

University of Trás-os-Montes and Alto Douro

**Potential of *Phaseolus vulgaris* L.: nutritional value, functional properties
and development of innovative tools for their assessment**

PhD thesis

- Agricultural Production Chains - From Fork to Farm -

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Vila Real, 2020

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To my family

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Resumo

As leguminosas (Fabaceae), são da segunda família mais importante das plantas a seguir à família das gramíneas (Poaceae). Entre as leguminosas, o feijão comum (*Phaseolus vulgaris* L.) é o mais consumido no mundo e o mais importante para consumo humano direto, devido ao seu rico valor nutricional e funcional. Proteína, fitoquímicos, fibra, tocoferóis, aminoácidos, amido, ácidos gordos e minerais são compostos importantes presentes no feijão que estão relacionados com benefícios para a saúde. A indústria alimentar tem demonstrado um crescente interesse na incorporação de leguminosas de baixo custo, mas ricas nutricionalmente, na preparação de vários produtos alimentares e rações, criando valor acrescentado. Deste modo, considerando a biodiversidade do feijão comum e a necessidade de conhecer mais detalhadamente a sua composição nutricional e compostos bioativos, justificam-se estudos que aprofundem estes conhecimentos, consequentemente a exploração desta informação e respetivo uso específico. Por outro lado, ao longo dos últimos anos, tem-se assistido ao desenvolvimento de metodologias inovadoras, que permitam uma avaliação da qualidade dos produtos alimentares, de modo a substituir as análises convencionais, reduzindo o uso de reagentes químicos, o tempo análise e minimizando a manipulação das amostras. Contudo, a aplicação de modelos preditivos para avaliação da qualidade do feijão, quer nos laboratórios quer na indústria alimentar é ainda limitada.

De acordo com este alinhamento, foram definidos como principais objetivos desta tese a caracterização nutricional, antinutricional e fitoquímicos do feijão e o estudo da sua utilização no enriquecimento de produtos alimentares, com valor acrescentado para a cadeia agroalimentar. A partir desta informação, fixou-se também como objetivo, desenvolver novos métodos de rotina em laboratório, para a determinação dos nutrientes, antinutrientes e fitoquímicos presentes nas cultivares de feijão, utilizando análises multivariadas, por forma a estabelecer correlações entre a composição de cada cultivar de feijão e seu espectro adquirido em Espectroscopia no Infravermelho por Transformada de Fourier (FTIR) na região do Infravermelho Médio (MIR) e Infravermelho Próximo (NIR).

Dos resultados obtidos destaca-se a relevância das condições climáticas na fase de crescimento da planta, que exercem influência determinante nos principais compostos nutricionais, antinutricionais e compostos fenólicos das cultivares de feijão. O feijão branco, preto e lindo revelaram ser fontes promissoras de proteína e aminoácidos, podendo ser utilizadas para enriquecimento nutricional. As cultivares de feijão vermelho e feijão canário oferecem as melhores propriedades funcionais devido ao elevado teor em composição

fitoquímica, atividade antioxidante e compostos fenólicos individuais. Este perfil funcional revela que o feijão comum, à semelhança de outras leguminosas, pode oferecer um papel importante na prevenção do desenvolvimento de doenças cardiovasculares, diabetes e cancro. Por outro lado, os resultados deste trabalho indicam que a análise através de FTIR permite a avaliação de parâmetros relevantes, não apenas em relação aos macronutrientes presentes nos alimentos, mas também para compostos presentes em baixas concentrações.

Em conclusão, os resultados desta tese permitiram um progresso no conhecimento da composição nutricional, antinutricional e compostos bioativos de cada cultivar de feijão e da forma como esta é influenciada pelas condições climáticas. Em função da composição nutricional, antinutricional, fitoquímicos e capacidade antioxidante de cada cultivar é possível aferir, com mais detalhe, o seu potencial para a indústria alimentar, bem como os benefícios para a saúde. Este trabalho deu também início à utilização do FTIR como metodologia inovadora e ecológica de rotina em laboratórios e indústria de alimentar.

Palavras-chave: Feijão comum, nutrientes, antinutrientes, fitoquímicos, aplicações alimentares, FTIR

Abstract

Legumes (Fabaceae) are the second most important family of plants after the grass family (Poaceae). Among legumes, common beans (*Phaseolus vulgaris*) are the most consumed worldwide and produced for direct human consumption, due to their rich nutritional and functional value. Protein, phytochemicals, fiber, tocopherols, amino acids, starch, fatty acids and minerals are important compounds present in beans which may provide health benefits. The food processing industry has shown a growing interest in low cost grain legumes for incorporation, but nutritionally rich, in food and feed, creating value-added. Hence, considering the biodiversity of beans and the need to know their nutritional and bioactive compounds in more detail, studies that deepen this knowledge are justified, consequently the exploration of their potential uses in food products. Concomitantly, over the past few years, have been developed innovative methodologies and approaches, which allow the quality evaluation of food products, in order to replace conventional analysis, reducing the use of chemicals, saving time and minimizing sample handling. However, the application of predictive models for quantification of beans' quality, in laboratories and food industry is still limited.

According to this alignment, the main objectives of this thesis are the nutritional, antinutritional and phytochemical characterization of beans and to study their uses in the enrichment of food products, with added value for the agrifood chain. From this information, the objective was also to develop new routine methods in the laboratory, for the determination of nutrients, antinutrients and phytochemicals present in bean cultivars, using multivariate analysis, in order to establish correlations between the bean composition and its spectrum acquired using Fourier Transform Infrared Spectroscopy (FTIR) approach in the Mid-infrared (MIR) and Near-infrared (NIR) region.

Our study highlights the relevance of climatic conditions during the growing seasons which has a determinant influence on major nutritional and antinutritional compounds and the respective phenolic compounds of bean cultivars. Navy, black and pink eyed cultivars were the most promising protein and amino acids sources, which can be used for nutritional enrichment. Red kidney and arikara yellow cultivars offer the best functional proprieties due to their higher phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds. This functional profile reveals that, similarly to other legumes, it can offer an important protective role in the prevention and treatment of cardiovascular diseases, diabetes and cancer. On the other hand, the results of this work proved that the FTIR techniques, allows the

evaluation of relevant compounds, not only regarding the macronutrients present in beans but also for compounds present in low concentrations.

In conclusion, the results of this thesis allowed a progress in the knowledge of the nutritional, antinutritional and bioactive compounds of each bean cultivar and how they are influenced by climatic conditions. Regarding to the nutritional, antinutritional composition and phytochemicals and *in vitro* antioxidant activity of each cultivar, it is possible to assess, in more detail, their potential for food applications, as well as the health benefits. This work also initiated the use of innovative and eco-friendly FTIR methodology as a routine method in laboratories and food industry.

Keywords: Common bean, nutrients, antinutrients, phytochemicals, food applications, FTIR

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List of abbreviations

ABTS- 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt

Ala- Alanine

AOAC- Association of Official Agricultural Chemists

ANOVA- Analysis of variance

Arg- Arginine

Asn- Asparagine

Asp- Aspartic acid

ATR- Attenuated Total Reflection

CE- Catechin equivalents

CV- Cross-validation

CWT- continues wavelet transform

DPPH- 2,2-diphenyl-1-picrylhydrazyl radical

DRIFT- Diffuse Reflectance Infrared Fourier Transformed

ECE- Epicatechin equivalents

EDTA- Ethylenediaminetetraacetic acid

FI- Full Interval

FRAP- Ferric reducing antioxidant power

FTIR- Fourier Transform Infrared spectroscopy

GAE- Gallic acid equivalents

GC-MS- Gas chromatography - mass spectroscopy

Gln- Glutamine

Glu- Glutamic acid

Gly- Glycine

HSI- Hyperspectral imaging

HF- High Frequency

His- Histidine

HPLC- High-Performance Liquid Chromatography

Ile- Isoleucine

IP₆- inositol polyphosphate

IR- Infrared spectra

LF- Low Frequency

Leu- Leucine

LOD- Limit of detection
LOO procedure- Leave-one-out procedure
LOQ- Limit of quantitation
MIR- Mid infrared
MLR- Multi-linear regression
MUFA- monounsaturated fatty acids
MSC- Multiplicative scatter correction
NaH₂PO₄- Sodium dihydrogen phosphate
NaOH- Sodium hydroxide
NIR- Near infrared
Nor- Norvaline
PCA- Principal component analysis
PUFA- polyunsaturated fatty acids
Phe- Phenylalanine
PLS-R- Partial least squares regression
PRESS- Prediction error sums of squares
RFOs- Raffinose family oligosaccharides
Ser- Serine
SFA- Saturated fatty acids
SNV- Standard normal variate
TDF- Total dietary fiber
TE- Trolox equivalents
Thr- Threonine
TIU- Trypsin inhibitor units
Trp- Tryptophan
TPTZ- 2,4,6-tripyridyl-S'-triazine
Tyr- Tyrosine
Val- Valine
VIP- Variable importance projection

Chapter 1

1.1.General introduction

1.1. General introduction

The recent awareness of the importance of food composition on human health has triggered a rising worldwide consumer interest in eating foods that are particularly important sources of nutrients and bioactive compounds (Calles et al., 2019, FAO 2019). Of all the potential foods, the present study focuses on legumes for important reasons.

Firstly, legumes represent a good and cheap alternative to partly replace the meat based food products due to their rich nutritional and phytochemical composition. In fact, the increased legume consumption is currently around 7 kg/person per year (FAO, 2016), whilst its also increasing production is concentrated in India, United Kingdom, Poland and Russian Federation (Figure 1) (FAOSTAT 2019).

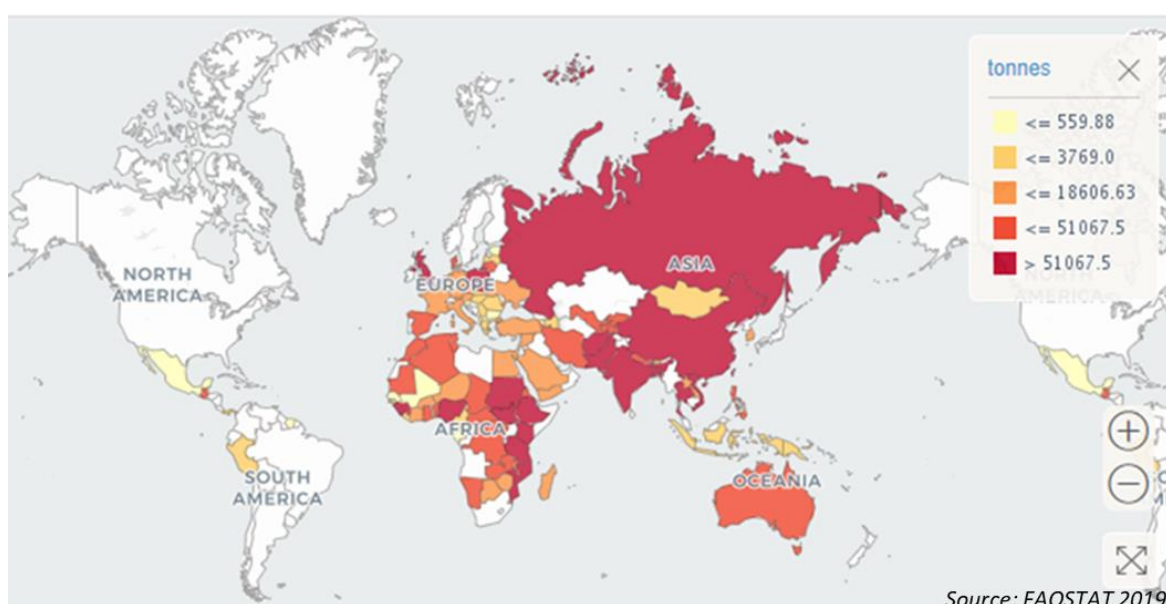


Figure 1 Average production (tonnes) in the world of legumes between 2010-2017.

Secondly, experts indicate that legumes play a key role in food security and climate challenges by i. providing proteins and lysine to complement cereals in human nutrition, ensuring a balanced diet for vegetarians and vegans, and ii. contributing to diversified crop rotations and nitrogen-fixing in the soil, thus reducing the use of fertilizers and greenhouse gas emissions.

Thirdly, it has received growing attention by food authorities. For instance, on the aftermath of the International Year of Legumes in 2016, several FAO activities were developed worldwide to promote awareness of the many benefits of legumes in food and nutrition security,

including opening up opportunities for information exchange and developing the necessary skills to enhance the production and trade of legumes (Calles et al., 2019).

Fourthly, legumes, particularly common beans are rich in protein, carbohydrates and dietary fiber, as well as a source of essential nutrients including vitamins, minerals, phenolic compounds and lower amounts of fat, all contributing to associated with health benefits (Kan et al., 2018). In addition and to some extent detrimental, beans are also known due to their antinutritional composition, which may affect the bioavailability and digestibility of some nutrients. However, several studies have identified beans antinutrients as beneficial to health (Carbonaro et al., 2015). Due their excellent nutrients and phytochemicals, beans have been used for protein-enriched products and gluten-free foodstuffs (Giuberti et al., 2015).

Lastly, the worldwide increasing bean consumption has the potential to greatly contribute to the sustainability of the agro-food system by reducing dependence on meat, as well as reducing the incidence and prevalence of a considerable number of degenerative diseases, including cardiovascular diseases and cancer (Dhillon et al., 2016, García-Lafuente et al., 2014).

If on one hand, this rising adoption of beans as high proteic alternatives for food and enrichment is beneficial, there is on the other hand recent concern about the use of rapid and eco-friendly methodologies in food industries and laboratories for assessment of beans quality. The evaluation of beans' quality is traditionally performed through the use of mainly high liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS) and spectrophotometry that are time-consuming, expensive, required the use of chemicals and are destructive for the samples. One of the current innovative methodologies is Fourier transform infrared spectroscopy (FTIR) which is simple to apply, faster, no chemicals used and non-destructive for samples. FTIR technique is based on chemometrics, establishing correlations between spectral data and the composition of bean flours, developing predictive models through multivariate analysis (Tao et al., 2017). This methodology focuses in two regions, the mid-Infrared (MIR) region $400\text{--}3600\text{ cm}^{-1}$ and near-Infrared (NIR) defined as encompassing the $4000\text{--}14000\text{ cm}^{-1}$, which the absorptions of spectrum are related to the vibrations of functional groups. The second motivation of this study is to apply the FTIR methodology to develop predictive models for evaluation of beans' quality and comparing the performance of both regions of infrared.

In summary, and given the legumes biodiversity between species and cultivars, a detailed study of the composition of different bean cultivars is essential to determine and their

potential uses in the food industry and the respective associated health benefits of the new food products. Associated to this need, there is a methodological challenge to develop and apply eco-friendly methodologies to quickly and non-destructively evaluation of the nutritional composition, antinutritional, phenolic compounds and *in vitro* antioxidant capacity of different bean cultivars.

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Chapter 1

1.2. *State of art*: “Meat of the poor” no more. Legumes: chemical composition, bioactive properties, innovative food products and health claims

1.2 “Meat of the poor” no more. Legumes: chemical composition, bioactive properties, innovative food products and health claims

Adapted from:

Bruna Carbas, Nelson Machado, Shivani Pathania, Carla Brites, Eduardo AS Rosa, Ana IRNA Barros. “Meat of the poor” no more. Legumes: chemical composition, bioactive properties, innovative food products and health claims. *Under review in Food Reviews International*

Abstract

Legumes are an integral part of the human diet and an important and sustainable source of protein, carbohydrates, dietary fiber, vitamins, and phytochemicals, categorizing them as a vital food resource. Nevertheless, legumes also contain some antinutritional compounds which deplete the bioavailability of these nutrients. Due to their composition, legumes have been used in enriched foodstuffs and for the development of gluten-free products. Such practices revealed legumes potential health effects. This overview is an effort to stimulate interest for the production and use of legumes by promoting the dissemination and a better understanding of their nutritional value, and the functional properties of flours and products developed with and from them.

Keywords: Nutritional composition, antinutritional components, health benefits, food products, innovative tools

1.2.1. Introduction

Legumes (Fabaceae) are annual leguminous crops and the second most important family of plant crops after the grass family (Poaceae) (FAO, 2017). Grain legumes represent 27% of the world crop production (Smýkal et al., 2015) and are represented mainly by dry beans (*Phaseolus vulgaris* L.), chickpeas (*Cicer arietinum* L.), dry peas (*Pisum sativum* L.), cowpeas (*Vigna unguiculata* (L.) Walp), lentils (*Lens culinaris* Medik.) and faba beans (*Vicia faba* L.) (FAO, 2019) (excluding the crops used mainly for oil extraction, such as soybean and groundnuts). The world leader regarding crop production are dry beans (31 Million tons) with India as the main producer of this crop, while faba beans (4 Million tons) represent the lowest world crop production and China is its main producer. Legumes are an essential food for human

diets in many parts of the world, and have a significant cultural and historical importance (Hant al., 2010).

The recent and growing awareness of the importance of food composition in human health has increased consumer interest in nutritious and bioactive rich legumes. The variation in nutritional and phytochemical compositions among legumes can be attributed to the differences in their genetics, cultivars, geographical location and climatic conditions (Aguilera et al., 2011). Legumes are important sources of proteins, dietary fiber, vitamins, minerals, carbohydrates and phytochemicals, important for human health (Parmar et al., 2017). The importance of consumption of legumes have gained special attention in the prevention of some diseases due to their low glycaemic index and slowly-digestible starch content, preventing sharp raises of sugar in blood and promoting weight loss (Becerra-Tomás et al., 2018), whereas flavonoids, phenolic acids and tannins have been associated with gastrointestinal functions, antioxidant activity, anti-inflammatory and anticarcinogenic properties (García-Lafuente et al., 2014). However, legumes contain substances which have been considered as antinutritional compounds, with dualistic functions, and ascribed with positive and negative health effects (Kalpanadevi & Mohan, 2013). An example is the oligosaccharides which are responsible for the flatulence. However, they have also been linked to prebiotic and potentially protective effects on gastrointestinal health (Sreerama et al., 2012).

Additionally, legumes are increasingly used in new product formulations for their nutritional and health-promoting properties such as high protein content, fiber content, and antioxidant properties. Legumes have gluten-free status and legume supplementation has been used to improve the nutritional status of gluten-free products (fastest growing new product trends), lower the glycaemic index of food products, enhances the antioxidant levels along with the functional properties such as water binding and fat absorption (Belghith Fendri et al., 2016). This review aims to describe the nutritional, bioactive and antinutritional composition of different legumes, pea, lentils, faba bean, bean, grasspea and cowpea. Their beneficial effects on health when included in human diet and the innovative food products with their incorporations. Finally, the use of innovative methodologies as an alternative to evaluate their composition.

1.2.2. Nutritional composition

A literature review of the available studies provided a range of components for legumes, which are listed in Table 1.

Table 1 Protein content, amino acids, ash, minerals, lipids, fatty acids, tocopherols and TDF of legumes species (g/100g dw).

	Pea	Chickpea	Lentils	Faba bean	Bean	Grass pea	Cowpea
Protein	21.0-24.9	18.5-28.3	20.6-28.1	20.9-28.0	16.7-32.6	22.4-31.7	17.2-25.7
Thr	0.64-0.88	2.70-3.50 [¤]	0.79-0.97	0.87-0.97	0.73-1.53	3.44-3.60 [¤]	0.71-0.92
Val	0.74-1.06	4.10-5.20 [¤]	1.08-1.29	1.19-1.31	0.00-0.10	3.52-4.02 [¤]	1.00-1.22
Met	0.03-0.05	0.80-1.30 [¤]	0.04-0.05	0.05-0.08	0.00-0.25	0.72-0.99 [¤]	0.09-0.16
Ileu	0.60-0.78	4.50-5.20 [¤]	0.85-0.92	0.92-0.97	0.67-1.21	2.81-3.17 [¤]	0.73-0.87
Leu	1.42-1.83	8.20-9.50 [¤]	1.98-2.37	2.16-2.26	1.09-2.25	5.01-5.82 [¤]	1.84-2.05
Phe	0.87-1.00	5.00-6.20 [¤]	1.18-1.44	1.00-1.12	0.88-1.59	3.28-3.77 [¤]	0.97-1.23
Lys	1.04-1.48	6.70-7.80 [¤]	0.90-1.38	1.41-1.61	1.02-2.00	5.52-5.94 [¤]	1.29-1.38
Tyr	0.15-0.33	2.60-3.10 [¤]	0.33-0.49	0.47-0.55	0.33-1.08	2.10-2.44 [¤]	0.31-0.41
Asp	1.58-2.23	10.20-11.50 [¤]	2.37-2.59	2.01-2.16	0.86-2.55	8.12-9.63 [¤]	1.75-2.19
Ser	0.63-0.87	3.30-4.20 [¤]	0.93-1.24	0.80-0.90	0.73-1.17	3.71-4.18 [¤]	0.71-1.05
Glu	2.86-3.79	16.50-17.80 [¤]	4.28-4.75	4.14-4.57	2.28-3.86	11.95-14.45 [¤]	3.74-4.12
Gly	0.56-0.71	3.40-4.00 [¤]	0.74	0.77-0.83	0.49-0.87	3.13-3.68 [¤]	0.62-0.64
Ala	0.55-0.78	4.70-5.20 [¤]	0.76-0.83	0.75-0.83	0.58-0.94	3.60-4.21 [¤]	0.66-0.72
Cys	0.05-0.08	0.40-0.80 [¤]	0.05-0.06	0.06-0.11	0.06-1.29	1.49-1.98 [¤]	0.06-0.07
His	0.34-0.42	2.90-3.20 [¤]	0.45-0.60	0.53-0.58	0.44-0.86	2.12-2.56 [¤]	0.49-0.59
Arg	1.26-1.95	8.00-8.50 [¤]	1.51-1.56	2.23-2.56	0.97-2.17	6.12-7.80 [¤]	1.17-1.42
Pro	0.52-0.71	3.50-4.00 [¤]	0.75-0.79	0.69-0.81	0.49-2.91	4.83-6.91 [¤]	0.48-0.76
Ash	2.7-3.6	2.3-3.8	2.4-3.5	3.1-3.6	3.0-5.7	3.1-3.6	3.2-3.7
Calcium	0.046-0.120	0.08-0.16	0.006-0.008	0.163-0.392	0.08-0.16	0.102-0.144	0.003-0.004
Iron	0.002-0.006	0.005-0.007	0.006-0.007	0.005-0.006	0.005-0.007	0.004-0.006	0.001-0.005
Potassium	0.563-0.939	0.99-1.06	0.987-1.024	0.925-1.026	1.22-1.44	0.803-1.185	1.171-1.908
Magnesium	0.0473-0.103	0.15-0.17	0.004-0.005	0.223-0.226	0.14-0.20	0.106-0.181	0.132-0.138
Phosphorus	0.163-0.373	0.39-0.45	0.114-0.301	0.178-0.198	0.36-0.50	0.535-0.662	0.312-0.422
Lipid	1.1-1.5	4.3-6.6	1.1-3.2	1.0-3.9	1.1-2.9	0.5-2.3	1.3-7.2
SFA	15.95-94.07	12.60-16.30	15.87-17.62	10.99-16.43	13.96-43.32	0.30-0.44	23.00-31.22
MUFA	5.93-28.10	20.10-31.90	22.72-22.77	20.80-27.04	8.09-15.96	0.11-0.17	8.72-16.32
PUFA	0.00-55.95	53.00-64.40	59.66-61.36	56.53-68.21	47.54-77.96	0.13-0.18	59.00-65.20
UFA	5.93-84.05	*	82.38-84.13	83.57-89.01	56.69-86.04	*	68.78-77.00
α -Toco ^c	0.07-0.15	1.76-2.11	0.25-0.40	0.49-0.84	*	0.07-0.16	0.01-0.04
β -Toco ^c	*	*	*	*	*	*	0.01-0.14
γ -Toco ^c	5.65-13.30	6.44-8.60	3.72-5.00	4.74-5.60	2.56-4.85	6.12-13.0	3.32-9.23
δ -Toco ^c	0.16-0.56	0.47-0.60	0.05-0.06	0.09-0.10	0.07-0.16	0.11-0.26	1.51-10.97
TDF	14.0-27.0	11.6-22.0	18.1-26.8	14.0-33.7	23.3-47.8	24.8-31.7	23.0-28.2
References	Boschin & Arnoldi, 2011; Pastor-Cavada et al., 2014; Grela et al., 2017; Millar et al., 2019; Witten & Aulrich, 2018; Harmankaya & Özcan, 2010;	Boschin & Arnoldi, 2011; Du et al., 2014; Sarmiento et al., 2015; Witten & Aulrich, 2018; Iqbal et al., 2006; Summo et al., 2019;	Boschin & Arnoldi, 2011; Pastor-Cavada et al., 2014; Du et al., 2014; Dueñas et al., 2016; Witten & Aulrich, 2018; Mousavi, 2016	Boschin & Arnoldi, 2011; Kan et al., 2018; Millar et al., 2019; Witten & Aulrich, 2018; Awad et al., 2014;	Boschin & Arnoldi, 2011; Dueñas et al., 2016; Baptista et al., 2017; Grela et al., 2017; Kan et al., 2017; Kan et al., 2018; Witten & Aulrich, 2018	Boschin & Arnoldi, 2011; Pastor-Cavada et al., 2014; Curiel et al., 2015; Sarmiento et al., 2015; Grela et al., 2017; Witten & Aulrich, 2018; Arslan & Arslan, 2017; Grela et al., 2012	Antova et al., 2014; Boschin & Arnoldi, 2011; Hussain & Basahy, 1998; Naiker et al., 2019; Witten & Aulrich, 2018

TDF- Total Dietary Fiber; Thr-Threonine; Val-Valine; Met-Methionine; Ile- Isoleucine; Leu-Leucine; Tyr-Tyrosine; Phe- Phenylalanine; Lys-Lysine; Asp-Aspartic acid; Ser-Serine; Glu-Glutamic acid; Gly-Glycine; Ala-Alanine; Cys-Cysteine; His-Histidine; Arg-Arginine; Pro-Proline; SFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids; UFA- Unsaturated fatty acids

^aexpressed as mg/kg; ^bexpressed as µg/100g; ^c expressed as mg/100 g of seeds; [¤] expressed as g/100g protein

Legume seeds are among the richest food sources of proteins and amino acids for human nutrition. Beans and grass peas contain proteins in the following concentrations: 16.7–32.9 g/100g and 22.4–31.7 g/100g, respectively (Table 1). Such concentration is higher than cereal crops (7.5–12 g/100g), eggs (12.8 g/100g) and meat (10–20 g/100g) (Kaur & Singh, 2007). The principal proteins in legumes are salt soluble globulins (80–90%) and water soluble albumins (10–20%). Legumin and vicilin are the main globulins whereas albumins are represented by enzymatic proteins, lectins, protease and amylase inhibitors. Essential amino acids composition and digestibility determine the nutritional quality proteins present in a given food (Carbonaro et al., 2015). Legumes are rich in lysine (1.02–2.00 g/100g dry weight (dw) in beans and 1.41–1.61 g/100g dw in faba bean) and leucine (1.09–2.25 g/100g dw in beans, 1.98–2.37 g/100g dw in lentils) (Pastor-Cavada et al., 2014; Kan et al., 2017). Other essential amino acids, such as methionine (0.00–0.25 g/100g dw in beans) is present in lower amounts (Kan et al., 2018). Non-essential amino acids present in higher amounts in legumes are aspartic acid (0.86–2.55 g/100g dw in beans) and glutamic acid (2.28–3.86 g/100g dw in beans), whereas cysteine (0.06–0.07 g/100g dw, 0.05–0.06 g/100g dw and 0.06–0.11 g/100g dw in cowpeas, lentils and faba bean, respectively) is present in lower amounts (Millar et al., 2019).

Lipids content of legumes is similar to rice and wheat (1.0 g/100g and 2.0 g/100g, respectively). Peas report low lipids content (1.1–1.5 g/100g) with the highest content being observed in chickpea, (4.3–5.2 g/100g). The fatty acids composition of legumes is generally divided in the following fractions: 50% of polyunsaturated fatty acids (PUFA), 30% of monounsaturated (MUFA), and 20% saturated fatty acids (SFA) (Caprioli et al., 2016). However, beans are reported to have higher amounts of SFA (25%) than MUFA (10%) (Caprioli et al., 2016; Kan et al., 2017). Among PUFA, linoleic acid and linolenic acid are essential fatty acids present in legumes in high amounts. For instance, these are the ranges of linoleic acid content in some legumes: lentils 43.9–46.1 g/100g; beans 20–44.6 g/100g; and chickpeas 46.8–49 g/100g (Caprioli et al., 2016). Oleic acid and palmitic acid are the main MUFA and SFA respectively present in fat composition of legumes.

Several studies demonstrated that the ash content of legumes ranges between 2.7–3.6 g/100g in peas and 3.6–5.7 g/100g in beans (Table 1). Ash content in legumes is slightly higher than in cereals. Legumes are also a good source of calcium, potassium, phosphorus, magnesium, iron, zinc and copper. Faba beans report 0.223–0.226 g/100g dw of magnesium, 0.163–0.392 g/100g dw of calcium, 0.005–0.006 g/100g dw of iron, and 0.925–1.026 g/100g dw of

potassium. Millar et al. (2019) reported that over 95% of calcium is in seed coat and 76–90% of potassium is in the embryo of bean seeds.

Among the several types of fiber present in legumes, long chain soluble and insoluble polysaccharides, galacto-oligosaccharides and resistant starch are the most important ones. Insoluble dietary fiber content represents the greatest fraction (70–88%) of the total dietary fiber of the legumes, while soluble dietary fiber represents only a small part (12–30%) of the total (Parmar et al., 2014). Dietary fiber imparts various physiological effects that have important health implications, mainly lower cholesterol levels and glycaemic indexes (Kan et al., 2018). Insoluble fiber is related with laxation whereas soluble fiber is associated to reducing cholesterol levels (Brummer et al., 2015). Total dietary fiber content in lentils and grass peas vary from 18.1 and 26.8 g/100g dw and 24.8–31.7 g/100g dw, respectively. Among all legumes the highest total dietary fiber (TDF) content (23.3–47.8 g/100g dw) was found in beans (Kan et al., 2018).

Legumes are also a good source of vitamins such as thiamine, riboflavin, niacin, vitamin B12 and folate (Rysová et al., 2010). Lentils have 0.647 mg/100g of thiamine, 0.062 mg/100g dw of riboflavin and 0.93 mg/100g of niacin. Faba beans report 0.253, 0.123, and 2.233 mg/100g dw of thiamine, riboflavin, and niacin, respectively. Lutein is a major carotenoid present in pea and chickpea, with its concentration ranging from 5.3–17.6 µg/g dw and 5.4–11.0 µg/g dw in both legumes, respectively (Ashokkumar et al., 2014; Serrano et al., 2017). In lentils the concentration of lutein ranged from 4.32–17.3 µg/g dw and zeaxanthin in minor amounts, between 0.32–2.73 µg/g dw (Zhang et al., 2014). Carotenoids include substances which can be converted into vitamin A, such as α - and β -carotene, as well as other compounds not convertible into vitamin A with more potent antioxidant properties.

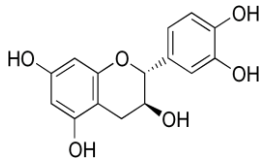
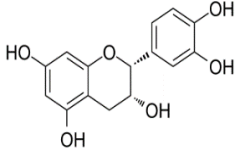
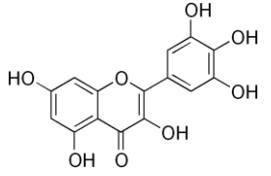
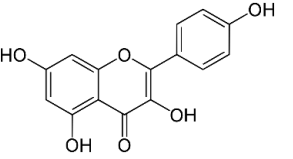
Another lipid soluble vitamin present in legumes is vitamin E comprising both tocopherols and tocotrienols, that are considered natural antioxidants (Zhang et al., 2014). Interestingly, tocopherols content is higher in legumes when compared to cereals, despite the fact that cereal grains are considered a good source of tocopherols in the diet. In general, α -tocopherol is the most abundant form, principal contributor to the vitamin E activity, followed by γ -tocopherol that has the highest antioxidant activity in food. In some studies, γ and δ -tocopherols reported higher quantities in legumes and α -tocopherol was found in lower amounts (Kan et al., 2018). Tocotrienols, in contrast are only reported in beans (Baptista et al., 2017). However, β -, γ -, and δ -tocotrienol were found in lower amounts in cowpeas (Antova, Stoilova, & Ivanova, 2014). The content of α -tocopherol in several legume species ranges as follows:

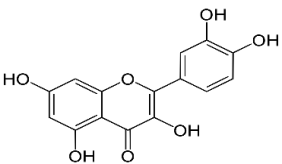
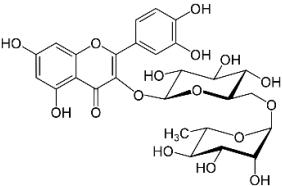
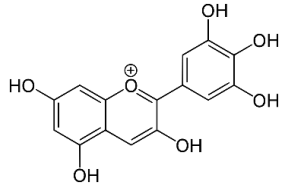
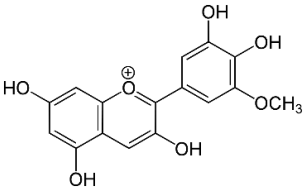
peas 0.07–0.15 mg/100g, chickpeas 1.76–2.11 mg/100g, faba beans of 0.49–0.84 mg/100g and lentils 0.25–0.40 mg/100g. In relation to γ -tocopherol, chickpeas (6.44–8.60 mg/100g) and peas (5.65–13.30 mg/100g) showed values higher than beans, faba beans and lentils, with ranges 2.56–4.85 mg/100g, 4.74–5.60 mg/100g, and 3.72–5.00 mg/100g, respectively. δ -tocopherol is present in lentils, beans and faba beans, reporting lower levels than chickpeas and peas (Baptista et al., 2017; Zhang et al., 2014). Sarmiento et al. (2015) reported values of α -, γ - and δ -tocopherol of 0.07–0.16 mg/100g, 6.12–13.0 mg/100g, and 0.11–0.26 mg/100g, respectively for grass pea. β -tocopherol was only found in cowpea samples and exhibited the highest amounts of δ -tocopherol (1.51–10.97 mg/100g) (Boschin & Arnoldi, 2011).

