Novel Food and Feed Safety

Safety Assessment of Foods and Feeds Derived from Transgenic Crops, Volume 3

COMMON BEAN, RICE, COWPEA AND APPLE COMPOSITIONAL CONSIDERATIONS

OECD

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Please cite this publication as:
OECD (2019), *Safety Assessment of Foods and Feeds Derived from Transgenic Crops, Volume 3: Common bean, Rice, Cowpea and Apple Compositional Considerations*, Novel Food and Feed Safety, OECD Publishing, Paris, *https://doi.org/10.1787/f04f3c98-en*.

ISBN 978-92-64-79779-6 (print)
ISBN 978-92-64-38646-4 (pdf)

Novel Food and Feed Safety
ISSN 2304-9499 (print)
ISSN 2304-9502 (online)

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Foreword

From their first commercialisation in the mid-1990s, genetically engineered crops (also known as transgenic crops) have been increasingly approved for cultivation and for entering in the composition of foods or feeds by a number of countries. To date, genetically engineered varieties of at least 33 different plant species (including agricultural crops, ornamental plants and flowers, as well as trees) have received regulatory approvals in OECD countries and other economies from all regions of the world. However, the vast majority of plantings remains for soybean, maize, cotton and rapeseed (canola), the four species having covered together more than 99% of the global area of transgenic crops in 2018. Over the 23-year period from 1996 to 2018, the surface cultivated with genetically engineered crops has drastically raised worldwide, resulting in a significant increase of their harvest in human food and animal feed (often designated as "novel" foods and feeds). Analyses and statistics from several sources, despite some differences in total estimates, concur in highlighting the same following trends:

1. general rising in volumes of genetically engineered commodities produced
2. still a limited number of producing countries (they were 26 in 2018) compared to those having granted some approvals for food or feed consumption (70 countries in 2018, including the 28 members of the European Union)
3. growth potential for genetically engineered crops at a global level in future years, in particular if a wider range of species are brought into cultivation.

For instance, the *Global Status of Commercialized Biotech/GM Crops in 2018*, issued by the International Service for the Acquisition of Agri-Biotech Applications, reports a record 191.7 million hectares of genetically engineered plants grown, representing a growth rate of 1% from the previous year. According to this study, the five main producers in 2018 were in ranking order the United States, Brazil, Argentina, Canada and India covering together more than 91% of the total area. Interestingly, developing countries grew more global transgenic crops (54%) than industrial countries (46%). Among the 26 countries having planted transgenic crops in 2018, only 7 of them were OECD countries, listed by decreasing area as follows: the United States, Canada, Australia, Mexico, Spain, Chile and Portugal. However, an additional group of countries does not produce transgenic crops but imports the produced commodities, for use in their feed industry in particular, as is the case in most jurisdictions of European Union member states and several other economies worldwide. Important volumes of genetically engineered commodities are already subject, every year, to international trade.

Information on these transgenic crops which have been approved for commercial release in at least one country (for planting and/or for use in foods and feeds processing) can be found in the OECD BioTrack Product Database (https://biotrackproductdatabase.oecd.org). Each transgenic product and its Unique Identifier are described, as well as information on approvals in countries.

In parallel to the expansion of genetically engineered crops developed for their resistance to pests and diseases, varieties are being developed by breeders for new types of traits:

adaptation to climate change, improved composition (biofortification), enhanced meat productivity, easier processing and many other applications. The range of biotechnology applications to agricultural plant breeding is widening and it seems that this trend will continue. Consequently, the volume of novel foods and feeds available on the market and exchanged internationally is expected to increase in the coming years.

Consumers from all over the world are requiring a high level of safety and full confidence in the products they eat. This is particularly important for the products of modern biotechnology, which are sometimes questioned and subject to diverse levels of acceptation among countries. The approvals of transgenic crops follow a science-based risk/safety assessment regarding their potential release in the environment (biosafety) and their use in foods or feeds (novel food and feed safety). The OECD has undertaken activities related to environmental safety aspects since the mid-1980s, while the development of scientific principles for food safety assessment was initiated in 1990. The OECD helps countries in their risk/safety assessment of transgenic organisms by offering national authorities a platform to exchange experience on these issues, identify emerging needs, collate solid information and data, and develop useful tools for risk assessors and evaluators.

The Working Group for the Safety of Novel Foods and Feeds (previously named the "Task Force") is composed of delegates from OECD member countries, other economies, international bodies and observer organisations involved in these matters, from all regions of the world. National participants and experts come from government ministries and agencies that have responsibility for the risk and safety assessment of novel foods and feeds in the respective countries. The main outputs of the working group are the "consensus documents", practical tools for helping with food and feed safety assessment, which compile science-based information and data relevant to this task. These publications address compositional considerations of crops subject to plant breeding improvement with modern biotechnologies. The key composition elements (nutrients, anti-nutrients, toxicants and, sometimes, other constituents) that they contain can be used to compare novel foods and feeds with conventional ones. These documents are published after consensus is reached among countries, providing a science-based set of information and data designed for use in the comparative approach as part of the safety assessment.

