da cevada é o efeito na redução dos níveis de colesterol e glicemia, devido à ingestão de fibras solúveis do tipo β-glucana. Acredita-se que este efeito depende da capacidade de formar uma camada viscosa na superfície do intestino delgado, retardando o esvaziamento gástrico, a digestão e a absorção de moléculas como a glicose e o colesterol dietético nos ácidos biliares. Além disso, a redução dos níveis de glicose no sangue está associada a ácidos graxos de cadeia curta, formados pela fermentação microbiana de carboidratos (Ames et al., 2016; Baldassano et al., 2018).

Diferentes artigos sugeriram uma modulação da saciedade e da liberação de hormônios intestinais pelas β-glucanas (Alminger & Eklund-Jonsson, 2008; Ames et al., 2015; Chillo et al., 2011; Pentikäinen et al., 2014). No geral, os estudos clínicos indicam que o consumo de alimentos com β-glucanas pode alterar os níveis de glicemia, lipídios e hormônios intestinais, auxiliando na prevenção da obesidade (Baldassano et al., 2018). Entretanto, O efeito sobre a glicemia pós-prandial depende do peso molecular das β-glucanas, da solubilidade e da quantidade ingerida (Ekstrom et al., 2017).

3.1.6.2 Ação biológica dos compostos antioxidantes

Radicais livres são moléculas que contém um ou mais elétrons desemparelhados no seu orbital e, portanto, apresentam altíssima avidez para reagir com átomos de outras moléculas. Os radicais livres são inativados por substâncias antioxidantes existentes, tanto no meio extracelular dos tecidos (albumina, ceruloplasmina, vitamina A, vitamina E, vitamina C, betacaroteno, superóxido dismutase extracelular e transferrina) como no meio intracelular (glutationa peroxidase, superóxido dismutase intracelular e catalase). O consumo

de certos tipos de alimentos pode auxiliar na prevenção de algumas doenças crônicas, como obesidade e diabetes. Acredita-se que estes benefícios estão associados a compostos antioxidantes presentes nestes alimentos, como carotenoides e flavonoides, que podem proteger os principais biomoléculas contra danos oxidativos. Estes danos ou estresse oxidativo é um processo que ocorre no metabolismo e influencia diretamente na sinalização, apoptose, expressão gênica e transporte de íons. Concentrações expressivas de espécies reativas de oxigênio (ROS - reactive oxygen species) podem ter efeito deletério em uma gama expressiva de moléculas, incluindo proteínas, lipídios, RNA e DNA. O estresse oxidativo também está associado à formação e progressão de placas de ateroma, por meio da oxidação da lipoproteína do tipo LDL, aumentando interações entre monócitos e células endoteliais e estimulando a proliferação de células musculares lisas e a síntese de fatores de crescimento (Cammerer et al., 2018).

O equilíbrio entre os processos oxidativos e níveis de antioxidantes constitui a condição normal da vida aeróbica. O desequilíbrio favorável ao estado pró-oxidante desencadeia o chamado estresse oxidativo. Quando uma cadeia de reações oxidativas não é devidamente contrabalançada pelos antioxidantes, resulta em estresse oxidativo pelo organismo (Rodrigues, 2007).

Estudos epidemiológicos têm associado também o consumo regular de cevada com seu potencial de reduzir o risco de certas doenças e esses potenciais terapêuticos são atribuídos à presença de componentes bioativos de vitaminas, minerais, fibras e outros fitoquímicos (Idehen et al., 2017).

3.1.6.3 Estudos clínicos com cevada

Há diversos estudos clínicos relatados em literatura com o objetivo de comprovar os efeitos funcionais da cevada, principalmente na redução do colesterol total e LDL, reduzindo, por consequência, o risco de doenças cardiovasculares.

Behall et al. (2004), fez intervenções em dezoito pacientes hipercolesterolêmicos, de ambos os sexos, com faixa etária de 28 a 62 anos, por meio da introdução de cevada na dieta, por sete semanas, sendo as duas primeiras uma etapa apenas de adaptação. O esquema alimentar na fase de intervenção substituiu 20% do total de calorias consumidas (2.800 kcal) por arroz integral e trigo integral, sendo este o grupo controle, ou cevada e arroz integral (primeiro grupo experimental), ou apenas cevada (segundo grupo experimental). Ao final da intervenção foram relatadas reduções de até 20% no colesterol total,

24% no LDL, 16% nos triacilgliceróis e aumento de até 18% do HDL (*high-density lipoproteins*) dos pacientes que compunham o segundo grupo experimental.

Coleman et al. (2009) desenvolveram metanálise com o objetivo de determinar uma correlação entre o consumo de cevada e a redução de colesterol no plasma sanguíneo de seres humanos saudáveis e seres humanos hipercolesterolêmicos. Oito estudos foram selecionados, com tempo de intervenção variando de quatro a doze semanas. Os alimentos oferecidos foram cevada perolada, concentrado líquido de cevada, extrato de β-glucana na forma de gel, farelo de cevada, farinha de cevada em formulações de pães e cevada em flocos. A quantidade de β-glucanas consumida diariamente nos diferentes estudos variou de 3 a 10 gramas. Após a compilação de dados, os resultados evidenciaram que os participantes que se alimentaram de cevada tiveram significativas reduções no colesterol total, sendo a média de redução igual a 13 mg/dL. Também os níveis de LDL e triacilgliceróis reduziram 10 mg/dL e 12 mg/dL, respectivamente, entre os participantes fora do grupo controle. Esta metanálise comprovou os efeitos funcionais da β-glucana presente na cevada, quando incorporada à alimentação de indivíduos saudáveis e também de indivíduos hipercolesterolêmicos.

Outra metanálise importante foi realizada por Abumweis et al. (2010). Onze estudos foram selecionados para a compilação de dados por meios estatísticos. O tempo de intervenção destes estudos variou entre quatro e doze semanas e o número de participantes variou de 14 a 62, incluindo homens e mulheres de diferentes idades. Os alimentos ofertados incluíam matrizes alimentares líquidas (sucos com concentrados) e sólidas (grãos, farinhas, *muffins* e pães). A ingestão diária de β-glucana entre os participantes variou de 3 a 10 gramas. Foi concluído que o consumo de cevada, ou da β-glucana proveniente da cevada, incorporadas em diferentes matrizes alimentares está associado à redução significativa de colesterol total e LDL.

Em estudo de intervenção com homens hipercolesterolêmicos entre 30 e 70 anos de idade, o consumo diário de cevada rica em β-glucanas reduziu, não só o colesterol do tipo LDL, mas também a gordura visceral destes indivíduos. No entanto, a gordura visceral foi reduzida quando as refeições continham 30% de cevada e o mecanismo para essa redução ainda não foi totalmente elucidado (Aoe et al., 2017).

Estudos epidemiológicos têm associado, também, o consumo regular de cevada com seu potencial de reduzir o risco de certas doenças, como doença cardíaca crônica, câncer de cólon, pressão alta e cálculos biliares. Há relatos do papel da cevada na manutenção de um cólon saudável, induzindo imunoestimulação e, geralmente, impulsionando o sistema imunológico. Esses potenciais terapêuticos são atribuídos à presença de componentes bioativos,

como: vitaminas, minerais, fibras e outros fitoquímicos. À β-glucana é creditado o principal efeito da cevada para a saúde; entretanto, evidências suficientes sustentam que os fitoquímicos também desempenham papéis importantes na diminuição de riscos do desenvolvimento de doenças crônicas (Idehen et al., 2017).

Em um estudo para avaliação da capacidade antinflamatória e funcional do consumo de cevada, foram estudados os efeitos da ingestão, por 30 dias, de macarrão enriquecido com 6% de β-glucana, consumindo-a apenas quatro vezes por semana. Ocorreu uma diminuição significativa de lipoproteína de baixa densidade (LDL), colesterol total e interleucina-6, confirmando a capacidade das β-glucanas de diminuir o estresse oxidativo e o estado inflamatório, por meio da quantificação de indicadores biológicos no sangue os voluntários (Barera et al., 2016).

3.1.6.4 Alimentos funcionais – esclarescimentos e alegações de saúde

Alimentos funcionais são produtos alimentícios que alegam propriedades metabólicas ou fisiológicas de algum de seus componentes, capazes de interferir no crescimento, desenvolvimento e manutenção das funções normais do organismo humano. Esses alimentos podem também ter alegações de saúde que consistam na redução do risco de doenças.

Para se registrar um alimento industrializado como funcional, é necessário, além da comprovação científica da funcionalidade, um pedido formal direcionado à Agência Nacional de Vigilância Sanitária (ANVISA), órgão público brasileiro que visa proteger o consumidor. Além de declarar a sua finalidade, a formulação do produto e a metodologia que avaliou o objeto da alegação, critérios mais elaborados devem comprovar cientificamente suas propriedades funcionais. O processo se inicia com a comprovação científica da funcionalidade, que deve ocorrer por meio de evidências obtidas em ensaios bioquímicos, clínicos, nutricionais, fisiológicos e toxicológicos. Além disso, a alegação pode ser reforçada por estudos epidemiológicos e o produto precisa, comprovadamente, não trazer danos à saúde da população no seu uso tradicional e sem supervisão médica. Na rotulagem, a alegação de funcionalidade do alimento não pode fazer referência à cura ou prevenção de doenças (somente à diminuição de risco para doenças), apresentar atributos ou propriedades que não possam ser demonstrados, indicar propriedades medicinais ou terapêuticas e ser diferente daquela aprovada para constar em sua rotulagem. Ainda, novos alimentos sem propriedades funcionais que requeiram registro na ANVISA devem declarar "O Ministério da Saúde adverte: Não existem evidências científicas comprovadas de que este alimento previna, trate ou cure doenças". Em

abril de 1999, o Ministério da Saúde, por intermédio da Agência Nacional de Vigilância Sanitária (ANVISA), aprovou os regulamentos técnicos para análise e comprovação de propriedades funcionais e/ou de saúde alegadas em rótulo de alimentos e para registro de alimentos com tais alegações (ANVISA, 1999).

Alimentos funcionais podem ser obtidos utilizando-se cereais como ingredientes principais em uma formulação (Ferrari et al., 2009). O aumento do interesse pela cevada, como ingrediente alimentar humano, resulta de estudos que mostraram que a cevada é uma excelente fonte de fibra alimentar, em particular, as β-glucanas. A relação entre consumo de alimentos ricos em fibras solúveis, especialmente β-glucana, e risco reduzido de doença cardíaca, levou à primeira alegação de saúde associada a um alimento específico pela Food and Drug Administration (FDA, 1997). O mesmo órgão declarou que a ingestão diária de 3 g de fibra solúvel (β-glucana) de cevada ou aveia pode reduzir o risco de doenças cardíacas, redução do colesterol total e da lipoproteína de baixa densidade colesterol (LDL-C) (FDA, 2005), o que também é aceito pela Autoridade Europeia para a Segurança dos Alimentos (EFSA, 2011).

No Brasil, a alegação funcional para produtos de aveia é reconhecida formalmente. De acordo com a ANVISA (1999), a β-glucana da aveia auxilia na redução da absorção de colesterol, desde que seu consumo esteja associado a uma alimentação equilibrada e hábitos de vida saudáveis. Porém, nacionalmente, a β-glucana da cevada não tem, ainda, sua alegação funcional formalmente reconhecida.

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4 CAPÍTULO II _ COMPARISON AND QUALITY EVALUATION OF HULL-LESS AND COVERED BRAZILIAN BARLEY FOR FOOD INDUSTRY APPLICATION

Abstract

Barley has a wide range of end uses. However, the technological characteristics expected from barley present different standards according to the destination of the cereal. Grain β-glucan content is the most important attribute for varieties destined for the food market due to blood glucose and cholesterol-reducing properties. High protein content, test weight, and huller rate may also add value to different end uses. In Brazil, the main destination for barley is malt production; however, not every lot achieves malting standards. To determine the quality of Brazilian barley for food industries, 9 covered barley cultivars and 8 hull-less barley breeding lines were studied. Thousand kernel weight (TKW), hectoliter weight (HW), huller rate (HR), protein, and β-glucan contents were analyzed. The hull-less breeding lines presented higher averages when compared to the covered group, except in protein content. Correlations between "β-glucan and HW", "β-glucan and TKW", and "TKW and HW" were positive. On the other hand, "HW and protein content" and "β-glucan and protein content" presented a negative correlation. There are bromatological quality differences between Brazilian hull-less breeding lines and covered varieties. Brazilian barley germplasm presents great industrial potential, not only for malt production and animal feed but also for human food applications.

Keywords: β-glucan; hectoliter weight; *Hordeum vulgare*; naked barley; protein.

COMPARAÇÃO E AVALIAÇÃO DA QUALIDADE DE CEVADAS BRASILEIRAS NUAS E CERVEJEIRAS PARA APLICAÇÕES EM INDÚSTRIA DE ALIMENTOS

Resumo

A cevada tem diversas aplicações como produto final. Suas características bromatológicas e tecnológicas determinam sua melhor finalidade. O conteúdo de β-glucana dos grãos é o atributo mais importante para a destinação ao mercado de alimentos, devido a propriedades funcionais de redução da glicemia e colesterolemia. A quantidade de proteína, testes de peso e taxa de descascamento também podem influenciar na destinação do cereal. No Brasil, a principal aplicação da cevada é o malte, porém, nem todos os lotes são aprovados pelos padrões exigidos pelas maltarias. Para determinar o potencial da cevada brasileira na indústria de alimentos, foram estudados 9 cultivares de cevada com casca e 8 linhagens sem casca. Foram analisados o peso de mil sementes (TKW), peso de hectolitro (HW), percentual de casca (HR), proteína e β-glucana. As linhagens sem casca apresentaram médias maiores nos atributos avaliados, exceto no teor de proteína. Correlações entre "β-glucana e HW", "β-glucana e TKW" e "TKW e HW" foram positivas. Por outro lado, "HW e teor de proteína" e "β-glucana e teor de proteína" tiveram correlação negativa. Ficou claro que há diferenças na qualidade bromatológica entre a cevada brasileira com e sem casca. Não obstante, o germoplasma brasileiro tem grande potencial industrial, não só para malte e ração animal, mas também para aplicações em alimentos para humanos.

Palavras-chave: β-glucana; peso do hectolitro; *Hordeum vulgare*; proteína; cevada nua.

4.1 INTRODUCTION

Barley is among the most ancient cereal crops grown in the world. Archeological evidence suggests the existence of barley in Egypt along the River Nile around 17,000 years ago (Idehen et al., 2017). A broad range of end uses, such as human consumption, malt for the brewing and distilling industry and animal feeding, makes barley one of the most important cereal crops in the world ranking as the fourth most produced cereal after maize, wheat, and rice (Ferreira et al., 2016). Generally, barley is classified according to its use. The most common classification is covered and hull-less barley, also known as naked, mainly used for human consumption. While in some countries, such as Japan, hull-less barley has several advantages over covered cultivars, including higher crop prices for the farmers and more stable demand from barley food manufacturers (Nagamine et al., 2012), in Brazil, hull-less varieties are only cropped in experimental scales and malt is the main destination for barley grain. An assessment of genetic diversity in Brazilian barley using SSR markers pointed out that the number of alleles detected in genotypes released in the 1980s was higher, whereas most of the cultivars released thereafter showed lower polymorphism information content, clustered in separate subgroups from the older cultivars (Ferreira et al., 2016). The same study recommended the use of a more diverse panel of genotypes in order to exploit new alleles in Brazilian barley breeding programs. Sayd et al. (2018) reported good performance of hull-less barley cropped under irrigation in the Brazilian savanna. However, since 1990, the acrage has been decreasing because when barley is not appropriated for malt production, the cereal is used for animal feed, which is not profitable for farmers (De Mori & Minella, 2015). High protein content is an undesirable quality issue for malt industries, which expect protein content between 10 to 12%. Grain size uniformity is also expected for malt, which could be a problem in six-row barley varieties (Baik et al., 2011).