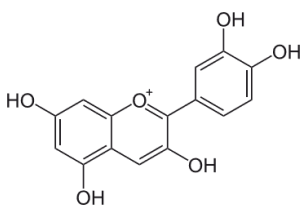
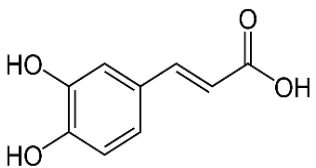
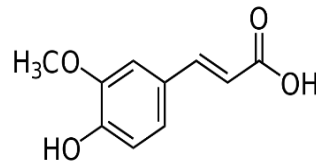
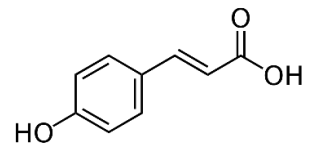
1.2.3. Phenolic composition

In addition to nutritional composition, legumes are also a good source of phenolic compounds which play a significant role in many physiological and metabolic processes of the human body. Phenolic compounds have, as a common characteristic, the presence of at least one aromatic ring with one or more linkages with hydroxyl groups (Wang et al., 2016). Depending on the number of phenolic hydroxyl groups involved and structural elements that link the aromatic rings, phenolic compounds can be classified in two classes: flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones and their glycosides) and non-flavonoids (phenolic acids and stilbenes) (Kan et al., 2018). Profile of phenolic compounds present in legumes are shown in Table 2.

Table 2 Flavonoids, anthocyanidins, hydroxycinnamic acids and hydroxybenzoic acids of legumes species (mg/g dw).

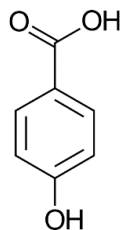
	Pea	Chickpea	Lentils	Faba bean	Bean	Grass pea	Cowpea
Flavonoids							
Catechin							
	n.d.	0.0-187.1	0.0-520.1	0.0-455.6	0.0-614.3	21.0-24.9	2.57-297.0
Epicatechin							
	n.d.	0.0-26.7	0.0-113.4	0.0-222.4	0.0-279.2	46.3-67.05	0.0-11.3
Myricetin							
	*	*	0.0-0.12	18.81-119.49	1.98-3.55	*	*
Kaempferol							
	n.d. ^a	0.0-7934.0 ^a	4.4-9.5 ^a	4.8-16.4 ^a	3.13-7.4 ^a	2.6-91.5 ^a	10.4-184.8 ^a

Quercetin							
	n.d.	0.0-1.0	0.0-90.8	n.d.	0.0-1486.3	n.d.	1.4-2.0
Rutin							
	n.d.	0.0-8.4	17.8-67.1	n.d.	82.2-104.8	0.0-7.14	n.d.
<i>Anthocyanidin</i> §							
Delphinidin							
	*	*	94.3-131.2	26.5-80.1	0.09-40.3	*	24.2-72.1
Petunidin							
	*	*	n.d.	n.d.	0.01-42.4	*	n.d.

Cyanidin							
	*	*	28.7-42.4	11.0-12.4	0.01-0.12	*	9.6-34.4
<i>Hydroxycinnamic acid</i>							
Caffeic acid							
	0.3-4.0	0.0-13.1	0.0-0.4	0.0-37.4	0.0-324	0.0-15.0	11.9-88.9
<i>Ferulic acid</i>							
	0.5-0.9	0.0-163.9	0.0-0.30	0.0-26.0	0.0-77.0	3.0-13.8	0.1-26.2
Coumaric acid							
	0.4-1.5	0.0-14.3	0.0-3.0	0.0-24.1	0.0-119.3	0.3-14.2	1.2-5.2

Hydroxybenzoic acid

Hydroxybenzoic acid



45.4-101.7

19.2-60.5

3.6-5.3

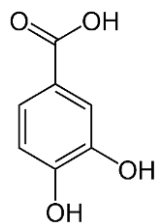
n.d.

0.02-26.8

*

1.0-81.6

Protocatechuic Acid



12.1-163.5

3.4-95.3

n.d.

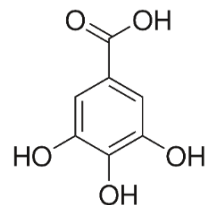
29.8-79.2

0-128.5

0.0-80.2

122.1-493.6

Gallic acid



n.d.

0-86.4

22.7-33.6

22.9-138.2

0-16.3

1.2-8.9

0.92-378.0

References	Giusti et al., 2017; Grela et al., 2017; Šibul et al., 2017; Grela et al., 2016	Grela et al., 2017; Šibul et al., 2017; Lonso et al., 2006	Fратиanni et al., 2014; Giusti et al., 2017; Grela et al., 2017; Lonso et al., 2006	Grela et al., 2017; Magalhães et al., 2017; Šibul et al., 2016; Lonso et al., 2006	Aguilera et al., 2011; López et al., 2013; Giusti et al., 2017; Grela et al., 2017; Ombra et al., 2016; Šibul et al., 2016; Lonso et al., 2006; Wang et al., 2016	Giusti et al., 2017; Grela et al., 2017; Šibul et al., 2016	Giusti et al., 2017; Teixeira-Guedes et al., 2019; Gutiérrez-Urbe et al., 2011; Lonso et al., 2006; Ojwang et al., 2013
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§ Expressed as mg/g; ^avalues of Kaempferol 3-glucoside *Not found in literature

Phenolic compounds in legumes depend on cultivar, edafo-climatic and processing conditions but generally their proportion is about 60% of flavonoids and 40% of non-flavonoids (phenolic acids) (Aguilera et al., 2011). However, these compounds are distributed differently in the seed coat (mainly flavonoids) and in the cotyledon (mainly phenolic acids) (Fратиanni et al., 2014).

The major flavonoids present in legumes are catechins, epicatechin, kaempferol 3-glucoside and quercetin (Table 2). Beans report amounts of 0.0–614.3 mg/kg dw of catechin and 0.0–279.2 mg/kg dw of epicatechin. In lentils, the following amounts of catechin and epicatechin were found: 0.0–520.1 mg/kg dw and 0.0–113.4 mg/kg dw, respectively (Giusti et al., 2017). In addition, procyanidins were also present in faba beans and lentils (Baginsky et al., 2013). Among flavonols, kaempferol 3-glucoside is present in highest amounts in chickpeas (0.0–7934.0 mg/kg dw) and beans (0.0–1486.3 mg/kg dw) (Giusti et al., 2017), although it was found in lower amounts in lentils (4.4–9.5 mg/kg dw).

The most abundant anthocyanidin present in legumes are delphinidin and cyanidin (Table 2), the highest amounts are found in lentils (94.3–131.2 mg/kg dw) and (28.7–42.4 mg/kg dw). Phenolic acids, another group of phenolic compounds, are divided into hydroxycinnamic and hydroxybenzoic acids. Hydroxycinnamic acids present in legumes are mainly caffeic, ferulic and coumaric acids (Wang et al., 2016). However, chlorogenic acid was found in 95% of bean samples analysed by Giusti et al. (2017), with amounts ranging from 24.0 to 239.2 mg/kg dw. Furthermore, Dueñas et al. (2015), reported that hydroxycinnamic acids represent nearly about 50% of all the phenolic content in beans. Among hydroxybenzoic acids, gallic, protocatechuic, and *p*-hydroxybenzoic acids are the most common in beans, lentils, peas and chickpeas (Magalhães et al., 2017; Telles et al., 2017). Gallic acid was found only in legumes with black coat, in beans (1.2–8.9 mg/kg dw), lentils (22.7–33.6 mg/kg dw), and chickpeas (0.0–86.4 mg/kg dw) (Giusti et al., 2017). Additionally, syringic acid was also found in chickpeas (Chen et al., 2015).

1.2.4. Antinutritional composition

Although legumes are nutritionally rich and exhibit important functional properties, they also contain a number of antinutritional compounds such as enzyme inhibitors, lectins, oligosaccharides, phenolic compounds, phytates, and saponins that affect the digestibility and bioavailability of nutrients in humans (Table 3). These antinutritional compounds decrease the nutrient bioavailability lessening the nutritional quality of legumes. Some antinutritional

compounds can even exert toxic effects in living organisms when consumed at high doses restraining legume utilization in human nutrition (Carbonaro et al., 2015).

Table 3 Antinutritional compounds of protein and non-protein origin in legumes (mg/g dw).

	Pea	Chickpea	Lentils	Faba bean	Bean	Grass pea	Cowpea
Trypsin inhibitor activity ^a	1.00–15.00	6.00–19.00	3.00–8.00	0.30–10.00	0.02–25.00	19.00–30.00	7.00
Acid phytic RFOs	6.40–8.30	9.60–12.01	4.50–9.30	4.00–13.00	2.07–16.20	8.65–17.00	3.60–14.00
Saponin	2.00–9.60	2.43–8.00	2.00–8.04	1.12–6.11	*	*	8.60
Tannins	0.30–1.80	0.71–4.00	0.63–1.14	0.01–4.03	0.97–7.86	*	*
References	0.03–7.00	0.04–3.78	3.00–8.72	0.10–10.00	0.90–1.53	0.90–5.00	1.10–8.20
	Grela et al., 2017; Millar et al., 2019	Wang et al., 2010	Grela et al., 2017	Grela et al., 2017; Millar et al., 2019	Shang et al., 2016; Grela et al., 2017; Valdez-González et al., 2017	Wang et al., 2010; Grela et al., 2017	Avanza et al., 2013; Sreerama et al., 2012

^a expressed as TIU/mg; TIU- Trypsin inhibitor units; RFOs- Raffinose family oligosaccharides; *Not found in literature

These compounds present in legumes, can be divided into two groups: antinutritional compounds of protein origin and non-protein origin.

1.2.4.1. Antinutritional compounds of protein origin

Antinutritional compounds of protein origin are usually the enzymatic inhibitors (trypsin/chymotrypsin and α -amylase inhibitors) and lectins. The toxic or undesirable physiological effects is owed to their interference with nutrients absorption (amino acids and minerals) (Carbonaro et al., 2015). Yet, trypsin inhibitors may have a positive nutritional role, due to their high content of sulphur-containing amino acids, in comparison to the majority of the seed proteins. Trypsin inhibitors have revealed a therapeutic effect in cancer and inflammatory diseases such as multiple sclerosis (Safavi & Rostami, 2012). Trypsin inhibitor activity in legume seeds represents between 0.30 and 10.00 TIU/mg in faba beans and between 19.00 and 30.00 TIU/mg in grass pea (Table 3).

Lectins are ubiquitous proteins exhibiting specific and reversible carbohydrate binding activities. The specificity of a lectin is defined in terms of the monosaccharide or simple oligosaccharide which inhibits the lectin-induced cell agglutination reaction. However, some lectins have hemagglutination activity. Lectins are similar to antibodies in their ability to agglutinate red blood cells, except they are not products of the immune system. Their structures are diverse and their specificity is restricted to carbohydrates (Lam & Ng, 2011).

1.2.4.2. Antinutritional compounds of non-protein origin

Among antinutritional compounds of non-protein origin there are phytic acid, raffinose family oligosaccharides, tannins, alkaloids, vicine, convicine and saponins.

Legumes are a good source of phytic acid (Kumar et al., 2010), grass pea being the one with wider range and higher values (8.65–17.00 mg/g) whereas lentils exhibited the lowest ones (4.50–9.30 mg/g) (Table 3). The antinutritional effects of phytic acid have been associated with obstructive absorption of elements such as P, Ca, Mg, Fe and Zn, and a worsening in the absorption of lipids and proteins. Phytic acid, like enzymatic inhibitors of protein origin, inhibits important digestive enzymes, such as amylase, pepsin and trypsin. However, phytic acid can also exert beneficial effects on human health. Scientific studies have reported a relationship between consumption of myo-inositol phosphates such as IP₆ from legumes and lower risk of cardiovascular disease and cancer (Kim et al., 2014).

Legumes are rich in RFOs (raffinose family oligosaccharides), also called α -galactosides, other antinutritional compounds and their values range from 1.12 to 6.11 mg/g in faba beans and from 2.00 to 9.60 mg/g in pea (Table 3). Consumption of raffinose has been related to flatulence and stomach discomfort. On the other hand, α -galactosidases in seeds have important physiological functions such as acquisition of desiccation tolerance during seed development and maturation, and seed longevity during dry storage. α -galactosidases are also associated with beneficial physiological effects including prebiotic activity, enhanced mineral absorption, stimulation of the immune system, regulation of lipid metabolism and attenuation of oxidative stress (Gangola et al., 2016).

Saponins present in legumes reduce bioavailability of iron, affect protein digestibility and inhibit various digestive enzymes (trypsin and chymotrypsin). Nevertheless, saponins are also related with several beneficial effects, as other antinutritional factors, on biological cell membranes such as the inhibition of the transport of active mucosal, facilitating the uptake of substances normally not absorbed and improving the permeability of intestinal mucosal cells (Couto et al., 2015). Beans report 0.97–7.86 mg/g and 0.71–4.00 mg/g of RFO in chickpeas and faba beans, respectively (Table 3).

Tannins content of legumes, as shown in Table 3, displayed a higher range in faba beans (0.10–10.00 mg/g), followed by lentils (3.00–8.72 mg/g), and the lowest amounts are commonly found in beans (0.90–1.53 mg/g). Tannins are water-soluble polyphenolic compounds, and their antinutritional effect is related to the interference with digestion by

binding to proteins or minerals. Tannins have the affinity to bind digestive enzymes and dietary proteins, forming complexes that are not easily digestible (Raes et al., 2014).

1.2.5. Beneficial effects of legumes consumption on human health

1.2.5.1. Diabetes

Diabetes is an epidemic disease defined by elevated concentrations of glucose in blood and is related with the development of heart diseases, blindness, kidney diseases and nerve damages (Mirmiran et al., 2019). Insulin, a hormone produced by pancreas, is responsible for the control of the levels of glucose in blood. Diabetes can be type I diabetes, where the pancreas is unable to produce insulin and type II diabetes, the most common, in which individuals produce so little insulin, that the body is unable to use it.

Development of type II diabetes has been related to obesity, reduced physical activity and a diet with high saturated and *trans* fatty acids (Hosseinpour-Niazi et al., 2015). Resistant starch, non-absorbable carbohydrates and oligosaccharides present in legumes are responsible for low glycaemic index (28-52) when compared with glycaemic index of glucose, that is 100 (Jenkins et al., 2012). These components retard the gastric emptying rate and slow digestion of starch in the small intestine. The reduction of blood glucose level and insulin can also be associated with phytic acid, lectins, amylase inhibitors or phenolic compounds (Winham et al., 2017).

The consumption of products with incorporation of beans have been associated with increased insulin production and glucose intestinal absorption (Pérez-Ramírez et al., 2018). Other studies reported that the consumption of legumes three or more times per week reduces the risk to develop type II diabetes (Becerra-Tomás et al., 2018). Another benefit associated to legumes consumption (chickpeas, peas, beans, and lentils) in overweight diabetic individuals, is the weight reduction and the reduction of plasma concentrations of inflammatory markers (Hosseinpour-Niazi et al., 2015).

1.2.5.2. Cardiovascular diseases

Coronary heart disease, in most industrialized countries, is a leading cause of death. The cardiovascular diseases can be reduced with consumption of carbohydrates and vitamins (B₉, C and E). For these reasons, the consumption of legumes can help to reduce the risk of cardiovascular diseases and total cholesterol (Pérez-Ramírez et al., 2018), low-density lipoprotein (LDL)-cholesterol and triglyceride concentrations by 10–15% (Hosseinpour-Niazi et al., 2015). Following the results of several epidemiological studies, the recommendation of

the consumption of legumes has increased. Legumes are a rich source of folic acid and a higher intake of folate (0.8 mg folic acid daily) might reduce the risk of stroke by 24% and ischaemic heart disease by 16% (Winham et al., 2017).

Another study investigating the relationship of legume consumption and cardiovascular disease, revealed that the risk of cardiovascular disease can decrease 34% in elder people, when the legume consumption is four or more times compared to less than once a week. Although, in the individuals below 55 years old it was not verified a significant influence of regularly legume consumption on cardiovascular disease (Nouri et al., 2016). Significant clinical reductions in serum cholesterol were found along a study in which the volunteers consumed a daily portion of cooked beans, lentils or chickpeas (Jenkins et al., 2012).

1.2.5.3. Celiac disease

Celiac disease is an auto-immune disorder caused by exposure to gluten, protein found in wheat, barley and spelt. In celiac patients, the gluten causes damage in the small intestine that interfere with the absorption of vital nutrients, consequently affecting all systems of the body (Volta et al., 2012). A gluten-free diet is the only therapy for people with celiac disease (Mulder et al., 2015). Nevertheless, there is a concern over the gluten-free dietary pattern because it is often associated with reduced consumption of proteins, vitamins B, iron and dietary fiber compared to products containing gluten (Morreale et al., 2018). The direct consumption of legumes, as well as their use as part of gluten-free products, can be a good alternative to satisfy the need of these nutrients as they are an excellent source of proteins and vitamins B (Pastor-Cavada et al., 2014). For instance, 100g of dry yellow pea flour can provide almost 70% of the daily requirements of folate (Muir et al., 2019). In the celiac disease, patients are recommended to intake a daily dose of 25–35 g of dietary fiber (Safavi & Rostami, 2012). However, gluten-free products are often low in fiber because wheat flour is mainly substituted by commercial starches, which usually do not significantly contribute to dietary fiber content (Larretxi et al., 2019). For this reason, it is necessary the enrichment of the dietary fiber in the gluten-free products. Flours of yellow peas, lentils and chickpeas can enrich gluten-free products with all natural dietary fiber that contains both soluble (33%) and insoluble fiber (26%) (Boukid et al., 2019; Rocchetti et al., 2017).

1.2.5.4. Cancer

Cancer is a major cause of death and its incidence has been associated with life style, mainly consumption of alcohol, smoking and diet (Hansen et al., 2013). Colorectal cancer is

one of the most commonly diagnosed. Higher consumption of soybean and legume fiber reduce the risk of colorectal cancer, due their content of isoflavones (Zhu et al., 2015). The consumption of legumes revealed an inverse relationship with cancer mortality. Papandreou et al. (2019) reported that the consumption of lentils, chickpeas and beans had a beneficial effect in the reduction of cancer mortality in diabetics and obese males, rather than in non-diabetics and non-obese females. Additionally, higher consumption of all legumes reduced the risk of cancer in 49%, in which 37% were from lentils meals. In other study, a diet rich in legumes, mainly chickpea, can reduce the risk of colorectal cancer due their levels of protein, albumins, that showed a good efficiency to inhibit the cell migration in colon carcinoma cells (Lima et al., 2016). The consumption of legumes, such as coloured beans, lentils and black soybean, proved to have inhibitory effects on the cells proliferation in prostate, ovarian and breast. This observation was realted with the legumes high phenolic compounds content and excellent dietary source of natural antioxidants (Xu & Chang, 2012). Zhong et al. (2018) reported that the higher amounts of isoflavones from legumes were related with a decrease in the risk of endometrial cancer.

1.2.6. Factors influencing the composition and properties of legumes

1.2.6.1. Climatic conditions

Environmental changes, mainly abiotic stress, influence negatively crop productivity in many legume species, while their quality can be influenced negatively or positively. Under environmental stress, several physiological stress reactions occur in plants, influencing biochemical and enzymatic pathways, which will irreversibly change the chemical composition of crops and consequently the quality of the harvested products (Stagnari et al., 2016). The impact of stress will vary according to different interacting factor, such as the intensity and exposure period, as well as the species. For instance, it is estimated a reduction in legumes and vegetables yield with an increase of 25% in ozone (O₃) and 22% of carbon dioxide (CO₂) concentrations, 25% in salinity and a decrease of 50% in water availability. Also, the average temperature will increase around 4°C until 2050 (Scheelbeek et al., 2018). The combination of all these factors will affect considerably the production, composition and quality of legumes and vegetables worldwide. In fact, the higher temperatures during the reproductive period, have an adverse impact in legumes, reducing crop yields and producing smaller seeds (Kaushal et al., 2014). The protein content, minerals and antioxidants increase under heat stress, while the lipids content reduce under stress conditions (Rana et al., 2016). The chickpea crop, under

extreme temperatures increases the transpiration during the day, losing water and consequently the crop will undergo a hydric stress. In sub-tropical areas, chickpea crop under drought stress reduce its productivity (Reichert et al., 2015). By other hand, the levels of starch, lipids and sensorial attributes are improved, therefore increasing the nutritional composition and phenolic compounds in stressed crops (Wang & Frei, 2011).

Recent plant breeding programs are progressing to develop strategies to produce higher quality crops under stressful environments, mainly under heat and drought conditions, which may be a component of an overall portfolio of climate smart agriculture technologies and practices to ensure the resilience of legumes cultivation that can maintain yields while also improving nutritional quality of seeds (Hummel et al., 2018).

1.2.6.2. Fertilization

The grain quality and composition of legumes are also influenced by the fertilization of soil. An important advantage of legume crops are considered as an effective biological nitrogen fixation (Humayun et al., 2019). The nitrogen element is one of the most important nutrient for crops, influencing directly their productivity. The nitrogen fertilization of soil, affect positively the seed yield and weight of seed in legumes, showing increases of 18.5% of seed weight and 20% of seed yield in chickpea crop with nitrogen fertilization during growth stage (Dhima et al., 2015).

Potassium shows a crucial role in water stress tolerance (Barłog et al., 2019). Potassium deficiency in soil have been associated with inhibition of nitrogen fixation mechanism and consequently lower protein content in seed legumes (Taha et al., 2016) and higher amounts of antinutrients in legumes (Mona et al., 2011).

Another important element in the nitrogen fixation is sulfur and the deficiency of this element in the soil decrease the yield and quality of legumes (Głowacka et al., 2019). Sulfur fertilization have been related with higher amounts of protein and amino acids, and adverse effects are increased of antinutrients such as tannins (Barłog et al., 2019; Neugschwandtner et al., 2015).

1.2.7. Innovative products with incorporations of legumes

The use of legumes in food products is largely determined by their functionality, which depends on physicochemical properties, and by food processing conditions. Additional factors that must be considered in many applications include fiber, colour and flavour due to their impact on sensory characteristics.

Various studies have already been done with the objective to evaluate sensory characteristics and consequently the overall acceptance by consumers of baked products and pasta with different incorporations of legumes.

Several studies related that baked goods such as baked rolls (Kohajdová et al., 2013a), biscuits, bread (Kohajdová et al., 2013b) and crackers with 10% lentil, pea and chickpea flour, respectively, were more acceptable in relation to sensory evaluation with no significant differences from wheat products. Higher levels of lentil and pea flours showed significant lower overall acceptance due to the reduction of sensory parameters such as shape, crust colour, crumb elasticity, and hardness of the final products. The crust colour of the baked rolls became darker because the legume flours have higher lysine content, which can develop Maillard reaction products during baking (Mohammed et al., 2012; Kohajdová et al., 2013b). The addition of lentil flour improved the functional properties such as water absorption capacity (from 59% to 75%) and dough development time, but dough stability was decreased. The physical parameters, such as volume, specific volume, and cambering of baked products reduced with increased levels of legumes (Hefnawy et al., 2012; Kohajdová et al., 2013a; Kohajdová et al., 2013b). Sensory evaluation showed that the most acceptable (93–94% of overall acceptance) baked rolls were those obtained with 10% of legumes incorporation. Higher levels of legume flours in the products adversely affected the shape, crust colour, crumb elasticity, and hardness of the final products (Kohajdová et al., 2013b).

On the other hand, toast bread with incorporations of 15% and 30% of chickpea flour had values similar to the control sample, turning them acceptable in terms of weight, volume, texture and crumb structure (Hefnawy et al., 2012). Through sensory analysis, it was found that the incorporation of lentil and chickpea flour influenced the colour, texture, flavour, and taste of crumb depending on the amount added. According to Bojňanská et al. (2012), bread with the incorporation of 20% of chickpea was totally accepted because it was considered as better or equally good as wheat bread, while bread with only an addition of 10% of lentil flour was considered as good as wheat bread. With the addition of 40% and 50% of chickpeas and/or lentil flour the sensory evaluation was unacceptable. The incorporation of 35% of beans in wheat biscuits was accepted by the consumers with similar scores as for the control produced with wheat and maize flour (Sparvoli et al., 2016). Additionally, pasta containing 70% of faba bean flour had similar scores of sensory attributes as in pasta with 100% of wheat semolina (Laleg et al., 2017).

1.2.7.1. Legume-enriched food products

Several studies have demonstrated the potential use of legumes as protein-enriching agents in baked products in the form of flours (Angioloni & Collar, 2012; Mohammed et al., 2012; Turfani et al., 2017). For instance, lysine is a limited amino acid in cereals but not in legumes and methionine reports a contrary trend. Therefore, the combination of cereals and legumes improves protein quality and essential amino acid balance (Kan et al., 2017). The incorporation of lentil flour in wheat bread increased the amounts of lysine, dietary fiber and phenolic compounds, and improved the antioxidant activity (Turfani et al., 2017). Another example of legumes supplementation is the incorporation of hydrolysates cowpea flours in the production of pasta made from wheat flour. The antioxidant capacity increased three times in cooked pasta with 10% of cowpea compared with pasta exclusively prepared with wheat (Drago et al., 2016). Abou-Zaid et al. (2011) obtained protein-rich cake with raw and germinated faba bean flour blended with wheat flour. The blends with a high source of protein increased the amino acid levels.

Millar et al. (2017) developed crackers with the incorporation of 40% faba bean and pea flours, increasing the protein and total dietary fiber content up to 55%. The phenolic content and antioxidant activity were significantly higher than the wheat crackers and the consumer preference studies revealed no negative effect on appearance, hardness and mouthfeel.

1.2.7.2. Gluten-free products

Legume flours are useful ingredients for the nutritional improvement of gluten-free products. A study reported that the consumption of gluten-free spaghetti formulated with bean flour can have positive implications in celiac patients, due to their increased nutritional quality (protein, ash, dietary fiber and resistant starch contents) and lower glycaemic index compared to control (100% rice) (Giuberti et al., 2015). In another study, gluten-free cakes with the incorporation of chickpea, pea, lentil and bean flour with rice flour (rice:legume, 50:50), reported higher total protein, minerals, fat and fiber than rice cake. The hardness and chewiness of the gluten-free layer cake increased with the incorporation of legumes, except with lentil flour. There was a decrease in the digestible starch and glycaemic index, except in those samples containing chickpea. In general, the lentil flour incorporation resulted in the best gluten-free cakes in terms of physicochemical properties and nutritional quality (Gularte et al., 2012).

Han et al. (2010) investigated the effect of 100% legumes flour (chickpea, green and red lentils, pinto bean, navy bean and yellow pea) to develop gluten-free cracker snacks. The compositional and nutritional profiles of the crackers were also very similar to the existing crackers available in the market with the exception of the percentage daily requirements of iron that were –three to six times higher in the chickpea crackers on a per serving basis.

1.2.8. Innovative methodologies for quality measurements

In the food industry, the evaluation of legumes' quality, have been performed through the use of conventional analysis, mainly, high liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS) and spectrophotometry. The conventional analyses are time-consuming, expensive, destructive for the samples and laborious. Therefore, there has been an increase on the concern to use innovative tools that can be managed easily, faster and being non-destructive to any type of matrices such as Fourier transform infrared spectroscopy (FTIR) and hyperspectral imaging (HSI) (Table 4).

Table 4 Summary of literature on spectroscopy and imaging methods for legumes evaluation.

Legume	Sample	Features	Methodology	Treatment	Accuracy R^2_{cal} ; R^2_{val}	Reference
Chickpea	Flour	Total phenols	MIR	-	0.98; 0.95	Kadiroğlu et al., 2018
		Water soluble protein			0.98; 0.97	
		ABTS			0.98; 0.96	
		Iron chelating activity			0.97; 0.98	
		Water binding capacity			0.98; 0.96	
		Oil binding capacity			0.98; 0.96	
Pea	Seed	Phenotype yield	HSI		-;-	Quirós et al., 2019
	Seed	Weevil infection	HSI	-	0.85–0.91; 0.83–0.84	Nansen et al., 2014
		Weevil non-infection			0.84–0.85; 0.83–0.84	
Faba bean	Flour	Protein	NIR	SNV	0.97; 0.94	Wang et al., 2014
		Starch		1 st deriv	0.93; 0.88	
		Oil		1 st deriv	0.81; 0.68	
		Total polyphenols		SNV	0.89; 0.78	
	Seed	Protein		1 st deriv	0.88; 0.76	
		Starch		MSC	0.89; 0.78	
		Oil		SNV	0.81; 0.67	
		Total polyphenols		SNV	0.84; 0.69	
Beans	Seed	Dietary fiber	NIR	SNV	-; 0.41	Plans et al., 2012
		Uronic acids		SNV+2 nd deriv	-; 0.51	
		Ash		SNV+1 st deriv	-; 0.76	
		Calcium		SNV+1 st deriv	-; 0.82	
		Magnesium		SNV	-; 0.44	
	Ground seed coat	Dietary fiber		SNV+2 nd deriv	-; 0.86	

			Uronic acids	SNV+2 nd deriv	-; 0.55	
			Ash	SNV+2 nd deriv	-; 0.92	
			Calcium	SNV+2 nd deriv	-; 0.92	
			Magnesium	SNV+2 nd deriv	-; 0.56	
	Flour		Seed weight	None	0.85; 0.74	Hacisalihoglu et al., 2010
		NIR	Protein	None	0.84; 0.82	
			Starch	None	0.51; 0.52	
	Flour		Protein	MIR: norm+2 nd deriv SG NIR: MSC	MIR: 0.97; 0.96 NIR: 0.97; 0.96	Plans et al., 2013
		NIR - MIR	Starch	MIR: norm+2 nd deriv SG NIR: MSC	MIR: 0.96; 0.95 NIR: 0.96; 0.95	
			Amylose	MIR: norm+2 nd deriv SG NIR: MSC	MIR: 0.95; 0.94 NIR: 0.95; 0.94	
	Raw		Aroma	SNV+2 nd deriv	0.34; 0.20	Plans et al., 2014
			Flavour	SNV+2 nd deriv	0.55; 0.39	
			Mealiness	SNV+2 nd deriv	0.43; 0.44	
			Seed-coat perception	SNV+2 nd deriv	0.03; 0.12	
			Seed-coat roughness	SNV+2 nd deriv	0.40; 0.33	
			Seed-coat brightness	SNV+2 nd deriv	0.59; 0.44	
	Cooked		Aroma	SNV+2 nd deriv	0.30; 0.13	
		NIR	Flavour	SNV+2 nd deriv	0.04; 0.03	
			Mealiness	SNV+2 nd deriv	0.57; 0.73	
			Seed-coat perception	SNV+2 nd deriv	0.04; 0.11	
			Seed-coat roughness	SNV+2 nd deriv	0.39; 0.69	
			Seed-coat brightness	SNV+2 nd deriv	0.52; 0.66	
	Soaked		Water uptake	2 nd deriv	0.72; 0.47	Mendoza et al., 2018
		HSI	Cooking time	CWT	0.98; 0.89	
	Unsoaked		Cooking time	CWT	0.94; 0.71	
Cowpea	Pods		Total phenols	MIR: 1 st deriv MN NIR: spectra	MIR: 0.99; - NIR: 0.82; -	Machado et al., 2017a
		MIR - NIR	Flavonoids	MIR: spectra NIR: spectra	MIR: 0.99; - NIR: 0.99; -	

Pods	<i>Ortho</i> -diphenols		MIR: spectra	NIR: spectra	MIR: 0.99; - NIR: 0.99; -	Machado et al., 2017b
	DPPH		MIR: spectra	NIR: spectra	MIR: 0.98; - NIR: 0.99; -	
	ABTS		MIR: 1 st deriv	NIR: 1 st deriv	MIR: 0.99; - NIR: 0.98; -	
	Protein		MIR: 1 st deriv SG	NIR: Spectra	MIR: 0.99; - NIR: 0.99; -	
	Threonine		MIR: -	NIR: 1 st deriv	MIR: - ; - NIR: 0.99; -	
	Histidine		MIR: -	NIR: 1 st deriv SG	MIR: - ; - NIR: 0.99; -	
	Valine		MIR: 1 st deriv	NIR: -	MIR: 0.99; - NIR: 0.84; -	
	Lysine		MIR: 1 st deriv	NIR: 1 st deriv	MIR: 0.99; - NIR: 0.93; -	
	Isoleucine		MIR: -	NIR: 1 st deriv	MIR: - ; - NIR: 0.78; -	
	Leucine		MIR: 1 st deriv	NIR: 1 st deriv	MIR: 0.53; - NIR: 0.84; -	
	Phenylalanine		MIR: -	NIR: 1 st deriv SG	MIR: - ; - NIR: 0.97; -	
	Aspartic acid + asparagine	MIR - NIR	MIR: 1 st deriv SG	NIR: 1 st deriv SG	MIR: 0.95; - NIR: 0.90; -	
	Serine		MIR: Spectra	NIR: 1 st deriv	MIR: 0.89; - NIR: 0.93; -	
	Glutamic acid + Glutamine		MIR: 1 st deriv	NIR: 1 st deriv	MIR: 0.56; - NIR: 0.44; -	
	Glycine		MIR: 1 st deriv	NIR: 1 st deriv	MIR: 0.93; - NIR: 0.95; -	
	Arginine		MIR: 1 st deriv	NIR: 1 st deriv SG	MIR: 0.62; - NIR: 0.86; -	
	Alanine		MIR: 1 st deriv	NIR: 1 st deriv	MIR: 0.99; - NIR: 0.99; -	
	Proline		MIR: 1 st deriv	NIR: 1 st deriv	MIR: 0.94; - NIR: 0.97; -	
	Tyrosine		MIR: spectra	NIR: 1 st deriv	MIR: 0.37; - NIR: 0.94; -	

SNV- Standard normal variate; 1st deriv - first derivative; 1st deriv MN - first derivative mean normalized; MSC - Multiplicative scatter correction; 2nd deriv - second derivative; CWT- continuous wavelet transform decomposition; 1st Deriv SG - first derivative Savitzky and Golay

1.2.8.1. Fourier transform infrared spectroscopy

FTIR is a technique, that can be divided in the Near Infrared (NIR) and Mid Infrared (MIR) intervals, the latter being assessed using the Attenuated Total Reflectance (ATR) accessory. The MIR region covers the range of 400–3600 cm^{-1} , and the peaks in spectrum are vibrational modes of specific groups. The NIR region is defined as encompassing the 4000–14000 cm^{-1} . The absorptions in this region correspond to “combination modes” of functional group vibrations. Combining X-H (where X = C, N, O) stretches with other fundamental vibrations, besides overtones, which are multiple of the fundamental vibrations of MIR.