To date, 24 OECD "consensus documents" relating to the safety of novel foods and feeds have been published. They provide solid information commonly recognised by experts and collate the reliable range of data available in the scientific literature at the time of the publication. In addition, reports on key events and documents of a broader nature aiming to facilitate harmonisation have been developed, for instance: animal feedstuffs derived from transgenic commodities (2003), designation of an OECD "Unique Identifier" for transgenic plants (2002, revised in 2006), molecular characterisation of transgenic plants (2010), proceedings of the OECD Workshop on High-throughput DNA Sequence in the Safety Assessment of Genetically Engineered Plants (2016).

Volume 3 of this series compiles the four consensus documents of the OECD Series on Safety of Novel Foods and Feeds issued between 2015 and 2019, dealing with the composition of common bean, rice (revising the original version of 2004), cowpea and apple. The presentation of the OECD work, originally published in 2006 and updated in 2014 with the previous compendia, was used as a basis for the introduction section that explains the purpose of the consensus documents, their relevance to risk/safety assessment and their preparation by the working group.

The consensus documents constituting the four chapters of this Volume 3 were approved by the OECD Joint Meeting of the Chemicals Committee and the Working Party on

Chemicals, Pesticides and Biotechnology (the "Joint Meeting") under written procedure and prepared for publication by the OECD Secretariat. The consensus document on the composition of common bean was approved on 18 December 2015 [ENV/JM/MONO (2015)49], the document on the composition of rice on 3 November 2016 [ENV/JM/MONO(2016)38], the document on the composition of cowpea on 7 December 2018 [ENV/JM/MONO(2018)36], and the document on the composition of apple on 12 July 2019 [ENV/JM/MONO(2019)23].

The present series (Volumes 1 to 3) offers ready access to those documents which have been published thus far. This set of science-based information and data, agreed by consensus and published by the OECD, constitute a solid reference recognised internationally. It is already widely used in comparative approach as part of the risk/safety assessment of transgenic products. As such, this publication should be of value to applicants for commercial uses of genetically engineered crops, to regulators and risk assessors in national authorities in charge of granting approvals to transgenic plant products for their use as foods or feeds, as well as to the wider scientific community.

Each of the consensus documents may be updated in the future as new knowledge becomes available. Three of them dealing with key crops (canola, soybean and rice) have already been revised and recently updated in order to maintain their full relevance to risk assessors using them. Users of this book are therefore encouraged to provide information or an opinion regarding the contents of the consensus documents or any of the OECD's other harmonisation activities. Comments can be sent to ehscont@oecd.org.

The published consensus documents are also available individually from the OECD Biotrack website (www.oecd.org/biotrack) at no cost.

Acknowledgements

This book is the result of the common effort of the participants in the OECD Working Group for the Safety of Novel Foods and Feeds. Each chapter is composed of a "consensus document" which was prepared under the leadership of one or several countries, as listed at the end of this volume. During the preparation of their successive drafts, useful input and suggestions for the documents were provided by a number of delegates and experts from the working group, whether from OECD member countries, other economies or observer organisations.

Each consensus document was issued individually, as soon as finalised and agreed on declassification, by the OECD Environment, Health and Safety (EHS) Division in the Series on Safety of Novel Foods and Feeds. This volume, containing the consensus documents issued from 2015 to 2019, was prepared by Eleonore Morena, with the contribution of Yoko Takasu. It was edited by Bertrand Dagallier, under the supervision of Peter Kearns, at the EHS Division of the OECD Environment Directorate.

The OECD is grateful to the scientists, regulators and authorities who participated in the development of these consensus documents on compositional considerations for new varieties of crop species and wishes to thank each of them.

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Abbreviations and acronyms

1-MCP	1-methylcyclopropene
AAS	Amino acid score
ADF	Acid detergent fibre
AIU	α–amylase inhibitory unit
AUDA NEPAD-ABNE	Biosafety Network of Expertise of the African Union Development Agency
BGMV	Bean golden mosaic virus
BIAC	Business and Industry Advisory Committee to the OECD
°C	Degree Celsius
CA	Controlled atmosphere (for apple storage)
Ca	Calcium
CGIAR	Consultative Group on International Agricultural Research
CIU	Chymotrypsin inhibitor unit
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats – associated protein 9
DNA	Deoxyribonucleic acid
DW	Dry weight basis
EHS	OECD Environment, Health and Safety Division, Environment Directorate
ELISA	Enzyme-linked immunosorbent assay
Embrapa	Brazilian Agricultural Research Corporation
°F	Degree Fahrenheit
FAO	Food and Agriculture Organization of the United Nations
FW	Fresh weight basis
g	Gramme
h	Hour
HU	Haemagglutinating unit
ILRI	International Livestock Research Institute
ILSI	International Life Research Institute
IRRI	International Rice Research Institute
IP5	Inositol pentaphosphate
IP6	Inositol hexakisphosphate
ISAAA	International Service for the Acquisition of Agri-Biotech Applications
Joint Meeting	OECD Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology
K	Potassium

kDA	KiloDalton (atomic mass unit)
kg	Kilogramme
kJ	Kilojoule
kt	Kilotonne
µg	Microgramme
mg	Milligramme
Mg	Magnesium
min	Minute
ml	Millilitre
Mt	Million tonnes
n	Number of samples
NDF	Neutral detergent fibre
NFE	Nitrogen-free extract
OECD	Organisation for Economic Co-operation and Development
P	Phosphorus
RASI	Rice alpha-amylase/subtilisin inhibitor
RBTI	Rice bran trypsin inhibitor
RFOs	Raffinose family oligosaccharides
TDN	Total digestible nutrients
TI	Trypsin inhibitor
TIU	Trypsin inhibitor unit
UCR	University of California
WG-SNFF	OECD Working Group for the Safety of Novel Foods and Feeds
WHO	World Health Organization