For food uses, barley grain is first abraded to produce pot or pearled barley, after which it can be further processed into grits, flakes, and flour. In Western countries, pearled barley, whole, flaked, or ground, are used in breakfast cereals, stew, soups, porridge, bakery flour blends, and baby foods. In Middle Eastern and African countries, barley is pearled and ground, and used in soup, flatbread, and porridge (Tamm et al., 2015). Interest in barley as a food crop has been renewed, caused mainly by the beneficial effects of β-glucan. Mixed linkage (1→3)(1→4)-β-D-glucans are the major non-starch polysaccharides present in various tissues of barley. Barley β-glucan has been associated with lowering plasma cholesterol, reducing the glycemic index, and reducing the risk of colon cancer (Idehen et al., 2017). The β-glucan content in barley is around 2% to 10%, depending on the genotype and conditions of the growing environment (Baik & Ullrich, 2008). Hull-less barley generally contains higher

concentrations of this soluble fiber (Šterna et al., 2017), when compared to covered barley, considering that hull-less varieties were developed for human consumption in foods (Takeda et al., 2004).

In the recent years, Brazil imported approximately 350 thousand tons of barley annually to supply domestic needs, and 75% of this was destined to produce malt for beer brewing (Ferreira et al., 2016). Over the years, Brazilian cultivars have been selected for the malt and brewery industry, which has contributed to low β-glucan concentrations in national barley, since fiber interferes negatively in the malting process (Lizarazo, 2003). However, despite being genetically designed, climate conditions can affect barley quality. After being rejected for malt production, due to high protein levels, high β-glucan concentration, or grain size variations, it is not clear if covered barley can or not be destined for the food industry to maintain the profits for farmers.

To the best of our knowledge, nothing has been reported in the open literature about the differences between Brazilian covered barley cultivars and hull-less Brazilian barley lines, their β-glucan content and industrial performance when considering applying grain for human consumption in food. The aim of this study was to evaluate the variation in food quality characteristics of hull-less and covered Brazilian barley, comparing quality standards for the food industry and malting processes, and to determine if covered Brazilian barley is suitable for human consumption in food rather than malt.

4.2 Material and Methods

Nine different covered barley cultivars (BRS Brau, BRS Cauê, BRS Elis, BRS Itanema, BRS Korbel, BRS Manduri, BRS Sampa, MN 6021 and BRS Aliensa) (Embrapa, 2019) and eight breeding lines of hull-less barley were studied**.** Field experiments were conducted during the winter of 2015, in three experimental trials on value for cultivation and use of Brazilian Agricultural Research Corporation (Embrapa Trigo) located in three field sites, Taquarivaí (SP), Passo Fundo (RS) e Vitor Graeff (RS), Brazil. Five cultivars (BRS Brau, BRS Korbel, BRS Elis, MN6021 and BRS Cauê) were cultivated in two different areas, Passo Fundo, RS (PF), and Victor Graeff, RS (VG). The barley cultivars were assessed using a completely randomized design, and each cultivar, at the site, had three replicates. Table 1 shows the cultivars accordingly to ecological and agronomical adaptation and its respective site location. The soil in the three site locations is classified as red latosol (Santos et al., 2018). Due to the limited number of seeds, the breeding lines were cultivated with one replication, at one site,

Passo Fundo, RS.

Barley samples were evaluated for bromatological characteristics at the Laboratory of Food Science of State University of Londrina, Londrina, PR.

Table 1 – Barley, site location and respectively environmental data

Tabela 1 – Cultivares e linhagens de cevada, seus respectivos locais de cultivo e dados ambientais

Cultivar/Line	Site Location	Latitude	Longitude	Altitude (m)	Precipitation* (mm)
BRS Sampa	SP	23º 55' 28" S	48º 41' 35" W	555	720
BRS Aliensa	SP				
BRS Mandurí	SP				
BRS Itanema	SP				
BRS Brau	VG	28º 33' 37" S	52º 44' 54" W	411	1050
BRS Korbel	VG				
BRS Elis	VG				
MN 6021	VG				
BRS Cauê	VG				
BRS Brau	PF	28º 15' 46" S	52º 24' 24" W	687	1050
BRS Korbel	PF				
BRS Elis	PF				
MN 6021	PF				
BRS Cauê	PF				
149853	PF				
149852	PF				
149857	PF				
149858	PF				
149846	PF				
149841	PF				
149859	PF				

*INMET, 2017

4.2.1 β-glucan Concentration

β-Glucans were determined with a mixed-linkage β-glucan detection assay kit Megazyme_International Ltd., Wicklow, Ireland), according to the AACC32-23.01 method (AACC, 1999). The moisture content of all samples was determined with a Precisa HA60 IR moisture analyzer (Precisa Instruments, Diekinton, Germany). Data were reported on a dry basis and are the mean values of six replications.

4.2.2 Protein

Nitrogen content in barley grains was determined by the Kjeldahl method (AOAC, 1995). Protein concentration was obtained using the 6.25 conversion index. Samples were analyzed in duplicate, and dry basis results were expressed in percentage.

4.2.3 Thousand Kernel Weight (TKW)

Thousand kernel weight was determined by weighing a hundred grains using an analytical balance (Kern-Sohn, Balingen, Germany) and multiplying the average by 10, according to the AACC method 55-10.1 (AACC, 1983). Six replicates were analyzed.

4.2.4 Hectoliter Weight (HW)

Hectoliter weight was determined six times in a hectoliter weight apparatus (Mediza, Panambi, Brazil) and expressed in kg hL^{-1} (Brasil, 2009). Hectoliter weight was calculated by multiplying the weight in kilograms of a quarter-liter of barley grains by 100 and dividing into 1 L volume.

4.2.5 Grain Size (G > 2mm)

Grains larger than two millimeters were determined by sieving 50 grams of barley grains for one minute using a 2 mm sieve, in Granutest equipment (T model, Produtest, Paulinea, Brazil). The weight of grains retained in the sieve was determined, and the results were expressed as a percentage of initial weight. Six replications were performed for the analysis.

4.2.6 Hulled Rate (HR)

Fifty grams of barley were hulled in a laboratory huller equipment (Codema Inc., Maple Grove, USA) for 75 seconds. After hulling, caryopsis weights were measured, and results were expressed as a percentage of initial weight. The analysis was performed six replications in each sample. However, in order to avoid false high efficiency in the dehulling process, caryopsis that were hulled at the end of the 75 seconds were separated and quantified as a percentage of total weight after the dehulling process. Results of hulled rate were expressed as two percentages: the percentage of weight that left the dehulling process and the percentage of dehulled caryopsis combining the two data. Six replications were performed in each sample for the analysis.

4.2.7 Statistical Analysis

Results shown in Table 2 had the data processed by the statistical software program Statistica 7 for ANOVA (analysis of variance). Significant differences between means were tested with the Tukey test ($p \leq 0.05$). Pearson correlation coefficients were calculated by the 3.4.1 version of R Core Team (2017) Statistical Software. The results shown in Table 4 were calculated by a completely randomized factorial design (2 x 5), with ten treatments by two different location (Passo Fundo and Victor Graeff) and five barley cultivars. Data were submitted to analysis of variance (ANOVA) and means were compared by the Tukey test at 5% of significance.

4.3 Results and Discussion

Results of thousand kernel weight (TKW), hectoliter weight (HW), hulled rate (HR), β-glucan, and protein concentrations are presented in Table 2. Results of grain size (G > 2mm) were not included as there were no differences between analyzed samples.

The percentage of grains larger than 2 mm varied from 96.84 to 100% with an average of 98.79 ±1.13%. Barley grain size is traditionally used to evaluate the commercial quality of covered barley. High quality malt is expected from large barley grains (Brasil, 1996). The coefficients of variation for the kernel size parameter were low, indicating that barley grain samples presented uniformity in size. For food industry engineering processes, such as air transport, drying, milling and malting, geometric features of cereal grain, including barley, are very important. Kernel size and shape influence the electrostatic separation of barley from extraneous material, as well as the development of sizing and grading machinery (Sykorova et al., 2009).

The thousand kernel weight (TKW) is a typical indicator of the mean kernel size (Sykorova et al., 2009). Thousand-kernel weight is a predictive physical analysis for cereal grains as it is directly proportional to starch content and grain filling, which might improve yield in industrial processes.

Table 2 – Results of Thousand Kernel Weight (TKW), Hectoliter Weight (HW), Hulled Rate (HR), β-glucan and protein concentration of covered and hull-less barley

Tabela 2 – Resultado de peso de mil sementes (TKW), peso hectolitro (HW), índice de descascamento (HR), concentração de β-glucanas e de proteínas nas amostras de cevadas com e sem casca

	Site location	TKW (g)	HW (kg hL^{-1})	HR (%)	β-glucan (%)	Protein (%)
BRS Sampa	SP	36.15 cdef (±0.20)	68.40 c (±0.57)	91.05 defg (±1.02)	3.87 bcd (±0.30)	10.96 b (±0.09)
BRS Aliensa	SP	39.12 efgh (±0.12)	66.40 bc (±0.57)	85.55 bcdefg (±3.51)	3.57 abcd (±0.04)	12.55 f (±0.04)
BRS Mandurí	SP	32.97 bcd (±0.20)	68.40 c (±0.56)	89.09 cdefg (±0.29)	3.49 abcd (±0.19)	10.88 b (±0.06)
BRS Itanema	SP	45.48 i (±0.17)	68.80 c (±5.65)	91.88 defg (±0.46)	4.16 d (±0.29)	11.16 bcd (±0.06)
BRS Brau	PF	35.36 cde (±0.21)	63.40 bc (±0.85)	80.97 abcdef (±1.04)	3.26 abcd (±0.16)	12.63 f (±0.03)
BRS Brau	VG	31.66 bc (±0.08)	60.40 abc (±0.57)	81.60 abcdefg (±1.33)	2.87 abc (±0.19)	11.71 e (±0.08)
BRS Korbel	PF	40.22 fgh (±0.10)	59.20 abc (±4.52)	76.71 abcd (±3.62)	2.82 ab (±0.14)	11.43 cde (±0.09)
BRS Korbel	VG	29.91 b (±0.12)	52.60 a (±1.41)	69.66 a (±4.04)	3.41 abcd (±0.28)	10.49 a (±0.09)
BRS Elis	PF	35.42 cde (±0.12)	57.80 ab (±7.07)	86.25 bcdefg (±1.22)	3.21 abcd (±0.04)	12.82 f (±0.06)
BRS Elis	VG	29.72 b (±0.13)	59.20 abc (±0.57)	73.34 ab (±0.31)	3.07 abcd (±0.14)	13.59 g (±0.04)
MN 6021	PF	36,57 defg (±0.22)	61.60 abc (±2.26)	79.57 abcde (±5.98)	3.68 abcd (±0.22)	12.76 f (±0.02)
MN 6021	VG	24.96 a (±0.19)	56.80 ab (±1.13)	78.87 abcde (±5.18)	3.35 abcd (±0.10)	12.53 f (±0.05)
BRS Cauê	PF	35.42 cde (±0.12)	57.00 ab (±1.41)	74.72 abc (±0.57)	2.98 abcd (±0.14)	ND (±0.08)
BRS Cauê	VG	35.42 cde (±0.12)	52.20 a (±0.85)	66.65 a (±3.19)	2.56 a (±0.15)	ND (±0.03)
AVERAGE of covered barley		34.87 A	60.87 A	80.42 A	3.31 A	13.03 A
149855	PF	40.31 fgh (±0.01)	78.2 d (±0.28)	97.43 fg (±0.04)	3.75 abcd (±0.10)	11.12 bcd (±0.12)
149853	PF	40.55 fgh (±0.06)	75.4 d (±0.28)	98.12 g (±1.98)	3.39 abcd (±0.30)	11.18 bcd (±0.25)
149852	PF	40.65 fgh (±0.03)	76 d (±0.54)	97.16 fg (±0.36)	3.92 bcd (±0.21)	11.43 cde (±0.08)
149857	PF	41.08 ghi (±0.08)	75.6 d (±0.56)	95.65 efg (±0.07)	4.15 d (±0.27)	11.07 bc (±0.12)
149858	PF	41.18 hi (±0.08)	76.4 d (±0.57)	96.56 fg (±0.31)	4.12 d (±0.24)	11.39 cde (±0.04)
149846	PF	41.31 hi (±0.03)	75.2 d (±0.56)	94.43 efg (±0.52)	4.03 cd (±0.28)	11.65 e (±0.04)
149841	PF	42.03 hi (±0.03)	78 d (±0.57)	97.96 g (±0.42)	3.44 abcd (±0.13)	10.46 a (±0.06)
149859	PF	42.31 hi (±0.12)	75.2 d (±0.57)	96.70 fg (±0.76)	3.96 bcd (±0.14)	11.44 de (±0.07)
AVERAGE of hull-less barley		41.18 B	76.25 B	96.75 B	3.85 B	11.21 B

SP: São Paulo, VG: Victor Graeff, PF: Passo Fundo / ND: not determined due to insufficient material

Means followed by the same lower letters in the column indicate that there was no significant difference ($p \geq 0.05$) between samples. Same capital letters in the column indicate there was no significant difference between averages of covered and hull-less barley grains.

Results of the TKW showed that hull-less grains are denser than the majority of covered barley analyzed, and the hull-less lines were not statistically different from each other. From the covered barley group, BRS Itanema presented great grain filling, and together with BRS Aliensa, BRS Korbel (PF) and MN6021 (PF) was similar to some hull-less varieties.

Sykorova et al. (2009) found TKW results between 38 and 50 grams for barley cropped in the Czech Republic, which matches some results found in this paper. MN6021 (VG) was the worst cultivar in grain filling with only 24.96 grams in TKW. Negamine et al. (2012) affirm that TKW had a negative correlation to the pearling time, which means that high TKW is good for food processing. TKW also has a positive correlation with grain size and barley quality (Nagamine et al., 2012), so a high-quality classification is expected in malting and food processes for BRS Itanema, followed by BRS Aliensa (PF) and BRS Korbel (PF). Although hull-less varieties presented high TKW, high quality classification is expected only in food processes, since husks are lost during harvest, which is a problem for malting processes (Baik et al., 2011). Rey et al. (2009) also found higher plump kernels in hull-less lines when compared them with covered varieties.

Volume weight is a key quality characteristic, especially for farmers, as one of the quality bonus characteristics. In Japan, the standard value for a quality bonus when purchasing barley for food industries is 840 g L^{-1} (Nagamine et al., 2012). Hectoliter weight is a measure of grain sample density, which can be an indicator of pre-harvest sprouting adversely affecting the grain. High hectoliter in barley samples indicates good performance in the malting process.

The Grain Industry Association of Western Australia determines barley as Class 1 for malt grain with HW up to 65.0 kg hL^{-1} and barley Class 2 grain with HW between 63.0 and 64.9 kg h L^{-1} (GIWA, 2010). According to this standard, BRS Sampa, BRS Aliensa, BRS Manduri, and BRS Itanema from the malting barley group were classified as Class 1, which means all samples cropped in Taquarivaí, (SP), indicating good climate conditions for barley production in São Paulo in 2015. Only BRS Brau (PF) was classified as Class 2, and other covered samples cultivated in the South of Brazil were Class 3, which is not desirable for farmers or the malt industry.

Hull-less samples had the highest HW in this research. Rey et al. (2009) also found higher HW in hull-less lines when compared to covered barley, which varied from 64 to

77 kg h L^{-1}. No hull-less samples achieved the malt standards. This is because hull-less varieties are not suitable for malt and loss of the husks affects HW results. However, in food industries, high HW helps raise yield processes (Baik et al., 2011). The general reason why hull-less lines may yield more than covered varieties is simply the weight of the husks, which are estimated to be 11 to 13% of the grain yield (Rey et al., 2009).

Husk not only protects barley grain but is also important in brewing. Grain damage can interfere negatively in the malting process. On the other hand, hulling is an important step for barley food products.