The principle of this method is based on chemometrics, that can generate correlations between the infrared spectra and the composition (nutritional, antinutritional, functional and physical properties) of the tested samples through multivariate data analysis since chemometrics can help extracting larger amounts of information from complex MIR and NIR spectra (Tao et al, 2017).

FTIR technique has been widely used as a fast and reliable method for qualitative and quantitative analysis in agriculture, food processing, petrochemical, and pharmaceutical fields (Wang et al., 2014). NIR and MIR have been successfully applied in for evaluation of legumes quality (Table 4). NIR has been successfully applied to determine protein, starch, oil, total polyphenols and tannins in faba beans (Wang et al., 2014). Hacısalihoglu et al. (2010) predicted protein, starch, and seed weight in intact common bean seeds. In that case the results were according to those from Wang et al. (2014), the statistical values were higher for flour samples than for unprocessed beans. Plans et al. (2012), reported NIR calibrations for dietary fiber, uronic acid, ash, calcium and magnesium in 90 ground seed coats of common beans. The authors concluded that NIR spectra of ground common bean seed coats can provide enough information about chemical components related with sensorial attributes. Thus, it can also help to monitor the sensory properties of marketable seeds. Additionally, NIR has been used to evaluate sensory properties of common beans (Plans et al., 2014), revealing in flavour it's mealiness, seed-coat roughness and seed-coat brightness to be roughly estimated in dried cooked and grounded beans. Other study has reported NIR calibrations to describe the different components such as crude protein, moisture and essential amino acids (methionine, cysteine, lysine, threonine, tryptophan, arginine, isoleucine, leucine, and valine) present in peas. Some studies concluded that 85–98% of amino acids variance in the samples can be explained using NIR (Fontaine et al. 2001). Font et al. (2006) concluded that NIR equations developed over chickpea seeds allowed accurate predictions of crude protein, oil and fatty acids (oleic and

linoleic acids). In the mentioned study, the authors suggested that NIR is an ideal tool for screening traits in plant breeding programs.

Even though, the MIR spectrum is rich in information that helps studying the composition of chemical molecules, only few studies have reported the use of MIR to determine legumes composition and also in comparison to NIR.

Plans et al. (2013) obtained partial least squares regression (PLS-R) models of NIR and MIR for protein, total starch and amylose contents in the common bean endosperm. The best PLS models for all the tested compounds were obtained using NIR, making these models suitable for screening and rough screening applications, satisfying the needs of plants breeders and germplasm banks. In other study, the use of both NIR and MIR intervals have been also assessed concerning the evaluation of the protein content and amino acids profile of legumes, with the first derivative being the best data treatment to be used in PLS-R (Machado et al., 2017b), as well as for phytochemical content of legumes and the evaluation of the impact of the storage period (Machado et al., 2017a). Calderon et al. (2009) used the whole sample sets (pea) and concluded that are usually better than heterogeneous sets (pea and triticum) for the development of calibrations for fiber and nitrogen in both MIR and NIR. That study showed that predictive calibrations were better in NIR than MIR when predicting for neutral detergent fiber (NDF) and nitrogen, but acid detergent fiber (ADF) calibrations were good for both spectral intervals.

1.2.8.2. Hyperspectral imaging

Hyperspectral imaging (HSI) is a technique that uses spatial images of a sample at numerous wavelengths (> 100), hence each pixel of image may be interpreted as a spectrum. This technique have been widely used as a novel tool for food quality evaluation, giving spectral and spatial information of a sample (Lorente et al., 2012).

HSI data can be obtained based on a step and acquire measuring mode, one single spectrum or line of spectra of each movement of sample, and also through the plane scan system without movement of the sample. The spectral imaging resultant is a three-dimensional data cube. Two of the dimensions represent each pixel (x, y) corresponding the spatial images, and the third dimension is the spectrum of λ wavelengths, which represents the spectral information. HSI data quality is defined with the regulation of illumination system and acquisition parameters (integration time) (Garrido-Novell et al., 2012). The principle of this methodology, as previous one, is based in chemometrical methods, including several methods of analysis such

as PLS, principal component analysis (PCA), artificial neural networks (ANN) and multi-linear regression (MLR) (Su et al., 2017).

HSI have been applied in different foodstuffs for different purposes (Lorente et al., 2012), although in legumes such application is still limited. In Table 4, is shown the applicability of HSI to detect defects in lentils (Nansen et al., 2014), for measure physical parameters of beans (Mendoza et al., 2018), and to obtain information about agronomic characteristics of peas and chickpeas (Quirós et al., 2019).

In the study of Nansen et al. (2014), it is explained the potential of HSI features for quality control systems for legumes and for a wide range of food products. The developed models for detection of pea weevil (*Bruchus pisorum* L.) in peas displayed great sensitivity (95%) (ability to detect the infestation) and specificity (100%) (ability to detect non-infestation).

This methodology exhibited a high potential to predict the cooking time of soaked beans ($R^2v= 0.89$) and for unsoaked beans ($R^2v= 0.71$), using a single feature method PLS regression (Mendoza et al., 2018). Additionally, the developed methods to evaluate the phenotype yield of green, yellow peas and chickpeas revealed higher potential for estimation in breeding programs, where yield cannot be assessed due to lower seed availability (Quirós et al., 2019).

1.2.9. Future perspectives

The higher consumption of products based on cereals (lower amounts of lysine, vitamins and phytochemicals) allied with a sedentary lifestyle is widely believed to be a major contributing factor in metabolic diseases (obesity, type II diabetes, cardiovascular disease and cancer) and gluten disorders. Legumes are excellent sources of nutrients and phytochemicals, which are influenced by genetic and agronomic factors. Therefore, it becomes imperative to promote an increased contribution of legumes in the diet in order to take advantage of their components that are nutritious and promote health, mainly in the prevention of some types of cancer and cardiovascular diseases. The literature shows that legumes are nutritionally dense but also comes packed with antinutritional components that have showed their beneficial effects on health. Nowadays, there is an increased concern about the applicability of eco-friendly, faster and cheaper methodologies such as FTIR and HSI. Such techniques allow the quantification and prediction of nutritional, antinutritional, phytochemicals and agronomic parameters in only one analysis. In fact, several food products have improved their nutritional composition due to legumes supplementation, becoming protein enriched products with improved functional

properties. Not only the incorporation of lower percentages of legumes but also the total replacement (gluten-free products), lead to higher sensory scores.

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Chapter 2

Objectives

Objectives

The overall objective of this Doctoral Thesis was to gather and evaluate the information on the nutritional, antinutritional and phytochemical characteristics of beans, using novel methods to assess its composition. Simultaneously and stimulated by the growing food industry interest, the present work also explored the applicability of high-quality bean flour as a functional element for food products enrichment, with added value and health benefits. The following work aims and corresponding expected contribution and novelty were targeted:

- Characterization of the nutritional, functional and antinutritional composition of different bean cultivars from two harvest years and their potential for food applications. Novelty: characterization of 21 bean cultivars, to be used in food products. Contribution: identify the most distinctive nutrients from each cultivar to be used as functional ingredient, gluten-free and protein enrichment products.
- Evaluation of the potential of FTIR techniques as an improved tool to assess the compositional feature of bean flours, including a comparison of the efficiency of the NIR and MIR intervals. Novelty: development of predictive models to measure the nutritional, antinutritional and phytochemical parameters of bean cultivars. Contribution: application of FTIR spectroscopy methodology as a routine method in food industry and/or laboratory.

The aims are then reflected in each chapter specific objective:

- i) determination of protein content, antinutritional (tannins and phytic acid) and amino acids composition, phytochemical composition (total phenols, *ortho*-diphenols, and flavonoids), *in vitro* antioxidant activities and individual phenolic compounds in different common bean cultivars harvested in 2013 and 2014 (**Chapter 3.1**).
- ii) evaluation of the potential use of each bean flours for developing novel legume-based foods with health benefits to be used in the food industry (**Chapter 3.1**).
- iii) association between the predictive performance of NIR and MIR spectroscopies for the assessment of protein, lipids, tannins, phytic acid and specific amino acids' contents in bean flours (**Chapter 4.1**).
- iv) understanding the performance of FTIR techniques for the assessment of bean flours, regarding phytochemical composition (total phenols, *ortho*-diphenols and flavonoids), *in vitro* antioxidant activity (DPPH, ABTS and FRAP), and individual phenolic compounds with relevance as phytochemicals (**Chapter 4.2**).

Chapter 3

3.1. Nutrients, antinutrients, phenolic composition and antioxidant activity of common bean cultivars and their potential for food applications

3.1. Nutrients, antinutrients, phenolic composition and antioxidant activity of common bean cultivars and their potential for food applications

Adapted from:

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Abstract

Phaseolus vulgaris L. is the most commonly consumed legume in the world, given its high vegetable protein content, phenolic compounds and antioxidant properties. It also represents one of the most sustainable, low-carbon, sources of food available at present to man. This study aims to identify the nutrients, antinutrients, phenolic composition and antioxidant profile of 10 common bean cultivars (arikara yellow, butter, cranberry, red kidney, navy, pinto, black, brown eyed, pink eyed, and tarrestre) from two harvest years, thereby assessing the potential of each cultivar for specific applications in the food industry. Navy and pink eyed beans showed higher potential for enrichment of foodstuffs and gluten-free products due to their higher protein and amino acids contents. Additionally, red kidney, cranberry and arikara yellow beans had the highest contents of phenolic compounds and antioxidant properties, which can act as functional ingredients in food products, thus bringing with health benefits. Our study highlights the potential of using specific bean cultivars in the development of nutrient-enriched food and as functional ingredients in diets designed for disease prevention and treatment.

Keywords: *Phaseolus vulgaris* L., protein, amino acids, phytochemicals, antioxidants

3.1.1. Introduction

Common beans (*Phaseolus vulgaris* L.) are amongst the most essential food legumes available for human consumption in the world, representing an excellent gluten-free ingredient and fortifying agent for food products such as canned food (Ramírez-Jiménez et al., 2018). They are also an everyday staple in plant-based diets recently identified by the Intergovernmental Panel on Climate Change (IPCC) as promoters of climate change mitigation by reducing meat consumption and its concomitant production costs (IPCC Climate Change and Land Report, August 2019). Notably, a worldwide increase in legume consumption and

their nutritional improvement can significantly promote food security and soil fertility, while simultaneously enhancing global health trends (Foyer et al., 2016). Their worldwide consumption is associated with their advantageous nutritional and health-promoting properties.

Nutritionally, beans represent a rich and inexpensive source of proteins, amino acids, carbohydrates, dietary fibre, vitamins (Kan et al., 2018), phenolic acids and flavonoids (Giusti et al., 2017). At the same time, beans also contain antinutrients such as tannins, lectins, phytic acid and oligosaccharides (Kan et al., 2017). These antinutrients influence the bioavailability and digestibility of nutrients and minerals (Wang et al., 2010). Several health benefits are linked to this nutritional profile (Dueñas et al., 2015): i) the proteins of beans have shown good digestibility, are gluten-free and promote cholesterol reduction and regulation of diabetes (Toews & Wang, 2013); ii) the phenolic composition of beans promotes several benefits including the reduction in the incidence of cancer and cardiovascular diseases (Cominelli et al., 2018), antioxidant and anti-inflammatory effects (Chen et al., 2017); iii) the phenolic acids and flavan-3-ol by reduce the risk of diseases of the digestive tract (Moreno-Jiménez et al., 2015); and, iv) its high amounts of resistant starch reduce the glycemic index and risk of chronic diseases (Mojica et al., 2017), and improve satiety in patients with metabolic syndromes (Reverri et al., 2017).

Within the industrial context, beans are used in the development of many different food products: i) gluten-free products (Giuberti et al., 2015); ii) biscuits with lower antinutrient content (Sparvoli et al., 2016); iii) extruded biscuits (Siddiq et al., 2013); iv) bread with higher nutritional and mineral composition (Bhol & Don Bosco, 2014); and, v) spaghetti with higher contents of phenolic compounds (Gallegos-Infante et al., 2010). Recently, their potential as stabilisers of frozen products and as food preservatives has been highlighted due to their higher thermal stability (Carbas et al., 2018), and their useful properties for gel and film manufacture have also been recognised (Yang et al., 2018).

Given their broad and increasing applicability of legumes to food products and globally, it is essential to identify their nutritional, phenolic composition, in addition to their and *in vitro* antioxidant activities, extending research to include common bean cultivars (Messina, 2014). The variation in the nutritional profile of the cultivars corresponds to differences in their bioactivities and associated health effects (Pastor-Cavada et al., 2014). Therefore, this study evaluates the protein content, antinutritional (tannins and phytic acid) and amino acids composition, the phenolic composition (total phenols, *ortho*-diphenols, and flavonoids), individual phenolic compounds and *in vitro* antioxidant activities (2,2-diphenyl-

1picrylhydrazyl radical (DPPH), (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), and ferric reducing antioxidant power (FRAP)); 10 widely used common beans cultivars were selected (arikara yellow, butter, cranberry, red kidney, navy, pinto, black, brown eyed, pink eyed, and tarrestre) from two harvest years. The aim was to gather essential nutritional and phytochemical information enabling future applications of these important cultivars as new and improved legume-based foods products with associated health benefits.

3.1.2. Material and Methods

3.1.2.1. Sampling

The common bean samples used (n=21) were classified according to their morphological characteristics resulting in 10 common bean cultivars clustered as: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M). The seeds were selected from the collection held at the Research Unit of Biotechnology and Genetic Resources (INIAV, Oeiras, Portugal) and were cultivated in Cabrela (near Sintra, Portugal) in 2013 and 2014. All harvested bean seed samples (n=42) were ground through the Falling Number 3100 mill (Pertin, Hägersten, Sweden) using a 0.8 mm screen and stored at room temperature in sealed plastic bags until further analysis.

3.1.2.2. Climatic conditions

The climate data for average values for 1985–2014 was obtained from the E-OBS gridded dataset (Haylock et al., 2008). The main climate data for the 2013 and 2014 seasons of production site were taken from an automatic weather station in the orchard and is presented in Figure 2.

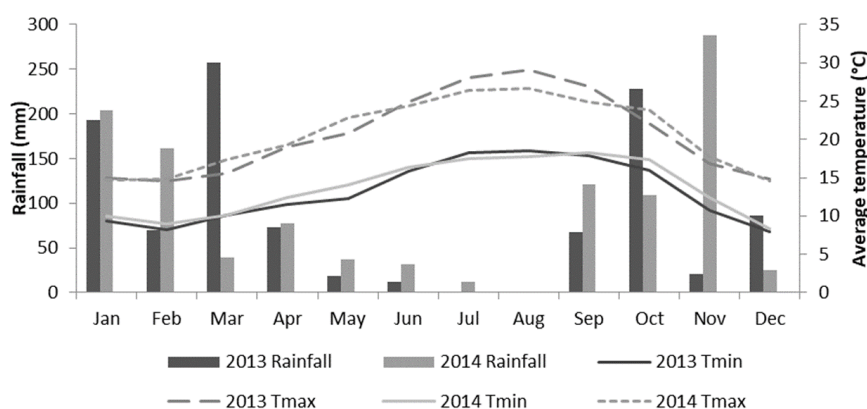


Figure 2 Climatic conditions (Tmin, Tmax and Rainfall) in 2013 and 2014 in Sintra, Portugal.

During the first months of the vegetative cycle (May and June), the Tmin and Tmax were higher in 2014 (variation between 14.0–26.6 °C) than in 2013 (variation between 12.3–29.1 °C). Later in the vegetative cycle (July, August and September), the opposite occurred, with higher Tmin and Tmax in 2013 compared to 2014. The average rainfall in the vegetative cycle in 2014 (40.4 mm) was twice the 2013 value (19.4 mm). Hence, there were considerable differences in rainfall between years, which were found to influence the quality of the common bean seed. Nevertheless, samples shared an irrigation regime that assured a standard supply of water to the plants.

3.1.2.3. Chemicals and reagents

Sulphuric acid, sodium hydroxide, acetonitrile (Ultra high performance liquid chromatography (UHPLC) grade), ethylenediamine tetraacetic acid (EDTA), sodium borate and hydrochloric acid were obtained from Panreac (Castellar del Vallés, Barcelona, Spain). Sodium acetate (anhydrous), trimethylamine (TEA), phosphoric acid, ammonium molybdate and methyl cellulose were purchased from Sigma-Aldrich (Darmstadt, Germany). Standard of amino acids (L-arginine (Arg), L-alanine (Ala), L-asparagine (Asn), L-aspartic acid (Asp), glycine (Gly), L-glutamic acid (Glu), L-glutamine (Gln), L-histidine (His), L-isoleucine (Ileu), leucine (Leu), L-lysine (Lys), L-norvaline (Nor), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr) and L-valine (Val)) were purchased from Sigma-Aldrich (Darmstadt, Germany). All the other chemicals and reagents used were of analytical grade.

3.1.2.4. Nutritional composition

3.1.2.4.1. Protein content

The protein content of samples was determined by the Association of Official Agricultural Chemists (AOAC) international, method 954.01 (AOAC, 2005). The results are presented as grams of protein per hundred grams of sample dry weight (g/100 g dw).

3.1.2.4.2. Amino acids composition

Except for tryptophan and tyrosine, all amino acids were quantified by HPLC (Machado et al., 2017). For quantification of tryptophan and tyrosine, 25 mg of each sample was weighed into a 5 mL tube with 5.0 M NaOH, and then the samples were hydrolysed at 120 °C for 12h.

After this period, the samples were cooled to room temperature, and the pH was adjusted to 2.0 with 6.0 M HCl and transferred to a 50 mL volumetric flask. 100 μ L of internal standard (Norvaline at 500 μ g/mL) were added to the flask, and the volume was made up to 50 mL with ultrapure water. 500 μ L of the resulting solution was filtered through a 0.22 μ m syringe filter into an HPLC vial. The mobile phase was 50 mM NaH₂PO₄ (phase A) and acetonitrile (phase B). The gradient programme started at 5% phase B and increased to 60% phase B for 8.0 min; it was maintained for 1.0 min and then returned to the initial conditions after 0.5 min. These conditions were maintained for 3.5 min. The injection volume was 5 μ L, and the column oven was set to 40 °C. Fluorescence detection was performed according to the following timetable: Excitation– 274 nm, emission– 304 nm at 0.0 min (for tyrosine with retention time of 2.2 min); changing to excitation– 280 nm, emission– 340 nm at 3.0 min time (at a retention time of 4.15 min for tryptophan); and finally changing to excitation– 202 nm, emission– 296 nm at 5.0 min (for tramadol with retention time of 6.37 min). The Chromeleon (version 7.2) software (Thermo Fisher Scientific, Inc., Waltham, USA) was used to analyse the results.

3.1.2.4.3. Antinutritional composition

Total tannins content was quantified using the methyl cellulose precipitable tannin (MCT) assay (Damberg et al., 2012), with slight modifications. Briefly, 200 μ L of each sample were transferred into a 1.5 ml tube, and 600 μ L of methyl cellulose solution (0.04%) were added, and the volume was weighed with distilled water. Then, the tubes were manually stirred and left at room temperature for 2–3 minutes. 400 μ L of saturated ammonium sulphate solution was added, followed by 2.0 mL of distilled water. After, the mixture was stirred in a vortex and held at room temperature for 10 min. The reaction was terminated by centrifuging the contents at 9167 g for 5 min (centrifuge Sigma-2-16 K, Steinheim, Germany). The absorbance of the supernatant was read at 280 nm. Epicatechin was used as standard, and the results were expressed as grams of epicatechin equivalents per hundred grams of sample (g ECE/100g).

The phytic acid content of the bean flours was measured using the standard assay procedure of K-PHYT (Megazyme, 2015), with the colourimetric determination of phosphorus and the absorbance determined at 655 nm. The results were expressed as grams of phytic acid per hundred grams of sample dry weight (g/100g dw).

3.1.2.5. Phenolic composition

Each bean flour sample (40 mg) was added to 1.5 mL of methanol/water (70:30, v/v) and placed in an orbital shaker at room temperature for 30 min. Then, the extracts were

centrifuged at 2951 g for 5 min at 4°C (centrifuge Sigma-2-16 K, Steinheim, Germany). The supernatant was recovered; and the procedure was repeated three times, and the final volume was adjusted to 5.0 mL. The extracts were filtered through a 0.45 µm polyvinylidene fluoride filter (Millex HV13; Millipore) and stored at 4°C. All analyses were determined at microscale using 96-well microplates using a Multiscan FC microplate reader (Thermo-Fisher Scientific, Inc., Waltham, USA).

3.1.2.5.1. Total phenols

The quantification of the total phenols content of each bean extract was determined according to the method of Mena et al. (2011), with minor modifications. Briefly, 20 µL of extract, 100 µL of Folin-Ciocalteu reagent (10%) and 80 µL of Na₂CO₃ (7.5%) were homogenised and incubated in the oven at 40–45°C for 30 min in darkness. The absorbance was measured at 750 nm, and the results expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g dw).

3.1.2.5.2. *Ortho*-diphenols

The *ortho*-diphenols content of each bean extract was evaluated though the method described in Mateos et al. (2001), with minor modifications. Briefly, 160 µL of bean extract was mixed with 40 µL of Na₂MoO₄ (5%). Mixtures were incubated at room temperature for 15 min and kept in dark conditions. Absorbance was measured at 375 nm, and the results were expressed as mg GAE/g dw.

3.1.2.5.3. Flavonoids

The flavonoids content of bean extracts was determined using the method described by Zhishen et al. (1999). Briefly, to 24 µL of bean extract was added 28 µL of NaNO₂ (5%). Five minutes later, 28 µL of AlCl₃ (10%), and then the mixture was left to react for 6 min. Then, 120 µL of NaOH (1M) was added to the mixture and shaken for 30s. The absorbance was read at 510 nm, and the results were expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g dw).

3.1.2.6. Individual phenolic compounds

The individual phenolic compounds were determined following the method described by Lin et al. (2008), with slight modifications. The main individual phenolics determined were myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-(6''-*O*-manolyl)glucoside, kaempferol 3-*O*-glucoside, myricetin, kaempferol 3-*O*-(6''-*O*-manolyl)glucoside, kaempferol

3-*O*-(malonyl)glucoside, kaempferol, gallic acid, protocatechuic acid and catechin. HPLC analyses were performed using a Thermo Finnigan Surveyor HPLC-DAD system. The separation was achieved on an ACE 5 C18 column (5µm, 250 × 4.6 mm I.D.) and oven temperature was set at 25°C. Injection volume was 20 µL, and the flow rate was 1.0 mL/min. The mobile phase contained A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient program started with 10% of phase B at 0 min, 26% B at 40 min, 65% B at 70 min, 100% B at 71 min. The results were monitored at 280, 310 and 520 nm, while a UV/VIS spectrum from 2010–6500 nm was continuously collected. Chromatograms were analysed using the Xcalibur software (Thermo Fisher Scientific, Inc., Waltham, USA).

3.1.2.7. *In vitro* antioxidant activities

The free radical scavenging (DPPH and ABTS) assays and ferric reducing antioxidant power (FRAP) assay were performed according to the methods described by Espín et al. (2000), with slight modifications. The measurements were performed at microscale using 96-well microplates and measured by a Multiscan FC microplate reader (Thermo-Fisher Scientific, Inc., Waltham, USA). In DPPH and ABTS assays, 12 µL of bean extract was added to 188 µL of working solution to complete the volume reaction of 200 µL. The absorbance was measured at room temperature after 30 min of reaction at 520 nm for DPPH and at 734 nm for ABTS. In the FRAP assay, the total reaction volume was 300 µL, corresponding to 12 µL of bean extract and 288 µL of working FRAP solution. The mixtures were incubated for 30 min at 37 °C in the dark and read at 593 nm. The results of all procedures were measured as µM of trolox equivalents per gram of dry weight (µM TE/g dw).

3.1.2.8. Statistical analysis

All the experiments were performed in triplicates. Data was expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by the Tukey test, was used to assess significant differences between samples. Differences were considered significant at $p < 0.05$. The Partial Least Squares regression (PLS-R) approach was applied, resorting to the Wold's interaction, and using the analytical parameters assessed as independent variables, while the type of beans was considered as the dependent variable. The results were presented as two-dimensional plots of the scores obtained for the distinct samples, concerning Factor 1 and Factor 2. Statistical analyses were carried out with the SPSS Statistics 21.0 software (IBM Corporation, New York, USA).

3.1.3. Results and discussion

3.1.3.1. Nutritional composition

3.1.3.1.1. Protein content

The protein content varied between years with levels in 2014 (22.0–31.3 g/100g dw) significantly higher than in 2013 (19.5–24.8 g/100g dw), except for pink eyed cultivars (Figure 3).

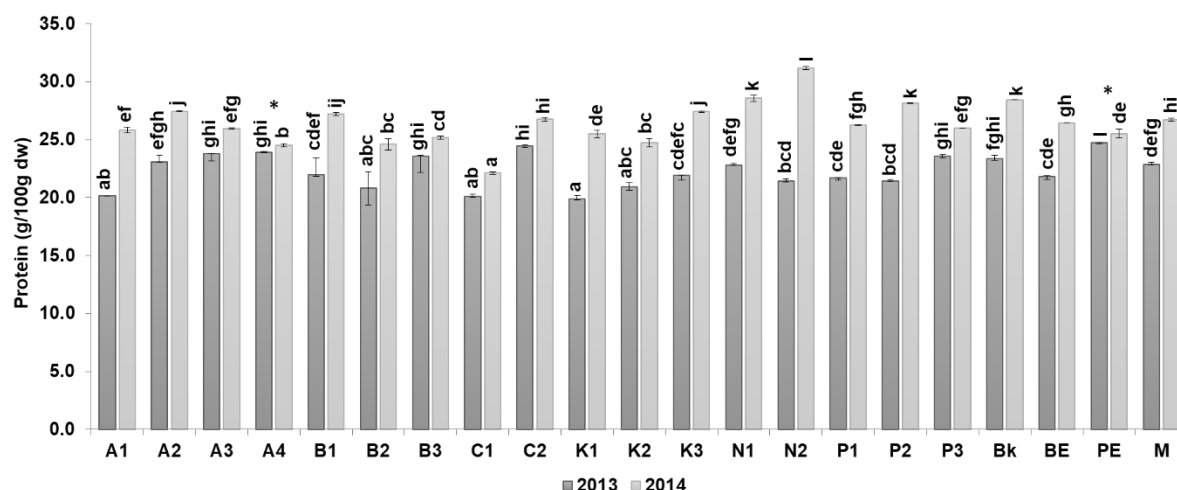


Figure 3 Protein content of 10 common bean cultivars for 2013 and 2014, grouped according to their morphological characteristics. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M)”. The absence of common letters indicates significant differences at $p < 0.05$; the Tukey's multiple range tests were performed for each year separately. * indicates that does not differ significantly at $p < 0.05$ between two harvesting years.

This tendency could be due to the influence of climatic parameters, mainly rainfall and temperature, which positively influence the content of protein relative to other constituents (Heinemann et al., 2016). In our study the highest amounts of protein were respectively found in navy > black > pink eyed > butter > tarrestre > pinto > arikara > brown eyed > red kidney > cranberry. A similar tendency was verified by Du et al. (2014). However, Wang et al. (2010), found dark red kidney to have the highest protein content (27.1 g/100g dw) whilst the lowest was reported for black bean (23.9 g/100g dw) and pinto bean (22.0 g/100g dw). Our study suggests navy (26.0 g/100g dw) and black (25.9 g/100g dw) cultivars as the best positioned to be used as protein enrichment sources in food products or for incorporation into plant-based diets. Other food enhancement effects are associated with these two beans: navy beans flour can confer additional health benefits by contributing to the prevention of colorectal cancer

(Borresen et al., 2014), and black bean food products (mixed with rice) are effective in infant weaning diet products. Curiously, the pink eyed cultivar showed no differences in protein content between years, suggesting that this cultivar is environmentally stable in relation to this parameter.

3.1.3.2.2. Amino acids composition

The content of essential and nonessential amino acids was higher ($p < 0.05$) in 2014 (0.1–1.6 g/100g dw and 0.3–2.7 g/100g dw, respectively) than in 2013 (0.1–1.2 g/100g dw and 0.2–2.2 g/100g dw, respectively) as expected due to their protein contents (Figure 4).

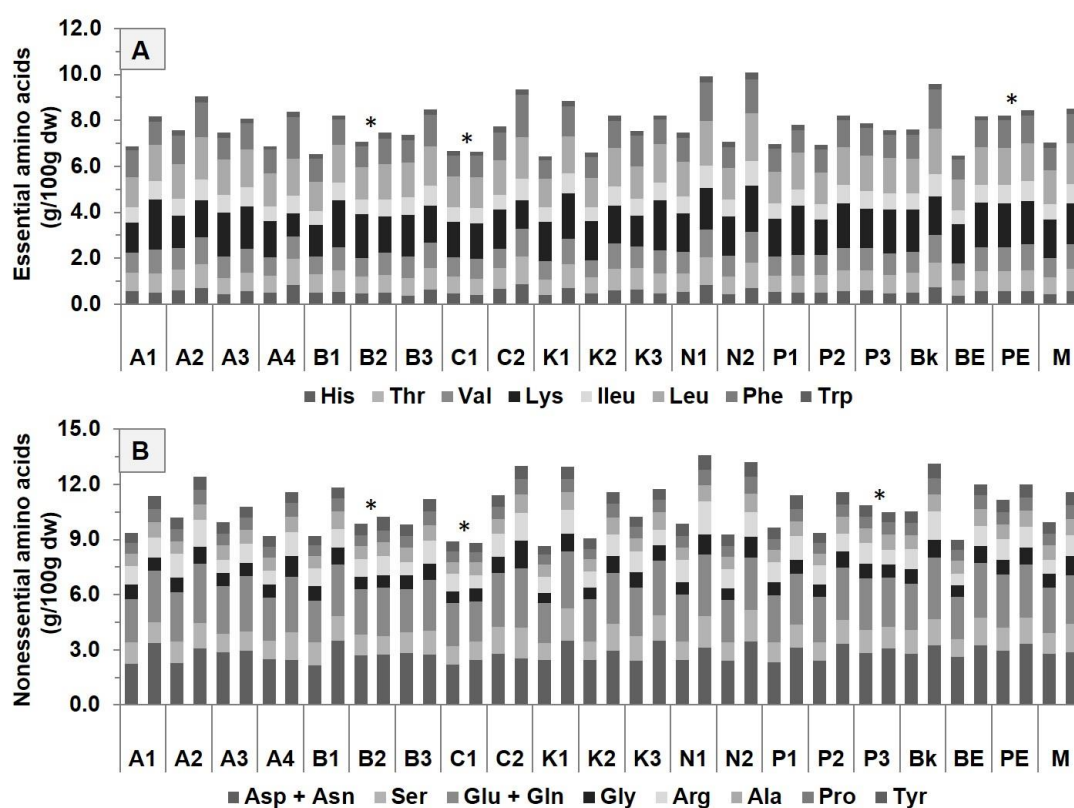


Figure 4 Amino acids of 10 common bean cultivars, grouped according to their morphological characteristics: (A) essential amino acids and (B) nonessential amino acids for 2013 (for each sample on the left) and 2014 (for each sample on the right) harvest years. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M)”. *indicates that does not differ significantly at $p < 0.05$ between two harvesting years.