Executive summary

This document constitutes the third volume of the OECD Series on Novel Food and Feed Safety. It is a compendium collating in a single publication the individual "consensus documents" on the composition of crops published by the OECD Working Group for the Safety of Novel Foods and Feeds from 2015 to 2019. The plant species covered by this Volume 3, presented in the order of their initial publication, are common bean, rice, cowpea and apple. The four crops are of highly significant importance in global agricultural production and the human diet.

The consensus documents on common bean, cowpea and apple composition are new to the series, while the publication on rice composition revises and updates the original issue of 2004, therefore replacing the rice chapter previously included in Volume 1.

The consensus documents prepared by the working group focus on compositional considerations for plants that can be subject to genetic engineering and development of "transgenic" crop varieties. Each chapter opens with background information on the species under consideration: its production, transformation process and uses for foods and feeds, followed by a brief summary on appropriate comparators for testing new varieties and screening characteristics used by breeders. Then the core of the chapter collates detailed information and solid data on compositional elements: key nutrients and anti-nutrients, toxicants, other metabolites and allergens where applicable. The main nutrients identified for each crop include usually proximate elements, carbohydrates, fibres, proteins, lipids, minerals and vitamins. Depending on the considered species, the important anti-nutrients and other constituents might be for instance phytic acids, tannins, saccharides, alkaloids, polyphenols or inhibitors, including allergenic elements in some cases. The final section of each chapter suggests key products and constituents for analysis of new varieties for food use and feed use, these analyses being conducted in a comparative approach as part of a safety assessment.

Modern biotechnologies are applied to plants, and also trees, animals and microorganisms. The safety of the resulting products represents a challenging issue, in particular for genetically engineered crops that are increasingly cultivated and foods or feeds derived from them marketed worldwide. The novel products should be rigorously assessed by governments to ensure high safety standards for the environment, human food and animal feed. Such assessments are considered essential for healthy and sustainable agriculture, industry and trade.

Since 1999, the OECD Working Group for the Safety of Novel Foods and Feeds assist countries in evaluating the potential risks of transgenic products, foster communication and mutual understanding of relevant regulations in countries, and facilitate harmonisation in risk/safety assessment of products from modern biotechnology. This is intended to encourage information sharing, promote harmonised practices and prevent duplication of efforts among countries. Therefore, the working group's programme contributes to reducing costs and potential for non-tariff barriers to trade, while consolidating high food and feed safety standards. Focused on novel foods and feeds derived from genetically engineered organisms, the working group's activities and outputs are directly

complementary to those of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology, which deals with environmental safety.

OECD member countries, other economies, international bodies and observer organisations from all regions of the world take part in the working group. National participants and experts come from the ministries and agencies responsible for the risk and safety assessment of novel foods and feeds. Delegates exchange experience and information, identify new needs and develop practical tools for helping the assessment. The main outputs are the "consensus documents", which compile science-based information and data relevant to this task. The key composition elements that they contain can be used to compare novel foods and feeds with conventional ones.

These documents, agreed by consensus among countries and published by the OECD, constitute a solid reference recognised internationally. They are widely used in comparative approach as part of the risk/safety assessment of transgenic products. As such, this publication should be of value to applicants for commercial uses of genetically engineered crops, to regulators and risk assessors in national authorities in charge of granting approvals to transgenic plant products for their use as foods or feeds, as well as to the wider scientific community.

Introduction to OECD work on novel food and feed safety

OECD activities on novel food and feed safety

The OECD Task Force for the Safety of Novel Foods and Feeds was established in 1999, with the primary goal to promote international regulatory harmonisation in the risk and safety assessment of biotechnology products among member countries, by addressing aspects of the assessment of human food and animal feed derived from genetically engineered crops. This body was renamed the Working Group for the Safety of Novel Foods and Feeds (WG-SNFF) from 1 January 2017.

The terms "novel foods and feeds" usually relate to foods and feeds derived from transgenic organisms, that is, partly or fully composed of such ingredients. By extension, these terms can also be understood as foods and feeds containing products obtained from other modern biotechnology techniques. Regulatory harmonisation is the attempt to ensure that the information used in risk/safety assessments, as well as the methods used to collect such information, are as similar as possible. It could lead to countries recognising or even accepting information from one another's assessments. The benefits of harmonisation are clear: it increases mutual understanding among member countries, which avoids duplication, saves on scarce resources and increases the efficiency of the risk/safety assessment process. This, in turn, improves food and feed safety while reducing unnecessary barriers to trade (OECD, 2000).