The hulled rate in hull-less barley was up to 95% (Table 2) since many caryopses from hull-less barley lose husk during harvest (Baik et al., 2011). The American standard for winter cereals is at least 74% of de-hulled grains. However, this standard may vary according to cereal use, year, climate conditions, and cultural habits (Tamm et al., 2015). According to this American standard, only hull-less barley would be well accepted in food industries. Although some covered barley presented more than 85% yield in dehulling process, this high efficiency is not true due to low percentages of dehulled grain (BRS Sampa (SP), BRS Aliensa (SP), BRS Manduri (SP), BRS Itanema (SP) and BRS Elis (PF)). These covered varieties tend to maintain husks tightly, which is suitable for the malting process but not for food processes. Food industries that accept covered barley for food use should consider more aggressive dehulling machines, such as polishing and lapping equipment, which generally demands high-energy supplies and sophisticated machine regulation in order to maintain the fibers and whole grain composition (Baik et al., 2011).

The β-glucan content is important information for barley grain utilization. In beer production processes, β-glucans determine wort viscosity and, beer filtration rates, and form a barrier for hydrolytic enzymes attacking starch and protein within the cell walls. Accordingly, low β-glucan content of grain and/or its breakdown during malting are critical issues in brewing. The importance of a minimal amount of β-glucans has been reported for foam stability of beer (Baik & Ullrick, 2008).

For food application, high β-glucan concentration is desirable due to its functional effects (Šterna et al., 2017). On the other hand, the technological properties of β-glucans in food processing and end-use quality, except for malting and brewing, are little known. A close positive relationship between total β-glucan content and grain hardness was determined and this may be related to thicker endosperm cell walls in high β-glucan lines (Baik & Ullrick, 2008). Although hull-less barley is described in the literature as having higher β-glucans content compared to covered cultivars (Soares et al., 2007), in our study, some hull-

less and covered grains showed no differences in β-glucan quantification when comparing samples one by one. On the other hand, when analyzing averages from covered and hull-less groups, there were significant difference between them (Table 2), which matches reports in the literature. Rey et al. (2009) reported that hull-less cultivars and lines had an average β-glucan value of 5.5% compared with 3.5% for hulled cultivars/lines. Šterna et al. (2017) found that β-glucans in hull-less and covered barley grains in Poland ranged from 3.44 to 4.97%. Helm & Francisco (2004) reported the β-glucan content of some Brazilian hull-less barley as 3.70 - 3.77%, which is quite similar to the average of 3.85% found in this study.

The U.S. malting and brewing industry currently specifies a range of protein from 11.5 to 13.5% (Rey et al., 2009). In Brazil, for malting process, levels from 10 to 12% are expected, in order to guarantee foam stability and sensorial properties in beer (Minella, 2001). Although higher grain protein should be a desirable attribute in animal feed and human food, there are currently no premiums paid for high protein in barley grains (Rey et al., 2009).

BRS Aliensa, BRS Brau (PF), BRS Elis (PF and VG) and MN6021 (PF and VG) were not suitable for malt due to high protein concentration (Table 2). This characteristic makes the malting process longer and results in beer with low stability (Minella, 2001). Šterna et al. (2017) found that crude protein content in barley grain samples cropped in Poland ranged from 10.5 to 13.9%, which matches the results found in this study. On the other hand, Brazilian authors reported protein from 12.55 to 15.92 in hull-less lines (Helm & Francisco, 2004), which is higher than the findings in the current study. Higher crude protein content in barley was usually accompanied by lower starch and dietary fiber content (Šterna et al., 2017). Starch and total dietary fiber were not quantified in this study; however, this could be one of the reasons why the average of the total crude protein in hull-less barley group is lower than average in the hulled barley group, with statistical difference. BRS Cauê was not analyzed due to insufficient quantities of material.

Correlations among grain parameters are shown in Table 3. The high HW in hull-less lines suits the TKW results (Table 2) since TKW and HW have a positive correlation (0.73*) as TKW and HW are both weight measurements. Similar results were reported by Rey et al. (2009).

Table 3 – Pearson's Correlations among the analyzed properties of barley samples

Tabela 3 – Correlação de Pearson entre as propriedades analisadas das amostras

	TKW	HW	Protein	β-glucan
TKW	1	0.73 *	-0.23 ns	0.48 *
HW	-	1	-0.51 *	0.63 *
Protein	-	-	1	-0.45 *
β-glucan	-	-	-	1

TKW: thousand kernel weight, HW: hectoliter weight, *Significant at 5% level; ns: not significant

β-glucan presented a positive correlation with TKW (0.48*) and HW (0.63*), which is in accordance with Elfverson (1999), who affirms that grain size and cell wall thickness seems to positively influence β-glucan concentration.

The correlation between β-glucan and protein concentrations was negative (-0.45*). Ehrenbergerová et al. (2008) did not find a significant correlation between β-glucan and protein, although Rey et al. (2009) and Šterna et al. (2017) observed a positive correlation between these factors, which seems to vary depending on climate conditions and varieties.

The correlation between protein and HW was negative (-0.51*) as grains that have high HW also have great grain filling, meanings that starch is available in high concentrations. This high starch concentration reduces the percentage of protein when expressing grain composition. Whereas, Nagamine et al. (2012) found a positive correlation between grain protein content and volume weight only in barley grains cropped in 2008 but not in grains from 2009. The amount of divergent data reported in the literature emphasizes that correlations among grain characteristics can be affected by many factors, such as the wide range of environmental conditions and genetic attributes.

In Table 2, divergent results can be observed within the same cultivar grown in different areas, making it clear that not only genetic determines grain characteristics. Despite the fact that cultivars were genetically designed for specific environmental conditions, which is the reason why the same cultivar is not cropped and suitable for both states, São Paulo and Rio Grande do Sul, even when cropped in different areas located in the same state of Rio Grande do Sul (Passo Fundo and Victor Graeff), different characteristics were observed.

Environmental conditions create interactions between genotype and the same variety can express different characteristics when cropped in different areas, which is known as phenotype (genotype combined with the environment). Whereas, when considering the environment and genotype, it is also possible to identify an additional effect: the interaction between them. The influence of the environment demonstrates that cultivars developed

differently in different locations, and that genetic factors are not the only factors that determine grain characteristics (Tamm et al., 2015). To better demonstrate the influence of the environment in the same cultivar, results from barley varieties that were cropped in two different areas are compared in Table 4.

Table 4 – Comparison between the same cultivar in two different crop location and between averages of samples cropped in Passo Fundo and samples cropped in Victor Graeff

Tabela 4 – Comparação entre o mesmo cultivar de cevada em dois locais distintos de cultivo e entre as médias das amostras cultivadas em Passo Fundo e amostras cultivadas em Victor Graeff

	TKW (g)		HW (kg hL^{-1})		β-glucan (%)		Protein (%)	
	Passo Fundo	Victor Graeff	Passo Fundo	Victor Graeff	Passo Fundo	Victor Graeff	Passo Fundo	Victor Graeff
BRS Brau	35.36 a	31.66 a	63.40 a	60.40 a	3.26 a	2.88 b	12.62 a	11.71 b
BRS Korbel	40.22 a	29.91 b	59.20 a	52.60 a	2.82 a	3.41 a	11.43 a	10.49 b
BRS Elis	35.43 a	29.72 b	57.80 a	59.20 a	3.21 a	3.07 a	12.82 a	13.59 b
MN 6021	36.57 a	24.96 b	61.60 a	56.80 a	3.68 a	3.35 b	12.76 a	12.53 b
BRS Cauê	35.42 a	35.42 a	57.00 a	52.20 a	2.98 a	2.56 a	ND	ND
Average	36.60 B	30.34 A	59.80 B	56.24 A	3.19 A	3.05 A	12.40 B	12.08 A

TKW: thousand kernel weight, HW: hectoliter weight, ND: not determined due to insufficient sample quantities. Same cultivars and characteristics followed by the same letters are not statically different ($p \geq 0.05$). Capital letters were used to compare averages of the same characteristics in a different area (PF and VG).

Whereas samples cultivated in Victor Graeff had statistically lower Thousand Kernel Weight (TKW) than those cropped in Passo Fundo, BRS Brau and BRS Cauê did not show differences in TKW at different locations (Table 4). Hectoliter weight (HW) was not different when comparing the same cultivar in both places. However, when analyzing averages, the group of cultivars cropped in Passo Fundo had higher HW than the group of the same cultivars cropped in Victor Graeff. No differences in the β-Glucan concentrations were observed between the averages of the groups from Passo Fundo and Victor Graeff. Only BRS Brau and MN6021 demonstrated higher β-glucan content when cropped in Passo Fundo. It is estimated that 66% of the variability in β-glucan content was attributable to genotype (Rey et al., 2009). Our results support the assertion that genetics is more important than environment in β-glucan content in grain. However, the environment may also influence β-glucan content; higher precipitation during the flowering time and grain filling period and lower temperatures during the flowering time had negative effects on the concentration of β-glucans (Ehrenbergerová et al. 2008). BRS Brau and MN 6021 showed statistical differences in β-glucan content when cropped in Passo Fundo, (RS) and Victor Graeff (RS) (Table 4), which reinforces some influence of environment in β-glucan content in these two varieties.

Šterna et al. (2017) and Wamser & Mundstock (2007) showed that protein content was significantly influenced by variety, year, and nitrogen fertilizer rates. High protein concentration in grains can be enhanced by using fertilizers that contain nitrogen, which means that the environment and crop management influence protein content. In addition, it has been reported that protein content in barley of the same genotype varied from 8.1 to 14.7% at different locations with similar nitrogen fertilization levels (Šterna et al., 2017). Table 4 shows that all varieties cropped in both location (Passo Fundo and Victor Graeff) had different protein content and those cropped in Passo Fundo showed higher protein concentration than those cropped in Victor Graeff, except BRS Elis (Table 4). Our results match those found in the literature.

According to ANOVA, genotype and environment were significant for all study grain characteristics. On the other hand, the interaction between genotype and environment affected only Thousand Kernel Weight and Protein content (Table 5).

Table 5 – Summary of ANOVA for influence of the environment on barley characteristics
Tabela 5 – Resultados da ANOVA sobre a influência do ambiente nas características da cevada

Characteristics	Sum square				VC (%)
	Genotype	Local	Genotype x local	Error	
TKW	14.46*	196.23*	22.77*	1.07	3.09
HW	35.78*	133.13*	2.27	5.51	4.08
Protein	44.49*	5.85*	3.34*	0.005	0.5
β-glucan	0.59*	0.63*	0.11	0.06	7.78

TKW: thousand kernel weight, HW: hectoliter weight, * Statistically significant (5%) in F Test. VC: variation coefficient.

Passo Fundo produced barley with better quality characteristics for malt and food industry, such as higher HW and TKW. For food industries, Passo Fundo is also the better place to crop barley due to the higher protein content of the grains. On the other hand, Victor Graeff is better for producing barley for malting since high protein content in grains is a technological problem. The reasons why Passo Fundo performed differently from Victor Graeff might involve altitude and crop management, since rain was not different between the areas.

4.4 CONCLUSION

Although hull-less barley lines had higher TKW, HW, HR and β-glucan content, they also had lower protein content when comparing the average of hull-less and covered groups (Table 2). The hull-less and covered barley differed in chemical composition and physical properties.

Our research indicates that there is a significant environmental interference that can discard barley for malt. However, the barley might still be suitable for food industries. Approval of the barley health claim may increase interest in, and markets for, food barley. To meet this demand, barley processors are likely to require the production of barley cultivars due to their high β-glucan contents, which could be a potential market for hull-less lines and for covered barleys that are not suitable for malt. Barley can be used for feed, malting, and food, and there is great potential to improve barley for all these uses.

Overall, the data of this study demonstrated the main differences between Brazilian hull-less lines and covered varieties. However, in order to discuss further the effects of genotype, environment, and their interactions, which could contribute to variation in grain composition, particularly protein content, more than one harvest is necessary, in different site locations.

This study contributes to understanding of the composition and physical properties of Brazilian barley varieties and points out characteristics that could indicate the most suitable end uses.

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5 CAPÍTULO III _ EFFECT OF BARLEY PRODUCTS INTAKE ON BIOCHEMICAL INDEXES AND PLASMA INDICATORS OF OXIDATIVE STRESS IN HEALTHY HUMAN SUBJECTS: A RANDOMIZED BRAZILIAN STUDY

Abstract

Nutritional diets are associated with lower incidences of certain chronic diseases and long-life expectancy. The reason lays on their different contribution in fibers and antioxidant compounds. Barley is high fiber cereal that contains high amount of β-glucans and insoluble fibers that it was recognized having healthy benefits. Whole grain barley also contains phytochemicals including phenolic acids, flavonoids, lignans, tocols, phytosterols, and folate. These phytochemicals exhibit strong antioxidant, antiproliferative, and cholesterol lowering abilities, which are potentially useful in lowering the risk of certain diseases. Therefore, the high concentration of phytochemicals in barley may be largely responsible for its health benefits. The aim of the study was to evaluate the effects of 3 g/day barley β-glucans intake (by eating whole grains and barley cookies) on glycemic response, cholesterol and anthropometric indexes by a randomized, double-blind, wheat-controlled intervention study conducted with 39 Brazilian individuals for 45 days. The same clinical intervention aimed to compare oxidative responses of wheat-based diet and barley-based diet by measuring stress oxidative indicators in blood samples. Both groups showed no cholesterol reductions but barley group at the end of intervention time (T45) showed decrease in glycemia when compared to T0. Oxidative stress indicators showed better results for malonaldehyde (MDA), plasma total antioxidant capacity (TRAP), catalase (CAT), reduced glutathione (GSH) and oxidized glutathione (GSSG) in wheat group. On the other hand, in barley group, only MDA and TRAP improved during diet intervention. The conclusion was that a regular intake of barley β-glucan led to significant and safe reductions in glycaemia but wheat-based products seems to improve better the oxidative responses when added regularly in human diet.

Keywords: oxidative stress; cholesterol; functional food; β-glucan; whole grain.

5.1 INTRODUCTION

Consumption of fiber-rich or whole grain cereal products is implicated with several beneficial nutritional and health outcomes (Priebe & Mc Monagle, 2016). Cereals such as oats and barley are rich in soluble fibers, e.g., β-glucan, which is a non-starch polysaccharide composed of linear chains of glucose with β-(1/3) and β-(1/4) linkages (Tiwari & Cummins, 2011).

Barley β-glucan are well recognized for their many health claims and current research is focused on increasing soluble fiber consumption through dietary intervention to address growing consumer awareness. The Food and Drug Administration (FDA) authorizes the use of a health claim on oat or barley whole grains (and derivatives) because β-glucan from these sources decreases the risk of coronary heart disease (FDA, 2005). Whereas, in Brazil, the Brazilian Health Regulatory Agency (Anvisa) does not yet recognize barley as a functional food or ingredient.

Epidemiologic studies have reported that the intake of soluble fiber (β-glucan) lowers lipid absorption and in turn decreases the risk of cardiovascular diseases. Consequently, due to its physiologic effects, many researchers have incorporated barley β-glucan into various food products, including breakfast cereals, beverages, bread, and infant foods, to improve the nutritional and health benefits (Tiwari & Cummins, 2011).

Tiwari and Cummins (2011) performed a meta-analysis examining the cholesterol lowering effect of barley β-glucan in 126 clinical studies and concluded that consumption of 3 grams every day of barley β-glucan is enough to decrease blood cholesterol.

In addition, many intervention studies have reported that consuming barley on a regular basis as part of a low glycemic response diet may be one additional strategy in helping to minimize blood sugar spikes and prevent insulin resistance (Tosh, 2013). Ekstrom et al. (2017) study three commercially available β-glucans from barley and oat were baked into yeast leavened bread products. The three levels of oat β-glucans reduced the glycaemic index and glucose iPeak by 32–37% compared to a white wheat reference bread. In 2016, a meta-analysis reaffirmed that consumption of barley and barley β-glucan lowered postprandial glycaemic response, and that the magnitude of reduction in pos prandial glycaemic response was large enough to be considered a physiologically relevant change (Ames et al., 2016).