Navy (9.3 g/100g dw), black (9.2 g/100g dw) and pink eyed (8.9 g/100g dw) had the highest amounts of total essential amino acids, while pinto (8.1 g/100g dw), butter (8.1 g/100g dw) and brown eyed (7.9 g/100g dw) had the lowest. Lys and Leu were the most predominant

essential amino acids in the cultivars, showing 1.3–1.9 g/100g in 2013, 1.0–2.2 g/100g in 2014 and, 1.4–1.8 g/100g in 2013 and 1.5–2.3 g/100g in 2014, respectively. These results are consistent with those previously reported by Kan et al. (2018). Essential amino acid composition and digestibility determine the nutritional quality of food proteins (Carbonaro et al., 2015). Hence, our results suggest that navy, black and pink eyed combine the best protein-essential amino acid contents and should therefore be prime candidates for protein-enhanced food products. This combination can be further explored in food products. Cereals and vegetables have lower amounts of Lys and higher amounts of Trp. Consequently, the combination of legumes with cereals and vegetables has gained importance due to the improvements in protein quality in food products (Pastor-Cavada et al., 2014). Therefore, these cultivars with the best characteristics (highest amounts of Lys and Trp) can provide a balanced source of dietary protein when combined with cereals and vegetables. Additionally, the low viscosity of bean flours has revealed a good thermal stability and lower tendency to retrograde in food incorporations, principally rice and bean blends (Carbas et al., 2018).

Regarding the nonessential amino acids, Asp+Asn and Glu+Gln were the predominant nonessential amino acids for all cultivars in both years. Asp+Asn ranged from 2.2–2.9 g/100g dw in 2013 and 2.4–3.5 g/100g dw in 2014. For Glu+Gln, the concentration ranged from 2.2–2.9 g/100g dw in 2013 and 2.2–3.4 g/100g dw in 2014. Samples A4 and K3 had the highest amounts of Asp+Asn (3.4 and 3.5 g/100g dw, respectively). The highest amounts of Glu+Gln were found in N1 (3.4 g/100g dw) and Bk (3.3 g/100g dw). All cultivars were poor in the nonessential amino acids Tyr and Pro.

3.1.3.3. Antinutritional composition

The tannins content decreased ($p < 0.05$) in 2014 (0.2–1.1 g ECE/100g dw) compared to 2013 (0.8–1.7 g ECE/100g dw), except for butter (B2), navy (N1, N2) and pinto (P3) cultivars (Figure 5). This tendency could be due to the higher amounts of protein observed in the second harvest year given their apparent negative correlation (except in A4).

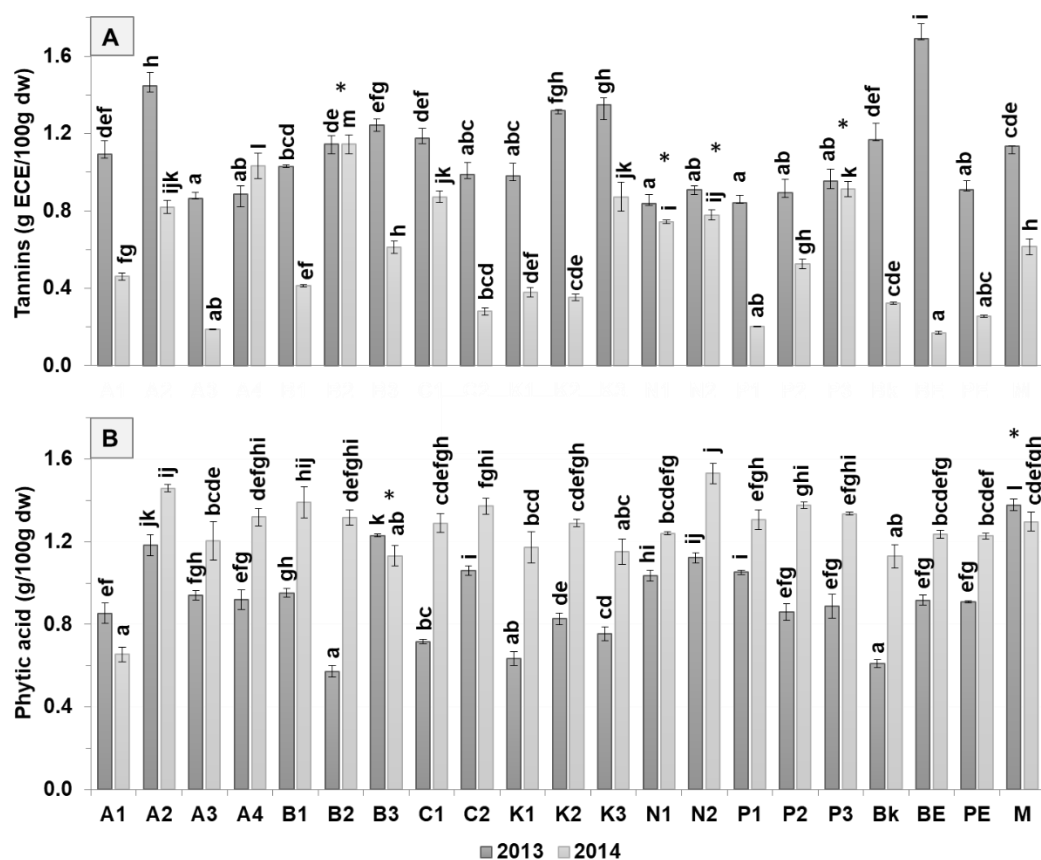


Figure 5 Antinutritional composition of 10 common bean cultivars, grouped according to their morphological characteristics: (A) tannins and (B) phytic acid content for 2013 and 2014. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M)”. The absence of common letters indicates significant differences at $p < 0.05$; the Tukey's multiple range tests were performed for each year separately. *indicates that does not differ significantly at $p < 0.05$ between two harvesting years.

Considering the tannins and phytic acid contents, the cultivars butter (B2), navy (N1, N2) and pinto (P3) are potentially better adapted to different climatic conditions since they exhibited similar values for both years. Brown eyed (0.9 g ECE/100g dw) and butter (0.9 g ECE/100g dw) cultivars had the highest average amount of tannins content, while pinto bean (0.7 g ECE/100g dw) and pink eyed (0.6 g ECE/100g dw) cultivars exhibited the lowest amounts. Grela et al. (2017) reported low amounts (0.1 g ECE/100g dw) for the Mela bean cultivar.

Concerning the phytic acid content, higher contents were found in 2013 than in 2014, ranged from 0.6–1.4 g/100g dw in 2013 and 0.6–1.5 g/100g dw in 2014, except arikara yellow (A1) and tarrestre (M) which revealed lower amounts in 2014. Butter (B3) and tarrestre (M)

samples showed good stability for varying climatic conditions, by displaying similar amounts of phytic acid (1.2 g/100g dw for B3 and 1.3 g/100g dw for tarreste (M) in both years (Figure 5). The highest amounts were found in the group of navy (1.2 g/100g dw) and tarreste (1.3 g/100g dw) cultivars, while the lowest amounts were found in black (0.9 g/100g dw) and red kidney (1.0 g/100g dw) beans. Wang et al. (2010) reported similar amounts for cranberry (1.2 g/100g dw), higher amounts for kidney (1.4 g/100g dw), and lower amounts for pinto beans (0.9 g/100g dw).

Regarding tannins vs protein content of distinct bean cultivars for different harvest years, and for 2013 (Figure 5A), the samples are more distributed along the protein axis and ranging 20–25 g/100g dw, while the tannins content is between 0.8–1.2 g ECE/100g dw.

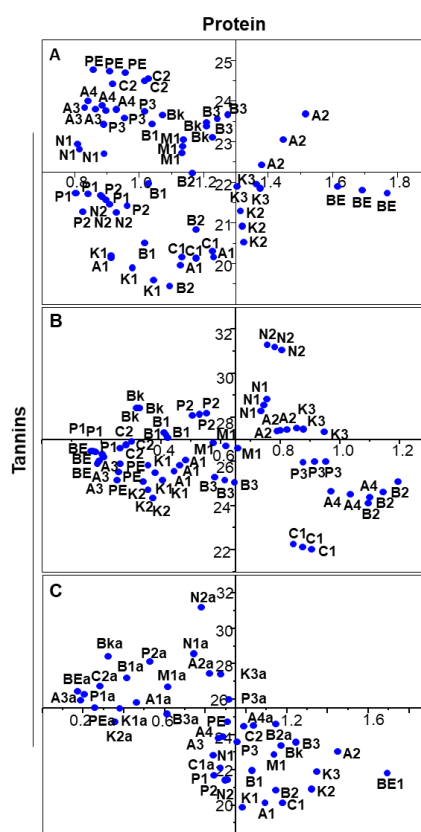


Figure 6 Plot of tannins vs protein content of 10 common bean cultivars, grouped according to their morphological characteristics. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarreste (M)”. (A) Triplicates of each sample from 2013, (B) triplicates of each sample from 2014, (C) average of each sample in both years, the samples being divided according to their harvest years.

Arikara yellow (A), black (Bk), red kidney (K), navy (N) and pinto (P) beans clustered, indicating that they have similar values among samples of the same bean group. Regarding the 2014 harvest year (Figure 6B), although the cultivars are more scattered than in the plot of 2013, the navy and red kidney beans are still clustered. Contrary to the 2013 plot, here the samples are distributed along the tannins' axis between 0.2–0.8 g ECE/100g dw, and the protein content ranged from 24 g/100g dw to 30 g/100g dw.

Regarding the average values of the samples from both harvest years (Figure 6C), the protein amounts are negatively correlated, matching the pattern whereby samples with higher protein content exhibit lower tannins amounts. However, the samples clustered according to bean group such as navy (in both years, (N; Na)), arikara yellow (2013 (A)), pinto (2013 (P)), red kidney (2013 (K)), as well as black (both years, (Bk; Bka)). Navy bean cultivars from both years cluster given their similarity between both years (Figure 6A).

Data observed in the present study has shown here that climatic cultivation conditions could have a substantial effect on the antinutrients content of several cultivars. Regarding the application of these cultivars to food products, the results showed that pinto, pink eyed, black and red kidney cultivars have the lowest amount of antinutrients and hence are the best candidates. The navy and red kidney bean flour extrudates have been shown to reduce the antinutrients content of pasta (Segura-Campos et al., 2014). Antinutrients such as tannins and phytic acid can affect the digestibility and bioavailability of nutrients (Campos-Vega et al., 2010). Interestingly, antinutrients such as phytic acid have also been related to health benefits (Cominelli et al., 2018), evidencing the need for further health studies before a cultivar may be chosen for food products applications.

3.1.3.4. Phenolic composition

The contents of total phenols, *ortho*-diphenols and flavonoids decreased in red kidney, black and brown eyed beans from the first harvest year to the second harvest year, while in the butter, navy and pink eyed cultivars an opposite trend was found (Table 5). Nevertheless, the amounts of flavonoids were not significantly different between years in almost all cultivars.

Table 5 Total phenols (mg GAE/g dw), *ortho*-diphenols (mg GAE/g dw) and flavonoids (mg CE/g dw) of 10 common bean cultivars for 2013 and 2014 harvest year, grouped according to their morphological characteristics. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M)”.

Cultivars	Total phenols		<i>Ortho</i> -diphenols		Flavonoids	
	2013	2014	2013	2014	2013	2014
A1	2.97 ± 0.24 jk*	2.98 ± 0.08 ijk	6.46 ± 0.34 ij*	5.07 ± 0.17 k	2.63 ± 0.12 i*	2.47 ± 0.22 hi
A2	2.32 ± 0.22 hi*	2.79 ± 0.22 hi	2.54 ± 0.11 def*	2.78 ± 0.07 defg	2.18 ± 0.08 g*	2.30 ± 0.12 ghi
A3	2.27 ± 0.05 gh	1.16 ± 0.11 b	6.16 ± 0.07 i	3.26 ± 0.17 ghi	1.62 ± 0.04 def*	1.36 ± 0.12 bc
A4	2.30 ± 0.05 h	1.36 ± 0.07 bc	5.53 ± 0.08 h	3.00 ± 0.06 efgh	1.33 ± 0.04 cd *	1.19 ± 0.00 ab
B1	1.38 ± 0.06 de	2.87 ± 0.21 ij	1.93 ± 0.03 bc	3.97 ± 0.05 j	1.76 ± 0.05 ef*	1.93 ± 0.01 def
B2	1.89 ± 0.18 fg	2.67 ± 0.26 ghi	2.42 ± 0.11 de*	2.84 ± 0.06 defg	2.28 ± 0.14 gh*	2.17 ± 0.14 fgh
B3	1.41 ± 0.10 de	3.03 ± 0.14 ijk	2.39 ± 0.07 de	3.00 ± 0.20 efgh	1.70 ± 0.09 ef	2.53 ± 0.22 i
C1	3.17 ± 0.18 k*	3.39 ± 0.30 kl	2.23 ± 0.03 cd	2.73 ± 0.24 cdef	3.90 ± 0.13 k*	4.02 ± 0.04 l
C2	2.75 ± 0.05 j*	2.61 ± 0.17 fghi	2.87 ± 0.16 f	2.06 ± 0.03 ab	2.53 ± 0.15 hi	2.14 ± 0.01 efg
K1	2.70 ± 0.16 ij	3.37 ± 0.23 jkl	2.50 ± 0.06 def	3.42 ± 0.27 hi	3.10 ± 0.05 j*	3.00 ± 0.16 j
K2	4.03 ± 0.19 l	3.31 ± 0.11 jk	6.69 ± 0.27 j	4.86 ± 0.38 k	2.81 ± 0.02 ij	2.19 ± 0.04 fgh
K3	4.59 ± 0.24 m	3.84 ± 0.13 l	4.70 ± 0.15 g	3.40 ± 0.10 hi	4.33 ± 0.25 l	3.35 ± 0.08 k
N1	0.11 ± 0.00 a*	0.43 ± 0.01 a	0.89 ± 0.06 a	2.37 ± 0.07 abcd	0.80 ± 0.03 a*	0.97 ± 0.04 a
N2	0.35 ± 0.01 ab	2.13 ± 0.13 def	1.07 ± 0.07 a	2.23 ± 0.09 abc	0.98 ± 0.03 ab*	0.91 ± 0.02 a
P1	1.39 ± 0.02 de	2.06 ± 0.04 de	2.57 ± 0.04 def	3.53 ± 0.17 ij	1.78 ± 0.11 ef	1.29 ± 0.03 b
P2	1.30 ± 0.02 de	2.04 ± 0.18 d	1.72 ± 0.06 b	3.25 ± 0.29 fghi	1.59 ± 0.08 def*	1.71 ± 0.07 d
P3	1.53 ± 0.02 ef	2.30 ± 0.20 defgh	2.28 ± 0.13 cde	3.58 ± 0.21 ij	1.65 ± 0.01 ef*	1.83 ± 0.15 de
Bk	2.25 ± 0.06 gh	2.21 ± 0.12 defg	2.91 ± 0.08 f	2.87 ± 0.07 defg	1.78 ± 0.01 ef	1.67 ± 0.06 cd
BE	1.03 ± 0.03 cd*	0.65 ± 0.02 a	2.58 ± 0.20 def	1.85 ± 0.07 a	1.54 ± 0.06 de	1.09 ± 0.08 ab
PE	0.67 ± 0.06 bc*	1.84 ± 0.12 cd	1.53 ± 0.04 b	2.52 ± 0.04 bcde	1.23 ± 0.01 bc*	2.04 ± 0.09 efg
M	1.96 ± 0.18 gh	2.56 ± 0.10 efghi	2.68 ± 0.11 ef	2.40 ± 0.01 bcd	1.84 ± 0.10 f	2.23 ± 0.03 fghi

The absence of common letters (^{a-l}) indicates significant differences at $p < 0.05$; the Tukeys’s multiple range test was performed for each year, separately. *indicates that does not differ significantly at $p < 0.05$, between two harvesting years

Total phenols content ranged from 0.11 to 4.59 mg GAE/g dw in 2013 and 0.43 to 3.84 mg GAE/g dw in 2014. Cranberry and red kidney cultivars exhibited the highest amounts of total phenols (2.98 and 3.64 mg GAE/g dw, respectively). Navy and brown eyed beans showed the lowest amounts (0.75 and 0.84 mg GAE/g dw, respectively). These results were expected as dark coloured beans have higher phenolic composition and antioxidant capacity than the uncoloured beans (Chavez-Mendoza et al., 2019). Previous studies found lower amounts of total phenols (0.47–2.38 mg GAE/g dw) in 26 kidney beans analysed (Kan et al., 2017). Giusti et al. (2017) reported similar amounts for kidney beans (3.00 mg GAE/g dw) and cranberry

beans (2.24 mg GAE/g dw), although lower amounts were found for kidney (0.96 mg GAE/g dw) and black (0.90 mg GAE/g dw) beans by Rocchetti et al. (2019). The flavonoids ranged 0.80–4.33 mg CE/g dw in 2013 and 0.91–4.02 mg CE/g dw in 2014. Finally, the *ortho*-diphenol content ranged from 0.89–6.69 mg GAE/g dw in 2013 and 1.85–5.07 mg GAE g⁻¹ dw in 2014. The highest amounts were found in arikara yellow (4.35 mg GAE/g dw) and red kidney (4.26 mg GAE/g dw) beans, while navy and pink eyed revealed the lowest amounts of *ortho*-diphenols among all cultivars. Therefore, the highest amounts were exhibited for the cultivars with highest total phenols content, red kidney (3.64 mg GAE/g dw) and cranberry (2.98 mg GAE/g dw), and the lowest amounts were also found in navy (0.75 mg GAE/g dw) and brown eyed (0.84 mg GAE/g dw). The phenolic composition was higher in red kidney, black and cranberry cultivars in comparison to the uncoloured samples (navy and butter cultivars), a tendency found in accordance with other studies (Giusti et al., 2017), although the arikara yellow cultivar (yellow colour), also exhibited a substantial content in phenolic compounds.

The phenolic composition of different groups can be attributed to genetic factors, climatic conditions and variation in colour of coats between cultivars (Zhao et al., 2014). Our results support the previous assumption and further shows that the coloured red kidney, cranberry and arikara yellow are the most promising cultivars for phytochemical enrichment of food products. This is particularly important because the phenolic composition of cereals and pseudocereals is significantly lower than in legumes and the total phenols content of common beans is one of the highest amongst legumes (Zhao et al., 2014). Recent studies have reported that adding bean flours to snack formulations with oat flour improves the bioactive profile, mainly total flavonoids and *in vitro* antioxidant activity (Ramírez-Jiménez et al., 2018). This emphasises the advantageous use of bean flours or their combinations with (pseudo)cereals as functional ingredients in the enrichment of food products.

3.1.3.5. Individual phenolic compounds

The most predominant individual phenolic compound was catechin, present in all samples of both years, ranging from 324.7 to 1057.0 µg/g dw in 2013 and 202.0 to 1412.3 µg/g dw in 2014 (Table 6).

Table 6 Individual phenolic compounds of 10 common bean cultivars by HPLC-DAD for 2013 and 2014 harvest years, grouped according to their morphological characteristics, and expressed as $\mu\text{g/g}$ of dry weight sample. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M)”.

Cultivars	Myricetin 3- <i>O</i> -glucoside		Quercetin 3- <i>O</i> -glucoside		Quercetin 3- <i>O</i> -(6''- <i>O</i> -manoyl) glucoside		Kaempferol 3- <i>O</i> -glucoside		Myrcetin		Kaempferol 3- <i>O</i> -(6''- <i>O</i> -manoyl) glucoside		Kaempferol 3- <i>O</i> -(malonyl) glucoside		Kaempferol		Protocatechuic acid		Gallic acid		Catechin	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
A1	nd	nd	126.9	41.7	nd	nd	1767.1	974.6	nd	nd	89.8	119.0	5.2*	5.7	10.3	5.6	nd	nd	nd	nd	324.7	370.5
A2	nd	10.4	6.0*	5.7	nd	nd	184.1*	114.0	nd	nd	43.7*	45.4	nd	nd	48.3	31.3	nd	nd	nd	nd	625.0	554.9
A3	nd	nd	104.1	12.0	nd	nd	1322.6	570.2	nd	nd	62.3	40.0	nd	nd	16.8	8.9	15.4	17.7	nd	nd	1562.8	381.4
A4	nd	nd	78.2	11.2	nd	nd	1171.4	504.4	nd	nd	79.4	61.1	nd	4.7	10.1	nd	nd	nd	nd	nd	765.1	550.6
B1	nd	nd	37.1*	36.0	5.3	8.1	49.0*	37.4	nd	nd	20.6*	24.0	nd	nd	nd	nd	nd	nd	nd	nd	1004.4	1412.3
B2	nd	nd	61.3	30.3	9.0	6.5	73.5	46.0	nd	nd	22.6	33.3	nd	nd	nd	nd	nd	nd	nd	nd	856.8	433.3
B3	nd	nd	1.6	nd	nd	nd	74.8	41.3	nd	nd	37.8*	39.2	8.6	nd	163.4	93.3	nd	nd	nd	nd	253.5*	209.8
C1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	594.4	740.0
C2	nd	nd	nd	nd	nd	nd	16.8	8.6	nd	nd	16.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	1569.9	543.0
K1	nd	nd	13.8	44.7	nd	3.3	56.2*	56.4	29.7	19.3	5.6*	12.2	nd	nd	nd	nd	nd	nd	nd	nd	380.6*	373.6
K2	nd	nd	90.4	35.8	2.7	nd	1740.6	761.2	11.6*	12.4	199.0	132.5	10.2	6.8	19.5	9.3	nd	nd	nd	nd	668.1	455.3
K3	nd	nd	53.2	47.3	nd	3.3	96.3*	75.9	25.4	18.9	9.5*	12.8	nd	nd	nd	nd	nd	nd	nd	nd	724.6	380.1
N1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	54.0	63.2	763.4*	761.2
N2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	67.9	63.7	658.4*	555.0
P1	nd	nd	8.2	3.0	nd	nd	158.5	74	nd	nd	18.5*	20.9	nd	nd	14.3*	14.1	nd	nd	nd	nd	1380.7	1027.7
P2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	563.1*	559.0
P3	nd	nd	33.8	nd	nd	nd	245.2	516.9	nd	nd	19.1	75.9	nd	3.6	5.3	nd	nd	nd	nd	nd	1057.0	202.0
Bk	150.2	79.7	22.2	14.7	nd	nd	17.5*	12.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1003.2	206.5
BE	nd	nd	nd	nd	nd	nd	nd	15.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	666.3	268.4
PE	11.7	22.6	31.2	87.3	nd	3.0	18.6	75.0	nd	10.2	nd	12.9	nd	nd	nd	nd	nd	nd	nd	nd	759.0	119.1
M	nd	nd	4.8	nd	nd	nd	140.6	40.1	nd	nd	32.7	21.0	nd	nd	44.9	12.1	nd	nd	nd	nd	737.0	309.3

nd – not detectable; the signal was lower than limit of quantitation (LOQ). * indicates that does not differ significantly at $p < 0.05$, between two harvesting years.

Quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside and kaempferol 3-*O*-(6''-*O*-malonyl)glucoside were present in 75% of samples, while myricetin 3-*O*-glucoside, protocatechuic acid and gallic acid were only present in 5–10% of samples. In general, the amounts of individual phenolic compounds were lower in samples of the second harvest year, as observed for phenolic composition (Table 5).

Whilst the darker coloured red kidney and pink eyed bean showed the most significant number of individual phenolic compounds, the arikara yellow bean sample group (pale colour) exhibited the highest amount of individual phenolic compounds (3215.0 µg/g dw), around 50% more than red kidney (2188.2 µg/g dw). Among all groups, A3 and K2 samples revealed the highest total content of individual phenolic compounds, 4114.2 µg/g dw and 4155.4 µg/g dw, respectively, where kaempferol 3-*O*-glucoside and catechin were the individual phenolic compounds present in highest concentrations. As was verified in the study of Mecha et al. (2019), flavonols, namely as catechin, is the main phenolic compound found in the coat of beans. The brown eyed bean sample showed the lowest amounts of total individual phenolic compounds, having only two phenolic compounds (kaempferol 3-*O*-glucoside and catechin) in its matrix.

Gallic acid was only present in the navy cultivar, and myricetin was found only in red kidney and pink eyed beans. Furthermore, A3 is the only sample that had protocatechuic acid in its phenolic composition. Giusti et al. (2017) studied 36 pulses, including 21 bean varieties from different origins, cultivated in Italy from the 2014 harvest, and in their study the authors found similar amounts of catechin for cranberry beans and lower amounts of catechin for pinto (322.3 µg/g dw), black (14.1 µg/g dw) and red kidney (60.6 µg/g dw) beans. Giusti et al. (2017) also reported gallic acid only in black and streghoni beans samples. Also, the authors found kaempferol 3-glucoside in 30% of bean samples and therein, pinto samples exhibited 191.0 µg/g dw and black samples 24.5 µg/g dw, although it was not detected in kidney and cranberry samples. An opposite trend was found in the present study, where kaempferol 3-*O*-glucoside was found in 75% of samples with 74.0–516.9 µg/g dw for pinto beans, 56.4–1740.6 µg/g dw for red kidney beans and 8.6–16.8 µg/g dw for cranberry beans. Aguilera et al. (2011) found similar quantities of Quercetin 3-*O*-glucoside (3.6 µg/g dw) and kaempferol 3-*O*-(malonyl)glucoside (7.2 µg/g dw) for pinto cultivars.

Altogether, these results suggested that red kidney beans could be a particularly good source of phenolic compounds. Furthermore, their food industry application would not be compromised by the cooking method, since these beans have been shown to retain over 83% of

the total phenolic acids, after cooking, and they even increase the antioxidant activity (Gallegos-Infante et al., 2010).

3.1.3.6. *In vitro* antioxidant activities

In vitro antioxidant activities were measure by free radical scavenging assays: ((2,2-diphenyl-1picrylhydrazyl radical (DPPH), and (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS); and ferric reducing antioxidant power (FRAP), are reported Table 7.

Table 7 *In vitro* antioxidant activities (DPPH, ABTS, FRAP, ($\mu\text{mol TE/g dw}$)) of 10 cultivars of common beans for 2013 and 2014 harvest years, grouped according to their morphological characteristics. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M)”.

Cultivars	DPPH		ABTS		FRAP	
	2013	2014	2013	2014	2013	2014
A1	8.93 \pm 0.20 de*	10.39 \pm 0.61 cde	19.94 \pm 0.58 f	14.93 \pm 0.70 ef	18.10 \pm 0.54 gh*	16.61 \pm 0.69 i
A2	11.39 \pm 0.80 gh	9.87 \pm 0.23 cd	16.39 \pm 0.35 e*	15.16 \pm 0.90 efg	11.14 \pm 0.65 cd*	10.15 \pm 0.06 cd
A3	10.01 \pm 0.24 efg	7.15 \pm 0.17 b	20.36 \pm 0.75 fg	9.10 \pm 0.44 bc	16.48 \pm 0.86 fg	7.50 \pm 0.62 b
A4	8.22 \pm 0.27 d*	7.19 \pm 0.05 b	13.25 \pm 0.56 cd*	13.72 \pm 0.69 de	9.96 \pm 0.15 cd	7.68 \pm 0.13 b
B1	10.13 \pm 0.49 efg*	9.16 \pm 0.59 c	14.21 \pm 0.40 cde	16.96 \pm 0.66 fg	11.64 \pm 0.33 d	14.13 \pm 0.36 g
B2	10.43 \pm 0.36 fg*	11.22 \pm 0.44 ef	15.39 \pm 1.06 de*	17.69 \pm 1.06 gh	14.73 \pm 0.23 ef*	14.33 \pm 0.34 gh
B3	8.88 \pm 0.50 de	11.34 \pm 0.21 ef	13.26 \pm 0.70 cd	21.00 \pm 1.14 i	9.96 \pm 0.36 cd	13.84 \pm 0.73 g
C1	15.82 \pm 0.06 j	14.81 \pm 0.70 g	23.87 \pm 1.11 hi	20.98 \pm 1.57 i	19.68 \pm 0.65 hi	11.14 \pm 0.40 de
C2	10.98 \pm 0.63 gh*	10.95 \pm 0.80 def	20.25 \pm 1.11 fg	14.04 \pm 1.11 de	5.15 \pm 0.48 ef	12.44 \pm 0.63 ef
K1	14.65 \pm 0.18 ij*	13.89 \pm 0.41 g	22.38 \pm 0.18 gh*	20.18 \pm 1.17 hi	19.84 \pm 0.39 hi	14.47 \pm 0.10 gh
K2	13.82 \pm 0.59 i	11.75 \pm 0.60 f	23.12 \pm 1.27 hi	17.51 \pm 1.57 g	20.20 \pm 0.65 i	13.45 \pm 0.71 fg
K3	18.31 \pm 1.05 k*	17.83 \pm 0.45 h	32.58 \pm 0.16 j	23.93 \pm 0.09 j	26.92 \pm 1.08 l	22.24 \pm 0.32 j
N1	5.06 \pm 0.19 b	3.23 \pm 0.06 a	3.39 \pm 0.19 a	7.45 \pm 0.48 ab	4.90 \pm 0.29 a*	5.71 \pm 0.40 a
N2	2.73 \pm 0.08 a*	2.14 \pm 0.07 a	6.30 \pm 0.31 b*	6.28 \pm 0.23 a	7.97 \pm 0.14 b	5.58 \pm 0.18 a
P1	9.24 \pm 0.61 def	7.28 \pm 0.06 b	15.58 \pm 0.58 de	11.77 \pm 0.07 d	23.85 \pm 1.46 k	11.78 \pm 0.35 e
P2	11.83 \pm 0.39 h	7.67 \pm 0.14 b	15.00 \pm 0.71 de*	14.87 \pm 0.14 ef	13.48 \pm 0.25 e*	12.30 \pm 0.42 ef
P3	6.80 \pm 0.34 c*	6.57 \pm 0.26 b	13.67 \pm 0.86 cd*	12.00 \pm 0.57 d	9.55 \pm 0.35 bc*	9.71 \pm 0.42 c
Bk	9.37 \pm 0.21 def	10.47 \pm 0.38 de	20.72 \pm 1.63 fg	13.99 \pm 0.50 de	20.97 \pm 0.33 ij	13.33 \pm 0.33 fg
BE	4.94 \pm 0.03 b	7.49 \pm 0.43 b	12.60 \pm 0.47 c	7.18 \pm 0.20 ab	10.71 \pm 0.40 cd	6.81 \pm 0.47 ab
PE	5.77 \pm 0.46 bc*	7.36 \pm 0.10 b	6.96 \pm 0.12 b	11.53 \pm 0.07 cd	5.96 \pm 0.25 a	15.62 \pm 0.42 hi
M	14.56 \pm 0.16 ij	12.09 \pm 0.36 f	24.90 \pm 0.50 i	15.58 \pm 0.94 efg	22.24 \pm 0.15 jk	11.80 \pm 0.32 e

The absence of common letters (^{a-l}) indicates significant differences at $p < 0.05$; the Tukeys's multiple range test was performed for each year, separately. *indicates that does not differ significantly at $p < 0.05$, between two harvest years

DPPH mean values ranged between 2.73 and 18.31 $\mu\text{mol TE/g dw}$ in 2013 and 2.14–17.83 $\mu\text{mol TE/g dw}$ in 2014. Red kidney bean type (15.04 $\mu\text{mol TE/g dw}$) and tarreste (13.32 $\mu\text{mol TE/g dw}$) had the highest antioxidant activity while the navy (3.29 $\mu\text{mol TE/g dw}$) and brown eyed cultivars (6.22 $\mu\text{mol TE/g dw}$) had the lowest capacity. Lower amounts of DPPH were reported for kidney beans, ranging between 1.10–7.50 $\mu\text{mol TE/g dw}$ (Kan et al., 2017). However, a common bean sample (Mela cultivar) analysed in the study of Grela et al. (2017) was found to display much higher DPPH activity than in the current study, which may be due to a the different genetic background. The variation in antioxidant activity quantified by ABTS was similar between years (3.39–32.58 $\mu\text{mol TE/g dw}$ in 2013; 6.28–23.93 $\mu\text{mol TE/g dw}$ in 2014). Kidney beans had the highest antioxidant capacity exhibiting values 50% higher than for DPPH.