The WG-SNFF comprises delegates from the 36 member countries of the OECD and the European Commission. The OECD member countries span the globe, from North and South America to Europe and Asia-Pacific. They include many of the world's most advanced countries but also emerging countries like Chile, Mexico and Turkey. A number of observer delegations and invited experts also participate in the WG-SNFF work, from Argentina, Colombia, the Russian Federation and South Africa, as well as the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the Business and Industry Advisory Committee to the OECD (BIAC) and other relevant organisms such as the ILSI Research Foundation and the Biosafety Network of Expertise of the African Union (AUDA NEPAD-ABNE). Since 2004, several additional partner countries (Bangladesh, Brazil, the People's Republic of China, Costa Rica, India, Indonesia, Kenya, Moldova, Paraguay, Philippines, Uruguay, Viet Nam, Thailand and others) have participated occasionally or on a regular basis in the WG-SNFF activities, invited under the auspices of OECD Global Relations Secretariat and its Global Forum on Biotechnology.

Typically, delegates of the WG-SNFF come from government ministries and agencies which have responsibility for the food or feed safety assessment of products of modern biotechnology. In some OECD countries, this is the Ministry of Health; in others, it is the Ministry of Agriculture. Other countries have specialised agencies with this responsibility. Often, it is a shared responsibility among more than one ministry or agency. The expertise that these delegates have in common is related to their experience with the safety assessment of novel foods and feeds.

The emergence of the concept of consensus documents on compositional considerations

By 1997, several OECD countries had gained experience with safety assessment of foods derived through modern biotechnology. An OECD workshop in Aussois, France, examined the effectiveness of the application of substantial equivalence in safety assessment. It was concluded that the determination of substantial equivalence provides equal or increased assurance of the safety of foods derived from genetically engineered plants, as compared with foods derived from conventional methods (OECD, 1997).

At this event, it was also recognised that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (e.g. key nutrients, key toxicants and anti-nutritional compounds) on a crop-by-crop basis, which should be considered in the comparison. It is recognised that the components may differ from crop to crop.

Following the Aussois workshop, the question of whether there was a need to undertake work on food/feed safety at the OECD, and if so, what that work would entail, was examined. The in-depth analysis was conducted by an Ad Hoc Group on Food Safety (established by the Joint Meeting).[1] It took into account the results of national activities and those of previous OECD work, as well as the activities of the FAO and the WHO.

As a result of the Ad Hoc Group on Food Safety's activities, the Joint Meeting established the WG-SNFF, with a major part of its programme of work being the development of consensus documents on compositional data. These data are used to identify similarities and differences following the comparative approach as part of a food and feed safety assessment. They should be useful to the development of guidelines, both national and international, and to encourage information sharing among OECD countries and beyond.

Participation from non-OECD countries is strongly encouraged by the WG-SNFF. In line with the biotechnology development in recent years, "an increasing emphasis has been placed on crops of interest to developing countries and countries in transition including those with tropical climates" (Kearns, Dagallier and Nikaido, 2017). As transgenic crops are grown in several of these economies, their commodities are traded internationally and widely used for food and feeds. This exchange has increased over the years, and now more actively involves their expertise in OECD work. For example: the consensus documents on the composition of cassava, grain sorghum, papaya and common bean were developed under the leadership or co-leadership of Brazil, South Africa and Thailand; the Philippines have actively contributed to the revision of the rice composition document; Nigeria provides expertise for the continuing revision of the potato composition document. This concrete enlargement to non-OECD members' input and competency broadens the expertise available to the WG-SNFF, while addressing a wider range of food and feed products that are of global interest.

Background and principles surrounding the use of consensus documents

The OECD "consensus documents on compositional considerations" are a compilation of current information that is important in food and feed safety assessment. Agreed by consensus among the WG-SNFF participants, they provide a technical tool for regulatory officials, industry and other interested parties, as a general guide and reference source. They complement those of the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology which deal with the environmental safety aspects (biosafety)

(OECD, 2017; 2016a; 2016b; 2006a; 2006b; 2010a; 2010b). They are mutually acceptable to, but not legally binding for, member countries and are also used as key references by other economies beyond the OECD for their assessment of novel foods and feeds. They do not intend to offer a comprehensive description of all the issues considered necessary for a safety assessment but provide a base set for an individual product that supports the comparative approach. In assessing an individual product, consideration of additional components may be required depending on the specific case in question.

The work of the WG-SNFF builds on previous OECD experience in biotechnology safety-related activities, dating back to the mid-1980s. Initially, much of the work concentrated on the environmental and agricultural implications of the use of transgenic crops. By the end of 1990, it was decided to develop scientific principles for food safety assessment of products of modern biotechnology. This work was undertaken in collaboration with the FAO and WHO.

In 1990, a joint consultation of the FAO and the WHO established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO, 1991).

In 1993, the OECD further elaborated this concept and advocated the safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology (as well as other methods of modifying a host genome, including tissue culture methods and chemical- or radiation-induced mutation).

In 1996, a Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety elaborated on compositional comparison as an important element in the determination of substantial equivalence. A comparison of critical components can be carried out at the level of the food source (i.e. species) or the specific food product. Critical components are determined by identifying key nutrients and key toxicants and anti-nutrients for the food source in question. The comparison of critical components should be between the modified variety and non-modified comparators with an appropriate history of safe use. The data for the non-modified comparator can be the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO/WHO, 1996). The comparator used to detect unintended effects for all critical components should ideally be the near-isogenic parental line grown under identical conditions. While the comparative approach is useful as part of the safety assessment of foods derived from plants developed using recombinant-DNA technology, the approach could, in general, be applied to foods derived from new plant varieties that have been bred by other techniques.