However, it is now widely believed that the actions of the fiber component alone do not explain the observed health benefits associated with the consumption of whole grain barley. Whole grain barley also contains phytochemicals including phenolic acids, flavonoids, lignans, tocols, phytosterols, and folate. These phytochemicals exhibit strong

antioxidant, antiproliferative, and cholesterol lowering abilities, which are potentially useful in lowering the risk of certain diseases. Therefore, the high concentration of phytochemicals in barley may be also responsible for its health benefits (Sang et al., 2017).

In order to test functional food properties, it is very important to guarantee the commitment of volunteers during the intervention period. Many authors have offered different foods based on the same functional ingredient aiming to diversify the intake, favouring the adherence of the participants. Kerckhoffs et al. (2003) test β-glucan effects on dyslipidemia using bread and cookies of oat (test group) and wheat (control/comparison group). In a second study, for the following 2 weeks, bread and cookies were changed by orange juice containing the same levels of oat β-glucan or wheat fibers.

It is also known that process can interfere in β-glucan activity *in vivo*. Food process can interfere on biological functionality of an active compounds, which makes tests on processed food products necessary. Researches had found that bread production decrease the molecular weight of β-glucan but freezing the bread did not affect the molecular weight. For bread, the molecular weight is lower than for oat bran or oat bran concentrate. The β-glucan in the frozen cookie had the largest molecular weight (Kerckhoffs et al., 2003). Steneryd (2016) concluded that consumption of ready-to-eat cereals with oat β-glucan seem to result in a greater cholesterol reduction than of bread, due to the more beneficial effects of processing on molecular weight and solubility of the β-glucan. The cholesterol-lowering properties of β-glucan have been shown to vary based on the processing and use in products. Processing of cereals is a necessity for human consumption, but some procedures have been reported to alter important characteristics of β-glucan, which resulted in a lacking effect on cholesterol levels (Andersson et al., 2014).

To the very best of our knowledge nothing has been reported in the open literature about covered Brazilian barley health benefits when consumed regularly. It is known that covered barley is genetically designed for application in brewer and malt, which has contributed for lower β-glucan concentration in grains when compared to naked varieties (Soares et al., 2007). Since covered barley is very popular in Brazil and naked varieties are nationally available only in academic scale, the aim of this study was to test the functionality of barley, in the form of two derived products (whole grain and cookies), evaluating its effects on glycemic index and lipidic profile of healthy human subjects. Additionally, the anti-oxidative responses promoted by these barley products were compared with those of similar products from wheat.

5.2 Material and Methods

5.2.1 Barley and Wheat Products

Whole wheat grain and flour were kindly provided by SL Cereais e Alimentos LTDA (Mauá da Serra, PR, Brazil). Barley grain (approx. 3,46% β-glucans, quantified by Megazyme® kit) was kindly provided by Groupe Soufflet (Taubaté, SP, Brazil) and milled by SL Cereais e Alimentos LTDA. Vegetal fat, sugar, baking powder, cinnamon and nutmeg were bought in local supermarket.

The cookies recipe of the test group contained whole barley flour (46,0%), while that of the control/comparison group contained the same level of whole wheat flour. The proportion of vegetable fat (34.32%), sucrose (19.32%), baking powder (0.23%), cinnamon (0.06%), and nutmeg (0.06%) was the same for the two formulations.

All ingredients were mixed in a G-Paniz Artisan (G-Paniz AR15, Caxias do Sul, RS, Brazil) for ten minutes. Dough was patterned in approximately 10 g cookie at room temperature and baked at 180^{o}C in a baking oven (G-Paniz FTE-240, Caxias do Sul, RS, Brazil) for 35 min. After baking, cookies were left to cool for 4 h and divided into portions of 80 g each and put into plastic bags.

Whole grains (wheat and barley) were divided into portions of 50 g each and put into plastic bags.

5.2.2 Clinical Intervention

Forty-seven individuals were selected at a cardiology clinic in Londrina, PR, Brazil to participated in this randomized, blind, longitudinal and controlled clinical trial. The inclusion criteria were patients older than 18 years old, both gender, with total cholesterol plasma levels ≥ 200 mg/dL and no diseases. The exclusion criteria were patients with gluten intolerance or allergy, pregnancy and use of any cholesterol-lowering drugs. All participants were informed of the procedures they would undergo, signed an informed consent document and were aware of the possibility of withdrawing from the study at any time. Volunteers answered some questionnaires about lifestyle, food frequency and routine with the aim of knowing better the volunteer's habits and identify possible changes since the intervention started. Body weight, height, abdominal circumference and blood pressure were measured to calculate body mass index (BMI) and cardiologic risks. This study and the consent terms were

approved by the ethics in human research committee at Londrina State University (nº 68744917.2.0000.5231).

Eligible volunteers were randomly distributed in two experimental groups to either consume 80 g of barley cookies and 50 g of whole barley grain /day over a forty-five-day period (barley group) or to consume 80 g of wheat cookies and 50 g of whole wheat grain /day over the same time (control/comparison group). The total β-glucan provided by the consumption of cookies and grain in the barley group was 3 g/day. Whole wheat was chosen as control because it has non-significant amounts of β-glucans in its composition for functional effects on biochemical indexes and it was also used to compare its effects on anti-oxidative responses when consumed regularly. Wheat contains tocopherols, alkylresorcinols, steryl ferulates and other phenolic compounds, which may interfere in oxidative stress (Patel, 2015).

The volunteers were instructed to consume cookies and grains as they wanted as long as the daily portion was consumed during the 24 hours every day, otherwise they were told to maintain their regular life-style. Volunteers also received a list of recipes as a consumption suggestion in order to easily cope with the whole grain daily portions. The cookies recipes are described in specific section.

Volunteers were accompanied by biweekly meetings. The subjects arrived in the laboratory at 07.00 a.m. after an overnight fast. At every meeting, volunteers blood samples were collected for testing total cholesterol, LDL, HDL, uric acid, glycaemic index and oxidative markers. Body weight, arterial pressure and abdominal circumference were measured biweekly too.

Eight selected volunteers were excluded from the study because they could not follow the diet for more than five days. At the end of the intervention, barley group was within nineteen members (12 female and 7 male), and control/comparison group was within twenty members (12 female and 8 male). Volunteers were between 32 and 69 years old and average was 55 years old. Table 6 shows the homogeneity between barley and wheat group at the beginning of intervention, despite the abdominal circumference that was smaller in male barley group than in male wheat group.

Table 6 - Biochemical markers between groups

Dependent Variables	Female		Male		F	df	p
	Barley	Wheat	Barley	Wheat[a]			
	T0	T0	T0	T0			
Weight (kg)	75.81±3.77	73.43±3.47	83.73±4.43	94.12±4.73	3.71	1	>0.05
SP (mmhg)	115.45±4.11	116.38±3.78	115.50±4.82	128.28±5.15	0.24	1	>0.05
DP (mmhg)	75.36±2.66	75.38±2.44	76.50±3.12	84.00±3.33	1.87	1	>0.05
AC (cm)	95.54±2.92	94.38±2.68	97.37±3.42 [a]	111.00±3.66	3.65	1	0.036 [a]
GLU (mg/dL)	102.00±3.51	113.90±3.35	100.16±4.54	109.00±4.24	1.57	1	>0.05
HDL-C (mg/dL)	58.10±3.61	52.23±3.16	55.37±4.06	41.14±4.13	1.06	1	>0.05
LDL-C (mg/dL)	147.76±13.69	140.36±12.01	136.12±15.31	105.45±16.36	0.63	1	>0.05
CHOL (mg/dL)	230.00±11.52	220.16±10.51	213.37±12.88	181.42±13.77	0.50	1	>0.05
TG (mg/dL)	123.20±18.52	132.92±16.25	105.00±23.92	167.33±23.92	1.24	1	>0.05
AUR (mg/dL)	5.37±0.45	4.77±0.40	6.76±0.51	7.10±0.54	0.91	1	>0.05

Data are expressed as means ± SEM. * p<0.05. Multivariate analysis**.** AC: abdominal circumference, SP: systolic pressure, DP: diastolic pressure, Glu: glucose, CHOL: total cholesterol, TG: triglycerides, AUR: uric acid. a: Barley and Wheat Male Groups were different in AC. HDL-C: high-density cholesterol, LDL-C: low-density cholesterol.

5.2.3 Clinical Analysis

Approximately 20 mL of fasting venous blood of each volunteer were collected after an overnight fast at baseline (day 01), and at 15, 30 and 45 days of intervention. The plasma was separated by centrifuging at 3000 rpm for 15 minutes using an Excelsa® centrifuge (Fanem, São Paulo, SP, Brazil). Samples were then aliquoted and stored at -80ºC until analyses.

Biochemical parameters of the blood lipid profile: triglycerides (TG), total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), uric acid and fasting glucose were assessed by Dimension® (SIEMENS RxL, Deerfield, Illinois, USA) biochemistry reagent kits and Dade AR (Newark, New Jersey, USA) equipment.

Total radical-trapping antioxidant parameter (TRAP) (Repetto et al., 1996), lipid hydroperoxides, i.e., FOX-LOOH (Jiang et al., 1991) and CL-LOOH (Flecha et al., 1991), MDA (Bastos et al., 2012), NOx (Navarro-Gonzálvez et al., 1998), SOD activity in erythrocytes (Marklund et al., 1974), CAT activity in erythrocytes (Aebi, 1984), SH groups (Hu, 1994), total plasmatic PON1 activity (Richter et al., 2008) and advanced protein oxidation products (AOPP) (Hanasand et al., 2012) were assayed by previously described methods. The formation of TNB (thionitrobenzoic acid) can be monitored spectrophotometrically at 412 nm through the supernatant added in flat bottom microplates, resulting from the reaction of two reduced glutathione (GSH) molecules and one of dithio-bis-nitrobenzoic acid. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) resulting from the first

reaction (or one already present in the sample) is reconverted into GSH, which is reoxidized to GSSG forming more TNB, and obtaining the amounts of total glutathione (GT) in the sample. The amount of GSSG was obtained by calculation through the subtraction of GT-GSH /2.

5.2.4 Statistical Analysis

Initially, normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene's test) were checked. If these criteria were reached ($p<0.05$), to intra group analysis, variables were evaluated by two-way Repeated Measures ANOVA (RMANOVA). Data that did not reach normal distribution and homogeneity of variance criteria were analyzed by the non-parametric test of Kruskal-Wallis complemented with Bonferroni 's test.

5.3 Results and Discussion

5.3.1 Anthropological and Biochemical Indexes

Tables 7 and Table 8 show biochemical markers and anthropometric data of barley and wheat groups, respectively, divided by gender. Table 9 shows biochemical and anthropometric data comparing results between T0 and T45 of each group. There were no differences during the intervention time among weight, systolic pressure (SP) and diastolic pressure (DP), when compared results from T0, T15, T30 and T45 in both groups when they were divided by gender (Tables 7 and 8). SP and DP had still non-significant changes when gender was not considered in statistics (Table 9). Whereas, weight was higher ($p=0.019$) in wheat group at the end of intervention time when gender was not considered (Table 9). Abdominal circumference (AC) was smaller at T15 ($p=0.011$) in females from barley group when compared to T45 results (Table 7). However, when gender is not considered AC does not show any differences during the intervention time, neither in barley or wheat group (Table 9).

Table 7 - Biochemical markers and anthropometric data of barley intervention group in different collection times

Dependent Variables	Barley								F	df	P
	Female				Male						
	T0[a]	T15[b]	T30[c]	T45[d]	T0[e]	T15[f]	T30[g]	T45[h]			
Weight (Kg)	75.81 ±3.77	75.40 ±3.73	75.73 ±3.69	75.85 ±3.66	83.73 ±4.43	83.47 ±4.37	83.47 ±4.32	83.70 ±4.29	1.96	3	>0.05
SP (mmHg)	115.45 ±4.11	113.63 ±4.07	115.45 ±4.33	116.36 ±3.08	132.50 ±4.82	131.25 ±4.77	136.25 ±5.08	126.25 ±3.62	0.41	3	>0.05
DP (mmHg)	76.36 ±2.66	75.45 ±2.98	76.36 ±2.72	71.81 ±3.10	82.50 ±3.12	83.75 ±3.50	90.00 ±3.19	87.50 ±3.64	0.71	3	>0.05
AC (cm)	93.54 ±2.92	93.18 ±2.84[d]	95.18 ±2.91	95.36 ±2.73	97.37 ±3.42	96.00 ±3.33	95.50 ±3.14	96.37 ±3.20	0.24	3	0.011 [d]
GLU (mg/dL)	102.00 ±3.51[c]	100.80 ±2.38[c d]	93.50 ±2.99	92.60 ±3.27	100.16 ±4.54	100.50 ±3.07	94.33 ±3.86	95.55 ±4.22	0.26	3	<0.05 [c] 0.046 [d]
HDL-C (mg/dL)	58.10 ±3.61	59.30 ±4.06	54.50 ±3.60	55.20 ±3.73	49.37 ±4.06	51.50 ±4.54	48.62 ±4.02	49.75 ±4.17	1.49	3	>0.05
LDL-C (mg/dL)	144.76 ±13.69	141.70 ±14.33	141.84 ±13.83	144.40 ±15.68	126.12 ±15.31	137.72 ±16.02	129.77 ±15.46	141.10 ±17.53	0.32	3	>0.05
CHOL (mg/dL)	227.00 ±11.52	226.00 ±13.98	221.80 ±13.23	225.90 ±12.90	228.37 ±12.88	233.50 ±15.64	232.87 ±14.79	239.75 ±14.42	2.47	3	>0.05
TG (mg/dL)	124.20 ±18.52	125.00 ±23.85	127.30 ±26.68	131.50 ±24.56	112.00 ±23.92	107.50 ±30.79	118.00 ±34.45	129.33 ±31.71	1.58	3	>0.05
AUR (mg/dL)	5.27 ±0.45	5.28 ±0.44	4.98 ±0.41	4.80 ±0.39	6.65 ±0.51	6.91 ±0.50[g]	6.22 ±0.45	6.30 ±0.44	1.62	3	0.042 [g]

Data are expressed as means ± SEM. [a,e] p<0.05 compared to T0; [b, f] p<0.05 compared to T15; [c, g] p<0.05 compared to T30; [d,h] p<0.05. Two-way RMANOVA. AC: abdominal circumference, HDL-C: high-density cholesterol, LDL-C: low-density cholesterol, SP: systolic pressure, DP: diastolic pressure, Glu: glucose, CHOL: total cholesterol, TG: triglycerides, AUR: uric acid. DF: degree of freedom

Table 8 - Biochemical markers and anthropometric data of wheat intervention group in different collection times

Dependent Variables	Wheat								F	df	P
	Female				Male						
	T0[a]	T15[b]	T30[c]	T45[d]	T0[e]	T15[f]	T30[g]	T45[h]			
Weight (Kg)	73.43 ±3.47	73.25 ±3.43	73.36 ±3.39	73.67 ±3.36	94.12 ±4.73	94.24 ±4.67	94.44 ±4.62	94.91 ±4.59	13.90	3	>0.05
SP (mmhg)	115.38 ±3.78	116.92 ±3.74	119.23 ±3.98	117.69 ±2.84	124.28 ±5.15	124.28 ±5.10	128.57 ±5.43	130.00 ±3.87	1.35	3	>0.05
DP (mmhg)	75.38 ±2.44	77.69 ±2.74	77.69 ±2.50	75.38 ±2.86	80.00 ±3.33	82.85 ±3.74	84.28 ±3.41	81.42 ±3.89	1.49	3	>0.05
AC (cm)	95.38 ±2.68	95.07 ±2.61	95.69 ±2.61	94.53 ±2.51	107.14 ±3.66	107.57 ±3.56	107.00 ±3.65	107.57 ±3.43	5.68	3	>0.05
GLU (mg/dL)	105.90 ±3.35	104.27 ±2.27	99.45 ±2.85	98.18 ±3.12	114.00 ±4.24	118.57 ±2.84[g]	111.57 ±3.57	114.57 ±3.91	15.38	3	0.035 [g]
HDL-C (mg/dL)	52.23 ±3.16	52.53 ±3.56	49.07 ±3.15	50.76 ±3.27	42.14 ±4.13[h]	39.57 ±4.95	36.42 ±4.30	34.14 ±4.45	0.23	3	0.05 [h]
LDL-C (mg/dL)	135.36 ±12.01	142.09 ±12.57	142.23 ±12.13	132.52 ±13.75	106.45 ±16.36	109.37 ±17.13	107.14 ±16.53	91.40 ±18.74	3.75	3	>0.05
CHOL (mg/dL)	203.16 ±10.51	218.08 ±12.77	216.25 ±12.08	205.41 ±11.77	180.42 ±13.77	193.14 ±16.71	188.14 ±15.81	192.71 ±15.42	6.46	3	>0.05
TG (mg/dL)	128.92 ±16.25	156.46 ±20.92	171.92 ±23.40	151.61 ±21.54	154.33 ±23.92[g]	179.33 ±30.79	219.33 ±34.45	199.00 ±31.71	4.32	3	0.049 [g]
AUR (mg/dL)	4.73 ±0.40	4.52 ±0.39	4.53 ±0.36	4.30 ±0.35	6.60 ±0.54	6.34 ±0.53	6.82 ±0.49[h]	6.17 ±0.47	0.80	3	0.013 [h]

Data are expressed as means ± SEM. Student's t-test. . [a,e] p<0.05 compared to T0; [b, f] p<0.05 compared to T15; [c, g] p<0.05 compared to T30; [d,h] p<0.05. AC: abdominal circumference, HDL-C: high-density cholesterol, LDL-C: low-density cholesterol, SP: systolic pressure, DP: diastolic pressure, Glu: glucose, CHOL: total cholesterol, TG: triglycerides, AUR: uric acid. DF: degree of freedom.