With the FRAP assay, the values ranged from 4.90 to 26.92 $\mu\text{mol TE/g dw}$ in 2013 and 5.58–22.24 $\mu\text{mol TE/g dw}$ in 2014. The navy cultivar had the lowest antioxidant capacity, although they showed amounts two times higher than the ones reported by DPPH. The highest values were found in kidney beans with an average of 19.52 $\mu\text{mol TE/g dw}$. These differences in antioxidant activity between cultivars, found also in other studies can result from genetics, the extraction procedure used, or environmental factors such as rainfall and temperature (Heinemann et al., 2016).

In all methodologies, the antioxidant activity of our samples decreased in the second harvest year, likely due to higher average temperatures in 2013 than in 2014 (Figure 2). The *in vitro* antioxidant capacity of coloured cultivars (kidney, tarreste and black bean) exhibited the highest values in all methodologies for determination of antioxidant activities, whereas the uncoloured samples (navy, pink eyed and brown eyed beans) showed the lowest *in vitro* antioxidant capacity. These results suggest that functional flours of coloured cultivars are advantageous options for food enrichment at the antioxidant activity level. Although few studies have evaluated the antioxidant enrichment of common bean-based foods, available evidence suggests that common bean flour enrichment can increase the antioxidant activity of spaghetti pasta (Gallegos-Infante et al., 2010).

Partial least squares regressions have been performed using the triplicates of each sample, for the years 2013 and 2014, and for both years together, and the results are presented through the plot of the scores obtained for the first two factors, regarding all samples. These explain respectively 89.5% and 86.2% of the total variability, in 2013 and 2014, respectively

(Figure 7A, B). For both years, and using the average of each sample, these two factors explain only 30.8% of total variability (Figure 7C).

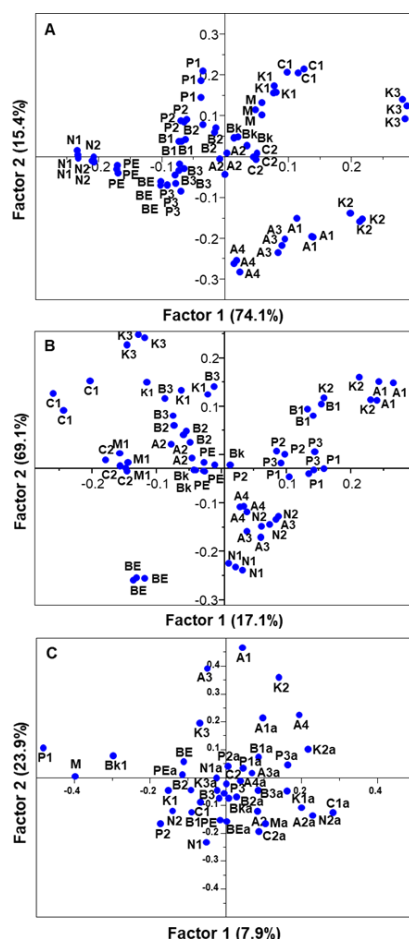


Figure 7 Plot of the scores of phytochemical composition and *in vitro* antioxidant capacity obtained for the first two factors extracted from the PLS regressions. (A) Triplicates of each sample from 2013, (B) triplicates of each sample for 2014 and (C) mean of each sample in both years. “Cultivars: arikara yellow (A1, A2, A3, A4), butter bean (B1, B2, B3), cranberry (C1, C2), red kidney (K1, K2, K3), navy (N1, N2), pinto (P1, P2, P3), black (Bk), brown eyed (BE), pink eyed (PE), and tarrestre (M)”.

In 2013, arikara yellow (A1, A3 and A4), butter bean (B1 and B2), navy (N1 and N2) and pinto (P1 and P2) are clustered, indicating a similar phenolic composition and *in vitro* antioxidant capacity. N1 and N2 showed the lowest amounts of total phenols, *ortho*-diphenols, flavonoids and *in vitro* antioxidant activity. Red kidney (K3) was separated from the other cultivars due to its highest amounts in total phenols and flavonoids, in spite of the higher results observed in all antioxidant activity methodologies. In 2014, the beans’ cultivars were more distributed in the plot, in comparison with the 2013 plot. Nevertheless, pinto and red kidney

cultivars were closer in the plot, showing a similar phenolic composition and *in vitro* antioxidant activity. Red kidney (K3) also exhibited the highest amounts of total phenols, flavonoids and *in vitro* antioxidant activities, while brown eyed (BE) showed the lowest amounts of *ortho*-diphenols. In the PLS regressions plot with both years (Figure 7C), the samples differed significantly between years in terms of phytochemical composition and *in vitro* antioxidant activities, except for the arikara yellow (A2) sample. These results further confirm that the coloured red kidney, and potentially the pinto cultivars, are preferential candidates for bean enrichment applications, especially for increasing the antioxidant activity.

3.1.4. Conclusion

Our study has identified *Phaseolus vulgaris* L. the cultivars as those which offer the best nutrient specific profile and functional proprieties for an increasingly requirement for healthy food. Navy, black and pink eyed cultivars were the most promising protein and amino acids sources for nutrient enrichment applications. Red kidney and arikara yellow cultivars offer better functional proprieties regarding phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds, associated with antioxidant effects. This functional profile indicates that common bean, similarly to other legumes, can offer an important protective role in the prevention and treatment of diet-dependent diseases.

Our study highlights the relevance of climatic conditions during the growing seasons which have a determining influence on major nutritional and antinutritional compounds, and the respective functional properties of bean cultivars. Further research is required to ascertain the best growing conditions to maximise the nutritional and functional capacities of the most promising cultivars identified in our study.

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Chapter 4

4.1. Comparison of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for the determination of nutritional and antinutritional parameters in common beans

4.1. Comparison of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for the determination of nutritional and antinutritional parameters in common beans

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Abstract

Common beans (*Phaseolus vulgaris* L.), represent the most consumed legume worldwide and constitute an important source of protein, being also known to contain antinutritional compounds, which compromise nutrients' bioavailability. However, the standard methodologies to assess these constituents are time-consuming and complex. Therefore, the present study evaluated the suitability of near-infrared (NIR) and mid-infrared (MIR) spectroscopies for the development of simple and reliable methods to assess protein, lipids, tannins and phytic acid contents, besides specific amino acids, in whole bean flours. Partial least squares (PLS) regression was used to develop analytical models, and external validation was performed.

NIR displayed better performance for the evaluation of protein, lipids, tannins and phytic acid contents, and MIR, for the assessment of specific amino acids. In both techniques, the use of the 1st derivative was the best data treatment. Overall, both techniques represent reliable methods to evaluate the proximate and antinutritional composition of bean flours.

Keywords: *Phaseolus vulgaris* L.; protein; lipids; tannins; phytic acid; amino acids; FTIR spectroscopy

4.1.1. Introduction

Grain legumes represent 27% of the world crop production, while common beans (*Phaseolus vulgaris* L.) are the most consumed in the world (Baptista et al., 2017), representing an excellent and sustainable source of protein and essential amino acids (mainly lysine) (Kan et al., 2017). Moreover, this foodstuff contains high contents in dietary fibre, minerals, vitamins

and phytochemicals, besides presenting low fatty acid contents, while its consumption has been lately related to anti-inflammatory activity, reduction of diabetes, and prevention of cancer and cardiovascular diseases (Campos-Vega et al., 2010; Thompson et al., 2012; Luzardo-Ocampo et al., 2018).

The main proteins present in legumes are albumins, responsible for structural and enzymatic functions in the human organism, and globulins, which are considered immune proteins, representing 80–90% of total protein in beans (Grela et al., 2017), while the nutritional quality of food proteins is commonly related to their specific composition, particularly regarding essential amino acids (Carbonaro et al., 2015). Furthermore, several studies have shown that the combination of cereals and legumes improves the protein quality and amino acid balance of food products, with legumes presenting high amounts of amino acids that are scarce in cereals (Kan et al., 2017). Therefore, the analysis of the specific amino acid contents in these matrices represents an issue of special relevance for the development of such foodstuffs (Pastor-Cavada et al., 2014).

Legumes are also known to contain minor compounds, which have been considered as antinutritional compounds that compromise the nutrients' bioavailability and, consequently, legume nutritional quality (Carbonaro et al., 2015), while these compounds may display positive or negative health effects, or even both (Campos-Vega et al., 2010). For instance, phytic acid, an antinutritional compound, interferes with minerals, lipids and proteins' absorption, while exerting beneficial effects on human health, mainly in the prevention of cardiovascular diseases and cancer (Cominelli et al., 2018). Furthermore, tannins, another family of antinutritional compounds, which bind to proteins and minerals, thus reducing the absorption and availability of these nutrients, also affect the digestibility of legumes (Díaz et al., 2010; Raes et al., 2014).

Regarding the nutritional composition assessment of bean flours, the standard evaluation methodologies display several limitations, being time-consuming and requiring technical expertise and the use of harmful reagents (Plans et al., 2013). On the other side, alternative spectroscopy based approaches, mainly using Fourier-transform infrared spectroscopy (FTIR), have been recently developed for an increasing number of matrices, resorting to multivariate analysis approaches, which allow to correlate the spectra with chemical data, thus retrieving analytical calibrations (Hell et al., 2016). In this sense, the use of such techniques has been widely spread in the last few years, due to the potential displayed as a simple, fast and reliable approach for quality assessment, mainly in the agro-food industries

(Ferreira et al., 2014). Furthermore, the FTIR technology can be applied through distinct techniques, taking advantage of two spectral regions, the near-infrared (NIR) and mid-infrared (MIR) intervals.

As the spectral features, in MIR, are due to fundamental vibrational modes, which are related to specific functional groups present in the samples, the spectral data can be used to develop analytical methods for the evaluation of several distinct matrices, such as legumes. For instance, Kadiroğlu et al. (2018) have recently reported the use of MIR to assess chickpea composition, successfully developing predictive models to evaluate the water soluble protein content in this product.

On the other side, in NIR, the absorptions observed correspond to combination modes or overtones from the fundamental vibrational modes (Xiaobo et al., 2010). This technique has been also successfully applied to measure compositional parameters such as moisture, protein, starch and lipids, in bean flours (Hermida et al., 2006), as well as seed weight in intact bean seeds (Hacisalihoglu et al., 2010). Plans et al. (2012) concluded that NIR spectra can provide enough information to evaluate the contents in dietary fiber, uronic acid, ash, calcium and magnesium, in ground seed coats of distinct bean cultivars, besides displaying potential to be applied in the screening of sensory attributes during breeding programmes (Plans et al., 2014).

To the authors knowledge, only one study outlined the comparison between distinct spectroscopy techniques to assess the composition of bean endosperm regarding protein, besides starch and amylose contents (Plans et al., 2013). Therefore, besides the evaluation of the suitability of such methodologies, the aim of the present study lies on the comparison between the predictive performance of NIR and MIR spectroscopies for the assessment of protein, lipids, tannins, phytic acid and specific amino acids' contents in bean flours.

4.1.2. Materials and methods

4.1.2.1. Solvents and Chemicals

Sulphuric acid, sodium hydroxide, acetonitrile (UHPLC grade), calcium disodium ethylene diamine tetraacetic (EDTA), sodium borate and hydrochloric acid were obtained from Panreac (Castellar del Vallés, Barcelona, Spain). Sodium acetate (anhydrous), trimethylamine (TMA), phosphoric acid, ammonium molybdate and methyl cellulose were purchased from Sigma-Aldrich (Steinheim, Germany). Standards of the amino acids (L-arginine (Arg), L-alanine (Ala), L-asparagine (Asn), L-aspartic acid (Asp), glycine (Gly), L-glutamic acid (Glu), L-glutamine (Gln), L-histidine (His), L-isoleucine (Ileu), leucine (Leu), L-Lysine (Lys), L-

norvaline (Nor), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr) and L-valine (Val)), were purchased from Sigma-Aldrich (Steinheim, Germany).

4.1.2.2. Sampling

Samples of bean (*Phaseolus vulgaris* L.) seeds, from 21 distinct cultivars, were obtained from the germplasm collection of Research Unit of Biotechnology and Genetic Resources, *Instituto Nacional de Investigação Agrária e Veterinária* (INIAV)-Oeiras. The beans were cultivated in Cabrela (near Sintra, Portugal), in two distinct years, resulting in a total of 42 samples. The dry bean seeds were ground through the Falling number 3100 mill (Perten, Kungens Kurva, Sweden) using 0.8 mm screen and stored at room temperature, in sealed plastic bags, until analysis.

4.1.2.3. Nutritional composition

The protein content of bean flours was determined by the Kjeldahl method ($N \times 6.25$); moisture, ash and lipids were determined according to the procedures described in AOAC methods (AOAC, 2005). The results are presented as grams per hundred grams of sample dry weight (g/100 g dw).

4.1.2.3.1 Essential and nonessential amino acid composition

All amino acids, except tryptophan and tyrosine, were quantified by high performance liquid chromatography (HPLC), through the method described by Machado et al. (2017a). Tryptophan and tyrosine contents of the samples were analysed by weighing 25 mg of each sample into a 5 mL tube with 5.0 M NaOH, which was posteriorly sealed with a cap. The samples were hydrolysed at 120 °C for 12h. After this period, the samples were cooled to room temperature and the pH was adjusted to 2.0 with 6.0 M HCl, and transferred to a 50 mL volumetric flask, 100 µL of internal standard (Norvaline at 500 µg/mL) were added, and the volume was made up to 50 mL with ultrapure water. After this step, 500 µL of the resulting solution were filtered through a 0.22 µm syringe filter into a HPLC vial. The mobile phase was 50 mM NaH₂PO₄ (phase A) and acetonitrile (phase B). The gradient program started at 5% phase B and increased to 60% phase B during 8.0 min; maintained for 1.0 min; then returning to the initial conditions in 0.5 min, these conditions being maintained for another 3.5 min for column re-equilibration. The injection volume was 5 µL and the column oven was set to 40 °C. Fluorescence detection was performed according to a timetable: Excitation– 274 nm, emission–

304 nm at time 0.0 (for tyrosine with retention time of 2.2 min); changing to excitation– 280 nm, emission– 340 nm at 3.0 min time (at a retention time of 4.15 min for tryptophan); and finally changing to excitation– 202 nm, emission– 296 nm at 5.0 min (for tramadol with retention time of 6.37 min).

For the quantification of amino acids, calibration curves were prepared for all compounds using analytical standards in the range of 0.00625–0.2 mM for each amino acid, except for tyrosine and tryptophan, which standard solutions were prepared in the range of 0.001–0.04 mM. For the calibration curves, the internal standard was added in the same concentration as for the samples. For all calibration curves an R^2 of at least 0.99 was obtained, and the Bias between the measured concentration and true concentration was within $\pm 15\%$ for all calibrators. The LOQ was assumed as the lowest point of the calibration curve, concentration which can be determined with adequate accuracy and precision. The LOD was not evaluated for this method, since the concentration range of the compounds in the samples was always within the calibration range.

4.1.2.4. Antinutritional composition

The total tannin quantities were evaluated using the methyl cellulose precipitable tannin assay, according to (Damberg et al., 2012), with slight modifications. Briefly, 200 μ L of each sample were transferred into a 1.5 mL tube, and 600 μ L of methyl cellulose solution (0.04%) were added and the volume weighed with distilled water; the tubes were then stirred manually and left at room temperature for 2–3 minutes. 400 μ L saturated ammonium sulphate solution was then added, followed by 2 mL of distilled water. After, the mixture was stirred in a vortex and held at room temperature for 10 min. The reaction was terminated by centrifuging the contents at 9167 g in the centrifuge (Sigma-2-16 K, Steinheim, Germany), for 5 min. The absorbance of the supernatant was read at 280 nm. Epicatechin was used as standard, and therefore, the results are expressed as grams of epicatechin equivalents per hundred grams of sample (g ECE/100g).

The phytic acid contents were measured using the colorimetric assay kit K-PHYT (Megazyme, 2015), with the phosphorus content being evaluated through the absorbance at 655nm. The results are presented as grams of phytic acid per hundred grams of sample dry weight (g/100g dw).

4.1.2.5. FTIR analysis

Infrared spectra (IR) of bean flours were collected in two regions (MIR and NIR), using a Thermo Scientific Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, USA), possessing both the Diffuse Reflectance Infrared Fourier Transformed (DRIFT) and attenuated total reflection (ATR) accessories, corresponding to NIR and MIR capabilities, respectively, being controlled by the Omnic software Version 9.2.28 (Thermo Fisher Scientific Inc., Waltham, USA). To register each MIR spectrum, a small amount of flour (~5 mg) was placed directly on the ATR accessory's crystal, the tip was closed, and the spectrum recorded. For NIR analysis, the window of the DRIFT accessory was totally covered with sample (~150 mg), and the spectrum was registered. The spectroscopic parameters for the acquisition of MIR and NIR spectra are presented in Table 12.

Table 8 Spectroscopic parameters for the acquisition of near-infrared (NIR) and mid-infrared (MIR) spectra of the bean flours.

	Spectral range (cm ⁻¹)	Resolution (cm ⁻¹)	Number of scans
MIR	400 - 4000	4	128
NIR	4000 - 10000	8	256

In both NIR and MIR, a background spectrum was registered between sampling and automatically subtracted from the final spectra. For each sample, 6 spectra were collected with each technique. Before the registration of each sample's spectra, the ATR accessory was cleaned, thoroughly scrubbed with ethanol, and dried with a soft paper tissue. The DRIFT accessory was cleaned with a brush and scrubbed with ethanol between the acquisitions of distinct samples' spectra.

4.1.2.6. Multivariate analysis

In MIR, baseline subtraction was performed, using 4th order polynomials and 50 iterations for convergence, resorting to the Omnic software package Version 9.2.28. NIR and MIR spectra were processed using the OriginPro 9.1 software (OriginLab, Northampton, USA), which has been also used for the multivariate analyses. The spectral treatments applied in both methodologies (MIR and NIR) comprised mean normalization (spectra), 1st derivative of spectra (1st deriv) and 1st derivative after mean normalization (1st deriv MN). Partial least squares (PLS) regression was used to correlate the spectra with analytical contents (Wold et al., 2001). The optimal models were evaluated through cross-validation (CV), using the leave-one-out (LOO) procedure, which indicates the optimal number of factors (NF). Whenever NF was

8, or higher, selected intervals (SI) have been defined for each parameter, resorting to the variable importance projection (VIP) plots and regression coefficients, in order to avoid the possible occurrence of over-fitting.

The accuracy of the models calibrated was evaluated through the values obtained regarding prediction error sums of squares (PRESS), and the determination coefficients observed between analytical and predicted values (R^2), which were calculated for both the calibration (R^2_c) and validation (R^2_v) sets (Xiaobo et al., 2010). For validation, two distinct sets of samples were constructed, a calibration (training) set with two thirds of the spectra (168 spectra), and an external validation set with one third of the spectra (84 spectra), which were randomly excluded from the complete set of spectra registered (252 spectra) (Wang et al., 2014; Plans et al., 2013).

4.1.2.7. Statistical analyses

The contents in specific amino acids, as well as nutritional and antinutritional compositions, were assessed in triplicate. The interpretation of the HPLC results obtained for all amino acids was performed using the Chromeleon (version 7.2) software (Thermo Fisher Scientific, Inc., Waltham, USA). The statistical analyses applied to the analytical results were performed using SPSS Statistics 21.0 software (SPSS inc., Chicago, IL, USA).

4.1.3. Results and discussion

4.1.3.1. Nutritional and antinutritional composition of the bean flours

The results obtained for the nutritional and antinutritional parameters assessed, are presented in Figure 8. The protein content observed ranges from 19.5 to 31.3 g/100 g dw, which is comparable to the values reported in previous studies (22.0–32.1 g/100 g dw) (Kan et al., 2017; Grela et al., 2017). Lipids content ranged 0.47–1.82 g/100 g dw, with similar amounts being previously found by Du et al. (2014), while higher values (2.91–5.30 g/100 g dw) were observed by Kan et al. (2018). The variation of the nutritional composition of grain and/or flours among legumes can be assigned to differences concerning variety, distinct genotypes within a cultivar, and edaphoclimatic conditions of each harvest (Kan et al., 2017).

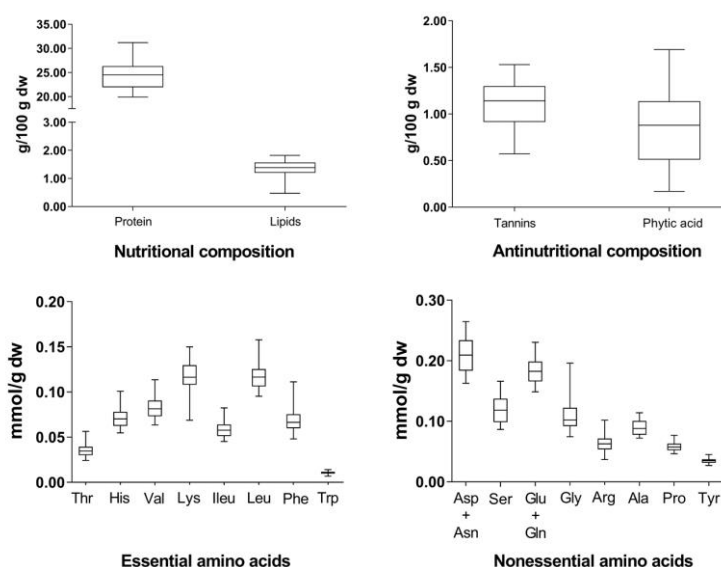


Figure 8 Descriptive statistics of nutritional composition (protein and lipids (g/100g dw), antinutritional composition (tannins (g ECE/100g dw) and phytic acid (g/100g dw)), essential and nonessential amino acids (mmol/g dw) in bean flours.

Concerning the antinutritional parameters, the total tannin contents ranged from 0.2 g to 1.8 g ECE/100g dw, being much higher than those observed by Valdez-González et al. (2017) (0.09 g ECE/100g dw). Phytic acid content ranged from 0.5 to 1.6 g/100g dw in the present work, with lower concentrations (0.21–0.51 g/100g) being reported by Shang et al. (2016), while a similar average (1.62 g/100g dw) was described by Valdez-González et al. (2017). These differences are probably due to specific edaphoclimatic conditions of the trials, and cultivars evaluated, since large variability has been found among distinct cultivars in the present study (Figure 8).

The main essential amino acids in the bean flours were Lys (0.059–0.156 mmol/g dw) and Leu (0.094–0.159 mmol/g dw), while the essential amino acid presenting the lowest concentration was Trp (0.006–0.014 mmol/g dw), with similar results being reported by Kan et al. (2017). The nonessential amino acids presenting the highest concentrations were Asp+Asn (0.150–0.290 mmol/g dw) and Glu+Gln (0.140–0.238 mmol/g dw), whereas Arg (0.036–0.106 mmol/g dw), Pro (0.046–0.080 mmol/g dw) and Tyr (0.026–0.046 mmol/g dw) displayed the lowest concentration values. These observations are consistent with those reported by Pastor-Cavada et al. (2014).

4.1.3.2. MIR and NIR spectral assignments

Representative MIR and NIR normalized spectra from a sample, and the 1st derivative values of the same spectral data, are displayed in Figure 9. In the MIR region, the interval 400–cm⁻¹ was considered, and the spectrum was divided in two distinct regions: low-frequency region (LF, 450–1800 cm⁻¹), and high-frequency region (HF, 2600–3700 cm⁻¹), while the simultaneous use of both regions has been denoted as full interval (FI, 450–1800; 2600–3700 cm⁻¹). The region between 1800–2600 cm⁻¹ was removed from the analyses, considering its signal to noise ratio, and the absence of IR absorption peaks of interest in this interval (Plans et al., 2013).

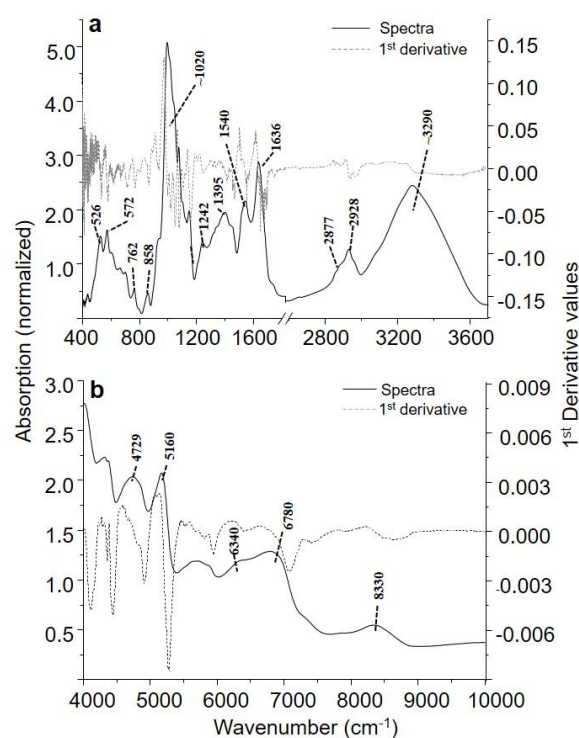


Figure 9 a- MIR-ATR normalized spectra and 1st derivative of bean flour sample obtained in the range between 400 and 3600 cm⁻¹. b- NIR spectra and 1st derivative values of bean flour sample, obtained in the range between 4000 and 10000 cm⁻¹.

In the low-frequency region, the weak intensity peaks found between 450–1000 cm⁻¹, are associated with CH₂ and CH₃ rocking vibrational modes. The most intense peak, around 1020 cm⁻¹, is related to C-O stretching modes, which are considered as fingerprint in the spectral range between 900 and 1200 cm⁻¹, because of the diversity of biological interesting molecules possessing the C-O bond feature (Ferreira et al., 2014). In the interval between 1150–1500 cm⁻¹

¹, C-O and C-C stretching modes, combined with (CH) and (OH) in-plane deformations, were found (Ferreira et al., 2014). In the interval above 1500 cm⁻¹, there are contributions from in-plane aromatic ring deformations, which do not present high intensities in IR. Besides, a strong peak is found around 1540 cm⁻¹, being related to the amide II mode, mainly composed of C-N stretching (Hell et al., 2016), while a second, at 1636 cm⁻¹, can be assigned to N-H bending and amide I modes, composed of C=O and C-N vibrational stretching (Barth, 2000).

In the HF region, the interval between 2600–2800 cm⁻¹ displays low absorption values, with very weak vibrational peaks being found, related to overtones, which are transitions to higher vibrational states, respecting the fundamental transition (Machado et al., 2017b). In the interval above 2800 cm⁻¹, two main peaks are found around 2900 cm⁻¹, related to the C-H stretching modes, besides a broad peak between 3000–3600 cm⁻¹ corresponding to N-H and O-H stretching modes, the former being mainly due to peptide bonds and amine groups found in amino acids, while the latter (3000–3600 cm⁻¹) is raised by distinct compounds, since hydroxylic groups are present in a diversity of biological molecules (Ferreira et al., 2014).

The typical NIR spectrum collected is represented in Figure 9b, while the interval registered within this region was 4000–10000 cm⁻¹, since the higher order overtones (> 3rd) correspond to very low absorption values (Xiaobo et al., 2010). Below 5500 cm⁻¹, the vibrational absorptions correspond to combination modes between fundamentals, which are related to X-H (where X=C, N, O) stretching modes combined with other vibrational motions. In the higher wavenumbers, above 5500 cm⁻¹, the spectrum is dominated by overtones, which are multiples of the fundamental vibrations observed in MIR (Ferreira et al., 2014). The peak found within the spectral interval between 4500–5000 cm⁻¹ can be assigned to combinations between N-H and C=O stretching, associated with protein contents (Plans et al., 2013), while the feature between 5000–5200 cm⁻¹ is related to combinations involving O-H functional groups, which are present in a diversity of biologically relevant molecules such as tannins, proteins and phytic acid. Besides, the absorption between 6100–6400 cm⁻¹ can be also related to phytic acid contents (Pande & Mishra, 2015).

4.1.3.3. Development of analytical models through PLS regressions

The results obtained with the PLS regressions developed for the MIR region, regarding the evaluation of nutritional and antinutritional compositions, and amino acids, are summarized in Table 13, while the results of the regressions developed for ash and moisture are only presented as supplementary material (Annexe I).

Table 9 PLS regressions for the prediction of the distinct constituents assessed, corresponding to the most effective MIR interval for each treatment, and to the optimal number of factors for each regression.

Analytical parameters	Treatment	PRESS (NF)	R ² c	R ² v	Analytical parameters	Treatment	PRESS (NF)	R ² c	R ² v
Nutritional Composition									
Protein	1 st deriv (FI)*	0.283 (4)	0.99	0.96	Lipids	1 st deriv (HF)*	0.840 (7)	0.98	0.93
	1 st deriv MN (LF)	0.316 (4)	0.98	0.96		1 st deriv MN (FI)	0.986 (6)	0.96	0.92
	Spectra (LF)	0.337 (5)	0.97	0.95		Spectra (SI) ^a	0.922 (7)	0.80	0.72
Antinutritional Composition									
Phytic Acid	1 st deriv (FI)*	0.650 (7)	0.99	0.88	Tannins	1 st deriv (HF)	0.675 (1)	0.77	0.74
	1 st deriv MN (LF)	0.684 (5)	0.95	0.82		1 st deriv MN (HF)	0.707 (1)	0.77	0.75
	Spectra (FI)	0.647 (6)	0.87	0.80		Spectra (FI)*	0.660 (1)	0.79	0.78
Essential amino acids					Nonessential amino acids				
Thr	1 st deriv (FI)	0.772 (2)	0.75	0.66	Asp+Asn	1 st deriv (LF)	0.611 (6)	0.88	0.88
	1 st deriv MN (LF)*	0.714 (7)	0.98	0.90		1 st deriv MN (HF)*	0.649 (4)	0.93	0.86
	Spectra (LF)	0.777 (5)	0.85	0.80		Spectra (LF)	0.649 (2)	0.81	0.77
His	1 st deriv (FI)*	0.935 (2)	0.61	0.51	Ser	1 st deriv (LF)	0.768 (2)	0.75	0.67
	1 st deriv MN (LF)	0.900 (1)	0.54	0.52		1 st deriv MN (HF)*	0.791 (4)	0.91	0.78
	Spectra (LF)	0.925 (2)	0.55	0.51		Spectra (HF)	0.778 (4)	0.74	0.71
Val	1 st deriv (HF)	0.523 (3)	0.93	0.88	Glu+Gln	1 st deriv (FI)	0.760 (2)	0.78	0.72
	1 st deriv MN (HF)*	0.530 (4)	0.95	0.88		1 st deriv MN (LF)	0.712 (1)	0.75	0.73
	Spectra (HF)	0.563 (6)	0.92	0.90		Spectra (LF)*	0.691 (2)	0.79	0.76
Lys	1 st deriv (LF)	0.975 (1)	0.40	0.36	Gly	1 st deriv (LF)*	0.762 (5)	0.95	0.89
	1 st deriv MN (HF)	0.969 (1)	0.45	0.30		1 st deriv MN (LF)	0.765 (5)	0.96	0.88
	Spectra (FI)*	0.978 (1)	0.41	0.37		Spectra (HF)	0.788 (2)	0.71	0.68
Ileu	1 st deriv (FI)	0.557 (2)	0.88	0.83	Arg	1 st deriv (FI)*	0.916 (2)	0.64	0.58
	1 st deriv MN (HF)	0.497 (3)	0.91	0.85		1 st deriv MN (HF)	0.923 (1)	0.58	0.53
	Spectra (HF)*	0.550 (6)	0.92	0.88		Spectra (LF)	0.933 (4)	0.63	0.56
Leu	1 st deriv (LF)*	0.319 (5)	0.99	0.96	Ala	1 st deriv (FI)	0.509 (3)	0.94	0.91
	1 st deriv MN (FI)	0.325 (4)	0.98	0.94		1 st deriv MN (FI)*	0.491 (4)	0.96	0.93
	Spectra (FI)	0.305 (7)	0.98	0.94		Spectra (FI)	0.517 (5)	0.92	0.88
Phe	1 st deriv (LF)*	0.881 (6)	0.97	0.87	Pro	1 st deriv (LF)*	0.458 (6)	0.99	0.95
	1 st deriv MN (LF)	0.856 (1)	0.60	0.59		1 st deriv MN (LF)	0.457 (5)	0.98	0.94
	Spectra (FI)	0.885 (2)	0.64	0.60		Spectra (LF)	0.501 (5)	0.94	0.91
Trp	1 st deriv (FI)*	0.790 (4)	0.90	0.84	Tyr	1 st deriv (SI)	0.793 (3)	0.87	0.80
	1 st deriv MN (FI)	0.807 (3)	0.85	0.79		1 st deriv MN (SI) ^{a*}	0.865 (4)	0.93	0.84
	Spectra (LF)	0.816 (7)	0.86	0.78		Spectra (LF)	0.742 (4)	0.83	0.79

SI- Selected intervals; FI- Full region; LF- Low-frequency region; HF- High-frequency region; NF- Number of factors; ^aSelected interval: Tyr- 2600–2882 cm⁻¹; Lipids- 1000–1799 cm⁻¹; *Treatment for which the standardized regression coefficients are presented

The best performance was observed for the prediction of protein, with the corresponding regressions conducting to the lowest PRESS values (< 0.340) and highest calibration and validation determination coefficients, for all treatments ($R^2c \geq 0.97$; $R^2v \geq 0.95$, respectively), using the FI and HF intervals (Table 13). These results are in good agreement with the $R^2c=0.97$ obtained by Plans et al. (2013). For lipids, the best predictive model was obtained with HF, using the 1st deriv, with the determination coefficients observed ($R^2c=0.98$; $R^2v=0.93$) presenting higher values than those observed by Ferreira et al, which reported $R^2c=0.91$ and $R^2c=0.67$ for protein and lipids, respectively, while the calibrations of that work were developed for soybean samples (Ferreira et al., 2014).