In 2000, the Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology (FAO/WHO, 2000) concluded that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process, although it is not a safety assessment in itself; it does not characterise hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The consultation

concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework.

Between 2000 and 2003, the ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology to the Codex Alimentarius Commission ("Codex Task Force") undertook work to develop principles and guidelines for foods derived from genetically engineered plants. The full report of the Codex Task Force included:

- principles for the risk analysis of foods derived from modern biotechnology
- a guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants
- a guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms (Codex Alimentarius Commission, 2003).

One notable feature of the principles is that they refer to a safety assessment involving the comparative approach between the food derived from modern biotechnology and its conventional counterpart. Annex II (safety assessment of foods derived from recombinant-DNA plants modified for nutritional or health benefits) and Annex III (safety assessment in a situation of low-level presence of recombinant-DNA plant material in food) were added to the guidelines in 2008.

The OECD WG-SNFF continues to collaborate with the Secretariat of the Codex Alimentarius Commission in order to strengthen their complementary activities.

The process for preparing consensus documents on compositional considerations

The consensus documents are prepared by the WG-SNFF on official proposals by countries. Typically, the focus is a food crop or vegetable for which modern biotechnology can be used in the plant-breeding process. New improved varieties of these species are being developed by researchers for future release in at least one country, or even exist already at a commercial level for some of them.

The WG-SNFF establishes ad hoc drafting groups, composed of officials and scientific experts of the species in interested countries. These drafting groups work with all this diversity of inputs, under the co-ordination of "lead countries". The successive revised drafts are reviewed by the full WG-SNFF, with careful examination of the proposed information, data, tables and figures. The several revisions and completions can require a few years, leading to a consensus from all delegations obtained on all elements. Following an OECD internal process for final approval, the document is published and becomes available online for worldwide users.

The OECD BioTrack website provides publications and news from the WG-SNFF, the Series on Novel Food and Feed Safety, contact details of national safety systems and other information. It links to the biosafety (environmental safety) publications, the Series on Harmonisation of Regulatory Oversight in Biotechnology. It also gives free access to the OECD BioTrack Product Database, available at www.oecd.org/biotrack.

Current and future trends

With the growing development of products from modern biotechnology, the production of transgenic crops has increased drastically in the last 23 years (ISAAA, 2018). It might even

expand in the future if new varieties adapted to emerging needs are adopted. Potential applications could encompass agriculture, industry, health and energy sectors.

Resistance to pests and diseases were introduced in plants from the early stages of genetic engineering and still constitute an essential feature of the varietal improvement for agriculture, horticulture and forestry. In parallel, breeders are also working on incorporating new traits in crops to gain other types of beneficial effects. Some of these varieties are about to enter the market or at initial stage of cultivation. In recent years, drought-tolerant varieties (maize, sugarcane) have been developed to contribute to climate change adaptation. "Innovation in plant breeding (including biotechnology) that aims to develop crop varieties that are more resilient to climate change impact (e.g. resistance to drought, soil salinity or temperature extremes) is part of a larger basket of possible adaptation options in agriculture" (Agrawala et al., 2012). Other innovative traits can have a direct beneficial impact on foods and feeds, and some are already promising: staple crops (rice, tubers, other species) offering nutritive improvements with increased content (biofortification) of elements such as pro-vitamins or micro-nutrients, feed plants (for example, maize, alfalfa) modified for higher digestibility and meat productivity, and many other products under development. The range of biotechnology applications to plant breeding continues to widen, leading to an expected increase of derived foods and feeds used and exchanged internationally in the coming future. In addition, strains of Atlantic salmon genetically engineered to include fast-growing trait have been recently approved for commercial production, the first animal species of which novel food can be marketed in a few countries.

Among the new breeding techniques developing at quick pace in recent years, genome editing refers to techniques able to modify specialised enzymes by insertion, replacement or removal of DNA fragment from a genome with a high degree of specificity. Genome editing, and its most discussed techniques such as the CRISPR/Cas9 system, has received increasing attention in the research sector and wider media. This advanced form of genetic engineering provides tools at relatively low cost for innovation in biomedicine, agriculture, industrial biotechnology and other sectors relating to the bioeconomy. Already successfully used in agriculture for plant crops and farm animals' husbandry, genome editing improves the efficiency of the breeding and offers new possibilities for the control of pests and diseases, as well as many other beneficial traits. The rapidly-growing use of genome editing has human health and environmental safety considerations, and policy implications that need to be understood. For facilitating the process in a first initiative, the OECD Conference on Genome Editing: Applications in Agriculture – Implication for Health, Environment and Regulation was organised in June 2018. The event aimed to review the situation and open discussion among policymakers, academia, innovators and other stakeholders involved in the topic. The proceedings of the event were published in 2019 (Transgenic Research, 2019). The WG-SNFF, which was actively involved in the preparation of the conference, keeps the item on its work agenda in order to continue the exchange of information, keep track of scientific and regulatory developments in countries, identify points of divergences and synergies, and contribute to better harmonisation.