Results of HDL-C and LDL-C were non-parametric, meaning that they did not depend on data belonging to any particular distribution. Total cholesterol (CHOL), HDL-

C, LDL-C and TG did not change during the intervention in barley group considering gender (Table 7) or not (Table 9). In wheat group, CHOL and LDL-C neither had changes during the intervention time but HDL-C was lower (p=0.038) at T45 when compared to T0 (Table 9). The same effect was observed in men (p=0.05) but not in woman who ate wheat products (Table 8). TG was higher in men from wheat group (p=0.049) at T30 when compared to T0 (Table 8). The same effect on TG was observed in wheat group when gender was not considered (Table 9) throw the intervention time (p=0.017).

Consumption of 3 g oat β-glucan per day is considered enough to lower serum LDL-C, but some studies have shown no effect (Wholever et al., 2010). The cholesterol-lowering effect of barley β-glucans is considered to depend on increased viscosity that reduces the reabsorption of bile acids and increases both the synthesis of bile acids from cholesterol as well as the fecal excretion of neutral sterols. Viscosity in the small intestine is determined by the concentration, molecular weight and solubility of the barley β-glucans (EFSA, 2011).

Results found in our study was not in accordance with many others found in the literature that tested barley diets effect on lipids indexes (AbuMweis et al., 2010). However, some of the clinical trials exhibited heterogeneity in the degree of cholesterol-lowering effects (Keogh et al., 2003; Behall et al., 2004; Queenan et al. 2007). In a study by Robards et al. (2007), 31 women and 31 men consumed 3.0 g of β-glucan from oat bran concentrate per day in oat bran cereal for 8-weeks. The control/comparison group consumed a wheat cereal product. No significant change in fasting plasma cholesterol or LDL-C between the two groups was observed and HDL-C levels were lowered, which is in accordance to what was found in our study. Byun et al. (2015) also fail to show significant lipid-lowering effects of barley sprout extract.

Aoe et al. (2017) study 100 Japanese individuals, who were randomly assigned to consume a mixture of rice and either high β-glucan barley (test group, 4.4 g/d) or β-glucan–free barley (placebo group) for 12 weeks. Authors found a tendency toward a reduction in LDL-C but HDL-C in their placebo group increased, compared with their test group.

In our study, we did not find a reason why only men who ate wheat had their HDL-C reduced and TG elevated during the intervention time, but women from the same group had no similar effects. Whereas, TG was meant to be the major fat molecules in human body. It is the form in which the fat is stored in the body and in the adipose tissue. Its main function is to store energy (Handelsman & Shapiro, 2016). Triglycerides are formed mainly from the carbohydrates and that is why it could be raised in wheat group, which is in accordance to the

increased weight (p=0.019) at the end of intervention time when gender is not considered (Table 9). It is important to consider that high TG is in accordance to low HDL-C due to Friedewald equation, which was observed in men from wheat group.

Table 9 - Biochemical markers and anthropometric data between initial and final intervention time

Dependent Variables	Barley		df	p	Wheat		df	p
	T0	T45			T0	T45		
Weight (Kg)	79.15±2.67	79.15±2.52	18	0.984	80.68±3.72	81.11±3.73	19	0.019*
SP (mmhg)	122.63±3.65	120.52±2.35	18	0.465	118.50±3.10	122.00±2.77	19	0.149
DP (mmhg)	78.94±2.00	78.42±2.56	18	0.790	77.00±2.06	77.50±2.70	19	0.825
AC (cm)	95.15±1.77	95.78±1.68	18	0.446	99.50±2.76	99.10±2.66	19	0.418
GLU (mg/dL)	101.70±2.02	93.94±1.92	18	<0.001*	109.05±3.09	104.55±3.34	17	0.204
HDL-C (mg/dL)	53.68±3.58	52.05±3.45	18	0.384	48.70±1.56	44.95±2.41	19	0.038*
LDL-C (mg/dL)	140.12±9.77	146.24±10.41	18	0.359	125.25±10.38	118.13±12.35	18	0.362
CHOL (mg/dL)	230.21±7.58	233.84±10.18	18	0.580	194.78±9.06	200.73±8.04	18	0.167
TG (mg/dL)	118.52±12.86	129.23±14.65	16	0.148	136.49±13.78	166.57±20.11	18	0.017*
AUR (mg/dL)	5.83±0.41	5.47±0.39	18	0.014*	5.38±0.31	4.95±0.26	19	0.016*

Data are expressed as means ± SEM. Student's t-test. AC: abdominal circumference, HDL-C: high-density cholesterol, LDL-C: low-density cholesterol, SP: systolic pressure, DP: diastolic pressure, Glu: glucose, CHOL: total cholesterol, TG: triglycerides, AUR: uric acid,*: statistically significant considering p<0.05. DF: degree of freedom.

Another potential effect that can interfere the biochemical results, is the β-glucan dose in the tested products. Trials with dose sizes of more than 5 grams of β-glucan were found to result in a greater reduction in CHOL and LDL-C (Ames et al., 2015). The β-glucan molecular weight also seems to play an important role on biochemical responses (Vannucci, Sima & Vetvicka, 2018). Wolever et al. (2010) studied the effect of oat β-glucan of different molecular weights incorporated to extruded ready-to-eat cereal. Products of lower molecular weight was made by increased temperature and shear and decreased moisture content during extrusion. After treatment and compared to the control group, all β-glucan cereals, except 4 g low molecular weight, significantly lowered LDL-C. The 3 g high molecular weight reduced CHOL as well. These studies may suggest that our absence of reduction in CHOL and LDL-C can be correlated to low molecular weight in barley β-glucan due to cookies process of production.

The glycaemic response results of the study varied depending on groups. In barley group glycaemic index (GLU) was reducing gradually in females among T0 and T30 (p<0.05) and T15 and T45 (p=0.046) (Table 7). Although reduction in GLU was not observed in men who ate barley products (Table 7), such reduction was still observed in barley group during the intervention time when gender was not considered (Table 9). In wheat group, GLU had no changes from T0 to T45 (Table 9) but at T15 it was higher (p=0.035) in men, when

comparing to results from T30 (Table 8). The fact that wheat group had GLU affected slighter than barley group could be assumed that the soluble fiber content rather than total fiber content is more responsible for lowering glycaemic index in foods. According to Thondre et al. (2012), who studied two barley grains that were different in their total fiber content but did not differ significantly in their glycaemic index, the reason for this could be the very similar β-glucan content in the two grains. Ames et al. (2015) suggested that β-glucan dose in the tested products was relevant for GLU results. Trials with dose sizes of more than 5 grams of β-glucan were found to result in a greater reduction in GLU.

Uric acid was lower at the end of intervention time in barley ($p=0.014$) and wheat ($p=0.016$) groups (Table 9). However, woman from both groups had no variances on uric acid during the study (Tables 7 and 8). Men in wheat group had their uric acid reduced in T45 when compared to T30 ($p=0.013$) while men in barley group had their uric acid reduced in T30 when compared to T15 ($p=0.042$). This was possibly because the whole grain portion brought more satiety to the volunteers, who reduced their portions of other protein-rich food groups, which was the same suggestion of Aoe et al. (2017).

5.3.2 Oxidative Stress

Table 10 shows a comparison between whole barley and whole wheat products intake on oxidative stress biomarkers considering results at the beginning and at the end of intervention time. In barley group, the values of tert-butyl hydroperoxide compound (CL-LOOH), malonaldehyde (MDA), nitric oxide metabolites (NOx), plasma total antioxidant capacity (TRAP) and paraoxanase 1 (PON 1) were statistically different.

In wheat group, only MDA, TRAP, catalase (CAT), reduced glutathione (GSH), oxidized glutathione (GSSG) and PON 1 differed statistically.

Table 10 - Oxidative stress biomarkers between initial and final collection time

Dependent Variables	Barley		df	p	Wheat		df	p
	T0	T45			T0	T45		
CL-LOOH (URL)	1.82±0.24	3.59±0.59	15	0.007*	2.59±0.39	3.59±0.51	19	0.169
MDA (μM)	2.06±0.08	1.62±0.07	18	<0.01*	1.95±0.06	1.55±0.09	19	<0.001*
FOX-LOOH (mM/L)	10.10±0.10	12.82±0.18	18	0.129	1.61±0.24	1.70±0.16	19	0.618
AOPP	99.84±1.33	116.49±1.63	17	0.115	107.30±9.49	133.89±16.42	19	0.061
NOx (μM/L)	4.81±0.41	5.71±0.51	16	0.013*	4.34±0.37	5.02±0.33	19	0.099
TRAP	149.49±5.50	179.92±7.84	18	<0.001*	158.54±5.07	176.22±7.10	17	0.009*
SOD (U/mg Hb)	56.85±4.73	49.49±3.26	18	0.230	50.99±2.64	47.48±4.18	19	0.478
CAT (U/mg Hb)	83.46±3.93	85.54±4.40	18	0.684	82.03±5.42	98.08±4.70	18	0.043*
GT (mMg Hb)	6.26±0.24	6.13±0.20	17	0.631	6.06±0.15	5.97±0.17	18	0.772
GSH (mMg Hb)	6.10±0.20	5.17±0.20	17	0.182	6.04±0.17	5.50±0.15	18	0.013*
GSSG (mMg Hb)	0.11±0.04	0.15±0.03	17	0.494	0.003±0.05	0.22±0.03	19	0.003*
SH (mM/mg prot.)	350.45±13.96	347.70±13.91	17	0.789	338.57±12.06	343.55±7.38	19	0.674
PON 1 (U/mL)	138.18±11.62	81.08±7.69	17	<0.01*	123.29±7.86	85.17±10.83	19	0.003*

Data are expressed as means ± SEM. p<0.05. Student's t-test**.** CL-LOOH: tert-butyl hydroperoxide compound; MDA: malondialdehyde; FOX-LOOH: hydroperoxides; AOPP: advanced protein oxidation products; NOx: Nitric oxide metabolites; TRAP: plasma total antioxidant capacity; SOD: superoxide dismutase; CAT: catalase; GT: total glutathione; GSH: reduced glutathione; GSSG: oxidized glutathione; SH: sulfhydryl group; PON 1: paraoxanase 1; URL: Relative Unity of Light; DF: degree of freedom. *: statistically different at p<0.05.

The CL-LOOH is a potent peroxyl radical builder. In biological membranes, these radicals attack lipids by generating lipoperoxides that can react with other lipids by oxidizing them. Thus, tert-butyl initiates a chain lipoperoxidation reaction with other lipids that can be detected through the photon emission that occurs during lipoperoxide formation (Flecha et al., 1991). Hydroperoxides increased significantly only in patients who consumed barley (Table 10) and this increase was relevant when compared T0 with T45 (p=0.007). It was noted that the volunteers who consumed barley started the intervention period with the hydroperoxides value lower (1.82) than the value presented in the wheat group (2.59 URL). In this topic, specifically, barley intake was not a good way to promote healthiness because CL-LOOH should be lower for indicating reduction in oxidative stress.

Malondialdehyde (MDA) is a secondary degradation product used for the quantification of lipid peroxidation. The higher its concentration in the sample, the higher the lipoperoxidation and the higher the oxidative stress. MDA decreased in the wheat and barley groups between T0 and T45, indicating a reduction in lipid peroxidation in volunteers. Zhao et al. (2012) also reported a significantly lower value of serum MDA content when investigated the effects of combined intake of tea polyphenols and *β*-glucan in STZ-induced diabetic rats.

While wheat group had no differences in nitric oxide metabolites (NOx), volunteers who ate barley had a significant increase in NOx, which is not a good result for oxidative stress (OE). It will further contribute to inflammatory and atherogenic processes. Reactive oxygen species (ROS) generated by oxidative stress and inflammation are associated

with age-related endothelial dysfunction. Endothelial dysfunction is characterized by a change in endothelial actions toward a proinflammatory state, reduced vasodilation, such as nitric oxide molecules, and increased vasoconstriction, such as endothelial molecules 1 and angiotensin II. These molecules play an important role in increasing arterial stiffness and oxidative stress, causing vascular damage (Chai et al., 2019).

Plasma Total Antioxidant Capacity (TRAP) evaluates the total antioxidant capacity of the sample using TROLOX (water soluble vitamin E) as a comparison. The higher the value of TRAP, the greater the antioxidant capacity of the sample. In this study, barley and wheat groups had increased TRAP when compared T0 and T45, which is a good result considering OE.

Catalase (CAT) is an intracellular enzyme. The higher the antioxidant activity of the sample, the higher CAT activity. In the wheat group, there was an increase in CAT activity comparing T0 with T45 ($p=0.043$). The same effect was not observed in the barley group.

While in barley group had no effects, in wheat group, reduced glutathione (GSH) decreased and oxidized glutathione (GSSG) increased during the intervention time, which suggest an improvement in OE effects. Another antioxidant enzyme that was analyzed is paroxonase 1 (PON 1). It is bound to high-density lipoprotein and plays a key role in protecting both low- and high-density lipoproteins from oxidation. Lowered PON-1 activity increases risk to neurodegenerative diseases as well as other related pathologies (Farias et al., 2016). Considering PON 1 decreased in both groups comparing T0 and T45, consuming wheat and barley did not help to reduce OE.

Hydroperoxides (FOX-LOOH), advanced protein oxidation products (AOPP), superoxide dismutase (SOD), total glutathione (GT) and sulfhydryl group (SH) had no changes during the intervention time in both groups (Table 10). It was reported that feeding diabetic mice with barley β-glucan improved HDL-C and increased their liver SOD activities while reducing their MDA levels (Patel, 2015); however, this was not observed in our study.