Regarding antinutritional composition, for phytic acid, the 1st deriv led to the best results ($R^2c = 0.99$; $R^2v = 0.88$), observed for the use of FI, while for tannins the best regression was obtained using the spectra, and also FI, showing $R^2c = 0.79$ and $R^2v = 0.78$. For the determination of tannins, it was always extracted one single factor, in all treatments, which justifies the lower determination coefficients observed respecting phytic acid, protein and lipids (Table 13).

Concerning amino acid contents, good quality prediction models have been calibrated for Val, Ileu and Trp, showing high determination coefficients for calibration (R^2c 0.85–0.95) and validation (R^2v 0.78–0.90), using all treatments. For Phe, high determination coefficients ($R^2c = 0.97$; $R^2v = 0.87$) were only observed for the use of the 1st deriv, which was also the treatment conducting to the best results for the prediction of Leu, Phe and Trp, using the LF interval (Phe and Leu), and FI (Trp) (Table 13). On the other hand, for Thr and Val, the best treatment was 1st deriv MN, whereas for Ileu, the use of the spectra led to the best results. Independently from the treatment, the calibrations developed for His (R^2c 0.54–0.61; R^2v 0.51–0.52) and Lys (R^2c 0.40–0.45; R^2v 0.30–0.37) did not produce suitable evaluation models. Among all constituents, the lowest determination coefficients were observed for Lys, despite its high concentration respecting other amino acids (0.059–0.156 mmol g⁻¹ dw), while Lys displays one of the highest variabilities among essential amino acids, which could have contributed for such result (Figure 9 and Table 13).

Concerning nonessential amino acids, high determination coefficients were observed for Asp+Asn, Ala, Pro and Tyr, for calibration and validation, using all treatments ($R^2v \geq 0.77$), while for Gly, the use of the spectra failed to provide a good calibration ($R^2v = 0.68$) (Table 13). The highest determination coefficients were observed for Pro ($R^2c = 0.99$; $R^2v = 0.95$), with 1st deriv displaying the lowest PRESS (0.458), while for Ala, the best treatment was the 1st deriv MN, corresponding to high determination coefficients ($R^2c = 0.96$; $R^2v = 0.93$). For the quantification of Tyr, the best model was developed using a selected interval (2600–2882 cm⁻¹), and 1st deriv MN ($R^2c = 0.93$; $R^2v = 0.84$). The lowest determination coefficients were observed for Arg ($R^2c < 0.65$; $R^2v < 0.60$), which also displayed the highest PRESS (> 0.915). In most cases, the best results were obtained with FI (450–1799; 2600–3699 cm⁻¹), which contains information regarding N-H, O-H and C=O groups, thus allowing the development of models for the quantification of amino acids, through the spectral data (Machado et al., 2017a). Nevertheless, the best models for Val, Ileu, Asp+Asn and Ser, ($R^2c > 0.90$; $R^2v > 0.77$), have been developed with HF (2600–3700 cm⁻¹), which includes the interval between 3000–3650 cm⁻¹, corresponding to amines and hydroxyl groups, present in amino acids and proteins (Table

13). On the opposite side, the best prediction model for Leu ($R^2c=0.99$ and 0.96), was obtained with LF ($450\text{--}1799\text{ cm}^{-1}$).

Generally, the best treatment was the 1st deriv, corresponding to the highest determination coefficients for calibration and validation, exception made to Thr, Ala and Tyr, for which the best results were observed for the 1st deriv MN, and some other cases, where the use of spectra led to the highest determination coefficients. For instance, for the regression models developed with spectra for Glu+Gln, both determination coefficients were higher ($R^2c=0.79$; $R^2v=0.76$), as well as for Ileu ($R^2c=0.92$ and 0.88), while in the cases of Val and Lys, only the determination coefficients retrieved from validation were higher than those observed for the use of the derivative. In the case of Lys, none of the calibrations developed corresponds to a suitable analytical model ($R^2v=0.37$) (Table 13).

The standardized regression coefficients, corresponding to the weight of each independent variable, in MIR, are displayed in Figure 10. The plots corresponding to the best regressions developed for tannins, Lys, Ileu, and Glu+Gln, undertaken with the spectra, display a distinct aspect, respecting cases where the plots correspond to the use of the derivative, presenting shapes that resemble spectral features. In these cases, three distinct spectral intervals conducted to the best regressions, FI in two cases (tannins and Lys), HF in the case of Ileu, and LF for Glu+Gln. Nevertheless, for all the other amino acids, protein, lipids and phytic acid, the best regressions have been obtained with either the 1st deriv or 1st deriv MN, with the plots extracted presenting a ‘noisy’ aspect, which is typical from regressions developed with derivative data (Kepper et al., 2018; Machado et al., 2017a).

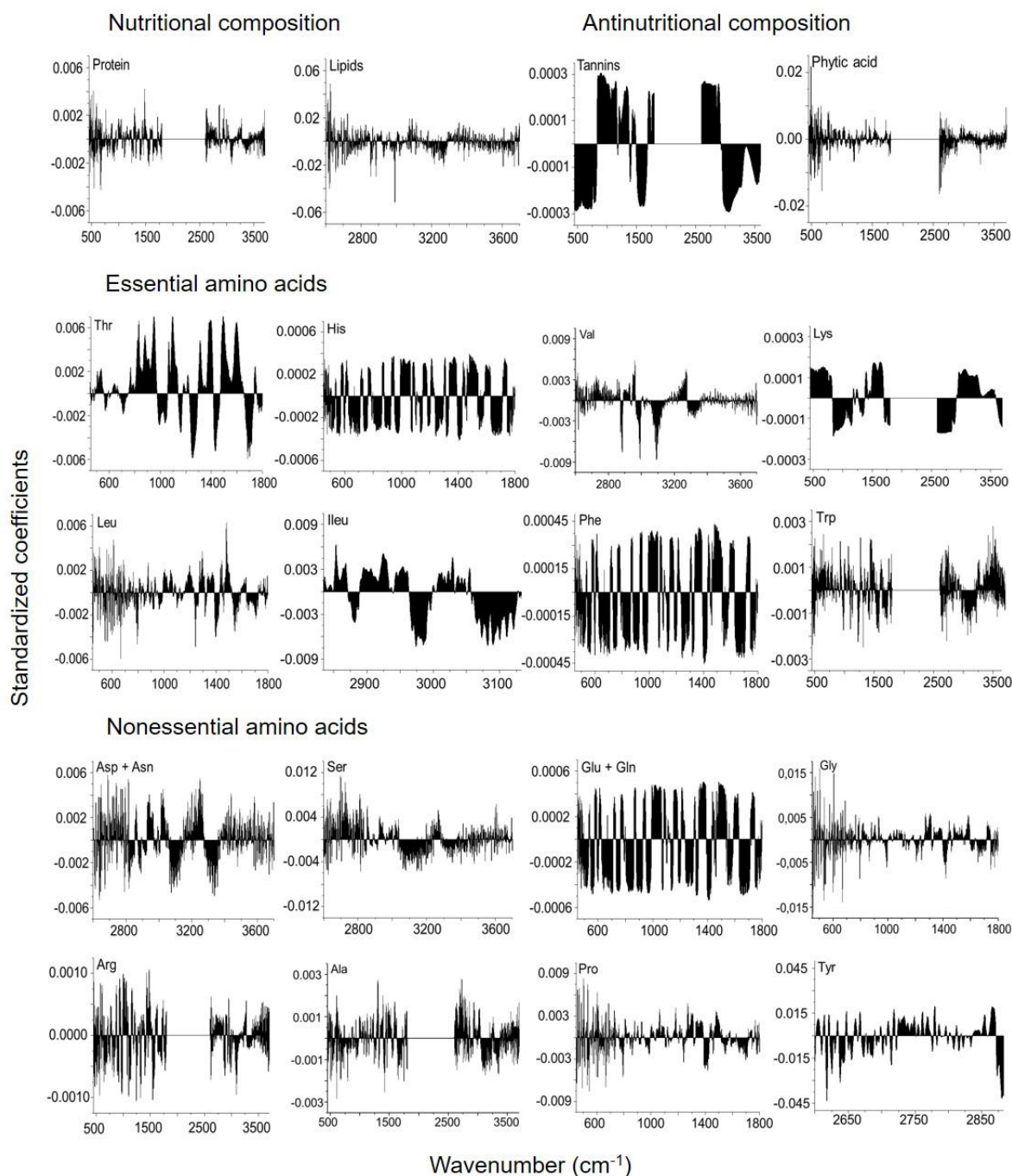


Figure 10 Standardized regression coefficients retrieved from the PLS procedure, corresponding to the best calibration developed with MIR data for each parameter.

The use of the derivative functions as a ‘zero-centering’ technique – the actual peaks assuming this value, thus, small spectral differences are exacerbated, which leads to high coefficients in the intervals below 800 cm^{-1} and between 2600–2800 cm^{-1} . In the second interval, only weak overtones are found, as well as Fermi resonances, while this spectral

window has been considered relevant for most of the regressions developed with FI, when the derivative is used (Figures 9a and 10).

In the cases of Phe and Thr, relevant intervals are scattered throughout the spectral window used (LF). Regarding Thr, where 1st deriv MN conducted to the best result, the coefficients are higher above 1200 cm⁻¹, which corresponds to a distinct trend respecting all the other cases where the use of either 1st deriv or 1st deriv MN led to the best regressions. This is probably related to the actual importance of certain intervals between 1000–1800 cm⁻¹, where peaks corresponding to functional groups related to amino acid contents can be found, while several distinct vibrational modes occur simultaneously in this region (Figures 9a and 10).

The results of the PLS regressions developed for NIR are summarized in Table 14. The model for the prediction of protein showed the highest determination coefficients for calibration ($R^2_c = 0.99$) and validation ($R^2_v = 0.98$) and the lowest PRESS value (0.284), which were obtained with the 1st deriv treatment, and correspond to a better model respecting MIR. Furthermore, these determination coefficients were higher than the coefficients published by Plans et al. (2013) ($R^2_c = 0.96$; 3 factors) and Hacısalıhoğlu et al. (2010) ($R^2 = 0.80$; 8 factors), also observed for the 1st deriv. Besides protein, this treatment also led to the best predictive model for lipids ($R^2_c = 0.99$ and $R^2_v = 0.97$) in the present work.

Table 10 PLS regressions for the prediction of the distinct constituents assessed, corresponding to the distinct treatments applied to the NIR data, and to the optimal number of factors for each regression.

Analytical parameters	Treatment	PRESS (NF)	R ² c	R ² v	Analytical parameters	Treatment	PRESS (NF)	R ² c	R ² v
Nutritional Composition									
Protein	1 st deriv*	0.284 (4)	0.99	0.98	Lipids	1 st deriv*	0.897 (7)	0.99	0.97
	1 st deriv MN	0.273 (3)	0.99	0.97		1 st deriv MN ^a	0.880 (4)	0.94	0.92
	Spectra	0.254 (6)	0.98	0.94		Spectra	0.940 (7)	0.71	0.68
Antinutritional Composition									
Phytic Acid	1 st deriv	0.748 (6)	0.99	0.89	Tannins	1 st deriv* ^a	0.700 (7)	0.99	0.91
	1 st deriv MN*	0.701 (6)	0.99	0.90		1 st deriv MN	0.710 (2)	0.81	0.78
	Spectra	0.787 (2)	0.71	0.70		Spectra	0.707 (2)	0.77	0.75
Essential amino acids					Nonessential amino acids				
Thr	1 st deriv	0.773 (1)	0.71	0.70	Asp+Asn	1 st deriv*	0.644 (2)	0.87	0.86
	1 st deriv MN*	0.748 (2)	0.77	0.75		1 st deriv MN	0.625 (2)	0.85	0.84
	Spectra	0.733 (4)	0.77	0.74		Spectra	0.626 (3)	0.83	0.82
His	1 st deriv	0.909 (1)	0.56	0.54	Ser	1 st deriv	0.773 (1)	0.72	0.71
	1 st deriv MN*	0.900 (2)	0.65	0.62		1 st deriv MN	0.730 (2)	0.79	0.78
	Spectra	0.913 (2)	0.59	0.56		Spectra*	0.727 (6)	0.81	0.78
Val	1 st deriv	0.609 (1)	0.84	0.84	Glu+Gln	1 st deriv*	0.716 (2)	0.85	0.83
	1 st deriv MN	0.595 (2)	0.87	0.86		1 st deriv MN	0.687 (2)	0.83	0.82
	Spectra*	0.530 (4)	0.89	0.88		Spectra	0.741 (1)	0.86	0.74
Lys	1 st deriv	0.974 (1)	0.45	0.43	Gly	1 st deriv	0.823 (1)	0.66	0.65
	1 st deriv MN	0.968 (1)	0.43	0.41		1 st deriv MN*	0.814 (2)	0.74	0.70
	Spectra*	0.962 (1)	0.46	0.44		Spectra	0.807 (4)	0.70	0.68
Ileu	1 st deriv*	0.629 (3)	0.91	0.89	Arg	1 st deriv	0.932 (1)	0.54	0.52
	1 st deriv MN	0.622 (2)	0.86	0.85		1 st deriv MN*	0.929 (2)	0.65	0.61
	Spectra	0.529 (4)	0.90	0.88		Spectra	0.882 (2)	0.64	0.60
Leu	1 st deriv	0.411 (5)	0.98	0.97	Ala	1 st deriv	0.503 (2)	0.92	0.91
	1 st deriv MN*	0.410 (4)	0.98	0.97		1 st deriv MN*	0.486 (2)	0.92	0.91
	Spectra	0.358 (6)	0.96	0.96		Spectra	0.494 (3)	0.90	0.89
Phe	1 st deriv	0.870 (1)	0.61	0.59	Pro	1 st deriv*	0.561 (2)	0.91	0.89
	1 st deriv MN*	0.876 (2)	0.68	0.65		1 st deriv MN	0.526 (2)	0.90	0.89
	Spectra	0.874 (2)	0.64	0.62		Spectra	0.508 (4)	0.91	0.89
Trp	1 st deriv*	0.859 (3)	0.82	0.77	Tyr	1 st deriv*	0.752 (5)	0.96	0.91
	1 st deriv MN	0.839 (2)	0.72	0.68		1 st deriv MN	0.722 (4)	0.94	0.90
	Spectra	0.771 (4)	0.75	0.45		Spectra	0.675 (5)	0.84	0.78

FI- Full region; LF- Low-frequency region; HF- High-frequency region; NF- Number of factors; ^aSelected interval: Lipids- 5002 - 8998 cm⁻¹; *Treatment for which the standardized regression coefficients are presented

Concerning antinutritional composition, the PLS regressions for the prediction of tannins led to satisfactory results with all treatments. The best performance ($R^2_c=0.99$; $R^2_v=0.91$) corresponded to 1st deriv, and a selected interval (3999–9152 cm⁻¹). Regarding phytic acid, higher determination coefficients have been found for the use of 1st deriv ($R^2_c=0.99$; $R^2_v=0.89$) and 1st deriv MN ($R^2_c=0.99$; $R^2_v=0.90$), respecting the use of the spectra ($R^2_v=0.70$) (Table 14). These prediction models for tannins and phytic acid, developed with NIR, retrieved better results than those presently developed for MIR, being comparable to the results ($R^2_v=0.96$) presented by Pande & Mishra, (2015), which was the single publication found in the

literature, regarding the quantification of phytic acid by NIR in pulses (green gram seeds) through PLS-R.

For the essential amino acids, the regressions undertaken for Val, Ileu and Leu, exhibited satisfactory results using all treatments, while the best model, developed for Leu ($R^2c= 0.98$; $R^2v= 0.97$), corresponds to the use of 1st deriv MN. For Val, the use of spectra corresponded to the highest determination coefficients, $R^2c= 0.89$ and $R^2v= 0.88$, and the lowest PRESS value (0.530). In the case of Ileu, 1st deriv was the treatment leading to the best results ($R^2c= 0.91$; $R^2v= 0.89$). The determination coefficients for His, Lys and Phe corresponded to unsuitable models, independently of the treatment, while the regressions developed for Thr and Trp, with the 1st deriv MN ($R^2v= 0.75$) and 1st deriv ($R^2v= 0.77$), respectively, conducted to acceptable results (Table 14).

Concerning nonessential amino acids, the models developed for Asp+Asn, Ala, Pro and Tyr, corresponded to the highest determination coefficients. For Asp+Asn, Pro and Tyr, and the best results were obtained with the 1st deriv, corresponding to ($R^2c= 0.87$; $R^2v= 0.86$), ($R^2c= 0.91$; $R^2v= 0.89$) and ($R^2c= 0.96$; $R^2v= 0.91$), respectively. In the case of Ala, 1st deriv MN represented the best treatment ($R^2c= 0.92$; $R^2v= 0.91$; PRESS = 0.486), the same being observed for Gly ($R^2c= 0.74$; $R^2v= 0.70$) and Arg ($R^2c= 0.65$; $R^2v= 0.61$). In the regression developed for Ser, the best results were observed for spectra ($R^2c= 0.81$; $R^2v= 0.78$; PRESS= 0.727), 6 factors being extracted, while for Glu+Gln, the 1st deriv led to the best result ($R^2c= 0.85$; $R^2v= 0.83$; PRESS= 0.716), using 2 factors, which reflects the tendency of the regressions developed for derivative data to extract smaller numbers of factors (Table 14).

Comparing both regions, MIR allowed the development of suitable calibrations for the assessment of Gly, while NIR failed to provide good calibrations ($R^2v\leq 0.74$). For Arg, none of the techniques allowed the development of analytical models ($R^2v\leq 0.61$) (Tables 13 and 14). Furthermore, in NIR, a greater number of unacceptable regression models (His, Lys, Phe, Gly and Arg) have been observed, respecting MIR (His, Lys and Arg). Generally, the regressions undertaken for compounds with higher ranges of concentration, such as Lys (0.06–0.16 mmol/g dw), displayed worse performance, while this effect seems more important in NIR, where the regressions failed for two additional amino acids presenting high variability, Phe (0.05–0.120 mmol/g dw) and Gly (0.07–0.205 mmol/g dw) (Figure 9, Tables 13 and 14). Fontaine et al. (2001) reported that NIR could be applied to quantify the essential amino acids Lys, Thr, Val, Ileu, Leu, Trp and the nonessential amino acid Arg, in soybeans and peas, whereas Lys and Arg are among the few amino acids for which no quality models have been developed in the present

work, though, the matrices were different, and the models proposed in that work were not fully validated.

The standardized regression coefficients, in NIR, are presented in Figure 11. Most of the plots correspond to regressions using 1st deriv (protein, lipids, tannins, Ileu, Trp, Asp+Asn, Glu+Gln, Pro and Tyr) or 1st deriv MN (phytic acid, Thr, His, Leu, Phe, Gly, Arg and Ala). Lys, Val and Ser, constitute the exception, with their best results being obtained with spectra, while their plots are similar, though presenting slight differences. For instance, the interval between 6000–7000 cm⁻¹, related to protein content (Wang et al., 2014), is relevant for both Val and Lys, while the interval between 8000 and 9000 cm⁻¹ is important for the determination of Val and Ser, though being negligible for the determination of Lys (Figure 11).

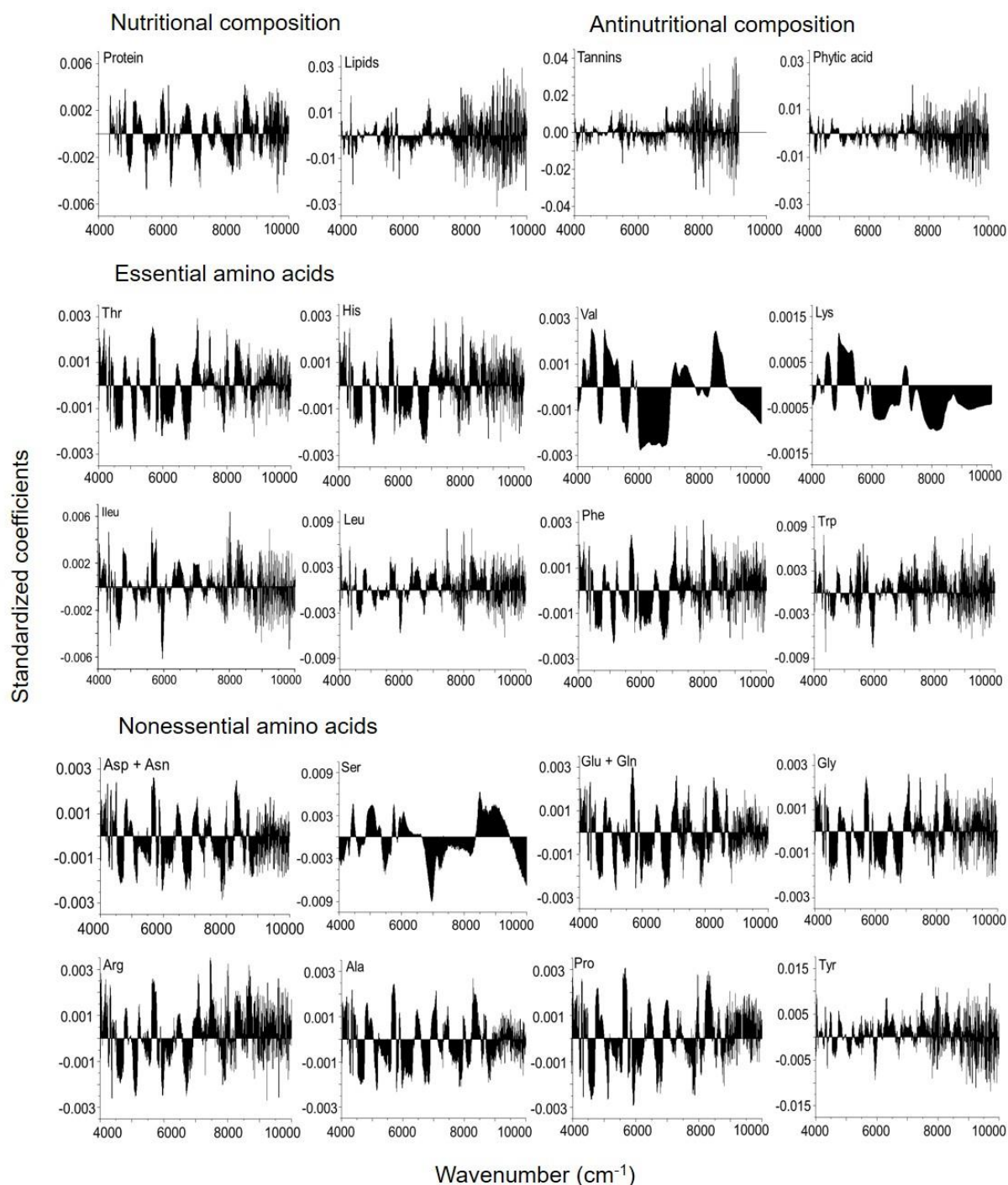


Figure 11 Standardized regression coefficients retrieved from the PLS procedure, corresponding to the best calibration developed with NIR data for each parameter.

For the plots corresponding to 1st deriv or 1st deriv MN, two distinct profiles can be discerned. The plots corresponding to the use of 1st deriv display two main intervals (7500–8000 cm^{-1} and $> 8800 \text{ cm}^{-1}$), while the most important intervals, in the regressions corresponding to 1st deriv MN, are evenly scattered throughout the spectral window used, with

the plots displaying intervals with negative or positive contribution, but similar intensities. The preponderance of the interval above 8800 cm^{-1} represents the only feature common to both 1st deriv and 1st deriv MN (Figure 12). Summarizing, these observations show that the most determinant intervals for these calibrations, as observed for MIR, vary with the data pre-processing applied, with distinct spectral features being exploited for the development of prediction models, if different treatments are applied (Figures 8–11).

4.1.4. Conclusions

The FTIR techniques MIR and NIR are becoming useful tools for the assessment of relevant parameters, not only regarding the macronutrients present in food, but also for minor compounds present in low concentrations. Both the MIR and NIR regions have been proven to represent reliable methods for the prediction of protein, lipids, tannins, phytic acid and most of the amino acids present in bean flours. According to the present results, the best treatment for the development of analytical models was the 1st deriv. Concerning MIR, the use of the full interval assessed ($450\text{--}1800$; $2600\text{--}3700\text{ cm}^{-1}$) represented the most reliable approach for the development of analytical models using these techniques.

Although PLS regression has failed to provide a suitable calibration in the cases of some amino acids, mainly in NIR, the determination coefficients regarding the assessment of nutritional and antinutritional composition were significantly higher, respecting MIR, especially for protein and tannins content. High correlations between predicted and measured values were obtained, even for amino acids present in low concentrations, such as Trp (0.01 mmol/g) and Tyr (0.04 mmol/g). The data obtained in the present study highlights the relevance and importance of studying the performance of both regions (MIR and NIR), as well as distinct treatments and specific intervals, to develop methods for the assessment of distinct constituents present in this food matrix.

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Chapter 4

4.2. Prediction of phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds of common beans using MIR and NIR spectroscopy

4.2. Prediction of phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds of common beans using MIR and NIR spectroscopy

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Abstract

The aim of the present study was the evaluation of the performance of analytical models developed with both mid-infrared (MIR) and near-infrared (NIR) spectral data, to assess the phytochemical composition and *in vitro* antioxidant activity, besides individual phenolic compounds determined by HPLC-DAD, of flours from 21 distinct cultivars of *Phaseolus vulgaris* L. Partial least squares (PLS) regression was used to develop the analytical models, which were validated with an external set of samples. In MIR, the best prediction models were developed using the first derivative after normalization (R^2c 0.86–0.99 and R^2v 0.75–0.94), while for NIR, the use of first derivative of the spectra led to the best results (R^2c 0.94–0.99 and R^2v 0.85–0.97). Both techniques allowed to ascertain the prediction models to ensure an accurate evaluation of the individual phenolic compounds in concentrations as low as $\sim 5 \mu\text{g/g}$, and *in vitro* antioxidant capacity till the lower limit of $2.1 \mu\text{mol/g dw}$.

Therefore, this study revealed that the spectroscopical methodologies may represent an accurate and rapid method for quantification of phytochemical composition, *in vitro* antioxidant activity, and individual phenolic compounds, of bean flours, thus, their applicability in the laboratories and food industry representing an alternative to the traditional approaches.

Keywords *Phaseolus vulgaris* L., phytochemicals, HPLC-DAD, near-infrared, mid-infrared

4.2.1. Introduction

Phaseolus vulgaris L., a species corresponding to several distinct cultivars, known as common beans, has a significant cultural and economic relevance for the human nutrition as sustainable source of protein, with applications in food industry, as functional food (Ramírez-

Jiménez et al. 2018), and as a gluten-free product (Han et al. 2010). The relevant nutritional properties and potential health benefits of *Phaseolus vulgaris* L. are attributed to its composition, representing an excellent source of protein, dietary fibre and carbohydrates, besides phytochemicals, namely, polyphenolic compounds, mainly represented by flavonoids (catechin, myricetin and kaempferol) and phenolic acids (gallic acid and coumaric acid) (Aguilera et al., 2011; Wang et al., 2016). According to previous works, the phytochemical composition of beans has been related to antioxidant, anti-inflammatory, anti-hypertensive and antiaging activities (García-Lafuente et al. 2014). Epidemiological studies have shown the positive effects of beans consumption in reduction of obesity, cardiovascular diseases and breast cancer (Dueñas et al., 2015; Mendoza-Sánchez et al., 2019).

In beans, as in other foodstuffs, antioxidant activity and phenolic profile and concentrations, are among the most essential aspects to evaluate the functional properties. In this sense, the main standard techniques to evaluate the phytochemical composition, *in vitro* antioxidant activity, and individual phenolic compounds, are colorimetric assays and High Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD) (Ramírez-Jiménez et al., 2014; Giusti et al., 2017), which are expensive, time consuming and require the use of extraction solvents and probes, generating laboratorial residues.

Fourier-transform infrared spectroscopy (FTIR), has been lately revealed as a good alternative to traditional techniques, for distinct applications in agriculture and food industry, due to its quick, reliable, and eco-friendly methodology, besides working with minimal technical supervision (Oliveira et al. 2014). Regarding the application of FTIR, this technology allows taking advantage of distinct Infrared intervals, namely, near-infrared (NIR) and medium-infrared (MIR), coupled with different instrumentation, such as the Attenuated Total Reflection (ATR) accessory, for MIR, and Diffuse Reflectance Infrared Fourier Transformed (DRIFT), for NIR, which leads these intervals to be designated as distinct spectroscopical techniques.

In MIR spectroscopy, fundamental vibrational frequencies, related to the functional groups present in the sample, are observed, occurring in the spectral range between 400–4000 cm^{-1} . Using this region, the quantification of phytochemical composition and antioxidant activity has been successfully performed in wine (Versari et al. 2010, Silva et al. 2014), olive oils (Gouvinhas et al. 2015), fruit (Park et al. 2015; Baptista et al., 2017), chickpea (Kadiroğlu et al. 2018) and vegetables (Lu et al. 2011), as alternative to spectrophotometry techniques. Moreover, recent studies reported the application of ATR spectroscopy as alternative to HPLC-

DAD, for the determination of catechin and epicatechin in chocolate (Hu et al. 2016) and honey (Tahir et al. 2017).

Concerning NIR spectroscopy, the spectral features observed are raised by overtones and combinations of fundamental vibrational modes, in the spectral range from 4000–10000 cm^{-1} (Hacisalihoglu et al. 2010). Several works reported the feasibility of NIR to be applied as alternative to colorimetric methods, for the assessment of phytochemical composition and antioxidant activity in distinct matrices, such as rice (Zhang et al. 2008), apples (Beghi et al. 2013; Schmutzler & Huck 2016), gluten-free grains (Wiedemair et al. 2019), faba bean (Wang et al. 2014) and grape pomace (Páscoa et al. 2015).

Although several studies are available using either MIR or NIR spectroscopies to evaluate phytochemical contents in distinct matrices, few works compared the performance of both techniques to quantify the phytochemical composition and antioxidant activity in the same matrix, exception made to works developed for medicinal plants (Schönbichler et al., 2014; Clara et al., 2016), red cabbage (Oliveira et al. 2018) and legumes (cowpea pods) (Machado et al. 2017). Indeed, the feasibility of FTIR methodologies to be applied as alternative to standard techniques, for the quantification of phytochemical composition, *in vitro* antioxidant activity, and individual phenolic compounds, in dry bean flours, has not been evaluated yet.

The main objective of this work has been the comparison between the performance MIR and NIR techniques, for the assessment of bean flours, regarding phytochemical composition (total phenols, *ortho*-diphenols and flavonoids), *in vitro* antioxidant activity (DPPH, ABTS and FRAP) and nine individual phenolic compounds (gallic acid, catechin, F1, F2, F3, F4, F5, F6 and F7).

4.2.2. Materials and Methods

4.2.2.1. Sampling

Common bean seeds ($n=21$) were selected by Maria Manuela Veloso from the collection held at the Research Unit of Biotechnology and Genetic Resources, INIAV-Oeiras, cultivated in Cabrela (near Sintra, Portugal) and harvested in 2013 and 2014, totalising 42 samples for analysis. All the bean grains' samples were ground through a Falling Number 3100 mill (Perten Sweden) using 0.8 mm screen. The samples were stored in room temperature until analysis.

4.2.2.2. Extraction of the phenolic fraction

40 mg of each sample were weighed and extracted with 1.5 mL of methanol/water (70:30, v/v) for 30 min in an orbital shaker at room temperature. The extracts were centrifuged

at 5000 rpm for 5 min at 4°C (Sigma-2-16 K; Sigma, Steinheim, Germany). The supernatant was recovered, and the previous procedure was repeated three times, the final volume of the resulting supernatant being adjusted to 5.0 mL. The extracts were filtered by 0.45 µm polyvinylidene fluoride filter (Millex HV13; Millipore) and stored at 4°C. All assays were measured in triplicate.

4.2.2.3. Phytochemical composition

Quantification of total phenols, *ortho*-diphenols and flavonoids, of bean extracts, were performed in microscale using 96-well microplates and measured by Multiscan FC microplate reader (Thermo-Fisher Scientific, Inc., Waltham, USA).

4.2.2.3.1. Total phenols content

Total phenolic content of bean extract was determined according to the method given by Mena et al. (2011) with minor modifications. Briefly, 20 µL of extract, 100 µL of Folin-Ciocalteu reagent (10%) and 80 µL of Na₂CO₃ (7.5%) were homogenized and incubated in the oven at 40–45°C, protected from light, for 30 min. For the quantification of total phenols, the calibration curve was prepared using gallic acid as analytical standards in the range of 2.5–200 mg/mL, with an $R^2 = 0.99$. The absorbance was measured at 750 nm and the results were expressed as mg of gallic acid equivalents g⁻¹ dry weight (mg GAE/g dw).