A reliable risk/safety assessment of novel foods and feeds is, therefore, more than ever a necessity for many world economies in the context of international trade of commodities. Release of such products should be based on solid information and appropriate tools for leading to national decision-making. Harmonised regulations, common practices and easy access to solid science-based compiled information are sought. The tools developed by the OECD Working Group for the Safety of Novel Foods and Feeds are designed to promote international harmonisation in the field of food/feed safety assessment. These outputs are

recognised and appreciated, and might play an increasing role in fulfilling the safety assessment needs in the future.

The WG-SNFF is continuing its work on a range of issues. The development of consensus documents on compositional considerations should still constitute the main area of the 2021-24 programme of work. Emerging topics are also considered in order to remain reactive to demand, for example other new biotechnology techniques including genome editing, innovative feed ingredients, animal composition data, all of them to be considered in light of food and feed safety issues.

In parallel, the consensus documents are reviewed periodically and updated as necessary to ensure that scientific and technical developments are taken into account. Users of these documents have been invited to provide the OECD with new scientific and technical information and to make proposals for additional areas to be considered. For example, the documents on the composition of low erucic acid rapeseed (canola), soybean and rice, originally published between 2001 and 2004, were completed and revised by the WG-SNFF, leading to updated issues in 2011, 2012 and 2016 respectively. The potato and maize composition documents (both issued in 2002) have initiated a revision process and others might follow in the coming years.

In order to enlarge dissemination of the scientific information and risk assessment tools produced by the WG-SNFF, the consensus documents are regularly collated in "compendia" published in the OECD Series on Novel Food and Feed Safety. Volume 1 and Volume 2 were issued in 2015, containing the consensus documents produced from 2002 to 2008 and from 2009 to 2014 respectively (OECD, 2015b; 2015a). This Volume 3 covers the 2015-19 period, dealing with the composition of common bean, rice (which revises the original version of 2004), cowpea and apple.

Note

[1] The Joint Meeting was the supervisory body of the Ad Hoc Group and, as a result of its findings, established the WG-SNFF as a subsidiary body. Today, its full title is the Joint Meeting of the Chemicals Committee and the Working Party ons Chemicals, Pesticides and Biotechnology.

References

Agrawala, S. et al. (2012), "Adaptation and innovation: An analysis of crop biotechnology patent data", *OECD Environment Working Papers*, No. 40, OECD Publishing, Paris, http://dx.doi.org/10.1787/5k9csvvntt8p-en.

Codex Alimentarius Commission (2003), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants*, CAC/GL 45/2003, Annexes II and III adopted in 2008, www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf (accessed on 28 Jan. 2015).

FAO/WHO (2000), *Safety Aspects of Genetically Modified Foods of Plant Origin,* Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology Food and Agriculture Organization, Rome, www.fao.org/fileadmin/templates/agns/pdf/topics/ec_june2000_en.pdf (accessed on 28 January 2015).

FAO/WHO (1996), "Joint consultation report, biotechnology and food safety", *FAO Food and Nutrition Paper No. 61*, Food and Agriculture Organization, Rome, www.fao.org/ag/agn/food/pdf/biotechnology.pdf (accessed on 28 January 2015).

ISAAA (2018), "Global status of commercialized biotech/GM crops in 2018: Biotech crops continue to help meet the challenges of increased population and climate change", *ISAAA Brief No. 54*, International Service for the Acquisition of Agri-Biotech Applications: ISAAA, Ithaca, New York, http://www.isaaa.org/resources/publications/briefs/54/default.asp (accessed on 5 September 2019).

Kearns, P., B. Dagallier and T. Nikaido (2017), "The sharing of information in risk assessment: How national authorities cooperate", in A.A. Adenle, E.J. Morris and D.J. Murphy (eds), *Genetically Modified Organisms in Developing Countries: Risk Analysis and Governance,* Cambridge University Press, Cambridge, https://doi.org/10.1017/9781316585269.012.

OECD (2017), *Safety Assessment of Transgenic Organisms in the Environment, Volume 7: OECD Consensus Documents*, Harmonisation of Regulatory Oversight in Biotechnology, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264279728-en.

OECD (2016a), *Safety Assessment of Transgenic Organisms in the Environment, Volume 6: OECD Consensus Documents*, Harmonisation of Regulatory Oversight in Biotechnology, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264253421-en.

OECD (2016b), *Safety Assessment of Transgenic Organisms in the Environment, Volume 5: OECD Consensus Documents*, Harmonisation of Regulatory Oversight in Biotechnology, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264253018-en.

OECD (2016c), "Report of the OECD Workshop on Environmental Risk Assessment of Products Derived from New Plant Breeding Techniques (February 2014)", *Series on Harmonisation of Regulatory Oversight in Biotechnology No. 61*, OECD, Paris, www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)5&doclanguage=en.

OECD (2015a), *Safety Assessment of Foods and Feeds Derived from Transgenic Crops, Volume 2, Novel Food and Feeds Safety*, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264180338-en.

OECD (2015b), *Safety Assessment of Foods and Feeds Derived from Transgenic Crops, Volume 1, Novel Food and Feeds Safety*, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264180147-en.

OECD (2010a), *Safety Assessment of Transgenic Organisms: OECD Consensus Documents: Volume 4*, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264096158-en.

OECD (2010b), *Safety Assessment of Transgenic Organisms: OECD Consensus Documents: Volume 3*, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264095434-en.