When looking at barley and wheat OE results, it seems that wheat and barley play a role in reducing oxidative stress by analyzing some blood indicator. In an *in vitro* study, extracts of dehulled highland barley showed excellent antioxidant activities determined by oxygen radical absorbance capacity and cellular antioxidant activity assays, as well as potent antiproliferative activity towards HepG2 human liver cancer cells (Zheng et al., 2015). The same authors reported that bound phenolics make a significant contribution to total phenolics content, antioxidant and anticancer activities in barley samples and suggested that highland

barley exhibits unique phenolic compound profile with potential health benefits, and it may serve as ingredients for functional foods; however, this was not observed in our study.

The present study was limited by the fact that barley food was only used without a dose–response study on total β-glucan content and authors did not have access to β-glucan molecular weight. Results obtained compared to other studies suggest that the dose of β-glucan consumed may have been lower than necessaire for functional effects and/or the barley food processing may have affected the functionality of the fiber.

5.4 CONCLUSION

Despite the fact that barley β-glucan lipid lowering capacity was not observed in this study, differences between barley and wheat groups were observed in GLU results, which may suggest that Brazilian barley-based diet can be used for controlling glycemic indexes. Increased knowledge of the cholesterol lowering effects of processed barley β-glucan could possibly adapt the use of effective control over diet on blood cholesterol concentrations. Wheat and barley seem to improve some OE indexes and barley has a possibility as a functional health food; therefore, future research is needed.

5.5 REFERENCES

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6 CAPÍTULO IV - COMPUTER VISION CLASSIFICATION OF BARLEY FLOUR BASED ON SPATIAL PYRAMID PARTITION ENSEMBLE

Abstract

Imaging sensors are largely employed in the food processing industry for quality control. Flour from malting barley varieties is a valuable ingredient in the food industry, but its use is restricted due to quality aspects such as color variations and the presence of husk fragments. On the other hand, naked varieties present superior quality with better visual appearance and nutritional composition for human consumption. Computer Vision Systems (CVS) can provide an automatic and precise classification of samples, but identification of grain and flour characteristics require more specialized methods. In this paper, we propose CVS combined with the Spatial Pyramid Partition ensemble (SPPe) technique to distinguish between naked and malting types of twenty-two flour varieties using image features and machine learning. SPPe leverages the analysis of patterns from different spatial regions, providing more reliable classification. Support Vector Machine (SVM), k-Nearest Neighbors (k-NN), J48 decision tree, and Random Forest (RF) were compared for samples' classification. Machine learning algorithms embedded in the CVS were induced based on 55 image features. The results ranged from 75.00% (k-NN) to 100.00% (J48) accuracy, showing that sample assessment by CVS with SPPe was highly accurate, representing a potential technique for automatic barley flour classification.

Keywords: machine learning; image processing; food quality; computer intelligence

6.1 Introduction

Barley is one of the most ancient cereal crops grown by humanity [1]. Over the years, some barley cultivars (e.g. malting or hulled barley) were selected for the malt and brewery industry, while other cultivars were selected to be used as food ingredients. These last cultivars are known as naked, or even hull-less or uncovered barley, generally containing higher amounts of soluble fiber [2,3]. Requirements concerning barley characteristics are quite different for malting and food industries. For brewery, grains with a low *b*-glucan concentration and barley kernels with a tough inedible outer hull still attached are required. High *b*-glucan levels interfere negatively in the malting filtration process. Furthermore, the loss of husks during malting processes leads to a reduction in malt quality. Such characteristics are inherent in hulled varieties [4]. On the other hand, barley cultivars with high levels of proteins and *b*-glucan (a functional ingredient) are preferred in the food industry, and some further specifications may vary depending on the requirements of each product. As an example, flours from naked types are preferably used for infant foods because they generally have fewer husk fragments [5].

Due to vast applicability, barley is one of the four significant grains, being used for various food materials [6,7]. Despite the genetic resource of a variety being the significant factor in determining its technological characteristics, it is well established that environmental conditions and interactions between environment and genotype can modify the expression of such characteristics [8]. Consequently, it is difficult to predict the best industrial destination for barley, or other cereal grains, without performing some physical and chemical analysis, which are generally expensive, time-consuming, and/or require specialized analysts and equipment [9]. The agricultural and food industries are often searching for fast and accurate technologies to increase processing performance, improving product quality. Imaging sensors and computer vision systems have been developed for grading product quality, discriminating among varieties, and detecting contaminants or added substances [10–12].

Quality evaluation can be performed by a Computer Vision System (CVS) based on an acquisition device (digital camera, inexpensive and broadly available) and prediction models using machine learning algorithms. This type of approach presents several advantages, including rapidity, low cost, and accuracy and can be applied to grains/seeds [13,14], flours [11], or other agricultural by-products. Being non-invasive methods and not employing chemical reagents, they can be considered as eco-friendly technologies. Product inspection is in high demand in the food industry, including quality inspection, process control,

classification, and grading. Manual inspection by visual examination demands a long time and is tedious and inefficient. Machine vision is suitable for this task, as computer vision provides an economical and fast alternative for food processing inspection [15].

The visual aspect is one of the most important parameters for the assessment of food quality. The general utilization of processing equipment in the industry has increased the risk of foreign material contamination [16]. Adulteration, contamination, or simply grading of products according to their visual characteristics are a common need in food processing. For instance, due to the resulting potential health threat to consumers, the development of a fast, label-free, and non-invasive technique for the detection of adulteration over a wide range of food products is necessary [17]. Hence, the food industry is interested in optimizing not only the nutritional characteristics of food products, but also their appearance, including color, texture, etc. It is essential to investigate objective methods that can quantify the visual aspects of food products [18].

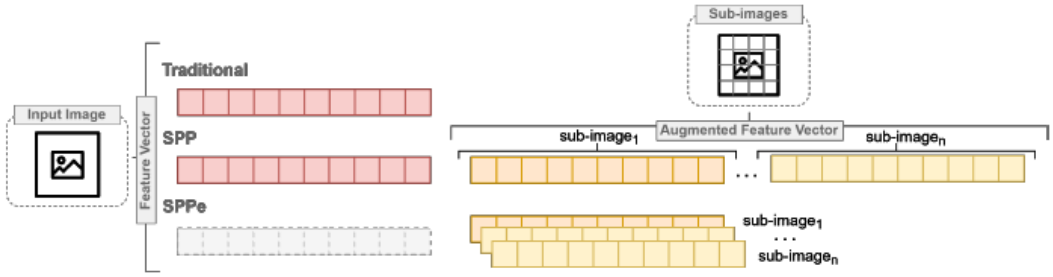
To meet the demand for high-quality produce, grains are classified according to their characteristics, before being sent for processing. Manual inspection of in-process products is difficult considering the sampling from processing lines [15]. Considering that barley grains are inhomogeneous, imaging techniques will have extensive practical applicability as analytical tools during industrial processing. Regarding all the chemical-free techniques available, there are still some common challenges before transferring recent research achievements obtained from a laboratory scale to industrial applications, such as building innovative data analysis algorithms that can thoroughly filter redundant information; exploiting appropriate statistical techniques for improving the model robustness for real-time operations; and decreasing the cost of the instrument [19].

In digital image analysis, spatial pyramid methods are very popular for preserving the spatial information of local features, focusing on improving the pattern description [20]. Sharma et al. [21] proposed Spatial Pyramid Partition (SPP) and highlighted that in many visual classification tasks, the spatial distribution carries important information for a given visual classification task. However, the proposed SPP method based on bag of features leads to enlarging the feature vector when several image descriptors take place, resulting in a highly dimensional problem and demanding feature extraction or feature selection methods.

Szczypinski et al. [9] classified barley grain varieties based on image-derived shape, color, and texture attributes of individual kernels. Considering barley flour classification, spatial information is required, but the original SPP is not feasible in our problem due to the characteristics of bag of features. In other words, barley grain and flour image-based

classification demand several features, while flour image analysis requires more robust pattern recognition approaches than SPP can provide. However, the increase of dimensionality arising from the application of SPP is a challenge in the machine learning scenario, and consequently for CVS applications. Therefore, we proposed the Spatial Pyramid Partition ensemble (SPPe), an ensemble technique fashioned on SPP towards supporting a suitable image pattern description in scenarios with a considerable number of features, as exposed in Figure 3.

Figure 3 - General overview highlighting the differences among traditional, SPP and Spatial Pyramid Partition ensemble (SPPe) approaches of feature vector composition.



A vast number of characteristics might rely on performance improvement of prediction tasks. The traditional feature extraction method considers the whole image at once for extracting its features, which possibly decreases important spatial information of some image descriptors from samples. As previously mentioned, the SPP was proposed to improve the problem task that requires some localized descriptors. However, it is based on a bag of features grounded on splitting images into sub-regions for supporting additional spatial information. Thus, SPP appraises a visual descriptor vector composed by the original image and its sub-regions from each sample. The proposed SPPe was evaluated in a CVS with a set of image features based on color, intensity, and texture, in comparison to SPP [21], directly using the features extracted from the Region Of Interest (ROI), as traditional CVS [13,22–24]. We compared the performance of four different machine learning algorithms: Random Forest (RF), Support Vector Machine (SVM), k Nearest Neighbor (k-NN), and J48 decision tree for modeling the classifier. These algorithms were employed to distinguish between naked and malting barley flour with image features extracted from 22 varieties acquired from five samples of each variety.

6.2 Related Work

Several studies presented CVS with machine learning methods applied to improve the prediction of a given parameter. Some CVS require sophisticated modeling to cope

with non-linearities and noisy and imbalanced datasets. The application of Machine Learning (ML) techniques for food attributes' prediction and quality evaluation has been widely investigated [10,22,25–30]. ML can be applied to extract non-trivial relationships automatically from a training dataset, producing a generalization of knowledge for further predictions [31]. Hence, machine learning promotes high performance as an alternative for an intensive agricultural operational process of the agri-technologies domain [32].

Random Forest (RF) [33], Support Vector Machine (SVM) [34], k-Nearest Neighbors (k-NN) [35], and the J48 decision tree algorithm [36] are well-established machine learning algorithms applied in many studies related to food quality analyses. RF was compared to SVM for an automated marbling grading system of dry-cured ham [37]. The SVM algorithm showed better performance with 89% of the samples correctly classified. Another application of SVM was described in Papadopulou et al. [27], achieving over 89% of accuracy for classification of beef fillets according to quality grades. For analyzing image features to evaluate the impact of diets on live fish skin, Saberioon et al. [38] applied four different classification methods, and SVM provided the best classifier with 82% of accuracy. Barbon et al. [23] proposed a CVS for meat classification based on image features, managed by an instance-based system using k-NN to classify meat according to marbling scores from image features. The authors presented an accuracy of 81.59% for bovine and 76.14% for swine samples, using only three samples for each marbling score by the k-NN prediction models. Granitto et al. [30] applied RF for the discrimination of six different Italian cheeses. In addition to reasonable accuracy, the RF model provided an estimation of the relative importance of each sensory attribute involved. The effectiveness of RF was also highlighted in a CVS used for predicting the ripening of papaya from digital imaging [22].

Considering barley applications, Nowakowski et al. [39] evaluated malt barley seeds using four barley varieties. The feasibility of image analysis was applied with machine learning and morphology and color features, achieving 99% accuracy. Kociolek et al. [40] classified barley grain defects using preprocessed kernel image pairs for feature extraction based on morphological operations. Pazoki et al. [41] identified cultivars using rain-fed barley seeds. The proposed method was applied with 22 features extracted from three varieties of samples, which fed a Multilayer Perceptron (MLP). The features of color, morphology, and shape were used for individual rain-fed barley seeds. Different network architectures were explored, including feature selection, resulting in 82.22% accuracy. Ciesielski and Nguyen [42] proposed to distinguish three different classes of bulk malt (made by barley grains). Image texture features were extracted and classification was performed with k-nearest-neighbor (k-

NN), achieving an accuracy of 77.00%. According to the authors, the classification through the evaluation of individual kernels is time-consuming, and many kernels are required to obtain a significant estimation of the modification index from a whole batch. Nevertheless, separating the samples in minimal milling portions is a booster alternative, aiding the evaluation of the difference between barley types. Lim et al. [7] explored Near Infrared Spectroscopy (NIRS) and a PLS-DA discrimination model to predict hulled barley, naked barley, and wheat contaminated with *Fusarium*. The authors achieved high accuracy at the cost of the complexity of NIRS equipment and signal processing.

Accordingly, the above studies have performed image analysis at different stages for varieties' identification for industrialization and improvement purposes. Integrating the industrial environment promotes a major role for developing an automated system for distinguishing agricultural raw-material products. The approach introduced in this paper is a CVS with an adaptation of the original SPP, modifying the overview perspective of sub-images that compose an original sample. The proposed approach is based on splitting each image into several sub-regions to predict a respective sample. We propose a method to improve prediction performance using CVS with machine learning, by applying the SPPe technique.

6.3 Materials and Methods

Twenty-two different barley varieties (cultivars) were provided by EMBRAPA Trigo (Brazilian Agricultural Research Corporation) in the city of Passo Fundo (Brazil). Barley samples were dehulled (Codema Inc. equipment, Maple Grove, MN, USA) during 75 s and milled (IKA A11 Basic Micro Miller, Osaka, Japan) for 75 s. Five different color images of flour were acquired from each of the 22 cultivars, in a total of 110 samples. Samples were collected and labeled by a specialist according to the source types of barley. After, all samples were classified either as malting Barley (B) or Naked barley (N). Fourteen of the cultivars were identified as malting barley, and eight were naked barley. Letters are followed by numbers in order to indicate differences from each specific barley variety (Table 11).

Table 11. Barley cultivars applied in the experimentation. B, malting Barley; N, Naked barley.

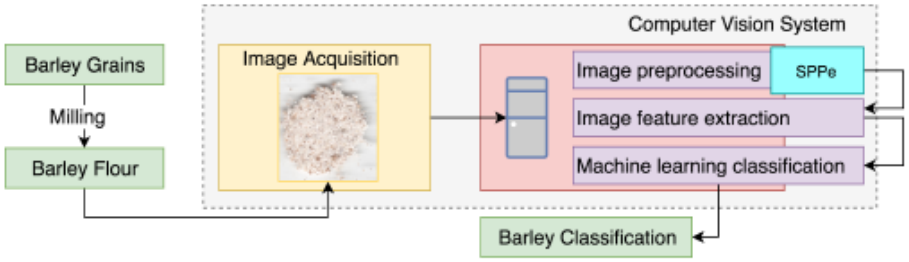
SAMPLE	CULTIVAR	TYPE
B01	BRS Aliensa	Malting
B02	BRS Itanema	Malting
B03	BRS Brau	Malting
B04	MN 6021	Malting

B05	BRS Sampa	Malting
B06	BRS Korbel	Malting
B07	MN6021	Malting
B08	BRS Elis	Malting
B09	BRS Korbel	Malting
B10	BRS Elis	Malting
B11	BRS Manduri	Malting
B12	BRS Brau	Malting
B13	BRS Cauê	Malting
B14	BRS Cauê	Malting
N01	149852	Naked
N02	149853	Naked
N03	149857	Naked
N04	149846	Naked
N05	149858	Naked
N06	149841	Naked
N07	149855	Naked
N08	149859	Naked

6.3.1 Computer Vision System

The CVS was constructed to classify samples as malting or naked barley, through the analysis of barley flour images. The employed CVS can be detailed as four main steps: acquisition, preprocessing, feature extraction, and classification (Figure 4).

Figure 4 - General overview of the proposed approach



It is important to highlight that the proposed SPPe is a technique to improve the classification performance grounded on a more informative strategy from the image sample before image feature extraction. SPPe requires interactive production of sub-images from an original sample image. These new sub-images had features extracted for enriching the dataset with complementary sources of information. Prediction of the original sample was based on a voting process for the sub-image samples' classification.