4.2.2.3.2. *Ortho*-diphenols content

The quantification of *ortho*-diphenols content of extract was performed using the method of Mateos et al. (2001) with minor modifications. 160 µL of bean extract were added to 40 µL of Na₂MoO₄ (5%). Mixtures were incubated at room temperature and kept away from light, for 15 min. For the quantification of *ortho*-diphenols, the calibration curve was prepared using gallic acid as analytical standards in the range of 2.5–200 mg/mL, with an $R^2 = 0.99$. Absorbance was measured at 375 nm and the results were expressed as mg GAE/g dw.

4.2.2.3.3. Flavonoids content

The flavonoids content of bean extracts were determined using a method described by Zhishen et al. (1999). 24 µL of bean extract were added to 28 µL of NaNO₂ (5%). After 5 min, 28 µL of AlCl₃ (10%) were added and the mixture allowed to react for 6 min. Then, 120 µL NaOH (1M) was added to the mixture, being shaken for 30 s, and the absorbance was read at 510 nm. For the quantification of flavonoids, the calibration curve was prepared using catechin

as analytical standards in the range of 2.5–200 mg/mL, with an $R^2 = 0.99$. The results were expressed as mg of catechin equivalents per gram of dry weight (mg CE/g dw).

4.2.2.4. *In vitro* antioxidant activity

The free radical scavenging (DPPH and ABTS), and ferric reducing antioxidant power (FRAP) assays, were performed according to the methods described by Mena et al. (2011) with minor modifications. *In vitro* antioxidant activity was determined in microscale using 96-well microplates (Thermo-Fisher Scientific, Inc., Waltham, USA). In DPPH, 10 μ L of bean extract were added to 190 μ L of DPPH solution, completing 200 μ L of total reaction volume. For ABTS assays, 12 μ L of bean extract were added to 188 μ L of ABTS working solution, to complete the volume reaction of 200 μ L. The absorbance was measured after 30 min of reaction at room temperature, at 520 nm for DPPH, at 734 nm for ABTS. For FRAP assay, the total reaction volume was 300 μ L, comprising 12 μ L of bean extract and 288 μ L of working FRAP solution. The mixtures were shaken, incubated at 37 °C in the dark, for 30 min, and read at 593 nm. For the quantification of DPPH, ABTS and FRAP, the calibration curves were prepared using trolox as analytical standards in the range of 0.034–0.625 mM for DPPH and FRAP, 0.034–0.200 mM for ABTS, with an $R^2 = 0.99$. The results of all procedures were measured as mmol of trolox g⁻¹ dry weight (mmol TE/g dw).

4.2.2.5. Individual phenolic compounds by HPLC

The individual phenolic compounds were determined following the reported method by Lin et al. (2008), with slight modifications. HPLC analyses were performed using a Thermo Finnigan Surveyor HPLC-DAD system. The separation was achieved on an ACE 5 C18 column (5 μ m, 250 x 4.6mm I.D.) and the column oven temperature was set to 25°C. Injection volume was 20 μ L and flow rate was 1.0 ml/min. The mobile phase contained A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient program started with 10% of phase B at 0 min, 26% B at 40 min, 65% B at 70 min and 100% B at 71 min. The results were monitored at 280, 310 and 520 nm while UV/VIS spectrum from 200–650 nm was continuously collected. Chromatograms were analysed using Xcalibur software (Thermo Fisher Scientific, Inc., Waltham, USA). The main individual phenolic components determined were gallic acid, catechin, F1 (Myricetin 3-*O*-glucoside), F2 (Quercetin 3-*O*-glucoside), F3 (Quercetin 3-*O*-(6''-*O*-mannosyl)glucoside, F4 (Kaempferol 3-*O*-glucoside), F5 (Myricetin), F6 (kaempferol 3-*O*-(6''-*O*-mannosyl)glucoside and F7 (kaempferol). For the quantification of all phenolic compounds, the calibration curves were prepared in the interval of 0.01–10

ug/mL. For all calibration curves a R^2 of at least 0.99 was obtained, and the Bias between the measured concentration and true concentration of the calibrators was within $\pm 15\%$ for all calibrators. The LOQ was assumed as the lowest point of the calibration curve, concentration which can be determined with adequate accuracy and precision. The LOD was not evaluated for this method, since the concentration range of the compounds in the samples was always within the calibration range. Identification of compounds in the samples was based on the retention time and UV/Vis spectra comparison to the analytical standards.

4.2.2.7. Spectroscopic analysis

FTIR spectra of each bean flour were recorded in the MIR and NIR regions, with Thermo Scientific Nicolet iS50 FTIR spectrometer, using Omnic software Version 9.2.28 (Thermo Fisher Scientific Inc., Waltham, USA). MIR analyses were performed by placing bean flour sample (~5 mg) directly in the crystal of the ATR accessory. Infrared spectra were obtained in the range of 400 (Thermo-Fisher Scientific, Inc., Waltham, USA). 4000 cm^{-1} , with spectral resolution of 4 cm^{-1} and 128 scans were acquired for each spectrum. In NIR analysis, the bean flour samples (~150 mg) were placed in the DRIFT accessory, and the spectra were measured between $4000\text{--}10000\text{ cm}^{-1}$ with a resolution of 8 cm^{-1} , 256 scans being acquired for each spectrum. In both techniques, NIR and MIR, the background spectrum was collected between sampling and subtracted to the spectrum of the sample. For each sample, 6 spectra were collected with each technique.

4.2.2.8. Statistical analysis

NIR and MIR spectra were processed using OriginPro 9.1 software (OriginLab, Northampton, USA). In the MIR region, baseline subtraction was performed in each spectrum, using 4th order polynomials and 50 iterations for convergence, and the chemometric analyses were developed with three intervals: low frequency ((LF) $450\text{--}1800\text{ cm}^{-1}$), high frequency ((HF) $2600\text{--}3700\text{ cm}^{-1}$) and the combination of both ((FI) $450\text{--}1800$; $2600\text{--}3700\text{ cm}^{-1}$). Concerning NIR, the complete spectral interval ($4000\text{--}10000\text{ cm}^{-1}$) was used, and no baseline subtraction was performed.

In both techniques (MIR and NIR), three distinct treatments, including mean normalization (spectra), first derivative after normalization (1st deriv MN), and first derivative of spectra (1st deriv), were tested. In partial least squares (PLS), Infrared spectral data have been used as X (independent) variable to predict the phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds as Y (dependent) variable. The most suitable

prediction models were evaluated by cross-validation (CV, leave-one-out), and their quality assessed with external validation, which was carried using two thirds of the spectra (168 spectra) as calibration set and the other third (84 spectra) as external (validation) set, which were randomly excluded from the complete set of registered spectra (252 spectra). (Clara et al., 2016; Plans et al., 2013). The accuracy of the prediction models was evaluated by the lowest values obtained for the Prediction Error Sums of Squares (PRESS) (<1.010), for each number of factors (NF), highest value for coefficients of determination for calibration (R^2c) and validation (R^2v), bias values, which should be close to zero (Kadiroğlu et al. 2018, Wang et al. 2014), and residual prediction deviation (RPD). RPD is the ratio between the standard deviation and the root mean standard error of prediction (RMSEP). Predictive models with RPD values lower than 2.3 cannot be used for further predictions; with an RPD value between 2.4 and 3.0 can be used for a rough prediction; and RPD values higher than 5 correspond to a very good prediction model (Williams, 2001). Selected intervals (SI) have been defined for each parameter, resorting to the variable importance projection (VIP) plots and regression coefficients, whenever the number of factors extracted from the PLS regression was equal to, or higher than 8, in order to avoid the possible occurrence of over-fitting.

The statistical analysis of all the samples was performed using SPSS Statistics 21.0 software (SPSS inc., Chicago, IL, USA). Phytochemical composition (total phenols, *ortho*-diphenols, flavonoids), *in vitro* antioxidant activity (DPPH, ABTS and FRAP) and individual phenolic compounds (gallic acid, catechin, F1, F2, F3, F4, F5, F6 and F7) concentration, were measured in triplicate.

4.2.3. Results and discussion

4.2.3.1. Phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds

The phytochemical composition (total phenols, *ortho*-diphenols and flavonoids), *in vitro* antioxidant activity (DPPH, ABTS and FRAP), and individual phenolic compounds (gallic acid, catechin, F1, F2, F3, F4, F5, F6 and F7), of the 21 bean flour cultivars, grown in two different years (2013 and 2014), is presented in Table 12.

Table 11 Phytochemical composition (total phenols, *ortho*-diphenols and flavonoids), *in vitro* antioxidant activity (DPHH, ABTS and FRAP) and individual phenolic profile (gallic acid, catechin, F1, F2, F3, F4, F5, F6 and F7) of bean flours from 2013 and 2014.

	2013				2014				Both years		
	Mean	Range	SD	SE	Mean	Range	SD	SE	Mean	SD	SE
Total Phenols ^a	2.0	0.1-4.8	1.1	0.2	2.4	0.4-4.0	0.9	0.2	2.2	1.0	0.1
<i>Ortho</i> -diphenols ^a	3.1	0.8-7.0	1.7	0.2	3.1	1.8-5.3	0.8	0.2	3.1	1.3	0.1
Flavonoids ^b	2.1	0.8-4.6	0.9	0.1	2.0	0.9-4.1	0.8	0.2	2.0	0.8	0.1
DPPH ^c	10.1	2.6-19.4	3.8	0.5	9.5	2.1-18.3	3.6	0.8	9.8	3.7	0.3
ABTS ^{c*}	16.9	3.2-32.8	6.8	0.8	14.6	6.0-24.0	4.8	1.0	15.7	5.9	0.5
FRAP ^{c*}	14.9	4.6-28.0	6.1	0.8	11.9	5.3-22.6	3.9	0.9	13.4	5.3	0.5
Gallic acid ^d	60.9	0.0-69.4	7.6	1.0	63.4	0.0-64.9	0.8	0.1	62.2	5.3	0.5
Catechin ^d	805.6	245.2-1731.1	355.2	54.8	495.8	144.1-1507.7	297.0	37.4	650.7	361.3	32.2
F1 ^d	80.9	0.0-150.2	75.9	9.6	37.6	0.0-79.7	32.0	3.9	54.9	55.9	5.0
F2 ^d	44.8	0.0-126.9	38.7	4.9	30.8	0.0-91.0	23.2	5.1	38.6	33.3	3.0
F3 ^d	5.7	0.0-9.5	2.7	0.3	4.8	0.0-8.2	2.2	0.5	5.2	2.4	0.2
F4 ^d	445.8	0.0-1906.2	633.1	79.7	230.8	0.0-976.6	301.2	63.3	335.1	500.2	44.6
F5 ^d	22.2	0.0-30.3	8.2	1.0	15.2	0.0-20.1	4.2	1.4	18.2	7.0	0.6
F6 ^d	46.9	0.0-210.7	49.6	6.2	46.4	0.0-137.5	37.6	8.4	46.7	43.7	3.9
F7 ^{d*}	37.0	0.0-168.1	47.9	6.0	24.9	0.0-94.2	29.7	4.6	31.7	41.0	3.6

SD- Standard deviation; SE- Standard error; ^a(mg GAE g⁻¹ dw); ^b(mg CE g⁻¹ dw); ^c(μmol TE g⁻¹ dw); ^d(μg g⁻¹ dw),

*indicates that does differ significantly at $p < 0.01$, between two harvest years

The total phenols content of bean flours ranged from 0.1–4.8 mg GAE/g dw in 2013, and 0.4–4.0 mg GAE/g dw in 2014, without significant differences between both years. These results are in agreement with Giusti et al. (2017) and Kan et al. (2017) who reported total phenols levels in common beans 0.6–3.4 mg GAE/g dw and 0.25–3.79 mg GAE/g dw, respectively, but higher than the levels (0.9–1.2 mg GAE/g dw) reported by Rocchetti et al. (2019) in common beans. *Ortho*-diphenols contents ranged 0.8–7.0 mg GAE/g dw, SE= 0.2 in 2013 and 1.8 – 5.3 mg GAE g⁻¹ dw, SE= 0.2 in 2014. The mean value of *ortho*-diphenols was the same in both years, 3.1 mg GAE/g dw and SE= 0.2. Lower SE indicate higher accuracy of mean sampling. Flavonoids levels were slightly lower in 2014 (0.9–4.1 mg CE/g dw) when compared to 2013 (0.7–4.6 mg CE/g dw), with no significant differences between years. The differences among cultivars can be attributed to the genetic factors and variation between cultivars (mainly colour of coats) (Zhao et al. 2014).

DPH and ABTS assays reveal free radical scavenging activity of foodstuffs, whereas FRAP assay displays their reducing power. In 2013, the samples showed 2.6–19.4 μmol TE/g dw through DPPH assay, 3.2–32.8 μmol TE/g dw in ABTS assay and 4.6–28.0 μmol TE/g dw in FRAP assay. For 2014, the amounts of *in vitro* antioxidant activity by DPPH, ABTS and FRAP ranged 2.1–18.3 μmol TE/g dw 6.0–24.0 μmol TE/g dw and 5.3–22.6 μmol TE/g dw, respectively. The low SE values for the *in vitro* antioxidant activity measures in both years

(SE= 0.8 for DPPH, SE= 1.0 for ABTS and SE= 0.9 for FRAP) revealed the accuracy of mean values for DPPH, for ABTS and FRAP. The average values of both years were $9.8 \mu\text{mol TE g}^{-1} \text{ dw}$ for DPPH, $15.7 \mu\text{mol TE g}^{-1} \text{ dw}$ in ABTS and $13.4 \mu\text{mol TE g}^{-1} \text{ dw}$ for FRAP, which were higher than the results ($1.7\text{--}7.5 \mu\text{mol TE g}^{-1} \text{ dw}$) of DPPH observed by Kan et al. (2017), and lower than the amount ($5.77 \text{ mmol TE g}^{-1} \text{ dw}$) reported by Grela et al. (2017), which a single common bean sample was analysed (cv. Mela). According to the previous authors, these discrepancies observed for *in vitro* antioxidant activities, in bean samples, might be due to the geographical origin of sample and environmental factors (Aguilera et al. 2011).

The individual phenolic compounds (gallic acid, catechin, F1, F2, F3, F4, F5, F6 and F7) were determined by HPLC. Catechin was present in all the flour samples, in both years, showing higher mean concentration in 2013 ($805.6 \mu\text{g/g dw}$) than in 2014 ($495.8 \mu\text{g/g dw}$), and a minimum concentration of $144.1 \mu\text{g/g dw}$, while the mean values were significantly different between years. The amounts of catechin observed in the current work were higher than the concentrations found in previous works, from $10 \mu\text{g/g dw}$ to $614 \mu\text{g/g dw}$ (Giusti et al. 2017). Regarding gallic acid, higher values were also found in 2013 than in 2014, $0.0\text{--}69.4 \mu\text{g/g dw}$ and $0.0\text{--}64.9 \mu\text{g/g dw}$, respectively. The amounts of F1 ranged from 0.0 to $150.2 \mu\text{g/g dw}$ in 2013 and $0.0\text{--}79.7 \mu\text{g/g dw}$ in 2014. For F2, the variation was between $0.0\text{--}126.9 \mu\text{g/g dw}$ in 2013 and $0.0\text{--}91.0 \mu\text{g/g dw}$ in 2014. The most abundant flavonol present in the flour samples was F4 (kaempferol-3-O-glucoside), ranging between $0.0\text{--}1906.2 \mu\text{g/g dw}$ in 2013 and $0.0\text{--}976.6 \mu\text{g/g dw}$ in 2014, with an average value, comprising both years, of $335.1 \mu\text{g/g dw}$, these results being comparable to previously reported concentrations ($0.0\text{--}1486.0 \mu\text{g/g dw}$) by Giusti et al., (2017). On the opposite side, F3 (Quercetin-3-O-(6''-O-manoyl) glucoside) and F5 (Myrcetin), exhibited the lowest mean concentrations comprising both years, $5.2 \mu\text{g/g dw}$ and $18.2 \mu\text{g/g dw}$, for F3 and F5, respectively, with lower values being observed in 2013, respecting 2014. F6 and F7 also showed lower amounts in 2013, with an average of $46.9 \mu\text{g g}^{-1} \text{ dw}$, SE= 6.2 and $37.0 \mu\text{g g}^{-1} \text{ dw}$, SE= 6.0, respectively, while, in 2014, the same compounds presented the concentrations of 46.4 and $24.9 \mu\text{g g}^{-1} \text{ dw}$, and SE ranging $4.6\text{--}8.4$. In general, the mean value of each individual phenolic compound found in our samples, is representative of its effective amount present in bean samples. Although the standard deviation for F1, F4 and F7 was higher than the average, the lower SE revealed the accuracy of mean sampling. In literature, no studies were found concerning the evaluation of most of these specific compounds in beans, while the differences verified among samples, regarding phytochemical contents, have been attributed to environmental conditions and variations between cultivars (Zhao et al. 2014).

Summarizing, all individual phenolic compounds decreased in 2014, respecting 2013, except for gallic acid, as well as *in vitro* antioxidant activity, supporting that these individual phenolic compounds are the main antioxidants present in the bean flours assessed.

4.2.3.2. FTIR spectral assignment

Typical MIR and NIR normalized spectra, obtained for the same cultivar in two distinct harvest years, and corresponding 1st derivative values, are presented in Figure 12a and b.

In MIR, the baselines have been subtracted, and three treatments (spectra, 1st deriv MN and 1st deriv) were tested for each region (LF, HF and FI). The 1800–2600 cm⁻¹ interval has been excluded, since no fundamental vibrational mode of interest is found in this region (Plans et al. 2013).

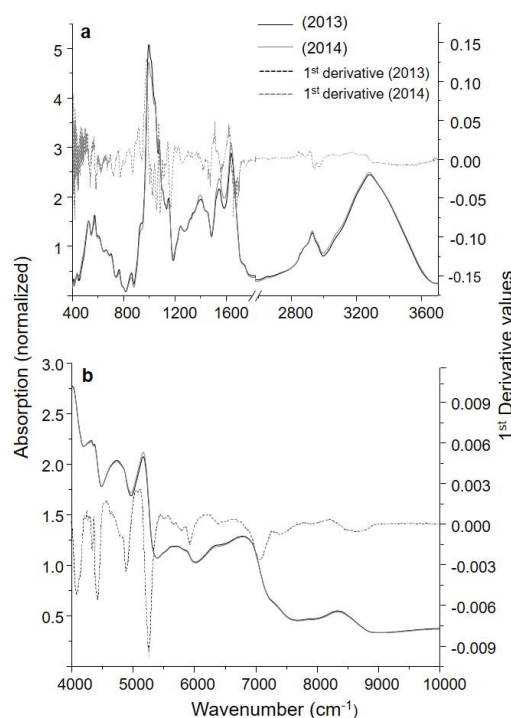


Figure 12 MIR normalized spectra and 1st derivative values of a bean sample from 2013 and 2014. b- NIR normalized spectra and 1st derivative of a bean sample from 2013 and 2014

In LF, the weak intensity peaks between 750–880 cm⁻¹ can be assigned to C-H deformations of aromatic rings, being thus related to phenolic contents (Batista et al. 2016). The most intense peak, around 1010 cm⁻¹, is associated to C-O stretching modes, presenting an important contribution from phenols (Masek et al. 2014). The spectral region between 1150–1500 cm⁻¹ is dominated by O-H, C-H and C=C (aromatic ring) in-plane deformations, therefore, reflecting the presence of flavanols and phenolic acids (Tahir et al. 2017). The peaks

in the regions 1550–1700 cm^{-1} have contributions from C=C (aromatic ring), stretch of phenyl (Lu et al., 2011), and C=O stretching of carbonyl groups, which are typical from phenolic compounds (Silva et al., 2014; Kadiroğlu et al., 2018). In the HF region, the weak peaks between 2800–2950 cm^{-1} were assigned to C-H stretching modes of aromatic ring, besides other molecules containing the CH moiety, and the wide spectral feature between 2950–3600 cm^{-1} is the result of O-H stretching modes (Hu et al. 2016; Samborska et al. 2019).

In the NIR region, the full spectral interval registered, 4000–10000 cm^{-1} , which was submitted to the three distinct treatments assessed, has been used for the PLS regressions, while distinct regions may provide information regarding the phytochemical contents. For instance, below 5400 cm^{-1} , the spectral features are dominated by combination modes between fundamentals, the interval between 4200–4900 cm^{-1} being associated to combinations between stretching and deformation modes of CH and OH groups belonging to phenolic rings (Wiedemair et al. 2019). The peak at around 5150 cm^{-1} contains combinations between modes related to the OH group in phenols and vibrations related to the corresponding aromatic ring, while the features in the interval 5400–6000 cm^{-1} , correspond to 1st overtones of C-H stretching modes from these rings (Oliveira et al., 2018). The features observed in the interval 6050–7200 cm^{-1} may also contain information on phytochemical contents, since the occurrence of 2nd order overtones of C=O stretching in flavonols, besides combinations involving OH groups, have been pointed to this interval. (Shi et al., 2012; Wiedemair et al., 2019), while this latter contribution for the spectral features has been specifically allocated to OH from phenols by other authors (Oliveira et al., 2018). Finally, in a previous study, conducted with faba bean, the most preponderant regions for the assessment of phenolic contents, were the intervals between 4200–5500 cm^{-1} and 6100–10000 cm^{-1} . In fact, these results are in good agreement with the other study in faba bean which identify the most preponderant regions for the assessment of phenolic contents in the intervals between 4200–5500 cm^{-1} and 6100–10000 cm^{-1} (Wang et al., 2014).

4.2.3.3. PLS regressions

A summary of the performance observed for the models developed to evaluate phytochemical composition, *in vitro* antioxidant activity, and individual phenolic compounds, in the MIR spectral region, is presented in Table 12. In none of the PLS regressions tested on validation more than 7 factors number were used, which was the limit considered, in order to avoid over-fitting, while in the cases where 8 or more factors number were extracted, only the

most promising intervals were used for the regressions, to diminish the number of factors necessary (Plans et al., 2013), except for ABTS, gallic acid, F3 and F7.

The results observed for the models developed for the assessment of total phenols and flavonoids, were $R^2c=0.95$ and 0.99 , respectively, similar $R^2v=0.86$, for both, while the PRESS value for total phenols was lower, 0.995 vs. 1.005 , bias $-6.8E-4$ – $6.8E-04$, slope $=0.96$ and $RPD>4.3$, these results being obtained using the low frequency for total phenols and high frequency for flavonoids, in the 1st deriv MN treatment (Table 2). Kadiroğlu et al. (2018) reported similar determination coefficients and number of factors ($R^2c=0.98$; $NF=8$), for the quantification of total phenols in chickpea samples. Concerning *ortho*-diphenols no suitable models have been developed ($R^2<0.60$) and $RPD<2.3$, independently from the data treatment, as observed in previous works (Batista et al., 2016). PLS regressions were successfully developed for the estimation of *in vitro* antioxidant activity, through the DPPH and ABTS assays, using LF and FI data. The best predictive model for DPPH ($R^2c=0.95$ and $R^2v=0.81$), was obtained with 1st deriv treatment, while spectra treatment led to the best model for ABTS ($R^2c=0.89$ and $R^2v=0.84$) (Table 2). In the case of FRAP, the best PLS regressions were developed with either FI or HF, though being unsuccessful independently from the data treatment ($R^2c<0.50$, Table 12).

Table 12 Statistical parameters for the results obtained with the PLS models developed to assess phytochemical composition, *in vitro* antioxidant activity, and individual phenolic compounds of 21 bean samples for two harvest years, using FTIR-MIR spectral data.

	Spectra							1 st deriv MN							1 st deriv						
	Int (NF)	PRESS	R ² c	R ² v	Bias	Slope	RPD	Int (NF)	PRESS	R ² c	R ² v	Bias	Slope	RPD	Int (NF)	PRESS	R ² c	R ² v	Bias	Slope	RPD
Total phenols §	LF(6)	0.860	0.82	0.80	-1.0E-06	1.03	3.95	*LF(6)	0.995	0.95	0.86	-6.8E-04	0.96	4.36	HF(6)	0.998	0.97	0.77	1.0E-04	0.96	3.16
<i>Ortho</i> -diphenols §	FI(5)	1.022	0.52	0.23	1.4E-03	0.82	1.39	*FI(1)	1.024	0.35	0.31	2.5E-03	1.26	1.54	FI(2)	1.020	0.43	0.28	7.8E-04	0.92	1.41
Flavonoids §	FI(7)	0.873	0.88	0.82	-8.6E-04	0.97	4.02	*FI(3)	1.005	0.99	0.86	-4.3E-04	0.96	4.30	HF(2)	0.991	0.79	0.71	-9.3E-05	0.94	2.97
DPPH §	FI(1)	1.019	0.25	0.24	-8.8E-04	0.58	1.13	LF(6)	0.830	0.95	0.81	2.6E-04	0.99	3.51	*LF(5)	0.776	0.85	0.85	2.6E-03	0.99	3.70
ABTS §	*FI(9)	0.766	0.89	0.84	3.8E-03	0.93	4.05	LF(5)	0.811	0.86	0.81	-3.3E-03	0.93	3.91	LF(5)	0.855	0.83	0.77	-2.6E-03	0.94	2.35
FRAP	HF(2)	1.024	0.36	0.23	-1.8E-03	0.86	1.56	HF(1)	0.993	0.42	0.30	5.3E-03	0.86	1.87	*FI(2)	0.993	0.50	0.43	5.1E-03	1.02	2.02
Gallic acid §	LF(11)	0.453	0.96	0.92	2.3E-02	0.94	9.24	*FI(7)	0.621	0.99	0.94	2.0E-01	1.28	10.12	LF(7)	0.573	0.94	0.89	7.4E-01	1.96	8.44
Catechin §	HF(4)	0.928	0.57	0.53	-6.7E-01	0.98	2.09	*HF(6)	0.929	0.98	0.89	-1.3E-01	0.97	9.47	FI(5)	0.932	0.93	0.78	-7.3E-01	0.92	5.26
F1
F2	*FI(4)	0.942	0.61	0.55	1.1E-01	1.33	2.03	HF(1)	0.991	0.43	0.39	1.3E-01	1.24	1.89	HF(2)	0.983	0.54	0.43	1.0E-01	1.26	1.91
F3 §	*LF(9)	0.955	0.82	0.78	2.5E-02	1.31	3.37	LF(3)	1.001	0.83	0.73	7.6E-03	0.94	4.25	LF(3)	0.960	0.72	0.55	2.5E-02	1.12	2.44
F4	FI(1)	1.021	0.28	0.29	6.5E-01	1.19	1.78	*HF(1)	1.001	0.41	0.38	7.0E-01	1.23	1.82	HF(2)	0.984	0.52	0.37	3.8E-01	1.11	1.89
F5	*LF(3)	1.023	0.46	0.36	1.7E-01	1.92	1.88	LF(2)	1.014	0.53	0.32	1.7E-01	1.76	1.82	LF(2)	1.015	0.51	0.35	1.8E-01	2.08	1.81
F6 §	*HF(2)	0.957	0.45	0.45	8.1E-02	1.11	2.01	HF(3)	1.017	0.68	0.36	9.2E-02	1.03	1.91	HF(2)	0.998	0.53	0.39	1.0E-01	1.10	1.79
F7 §	*FI(5)	0.950	0.94	0.84	-2.2E-01	1.19	7.03	LF(9)	0.941	0.99	0.71	-2.4E-02	1.00	3.17

NF- Number of Factors; LF- Low frequency; HF- High frequency; FI- Full interval; *Treatment for which the standardized regression coefficients are presented;

§Selected interval: Total phenols (Spectra: 753-1272 cm⁻¹; 1st deriv: 2600-3500 cm⁻¹); *Ortho*-diphenols (1st deriv MN: 815-3030 cm⁻¹; 1st deriv: 815-3799 cm⁻¹); Flavonoids (Spectra: 1034-1754 cm⁻¹; 1st deriv MN: 1000-2850 cm⁻¹; 1st deriv: 2600-2818 cm⁻¹); DPPH (Spectra: 1047-1748 cm⁻¹; 1st deriv: 865-1637 cm⁻¹); ABTS (Spectra, 1st deriv MN: 1041-1761 cm⁻¹; 1st deriv: 761-1591 cm⁻¹); Gallic acid (Spectra: 1049-1512 cm⁻¹; 1st deriv: 1175-1469 cm⁻¹); Catechin (Spectra: 3492-3699 cm⁻¹); F3 (1st deriv MN, 1st deriv: 862-1799 cm⁻¹); F6 (Spectra, 1st deriv: 2839-3028 cm⁻¹); F7 (1st deriv: 859-1799 cm⁻¹)

Concerning the individual phenolic compounds, the best regression results for all treatments, in the MIR region, were obtained for gallic acid, with $\text{PRESS} < 0.621$, R^2c ranging from 0.94–0.99, $R^2v \geq 0.89$, slope 0.94–1.96, bias = $2.3\text{E-}02$ – $7.4\text{E-}01$ and RPD ranging 8.4–10.12. The PLS models developed were not suitable for the quantification of F2, F4, F5, and F6 ($R^2v \leq 0.68$), while the regressions failed with all data treatments, and with spectra treatment, for the quantification of F1 and F7, respectively. For catechin and F7, the best models were obtained using 1st deriv MN, corresponding to ($R^2c = 0.98$, $R^2v = 0.89$) and ($R^2c = 0.94$, $R^2v = 0.84$), respectively. Concerning F3, spectra treatment displayed the best performance for the development of quantification models, with the HF interval leading to the best result. Nevertheless, in the cases where 1st deriv MN treatment presented the best performance, the quality of the optimal models was better (Table 2). The selection of intervals, which was undertaken regarding *in vitro* antioxidant activity (ABTS), gallic acid and F3 (spectra treatment), F7 (1st deriv treatment), did not improve the number of factors, although prediction models obtained were considered as acceptable ($R^2c > 0.95$) for gallic acid and F7 (Batista et al. 2016).

The predicted models in the MIR region with the best calibration coefficients from the correlation between measured and predicted values of total phenols, flavonoids, gallic acid and catechin are presented on Figure 13.

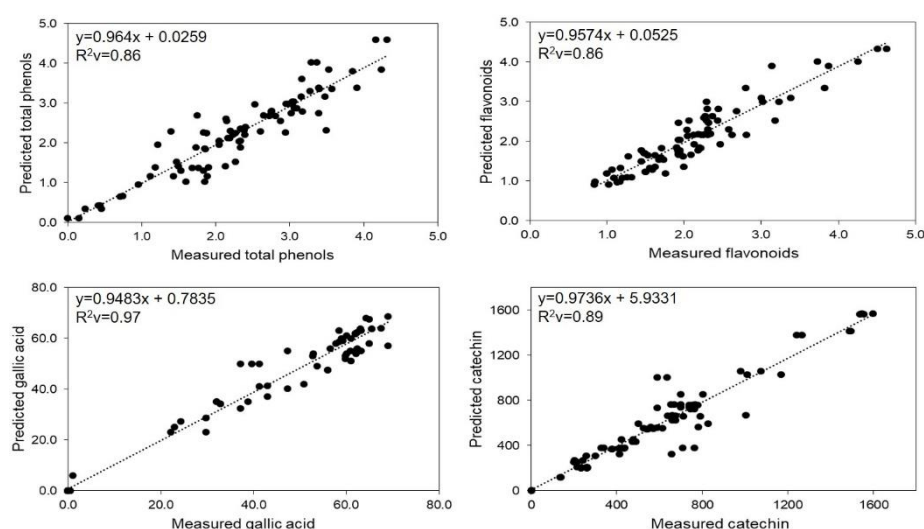


Figure 13 Correlation between the measured and predicted values by the calibration models obtained by MIR for the total phenols (1st deriv treatment), flavonoids (1st deriv MN treatment), gallic acid (1st deriv MN treatment) and catechin (1st deriv MN treatment)

The standardized regression coefficients, corresponding to the weight of each wavenumber, in MIR, are presented in Figure 14.