OECD (2009), *The Bioeconomy to 2030: Designing a Policy Agenda*, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264056886-en.

OECD (2006a), *Safety Assessment of Transgenic Organisms: OECD Consensus Documents: Volume 2*, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264095403-en.

OECD (2006b), *Safety Assessment of Transgenic Organisms: OECD Consensus Documents: Volume 1*, OECD Publishing, Paris, http://dx.doi.org/10.1787/9789264095380-en.

OECD (2000), *Report of the Task Force for the Safety of Novel Foods and Feeds*, Prepared for the G8 Summit held in Okinawa, Japan on 21-23 July 2000, C(2000)86/ADD1, OECD, Paris, www.oecd.org/chemicalsafety/biotrack/REPORT-OF-THE-TASK-FORCE-FOR-THE-SAFETY-OF-NOVEL.pdf (accessed on 31 January 2019).

OECD (1997), *Report of the OECD Workshop on the Toxicological and Nutritional Testing of Novel Foods*, Workshop held in Aussois, France, 5-8 March 1997, final text issued in February 2002 as SG/ICGB(98)1/FINAL, OECD, Paris, www.oecd.org/env/ehs/biotrack/SG-ICGB(98)1-FINAL-ENG%20-Novel-Foods-Aussois-report.pdf (accessed on 31 January 2019).

Transgenic Research (2019), "Proceedings of the 'OECD Conference on Genome Editing: Applications in Agriculture – Implications for Health, Environment and Regulation' held on 28-29 June 2018", *Transgenic Res. Volume 28, Issue Supplement*, August 2019, Springer, https://link.springer.com/journal/11248/28/2/suppl/page/2.

WHO (1991), *Strategies for Assessing the Safety of Foods Produced by Biotechnology*, Report of a Joint FAO/WHO Consultation, World Health Organization of the United Nations, Geneva, out of print.

Compositional considerations for new varieties of crop species: Key food and feed nutrients, anti-nutrients and other constituents

Chapter 1. Common Bean (*Phaseolus vulgaris*)

*This chapter deals with the composition of common bean (*Phaseolus vulgaris *L.). It contains elements that can be used in a comparative approach as part of a safety assessment of foods and feeds derived from new varieties. Background is given on bean production worldwide, common bean processing for industrial canning and other uses for human and animal consumption, followed by appropriate varietal comparators and characteristics screened by breeders. Nutrients in common bean seed, as well as main anti-nutrients, toxicants and other constituents, are then detailed. The final sections suggest key constituents for whole-grain analysis of new common bean varieties for food use and for feed use.*

This chapter was prepared by the OECD Working Group for the Safety of Novel Foods and Feeds, with Brazil as the lead country. It was initially issued in December 2015. FAOSTAT data on production and trade, including Table 1.1, have been updated.

Background

*General description of common bean (*Phaseolus vulgaris *L.)*

Common bean (*Phaseolus vulgaris* L.) is a major grain legume which is consumed worldwide for its edible seeds and pods (Heuzé et al., 2013) (Figures 1.1 and 1.2). Wild common bean [*Phaseolus vulgaris* L., tribe Phaseoleae, family Leguminosae (Schrire, 2005)] is present throughout Central and South America (Gepts and Debouck, 1991; Freytag and Debouck, 2002). All cultivated varieties grown in the world today originate from two independent domestication events of wild populations at different pre-Columbian times (Kaplan and Lynch, 1999; Piperno, 2012) in western Mexico (Kwak et al., 2009) and in central Peru (Chacón-Sánchez et al., 2005). Human selection has generated dozens of landraces in each region (Singh et al., 1991). After 1492, the common bean was taken to South-Western Europe (Rodiño et al., 2006), the Mediterranean region (Angioi et al., 2010), (mostly eastern) Africa (Westphal, 1974), parts of Asia (Zhang et al., 2008) and back to the Americas (Albala, 2007; Gepts et Bliss, 1988).

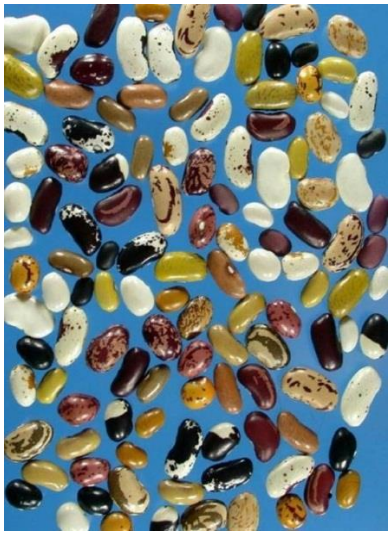
Figure 1.1. Pods of bush-type common bean

Source: Courtesy of D.G. Debouck, CIAT (2015).

Given this geographical and ecological expansion, the common bean is known by a variety of names under generic "bean" terms such as "frijol" in Spanish-speaking Latin America, "feijão" in Brazil, "judia" in Spain and "haricot" in French (Voysest and Dessert, 1991).