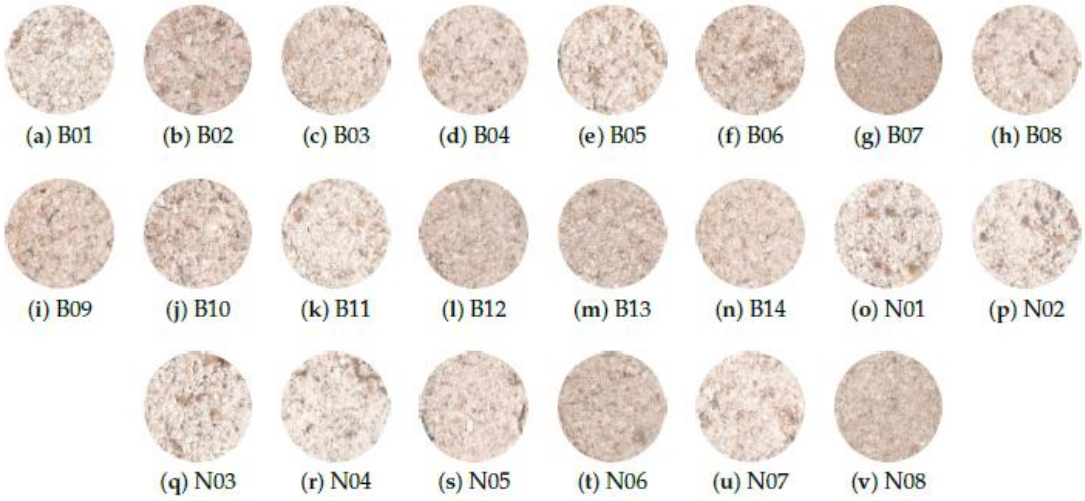
6.3.2 Image Acquisition and Preprocessing

The samples were collected from two different types for creating the image dataset: malting barley B and naked barley N (Figure 5).

Images (1200 dpi) were acquired by a computer vision system where each individual barley flour portion was scanned (HP Laser Jet M1120 MFP, Hewlett-Packard, Louveira, Brazil), using image acquisition software (HP Precision Scan Pro, Version 6.1, 2009). The images were acquired (14,028 _ 10,208 pixels) and stored as a .jpg file for further processing. A total of 110 barley sample images were collected, five from each cultivar, 40 from naked barley and 70 from malting barley. The ROI was cropped from the original image considering the largest square in individual portion of barley flour, removing the background and contour of each sample.

The main goal of preprocessing was background removal, keeping the ROI. To achieve this, the image was converted to the monochromatic space channel, and the background was removed using image thresholding. This threshold value was selected using Otsu's thresholding since it is one of the most widely-used methods for image segmentation. Since this image thresholding may lead to the removal of some pixels of the ROI, all the holes in the barley flour area were filled using a connectivity approach. At this point, the obtained image mask (representing the foreground) was used to find the center of mass of the object (barley flour samples). As the final step, the center of the mask found was used to grow a predefined square until reaching the object edge. The square mask was applied on to the original image, cropping the ROI.

Figure 5 - Samples of barley flour from malting (a–n) and naked (o–v) types



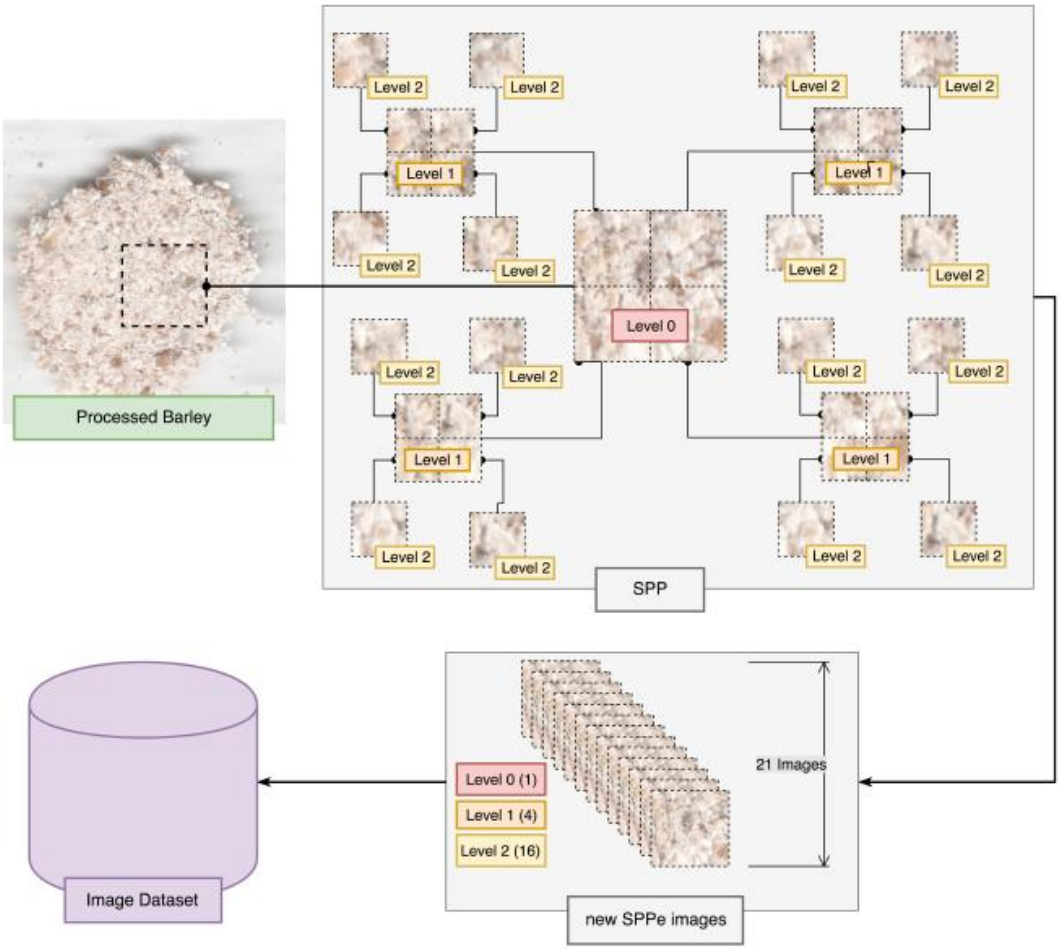
6.3.3 Spatial Pyramid Partition Ensemble

In the current work, we propose SPPe as part of the preprocessing step (Figure 6), to obtain a complete pattern comprehension of each sample. Our technique is a modification of the traditional SPP proposed in Sharma et al. [21]. Spatial Pyramid Partition (SPP) is based on splitting each image into a sequence of smaller sub-regions, extracting local image features from each image, and encoding their features into a vector [43,44]. In this sense, a given image is viewed as its low-level visual features extracted from all sub-regions. Each image is split into three levels, Level 0 being the image of the ROI by removing edges; Level 1 subdivides the ROI into four distinct parts, extracting its features; Level 2 subdivides each of the previous partitions into four other partitions, totaling 21 images from each ROI for extracting features to fine-tune the dataset. As a result, high-level and low-level features are extracted from the SPP image sequence to compose the image feature vector [21].

The proposed SPPe adapted the original SPP using an ensemble strategy to obtain the image classification. As opposed to traditional SPP, the aggregated image feature vector was not comprised of all sub-region, as a bag of features. Figure 6 presents an overview of the SPPe approach of creating sub-regions. Considering the description of image splitting, a new dataset was formed, which was composed of the sub-regions designated as Level 1 and 2. Thus, a feature vector was built from each sub-region without concatenating all regions. The ensemble strategy was applied to modify the dataset samples made up of smaller regions (Figure 6). Therefore, the sub-regions were used for problem modeling. After the prediction of a given sample from each sub-region, the scheme applied a weighing vote. In other words, we employed SPP with a subdivision strategy, to classify the Level 0 samples, and we considered each image separately for classification. Following the sub-regions' prediction, we aggregated them with the respective sample to analyze as Level 0. In this way, from a new sample image, each sub-region obtained from SPPe was classified, and the final decision was achieved by a voting step.

A single model was induced for predicting all sub-regions from different levels. The induction of the classification model was carried out in the Leave-One-Subject-Out (LOSO) scheme to avoid bias [45]. The method employed the LOSO procedure to bind the sub-regions and their image Level 0, keeping all of them together in the training or test phase. In other words, each sub-image was bound to the respective sample (Level 0) and received its label. Hence, the sub-regions were considered non-independent regions as part of the same sample. This methodology guarantees the model learns nothing about the subject to be predicted. Thereby, the technique to be applied decreases the learning bias, achieving accurate results.

Figure 6 - Spatial Pyramidal Partition ensemble (SPPe) for obtaining image samples



The SPPe output is based on the relation between the number of correct and incorrect sub-regions classified toward a majority decision as an ensemble prediction. Each level of partition by the SPPe method was assigned a voting weight. In the proposed experiment, for Level 1, it was assigned a weight of 1/3 and for Level 2, 1/12 for each ensemble member (image prediction). At the end of the iterations, the final result was computed considering each vote multiplied by the assigned weight. The final classification was obtained as the majority weighted vote from 20 sub-regions (4 from Level 1 and 16 from Level 2). This procedure creates a more reliable source of image classification by reducing overfitting, providing a robust description of barley based on several regions and dimensions.

We performed the original SPP proposed by Sharma et al. [21] in order to compare the SPPe performance improvements. SPPe avoids the high dimensional drawback, as in our scenario, SPP demands a total of 1155 image features per sample, while SPPe maintains only 55, both using only one classification model. Another important factor is related to the presence of visual components (e.g., husks) that could lead to noisy or biased features in the

image description vector. Using an ensemble technique such as SPPe, we could reduce the overfitting of the final model [33], since the visually undesired components are lost in the final decision by a minority vote.

Each image obtained from the SPPe method had its features extracted independently of the level by the same descriptors for further analysis.

6.3.4 Image Analysis and Feature Extraction

Step 2 is related to the image feature extraction in a sequence of previous procedures. The extracted features are groups of discriminatory properties suitable to distinguish the classes between naked and malting samples. We extracted a set of 55 image features based on color, intensity, and texture. The list including all image features used in our solution is presented in Table 12.

Table 12 - List of all image features used in the proposed SPPe approach for barley flour classification

Nº	Type	Name	Description
1	Color	meanH	Mean value of the H channel
2	Color	StdH	StdH Standard deviation of the H channel
3	Color	meanS	Mean value of the S channel
4	Color	stdS	Standard deviation of the S channel
5	Color	MeanV	Mean value of the V channel
6	Color	stdV	Standard deviation of the V channel
7	Color	stdHistH	Standard deviation of H channel histogram
8	Color	kurtHistH	Kurtosis of H channel histogram
9	Color	skewHistH	Skewness of H channel histogram
10	Color	stdHistS	Standard deviation of S channel histogram
11	Color	kurtHistS	Kurtosis of S channel histogram
12	Color	skewHistS	Skewness of S channel histogram
13	Color	stdHistV	Standard deviation of V channel histogram
14	Color	kurtHistV	Kurtosis of V channel histogram
15	Color	skewHistV	Skewness of V channel histogram
16	Color	meanL	Mean value of the L channel
17	Color	stdL	Standard deviation of the L channel

18	Color	meanA	Mean value of the A channel
19	Color	stdA	Standard deviation of the A channel
20	Color	meanB	Mean value of the B channel
21	Color	stdB	Standard deviation of the B channel
22	Color	stdHistL	Standard deviation of L channel histogram
23	Color	kurtHistL	Kurtosis of L channel histogram
24	Color	skewHistL	Skewness of L channel histogram
25	Color	stdHistA	Standard deviation of A channel histogram
26	Color	kurtHistA	Kurtosis of A channel histogram
27	Color	skewHistA	Skewness of A channel histogram
28	Color	stdHistB	Standard deviation of B channel histogram
29	Color	kurtHistB	Kurtosis of B channel histogram
30	Color	skewHistB	Skewness of B channel histogram
31	Intensity	meanInten	Mean value of intensity image
32	Intensity	StdInten	Standard deviation of Intensity image
33	Intensity	entropyInten	Entropy of intensity image
34	Intensity	stdHistInten	Standard deviation of Intensity image histogram
35	Intensity	kurtHistInten	Kurtosis of intensity image histogram
36	Intensity	skewHistInten	Skewness of intensity image histogram
37-46	Texture	lbp_0 - lbp_9	Vector of Local Binary Patterns (LBP) rotationally invariant features
47	Texture	entCoMatrix	Entropy of grey-level co-occurrence matrix
48	Texture	ineCoMatrix	Inertia of grey-level co-occurrence matrix
49	Texture	eneCoMatrix	Energy of grey-level co-occurrence matrix
50	Texture	corCoMatrix	Correlation of grey-level co-occurrence matrix
51	Texture	homCoMatrix	Homogeneity of grey-level co-occurrence matrix
52	Texture	eneFFT	FFT Energy
53	Texture	entFFT	FFT Entropy
54	Texture	ineFFT	FFT Inertia
55	Texture	homFFT	FFT Homogeneity

Concerning color descriptors, statistical moments from the CIE L*a*b* and HSV color spaces were used, similarly to Li et al. [43] and Campos et al. [46]. The image acquired was stored in the RGB format, where each pixel is based on three color space: R (red), G (green), and B (blue). Due to the brightness information presented in the whole color channel from RGB, a good practice is related to selecting a different color space able to isolate brightness. For this reason, the transformation of input images from RGB to CIE L*a*b and HSV was considered toward extracting color features. The CIE L*a*b* and HSV color spaces were explored in this study: L* (Lightness), a* (red-green), b* (yellow-blue), Hue (H), Saturation (S), and Value (V) color channels, respectively. The mean and standard deviation were calculated for each color channel. Moreover, we computed the standard deviation, kurtosis, and skewness from the histogram of each channel, comprising a total of 30 color features.

Likewise, the same five statistical moments were used to describe the intensity information of each image. The pixel intensity was calculated from the average of RGB values. Image entropy, which can be characterized as a statistical measure of the randomness, texture, and contrast of grey scale images, was calculated for the intensity channel [47].

Both color and intensity variations between samples can be observed in Figure 5. Therefore, those features were used to properly describe the samples, allowing the machine learning algorithms to find the correct relations between features and barley types.

The texture is an important feature to identify objects or the presence of patterns in an image [48]. In this case, texture features were used to distinguish between different types of barley. For example, the presence of husk fragments in milled barley affects some features and could characterize a specific type of barley flour. Thus, having general applicability, three texture descriptors were used: Local binary patterns [49], Grey Level Co-occurrence Matrix (GLCM) [48], for which distance d = 1 and angle 0_ considering 256 grey levels, and Fast Fourier Transform (FFT), this last to uncover frequency domain characteristics [50,51].

It is important to mention that we selected some traditional image descriptors to compose our feature vector, leveraging the comparison among the approaches for barley flour classification. Nevertheless, different image classification tasks can take more advantage of SPPe by employing alternative image features, e.g., features grounded on discrete wavelet transform [52] or fractal dimension [53].

6.3.5 Machine Learning

Features extracted from images are often used for classification and regression models, in order to identify samples from different classes or to predict quality parameters. In this way, machine learning algorithms can induce models from image features for automatic classification of barley flour. The modeling complexity of a machine learning system can vary greatly, allowing a high degree of customized freedom with appropriate trade-offs inherent in each specific scenario [54]. Some of the approaches include linear methods and non-linear machine learning algorithms, such as k-nearest neighbor, support vector machine, J48 decision tree, and random forest [46].

A brief description of the algorithms and the corresponding packages used to implement each ML algorithm are described in Table 13. In our experiments, the hyperparameters used were the default values of R packages in order to support a fair comparison among the algorithms.