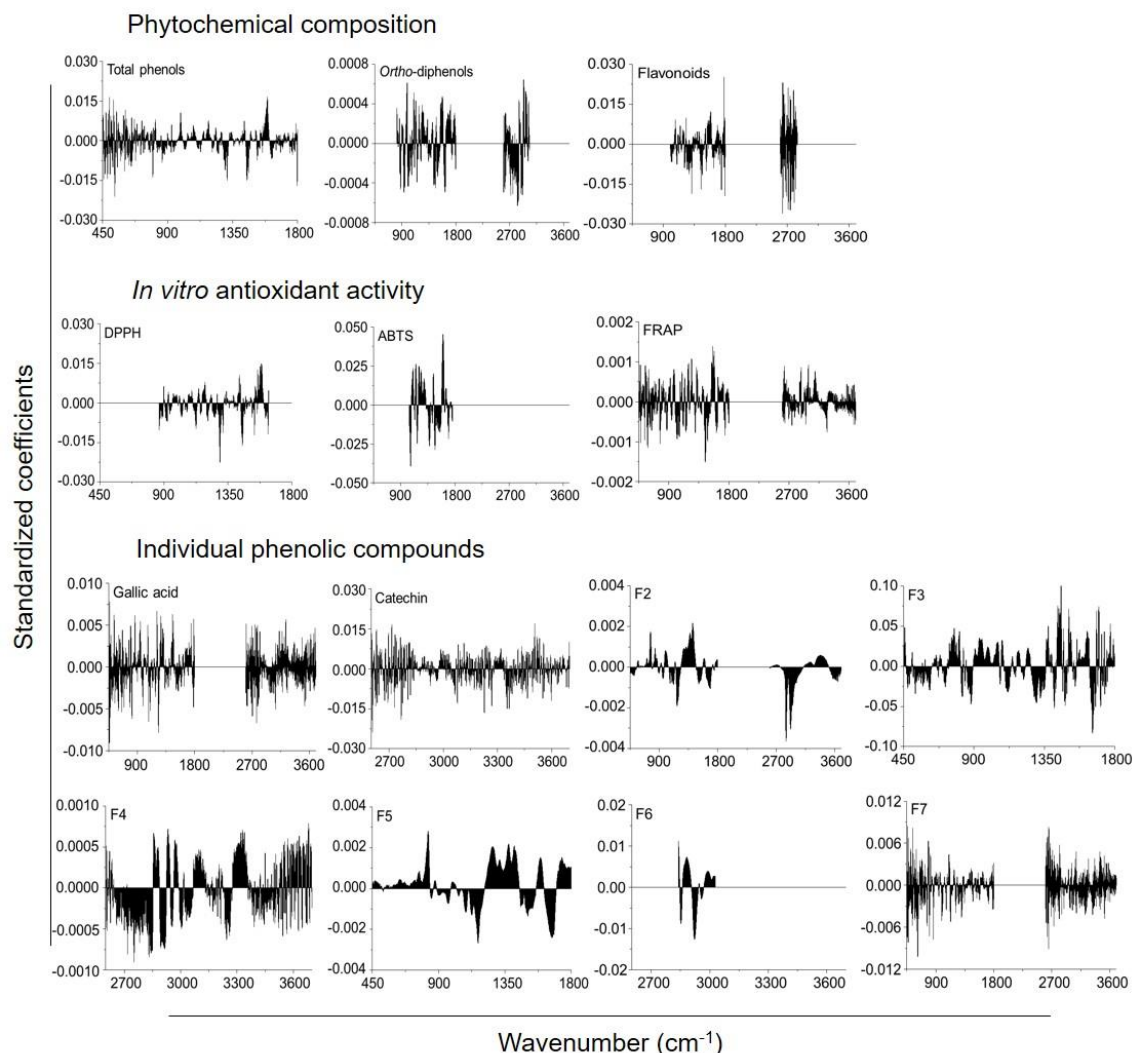


Figure 14 Standardized regression coefficients retrieved from the PLS procedure, corresponding to the best calibration developed with NIR data for each parameter

The best regressions have been obtained with 1st deriv MN (total phenols, *ortho*-diphenols, flavonoids, gallic acid, catechin, F4 and F7), and spectra (ABTS, F2, F3, F5 and F6). The plots undertaken with the spectra, display a distinct aspect, respecting cases where the plots correspond to the use of the derivative, presenting shapes that resemble spectral features. In these cases, three distinct spectral intervals conducted to the best regressions, LF in the case of F3 and F5, HF in the case of F6, and FI for in vitro antioxidant activity (ABTS) and F2, although for ABTS the selected interval was between 865–1637 cm^{-1} . For *in vitro* antioxidant activity (DPPH and FRAP), the best regressions were undertaken using normalized spectra. In these

cases, relevant intervals are scattered throughout the spectral window using low frequency, such as in total phenols, DPPH, F3 and F5. This is related to the actual importance of the interval 700–1800 cm^{-1} , where peaks corresponding to functional groups related to phenolic compounds and antioxidants are found, being considered the fingerprinting region for their quantification (Hu et al., 2016).

The PLS models developed for phytochemical composition, *in vitro* antioxidant activity, and individual phenolic compounds, in the NIR region, are shown in Table 13.

The best prediction models for total phenols ($R^2\text{c}= 0.98$, $R^2\text{v}= 0.91$, PRESS= 0.893 and NF= 4), *ortho*-diphenols ($R^2\text{c}= 0.98$, $R^2\text{v}= 0.85$, PRESS = 0.986 and NF= 4), and flavonoids ($R^2\text{c}= 0.94$, $R^2\text{v}= 0.90$, PRESS= 0.879 and NF= 3), were obtained with 1st deriv treatment. A single work, quantifying total phenols in legumes with NIR, was found in literature, showing determination coefficients of $R^2\text{c}= 0.89$ and $R^2\text{v}= 0.78$, obtained with normalized data, being slightly lower than the optimal model, but comparable to the values presently observed for the same treatment (Wang et al. 2014) (Table 13).

Regarding *in vitro* antioxidant activity, for DPPH and ABTS, similar PLS results were found among treatments, while 1st deriv led to the best results in both parameters (PRESS < 0.800, $R^2\text{c} > 0.96$ and $R^2\text{v} > 0.86$, bias ranged from -6.3E-04 to 1.7E-04, slope~ 1 and RPD > 4). For FRAP, the highest determination coefficients were observed for 1st deriv MN ($R^2\text{c}= 0.99$; $R^2\text{v}= 0.86$), in the 7286–10000 cm^{-1} selected interval.

Table 13 Statistical parameters for the results obtained with the PLS models developed to assess phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds of 21 bean samples for two harvest years, using FTIR-NIR spectral data.

	Spectra							1 st deriv MN							1 st deriv						
	NF	Press	R ² c	R ² v	Bias	Slope	RPD	NF	Press	R ² c	R ² v	Bias	Slope	RPD	NF	Press	R ² c	R ² v	Bias	Slope	RPD
Total phenols §	6	0.727	0.87	0.87	-1.0E-01	1.01	4.85	3	0.924	0.91	0.82	3.1E-03	1.04	4.52	*4	0.893	0.98	0.91	4.4E-05	1.06	5.20
<i>Ortho</i> -diphenols §	7	0.984	0.69	0.65	8.9E-05	0.97	2.02	3	0.942	0.96	0.85	-5.9E-04	1.01	4.72	*4	0.986	0.98	0.85	-6.3E-04	1.00	4.84
Flavonoids §	7	0.764	0.86	0.78	-1.4E-04	0.86	3.31	2	0.903	0.82	0.79	7.3E-04	0.83	3.99	*3	0.879	0.94	0.90	3.6E-04	0.96	5.18
DPPH §	7	0.793	0.85	0.80	7.9E-04	0.94	3.98	4	0.867	0.88	0.87	8.3E-04	0.98	4.02	*5	0.798	0.97	0.87	1.7E-04	0.96	4.64
ABTS §	6	0.751	0.86	0.86	-1.6E-03	0.93	4.09	4	0.754	0.89	0.89	-1.6E-04	0.97	4.73	*5	0.758	0.97	0.90	-6.3E-04	0.96	5.23
FRAP §	4	0.899	0.65	0.59	4.3E-03	1.09	2.09	*5	0.942	0.99	0.86	-2.1E-04	4.23	4.36	6	0.947	0.98	0.84	2.8E-03	1.05	4.83
Gallic acid §	12	0.408	0.97	0.95	1.2E-01	1.16	10.05	*7	0.659	0.99	0.96	4.2E-02	1.00	10.34	5	0.693	0.96	0.96	1.2E-01	1.16	10.25
Catechin	1	0.929	0.55	0.45	-6.7E-01	0.96	2.39	1	0.955	0.47	0.46	-5.7E-02	0.96	2.34	*1	0.968	0.48	0.48	-5.2E-01	0.99	2.38
F1 §	7	0.508	0.97	0.97	9.7E-03	0.98	9.25	2	0.592	0.97	0.97	3.9E-02	1.95	9.66	*2	0.618	0.99	0.97	5.4E-02	1.08	10.25
F2
F3 §	2	1.019	0.41	0.35	5.4E-02	2.16	2.01	5	0.941	0.96	0.88	1.7E-02	1.28	7.41	*6	0.996	0.98	0.90	2.3E-02	1.51	7.52
F4 §	1	1.005	0.33	0.30	9.9E-01	1.22	1.77	1	1.022	0.29	0.27	5.6E-01	1.19	1.72	*7	0.982	0.99	0.85	-4.1E-01	1.13	6.27
F5
F6 §	4	0.969	0.49	0.34	1.2E-01	1.09	1.85	2	0.972	0.88	0.88	-4.1E-02	1.02	6.62	*9	0.972	0.99	0.93	-3.7E-02	0.99	9.32
F7 §	6	0.989	0.94	0.83	3.6E-02	1.17	6.42	3	0.967	0.84	0.69	5.9E-03	1.35	2.10	*4	1.010	0.87	0.87	-2.2E-02	0.99	6.51

NF - Number of Factors; *Treatment for which the standardized regression coefficients are presented; §Selected interval: Total phenols (Spectra: 5338-6172 cm⁻¹; 1st deriv MN, 1st deriv: 7655-10000 cm⁻¹); *Ortho*-diphenols (1st deriv MN, 1st deriv: 7304-10000 cm⁻¹); Flavonoids (Spectra: 5333-6169 cm⁻¹; 6473-8600 cm⁻¹); (1st deriv MN, 1st deriv: 7399-10000 cm⁻¹); DPPH (Spectra: 5327-8525 cm⁻¹); ABTS (Spectra: 4826-7343 cm⁻¹); FRAP (1st deriv MN: 7286-10000 cm⁻¹); Gallic acid (Spectra: 5755-7024 cm⁻¹); F1 (Spectra: 8412-10000 cm⁻¹; 1st deriv MN, 1st deriv: 7646-10000 cm⁻¹); F3 (Spectra: 8357-8818 cm⁻¹); F4 (Spectra, 1st deriv: 7309-10000 cm⁻¹; 1st deriv MN: 7309-8520 cm⁻¹); F6 (1st deriv MN, 1st deriv: 7297-10000 cm⁻¹); F7 (Spectra: 4000-6153 cm⁻¹, 6509-9070 cm⁻¹)

Acceptable PLS models have been developed with all data treatments, for most of the compounds, except for catechin, F3, and F6, where at least one of the treatments failed to provide a quality model, whilst regarding F2 and F5, no regression was successful. Even though PLS regressions were developed with all treatments, regarding catechin, R^2c and $R^2v < 0.60$ and $RPD < 2.4$, none of the models presented enough quality to be used for quantification, while for F3 and F6, only the spectra treatment failed to provide an acceptable model (Table 13). Among all parameters, the best prediction models were obtained for F1, with all distinct treatments presenting the highest determination coefficients (R^2c 0.97–0.99 and $R^2v = 0.97$) and lowest PRESS 0.508–0.618. In the case of gallic acid, the best results were obtained with 1st deriv MN, showing determination coefficients of ($R^2c = 0.99$; $R^2v = 0.96$), while for F3 ($R^2c = 0.98$; $R^2v = 0.90$, $NF = 6$), the use of 1st deriv led to the optimal models. The only exception was F7, for which the highest determination coefficients ($R^2c = 0.94$, $R^2v = 0.83$, bias close zero, slope = 1.17 and $RPD = 6.42$) and 6 number of factors, were obtained with spectra treatment. The selection of interval for gallic acid on spectra treatment and F6 on 1st deriv treatment was not appropriate to reduce the number of factors, although the regression were considered acceptable with good PLS results.

In the Figure 15 are showed the correlations between the measured and predicted data of calibration for total phenols, *in vitro* antioxidant activity (ABTS), gallic acid and F1, which exhibited the best PLS results for models in the NIR region.

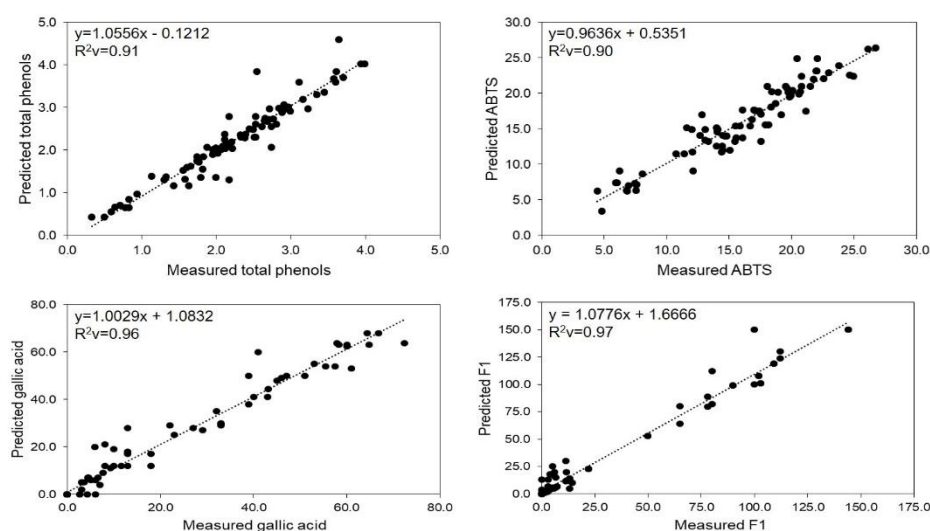


Figure 15 Correlation between the measured and predicted values by the calibration models obtained by NIR for the total phenols (1st deriv treatment), ABTS (1st deriv treatment), gallic acid (1st deriv treatment) and F1 (1st deriv treatment).

The standardized regression coefficients, in NIR, are presented in Figure 16. All plots correspond to regressions using 1st deriv (total phenols, *ortho*-diphenols, flavonoids, DPPH, ABTS, catechin, F1, F3, F4, F6 and F7), except for FRAP and gallic acid, for which the best results were obtained with 1st deriv MN.

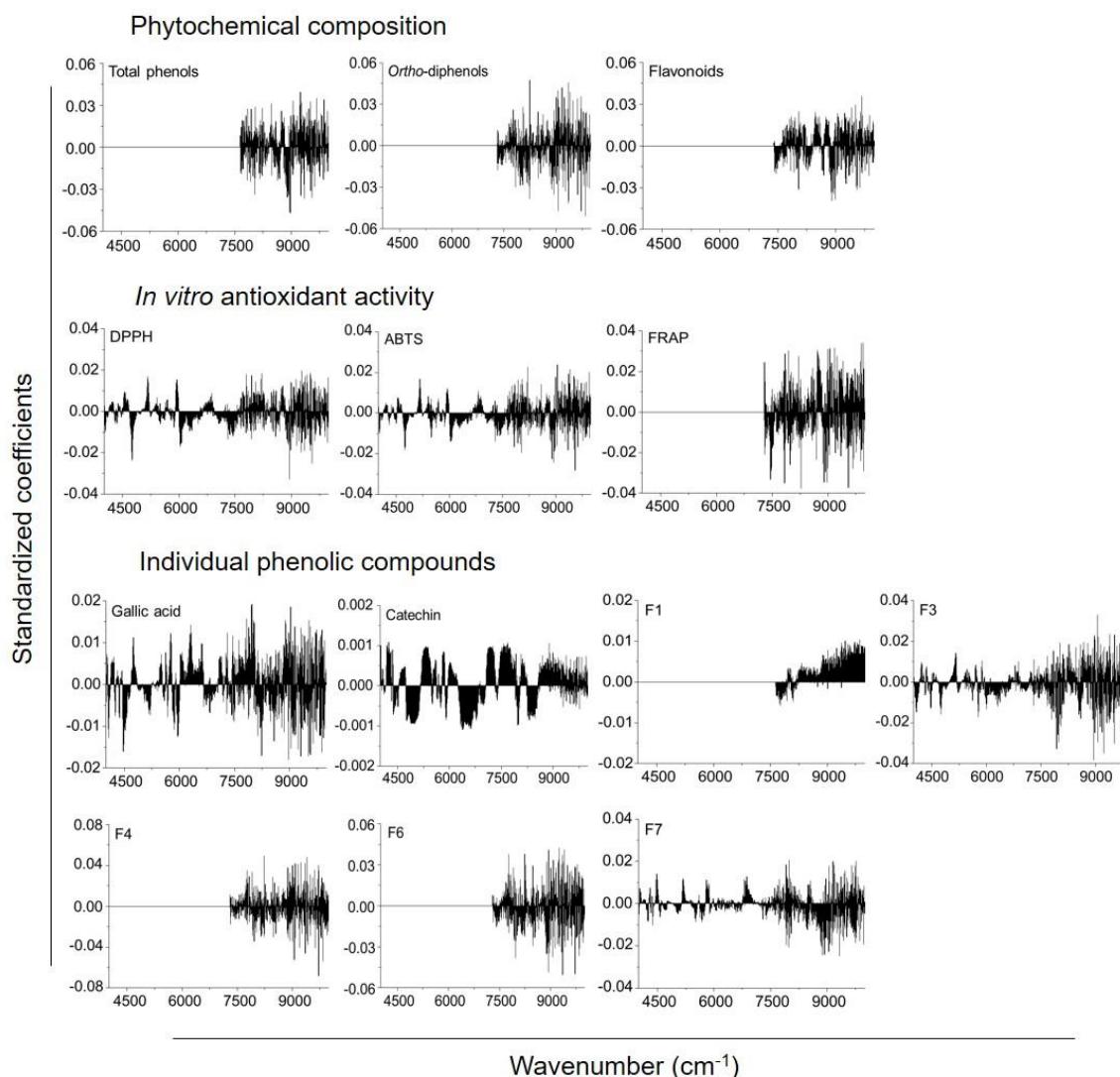


Figure 16 Standardized regression coefficients retrieved from the PLS procedure, corresponding to the best calibration developed with NIR data for each parameter

The most preponderant regions for the evaluation of phenolic contents have been described in the intervals between 4200–5500 cm⁻¹ and 6100–10000 cm⁻¹ (Wang et al., 2014), as observed for gallic acid, catechin, F3 and F7, corresponding to the use of 1st deriv and 1st deriv MN. For total phenols, *ortho*-diphenols, flavonoids, F1, F4 and F6, the dominant interval is above 7500 cm⁻¹, with regressions corresponding to 1st deriv. The plots corresponding to the

use of 1st deriv display two main intervals (7500–8000 cm⁻¹ and > 8800 cm⁻¹), while the most important intervals, in the regressions corresponding to 1st deriv MN, are evenly scattered throughout the spectral window used, displaying intervals with negative or positive contribution, but similar intensities (Figure 16). Summarizing, these observations show that the most determinant intervals for these calibrations, as observed for MIR, differ with the data pre-processing applied, with distinct spectral features being exploited for the development of prediction models, if different treatments are applied (Figures 14 and 16).

From the comparison between the performance of MIR and NIR, as well as the best approach, in each technique developed quantification models, it can be elated that the highest determination coefficients and lower PRESS, in MIR, were obtained from 1st deriv MN of the LF interval, while in NIR the best models were obtained with treatment 1st deriv. The PLS models for the estimation of *ortho*-diphenols, antioxidant activity (FRAP), F2, F4, F5 and F6, in the MIR region, were unsuitable for quantification, in all treatments ($R^2_v < 0.60$), the same occurring with catechin in NIR, which failed to provide regressions for F2 and F5, as observed for F1 in MIR (Tables 12, 13). Generally, PLS regressions failed to provide prediction models for the compounds presenting wider ranges of concentrations and lower mean values, which, to some extent, justifies the cases where no regression was successful. Summarizing, the models obtained with NIR data exhibited higher determination coefficients for calibration and validation, respecting MIR, except for catechin. Both FTIR techniques revealed to be suitable for the evaluation of individual phenolic compounds, in some cases, to determine rather low concentrations, such as in F3 (5.2 µg/g dw; $R^2_v = 0.84$), and *in vitro* antioxidant activity, for values as low as 2.0 µmol/TE g dw (Tables 11–13).

4.2.3.4. Classification of bean flour samples by PLS

PLS regressions were developed assuming the distinct samples as Y variable, to extract their variability to Factors, while the plots of the samples' scores, concerning these Factors, allowed assessing the associations between samples. The PLS plot obtained from the regression undertaken with phytochemical composition and *in vitro* antioxidant activity parameters as X variables, for the samples of the two harvest years, is presented in Figure 16. The first three Factors extracted explained 55.4% of total variability.

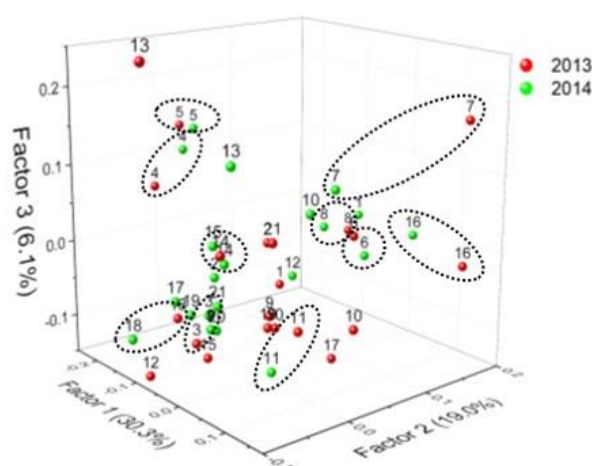


Figure 17 Plot of the results obtained for the PLS regressions developed with phytochemical composition and *in vitro* antioxidant activity of the bean samples from 2013 and 2014. The variability accounted by each Factor is presented between parentheses.

Concerning the cultivars 3, 4, 5, 6, 7, 8, 11, 13, 14, 16 and 18, the samples from the two distinct years are found together, as observed in Figure 17, showing that these cultivars present similar amounts of phenolic compounds and *in vitro* antioxidant activity in both years. Bean samples 6, 7, 8 and 16 present high scores for Factor 2, due to their higher phytochemical composition (total phenols, *ortho*-diphenols and flavonoids), *in vitro* antioxidant activity (DPPH, ABTS and FRAP), and high quantities of individual phenolic compounds (F6, F7, F8 and F9). The samples 4 and 5, displaying high negative scores for Factor 1, present lower *in vitro* antioxidant activity (FRAP), and low quantities of phytochemicals and individual phenolic (F1, F2 and F7). The negative scores for Factor 3, presented by samples 3, 11 and 18, are due to the high catechin contents (Table 11).

In the MIR region, the first three Factors extracted explained 70.8% of total variability, while the corresponding plot shows that bean samples from 2013 and 2014 are clearly separated, which is mainly due to Factors 1 and 2, one single exception being observed (Figure 18a). In NIR, the first three factors explained 73.0% of total variability. Generally, the bean flours were separated according to the harvest year, while concerning cultivars 8, 9, 11, 12, 14, 17 and 20, the samples from the distinct years are found together in the plane formed by Factor 1 vs. Factor 3, though being separated by Factor 2 (Figure 18b).

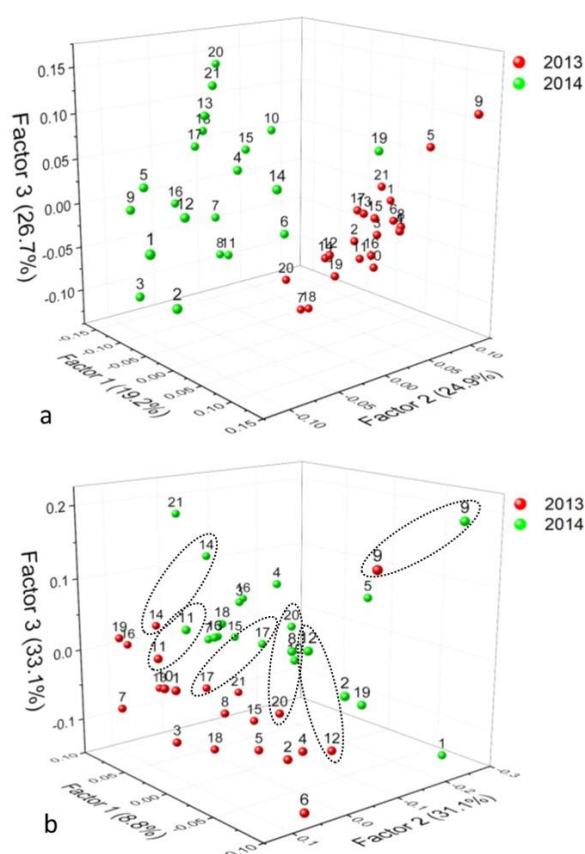


Figure 18 Plot of the results obtained for the PLS regressions developed with spectral data registered for the samples from both years: a) MIR region, treatment N; b) NIR region, treatment N. The variability accounted by each Factor is between parentheses.

These observations showed that, regarding the complete matrix, the yearly differences are larger than the variability between cultivars, which results in the separation observed between years, in the PLS regressions, since the spectra registered reflects all the composition of the flours, besides the parameters analysed (Kadiroğlu et al. 2018). Concerning the analytical parameters, the association observed between samples from the same cultivar, in distinct years, shows that the phytochemical parameters are more dependent on cultivar rather than harvest (Figure 18). Furthermore, in NIR, there are also visible association between samples of the same cultivar in distinct years, which is not observed in MIR, while this fact may be related to the best performance generally observed for NIR, regarding the models developed to predict the analytical parameters (Tables 12, 13, Figure 18).

4.2.4. Conclusions

The phytochemical composition (total phenols, *ortho*-diphenols and flavonoids), *in vitro* antioxidant activity (DPPH, ABTS and FRAP), and individual phenolic compounds (gallic acid, catechin, F1, F2, F3, F4, F5, F6 and F7), of bean flours, have been assessed, and the MIR and NIR spectra concomitantly registered, in order to develop spectroscopy based analytical approaches, resorting to PLS-R. Both techniques displayed suitability to be used for the quantification of phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds, while their performance, and use of distinct approaches, have been compared. In MIR, the use of 1st deriv MN treatment, applied to the restricted LF region (450 – 1799 cm⁻¹), has been the approach conducting to the best results. In NIR, the best models were developed using the 1st deriv treatment. The performance of NIR spectroscopy for the quantification of phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds was generally better, respecting MIR. The PLS regressions failed to provide quality models for the quantification of *ortho*-diphenols, FRAP, F1, F2, F4, F5 and F6 in MIR, the same occurring in NIR for catechin, F2 and F5. The regressions undertaken for compounds with higher ranges of concentration, such as *ortho*-diphenols, FRAP, F1, F4, F5 and F6 in MIR, and catechin in NIR, displayed worse performance, while regressions failed for individual phenolic compounds presenting high variability, F1 (0.0 – 150.2 ug g⁻¹) in MIR, and F2 (0.0 – 126.9 ug g⁻¹) and F5 (0.0 – 30.3 ug g⁻¹) in NIR. Nevertheless, both techniques exhibited good performances for the quantification of individual phenolic compounds, reaching concentrations as low as 5.2 ug g⁻¹ dw, as observed for F3.

Finally, further research is recommended to include additional bean cultivars, as well as samples from distinct harvests, with different concentration ranges, for the development of more comprehensive and reliable predictive models for the assessment of the phytochemical composition and *in vitro* antioxidant activities of common bean flours through this methodology.

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Chapter 5

Final remarks and conclusions

Final remarks and conclusions

There is currently a marked increase in the interest of food products with health benefits. Beans are a source of nutritional and phenolic compounds, used both in diet and in the prevention of many diseases. However, there is a considerable lack in the literature about the nutritional, antinutritional, bioactive compounds characterization and *in vitro* antioxidant activity of distinct bean cultivars, and hence hampering their potential uses in industry. At the same time, the research of specific compounds in food-like samples have been associated with time-consuming and massive use of chemicals. New infrared spectroscopic methodologies should be adopted in food laboratories and industry to solve this problem due to their easier procedures, faster application and chemical free features.

Within, chapter 3 was trying to address the lack in the nutritional, antinutritional and phytochemical characterization of different common bean cultivars to study their potential for food applications. According to the results shown in sub-chapter 3.1, navy, black and pink eyed beans showed the highest amounts of protein content and profile in amino acids, but concomitantly, black beans exhibited also lower amounts of antinutrients. The cultivars of type kidney and arikara yellow reported higher phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds, while the lower amounts were found in uncoloured cultivars including navy, brown eyed and pink eyed. Altogether, this detailed characterization of the distinct groups of beans enables a potential boost of the use of specific beans in the food industry for the development or enrichment of food products with health benefits. For instance, navy cultivars can be particularly used for nutritional enrichment of normal or for gluten-free products, mainly due to their higher protein content and essential amino acids (lysine). On the other hand, kidney bean cultivars showed considerable potential to be used as a functional ingredient to develop novel foodstuffs due to their higher amounts of phenolic compounds and antioxidant properties.

With the further objective of filling the gaps in the use of faster and eco-friendly innovative methodologies, in the chapter 4, two studies were performed using a fast and reliable spectroscopy method to evaluate the quality bean flours, for its application in laboratory and food industry, as an attempt to replace the traditional techniques. In both studies, FTIR techniques, MIR and NIR proved to be useful tools for the assessment of relevant parameters, not only regarding the macronutrients present in food but also for compounds present in low concentrations. In sub-chapter 4.1, both the MIR and NIR regions proved to be reliable

approaches for the prediction of protein, lipids, tannins, phytic acid and most of the amino acids present in bean flours. Hence, and according to the present results, the best treatment for the development of analytical models to quantify the protein, amino acids, tannins and phytic acids was the 1st derivative. Concerning MIR, the use of the full interval assessed (450–1800; 2600–3700 cm⁻¹) represented the most reliable approach for the development of analytical models. In sub-chapter 4.2., both techniques (MIR and NIR approaches) displayed suitability to be used for the quantification of phytochemical composition, *in vitro* antioxidant activity and individual phenolic compounds. The MIR approach providing the best results was the 1st derivative after normalization, applied to the restricted LF region (450–1799 cm⁻¹). As for NIR, the best models were developed using the 1st derivative treatment and its performance in assessing bioactive compounds was better than in MIR region. Nevertheless, both techniques exhibited good performances for the quantification of individual phenolic compounds, reaching concentrations as low as 5.2 ug/g dw, as observed for Quercetin 3-*O*-(6''-*O*-manoly)glucoside. It is recommended that this so far advantageous methodology is further tested with additional bean cultivars, as well as samples from distinct harvests, with different concentration ranges. This will enable the development of still missing (but desired) more comprehensive and reliable predictive models for the assessment of the phytochemical composition and *in vitro* antioxidant activities of common bean flours. Finally, our results revealed that the spectroscopical methodologies may represent an accurate and rapid method for quantification of nutritional, antinutritional, phytochemical characteristics and antioxidant activity of beans, thus, their applicability in the food industry representing an good alternative to the traditional approaches.

Chapter 6

Future perspectives

Future perspectives

This work secured still missing data on our current knowledge of the nutrients, antinutrients, phytochemicals and antioxidant properties of different bean cultivars and consequently, provided the foundation for a potential increase in their consumption and uses in the food industry. The next important contribution of the work was to showcase a novel and advantageous use of a spectroscopy methodology to evaluate the composition of beans. These exciting contributes nevertheless result in several open questions that can greatly benefit the field if answered such as:

- How to optimize the development of i) foodstuffs or formulations with specific bean cultivars to obtain food products with unique nutritional and bioactive characteristics and added-value health benefits and; ii) gluten-free products indicated to a specific group of consumers. These kinds of products could be used to help in the prevention of specific diseases (such as diabetes, cardiovascular disease, etc.) and make them adequate for people with celiac disease.
- The interesting antioxidant properties and phytochemical composition found in distinct cultivars of beans highlight the importance of evaluating its potential effects on health, mainly on diseases such as cancer, obesity and celiac disease. Hence, further *in vitro* and *in vivo* analyses are needed to evaluate the health potential of beans.

Based on the promising results regarding the application of spectroscopy methodologies to evaluate the macronutrients and micronutrients on beans, additional research is needed on its usefulness when applied to other characteristics of beans and to develop models for other legumes.

Annexes

Annexe I PLS regressions for the prediction of moisture and ash contents, corresponding to the most effective MIR interval for each treatment, and to the distinct treatments applied to the NIR data. All the models correspond to the optimal number of factors, which are indicated, for each parameter. Analytical data regarding both parameters are included.

Analytical Parameters (Range) (Mean \pm SD)	MIR				NIR			
	Treatment	PRESS (NF)	R^2_c	R^2_v	Treatment	PRESS (NF)	R^2_c	R^2_v
Moisture (13.01 - 15.51) (14.16 \pm 0.56)	1 st deriv (HF)	0.676 (3)	0.88	0.83	1 st deriv	0.628 (2)	0.84	0.83
	1 st deriv MN (FI)	0.699 (3)	0.88	0.80	1 st deriv MN	0.662 (2)	0.83	0.81
	Spectra (HF)	0.667 (6)	0.84	0.81	Spectra	0.664 (5)	0.84	0.82
Ash (2.94 - 3.61) (3.18 \pm 0.14)	1 st deriv (FI)	0.403 (6)	0.99	0.97	1 st deriv	0.419 (5)	0.99	0.98
	1 st deriv MN (FI)	0.419 (7)	0.99	0.98	1 st deriv MN	0.446 (3)	0.96	0.95
	Spectra (HF)	0.632 (7)	0.94	0.88	Spectra ^a	0.463 (6)	0.93	0.90

^a Moisture and ash content expressed as g/100g dw; FI- Full region; LF- Low-frequency region; HF- High-frequency region; NF - Number of factors; ^aSelected interval (6001 - 6502 cm⁻¹)