Table 13 - Machine learning algorithms used in the experiments and corresponding R packages

Algorithm	Description	R Package	Hyperparameters
K-Nearest Neighbor (k-NN)	A non-parametric lazy learning algorithm; the training data are not used for any generalization [55].	`RWeka`	Euclidean distance; k= 5
Decision Tree (J48)	A decision tree widely applied to represent series of rules that lead to a class or value [56,57].	`RWeka`	C = 0.25; threshold = 0.25; with pruning
Random Forest (RF)	A combination of decision tree models that provides more accurate prediction [33,58].	`RandomForest`	ntree = 100; mtry = 7
Support Vector Machine (SVM)	A statistical learning algorithm, used for supervised ML and food quality solutions [34,59].	`e1071`	kernel = polynomial; $\gamma = 0.02$, degree = 3

In our experiment, the algorithms were applied in the R environment to induce models for barley flour classification. In order to achieve a reliable evaluation, two datasets were created: cross-validation and prediction test set. The cross-validation set was used to induce the models, adjusting the hyperparameters 10-fold considering 1800 images (Levels 1 and 2), while the prediction set was employed to test the classification performance using 400 images (Levels 1 and 2). Separation of samples into training and test sets was made in order to minimize the risks of overfitting, using the Kennard–Stone algorithm [60]. It is important to mention that the samples were split into the training and testing set considering Level 0 (a group of sub-regions), 90 samples (81.8%) for training and 20 samples (18.2%) for testing.

6.3.6 Evaluation Metrics

Performance evaluation of the models from machine learning was done using the total accuracy method (accuracy matrix) [61]. It is computed through the confusion matrix, which is defined by Equation (1).

$$\text{Total Accuracy} = (TP + TN)/n \quad (1)$$

The total accuracy is calculated by the sum of the main diagonal values from the confusion matrix. These values are the True Positive (TP) and True Negative (TN), which are divided by the sum of the values from the whole matrix (n). Thus, it is possible to compute the performance of the image features and machine learning algorithms through the relation of the correctly-classified samples of barley flour. Recall (Equation (2)) and precision (Equation (3)) are often used to evaluate the effectiveness of classification methods based on False Negatives (FN) and False Positives (FP). In our work, we employed these metrics in order to support a fair comparison of the methods' quality.

$$\text{Recall} = TP/(TP + FN) \quad (2)$$

$$\text{Precision} = TP/(TP + FP) \quad (3)$$

Additionally, processing time from feature extraction to prediction was compared. Thus, it is possible to estimate overall job execution with an additional perspective of performance analysis. In the experiments, the time cost was calculated as the average of 30 runs. Dealing with descriptors, random forest importance was applied to this approach. The RF algorithm estimates the importance of a variable being observed when the prediction error increases if data for that variable are permuted, while all others are left unchanged. Based on the trees, as the random forest is constructed, RF's importance investigates each extracted image feature, measuring the impact of characteristic samples in order to predict them [33].

In order to evaluate features extracted from barley flour samples, the exposed metric of variable importance demonstrates the advantage of random forest permutation because it embraces the impact of each predictor variable individually, as well as in multivariate interactions with other predictor variables.

6.4 Results and Discussion

6.4.1 Algorithms and Image Processing Methods

The results of algorithm performance for the classification of naked and malting barley flour revealed the advantages of the proposed SPPe method, in comparison to SPP and traditional approaches. The experiments showed distinct performance values achieved with the techniques applied to this approach using machine learning algorithms. In order to establish a practical performance testing environment, the experiments were executed with IntelR Core i7-6700 CPU 3.40 GHz 16 GB memory. Table 14 summarizes the results obtained for prediction algorithms over the datasets considering performance measures such as: accuracy, precision, recall, and average processing time.

Table 14 - Performance measures in the comparison of the methods and algorithms (RF, k-NN, J48 and SVM) over the cross-validation and prediction dataset

Algorithm	Metric	Cross-Validation			Prediction		
		Traditional	SPP	SPPe	Traditional	SPP	SPPe
RF	Accuracy	90.00	91.00	100.00	90.00	95.00	95.00
	Precision	71.88	71.88	100.00	86.67	96.88	96.88
	Recall	68.93	69.43	100.00	86.67	90.00	90.00
	Time (s)	65.35 (±0.13)	281.63 (±1.09)	217.11 (±0.40)	62.53 (±0.12)	268.71 (±0.39)	207.07 (±0.34)
k-NN	Accuracy	77.56	70.56	95.56	80.00	60.00	75.00
	Precision	60.79	57.25	95.85	74.51	52.75	65.63
	Recall	58.79	53.88	94.81	66.67	53.33	63.33
	Time (s)	64.50 (±0.10)	279.34 (±0.94)	209.49 (±0.36)	62.44 (±0.15)	268.51 (±0.36)	206.11 (±0.29)
J48	Accuracy	89.00	88.00	100.00	85.00	85.00	100.00
	Precision	71.88	71.88	100.00	79.77	91.67	100.00
	Recall	68.43	67.93	100.00	83.33	70.00	100.00
	Time (s)	70.14 (±0.26)	353.37 (±2.31)	210.79 (±0.37)	62.61 (±0.10)	270.71 (±0.38)	206.32 (±0.33)
SVM	Accuracy	93.00	92.00	98.89	80.00	95.00	95.00
	Precision	70.42	72.50	99.11	89.47	96.88	96.88
	Recall	70.00	70.36	98.57	60.00	90.00	90.00
	Time (s)	64.62 (±0.15)	280.57 (±0.95)	213.40 (±0.43)	62.83 (±0.12)	268.66 (±0.37)	206.75 (±0.37)

Comparing the machine learning algorithms, k-NN provided the worst performance, with accuracy values equal to or below 80.00% for prediction using all methods investigated. Concerning only the results of the traditional CVS approach, (without SPP or SPPe) for the prediction set, RF obtained superior performance, with 90.00% of accuracy and precision/recall values of 86.67%, while SVM and k-NN presented similar accuracy (80.00%).

The original SPP presented superior results compared to the traditional method. SVM (92.00%) and RF (91.00%) reached superior results compared to J48 (88.00%) and k-NN (70.56%) for accuracy considering the cross-validation set. For the prediction set,

RF obtained superior results, similar to SVM (95.00%). The worst metrics evaluated for the prediction set using the SPP technique was k-NN (60.00%), followed by J48 (85.00%).

An improvement of classification accuracy was obtained by SPPe technique with ML algorithms (Table 14). The average performance of classification considering all machine learning algorithms was improved from 83.75% in the traditional method and original SPP to 91.25% in accuracy for prediction sets. It is important to highlight that J48 stood out with 100% accuracy, and k-NN maintained the lowest performance with 95.56% (cross-validation set) and 75.00% (prediction set). Likewise, the SPPe solution had the lowest processing time cost in comparison to SPP. Furthermore, traditional CVS provided better results than SPPe using k-NN.

Considering the processing time of the applied methods, CVS spent less time, being faster than SPP and SPPe, as expected. When comparing SPP and SPPe, our proposal was faster than SPP in all experiments. It is clear that the time cost tends to enlarge when the feature vector expands, as proposed by SPP and SPPe; however, the trade-off between predictive performance and processing time suggests the SPPe as a suitable solution when the main goal is the classification performance.

6.4.2 Evaluation of Image Features

The RF importance exposes the most relevant features in prediction tasks. The importance values are summarized in Figure 7. The most important features were from color: standard deviation values of the H and b* channels histogram (hue from HSV and yellow-blue CIE L*a*b* color spaces) were the most relevant explaining features with more scores higher than 50. Several statistic values from H, B*, and a* overcame texture and intensity features, although all features presented an impact for the classification procedure.

In order to characterize the types of barley flour, the mean and standard deviation of the grey-scale image, and hue HSV channel were the most discriminative image features. Moreover, the values of a* and b* channels gained higher importance, as well as the saturation. Texture features were significant for predicting the samples. Indeed, some texture features of the grey level co-occurrence matrix, and some LBP metrics were efficient at predicting variations of samples and also could be related to the granularity present in the barley flour.

Figure 7 - RF importance of image features

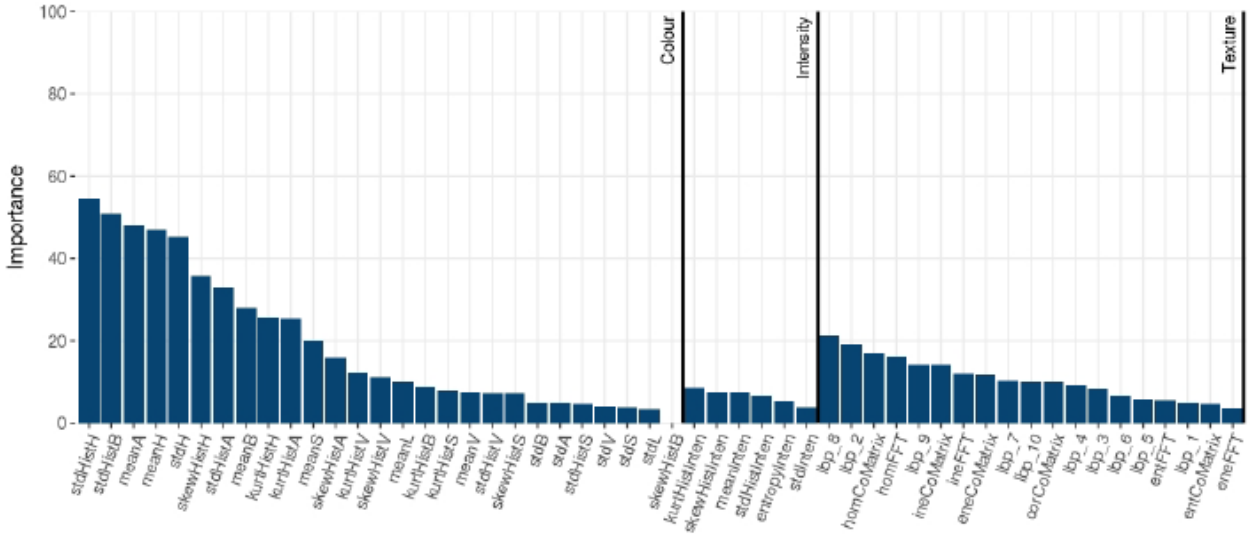


Figure 8 summarizes the results in which it is possible to observe some misclassified samples by comparing all performed techniques with five different repetitions of acquisition (A0, A1, A2, A3, and A4). Correctly-classified samples are presented in light blue, while dark blue shows the misclassified samples for each method. It is possible to observe that the naked class presented more misclassified samples, meaning it is more complex to predict. For some classification algorithms, it is possible to observe similar behaviors among the samples, with k-NN as the worst performance.

Analyzing the misclassified samples, it was possible to identify similar patterns in both naked and malting types. Observing the accuracy error, it is possible to conclude that naked sample N07 (37% error) presented similar characteristics to malting samples. Figure 9 presents an overview of the N07 cultivar, where it is possible to observe details by comparing the five samples as previously mentioned, and highlighted in the heat map shown in Figure 8. One possible explanation for the high error rate observed is the fact that Brazilian naked varieties were developed by using malting barley genes, so the studied features can be similar to those of malting barley varieties due to genetic origins [62].

Overall, SPPe demonstrated superior prediction ability compared to other methods, in addition to reduced overfitting and decreasing the high dimensionality present on the original SPP. Differences in composition/physical characteristics between the two barley groups (from naked and malting barley) were detected by the computer vision system, and classification accuracy was improved using SPPe.

Figure 8 - Accuracy heat map of J48, k-NN, RF, and SVM over the prediction dataset comparing traditional CVS, SPP, and SPPe techniques with repetitions A0, A1, A2, A3, and A4

Figure 9 - Samples of cultivar N07, the lowest accuracy of barley flour classification

6.4.3 SPPe in the Industry

There is an expressive advance when using the SPPe technique in comparison to the traditional CVS and SPP. The best result in the prediction set referred to J48 predictive performance and with low processing time in comparison to SPP. The proposed vision system was designed for an embedded process to provide high-level information for the barley flour industry environment. The system can be implemented by three sub-division steps:

The input image (acquisition) being extracted from the camera. Images are acquired by a camera placed at the scene under inspection;

The scene has to be appropriately illuminated and arranged, which promotes suitable reception of the image properties that are necessary for image processing (feature extraction and classification);

The processing system stage consists of a computer employed for processing the acquired images, resulting in classifying as naked or malting barley flour.

Combining the embedded technology with image processing, a future application in barley flour recognition types for quality control industry is possible.

Our proposal is a viable solution for barley flour industrial processing, as well as similar flour food products. More specifically, our proposal contributes to the industry in different stages of production. The CVS can be used as a quality control, observing specific supplier and providing financial advantages for high-quality flour. The proposed solution can be integrated into processing lines to identify barley according to the application, i.e., whether it is destined for infant formula, health food, and the malting industry, among other industrial production. It is important to highlight that the SPPe was fashioned with a minor feature vector in comparison with the SPP technique, spending less time to process, being faster and promoting its implementation in the production line.

6.5 CONCLUSION

This work proposed a system based on ML algorithms and computer vision developed to solve the automatic data analysis. A new proposed approach of Spatial Pyramid Partition ensemble (SPPe) provided better results for classification of barley flour into two different classes when compared to Spatial Pyramid Partition (SPP) and traditional CVS. Differences in barley composition cause variation of the flour's physical characteristics, which were detected by image analysis. The proposed method showed a significant improvement, by reducing overfitting, avoiding dimensional growth, and improving classification accuracy for several machine learning algorithms. The importance of all image descriptors (color, intensity, and texture) for providing helpful information to distinguish between malting and naked barley flour samples was identified. The best model was built using the SPPe with J48 decision tree, allowing the classification of 100% of samples. The results of this study are promising, and they could allow the development of an effective model in order to expand its use in the food industry, reducing costs and improving the effectiveness of automatic quality inspection.

6.6 References

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7 CONCLUSÃO

Este estudo contribuiu para o entendimento da composição e propriedades físicas das variedades brasileiras de cevada e aponta características que podem indicar os usos finais mais adequados. Há diferenças entre as linhagens de cevada nua e as cultivares cervejeiras plantadas no Brasil. Ademais, estas diferenças na composição da cevada causam variação das características físicas da farinha, que são detectadas por análise de imagem.

Apesar da cevada cervejeira brasileira não ter apresentado redução significativa dos níveis de colesterol total e LDL, ela ainda pode ser um ingrediente ou alimento que auxilia na promoção da saúde, principalmente no controle da glicemia. Uma possível aprovação da alegação de saúde da cevada poderia aumentar o interesse e a comercialização de cevada alimentícia. Porém, para isto, seriam necessários mais estudos *in vivo* utilizando cevada nacional.

ANEXOS

ANEXO A

Termo de Consentimento Livre e Esclarecido
"Cevada (*Hordeum vulgare*): prospecção de cultivares, desenvolvimento tecnológico e comprovação de características funcionais"

Prezado(a) Senhor(a):

Gostaríamos de convidá-lo (a) para participar da pesquisa **"Cevada (*Hordeum vulgare*): prospecção de cultivares, desenvolvimento tecnológico e avaliação de características funcionais"**, a ser realizada no Hospital Universitário da Universidade Estadual de Londrina e no Centro do Coração**.**

O objetivo da pesquisa é esclarecer o possível efeito benéfico à saúde do consumo da cevada brasileira**.** Sua participação é muito importante e ela se daria da seguinte forma: aderindo a pesquisa, você deverá consumir diariamente 10 bolachas (cada uma delas com 8 gramas) de cereal integral e mais uma porção de 50 gramas de grãos integrais descascados, que podem ser consumidos em substituição ao arroz ou outra fonte de carboidrato de costume. O tempo de intervenção será de 45 dias. No início da pesquisa e a cada quinze dias, você deverá comparecer ao Centro do Coração para coleta de sangue. No total, serão quatro coletas de sangue. Ressaltamos que durante a coleta pode ocorrer desconforto (dor) e, após o procedimento, pode haver hematoma no local de perfuração.

Esclarecemos que sua participação é totalmente voluntária, podendo você: recusar-se a participar, ou mesmo desistir a qualquer momento, sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Esclarecemos, também, que suas informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade. No final da pesquisa, as amostras de sangue serão descartadas, ou seja, não será arquivado nenhum material biológico.

Esclarecemos ainda, que você não pagará e nem será remunerado(a) por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação.

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