The common bean is an herbaceous vine. While it is an annual and monocarpic plant, some of its most primitive forms and wild relatives are pluri-annual and polycarpic vines in montane forests in Mexico and Central America (Freytag and Debouck, 2002). Cultivars vary widely, with bush determinate and vining indeterminate growth habits, and are selected for earliness. Further description of the common bean taxonomy, centres of origin and diversity, reproductive biology, genetics, hybridisation and introgression, general interactions with other organisms (ecology), common pests and pathogens, and biotechnological developments can be found in the Consensus Document on the Biology of Common Bean (*Phaseolus vulgaris* L.) (OECD, 2015).

Figure 1.2. Shape and colour diversity in common bean seed



Source: Courtesy of D.G. Debouck, CIAT (2015).

The common bean is typically cultivated in a mono-crop system and mechanically harvested (Figure 1.3). Although leaves and rarely flowers are consumed by humans (Purseglove, 1968), its main products are seeds, which are harvested either before or after physiological maturity as green pods such as snap beans (also known as "green beans") or dry beans respectively. Both forms have given rise to an important canning industry and, recently, frozen dried food products have also appeared on world markets. Most dry bean varieties are consumed after boiling; grains of some landraces, mostly central Andean, are consumed after toasting (Tohme et al., 1995). Dried stems and pods have been used as hay for animal feeding (Hendry, 1918; Westphal, 1974).

Figure 1.3. Large field of common bean crop (Pimampiro canton, Ecuador)

Source: Courtesy of D.G. Debouck, CIAT (2015).

Production

The common bean is produced in subtropical and tropical regions, most often by smallholders, and constitutes a major staple crop in both developing and developed countries. Mainly used for human consumption, the common bean is the most important grain legume in the human diet at global level. According to the Consultative Group on International Agricultural Research, the common bean provides protein, complex carbohydrates and valuable micronutrients for more than 300 million people in the tropics. In many areas, beans are the second most important source of calories after maize (CGIAR, n.d.).

Quantification of the world production of the common bean is difficult, first because a substantial part of the crop is consumed on-farm, with limited sale on local markets, and has not been documented. The second reason lies in the fact that some dry beans subject to national and/or international trade are not discriminated at the species level. As a result, a category reported as "pulses" or "beans" may include several legume species other than the common bean (*P. vulgaris* L.) such as other *Phaseolus* sp. beans and even some *Vigna* sp. (Lackey, 1981; Voysest, 1983; FAOSTAT, 2019). Finally, the diverse products of the common bean, while all derived from the same species, may be counted under different categories. For example, snap beans (green beans) may be tallied separately from dry beans (Voysest and Dessert, 1991).

According to FAO estimates, the global bean production (covering not only the common bean) has risen from 16.6 million tonnes (Mt) in 1988-90 (3-year-average) up to the record of 29.3 Mt in 2015-17. This significant growth results from the increase of both cultivation areas and yields over the past 30 years, with the Americas and Asia as the most important producing regions (Table 1.1). According to other sources, South America alone is producing 30% of the global common bean (Heuzé et al., 2013).

Table 1.1. Estimated global dry beans production, 1988-2017

In million tonnes (Mt)[1]

Region	Years (3-year average)[2]									
	1988-90	1991-93	1994-96	1997-99	2000-02	2003-05	2006-08	2009-11	2012-14	2015-17
Asia	8.05	7.77	7.63	7.17	8.07	8.97	9.67	10.57	11.20	14.03
Americas	5.59	6.14	6.77	6.46	6.79	6.86	7.43	7.23	7.39	7.50
Africa	2.30	2.75	2.55	2.72	3.33	3.41	4.08	5.27	6.21	6.70
Europe	0.60	0.53	0.52	0.63	0.57	0.47	0.38	0.44	0.56	1.01
Oceania	0.02	0.02	0.03	0.05	0.05	0.05	0.04	0.05	0.04	0.03
World	**16.56**	**17.21**	**17.50**	**17.03**	**18.82**	**19.77**	**21.60**	**23.56**	**25.40**	**29.27**

Notes: 1. Data on dry beans are aggregated and include several species: the common bean (*Phaseolus vulgaris*), other bean species (*Phaseolus* sp.) and, for several countries, some *Vigna* species.
2. Each column represents an average of three years, i.e. 1988-90 represents an average of the seasons 1988/89, 1989/90 and 1990/91 in the Southern Hemisphere.

Source: FAOSTAT (2019), "Crops – Beans, dry – Production quantity, years 1988 to 2017", http://faostat.fao.org (accessed on 10 July 2019). Aggregate may include official, semi-official, estimated or calculated data.

The five top producer countries of dry beans during the 2013-17 period were, in annual average, India (5.8 Mt), Myanmar (4.9 Mt), Brazil (3.0 Mt), the United States (1.3 Mt) and Mexico (1.2 Mt), followed in ranking order by the People's Republic of China and several African countries: the United Republic of Tanzania, Uganda, Kenya and Ethiopia (FAOSTAT, 2019).[1]

Common beans are mainly consumed in countries where they are produced. Countries with the highest rates of bean consumption per capita (mostly in Central and South Americas, the Caribbean, East Africa and some Asian economies) produce beans and also import them at varying levels, depending on the harvest, for meeting internal demand. Considering the global imports and exports of dry beans between 2012 and 2016, it seems that 12% to 18% of the world annual production (around 3.9 Mt on average) is traded internationally. China, Myanmar and the United States are the main exporters, with India and the European Union being the largest importers (FAOSTAT, 2019).

Processing

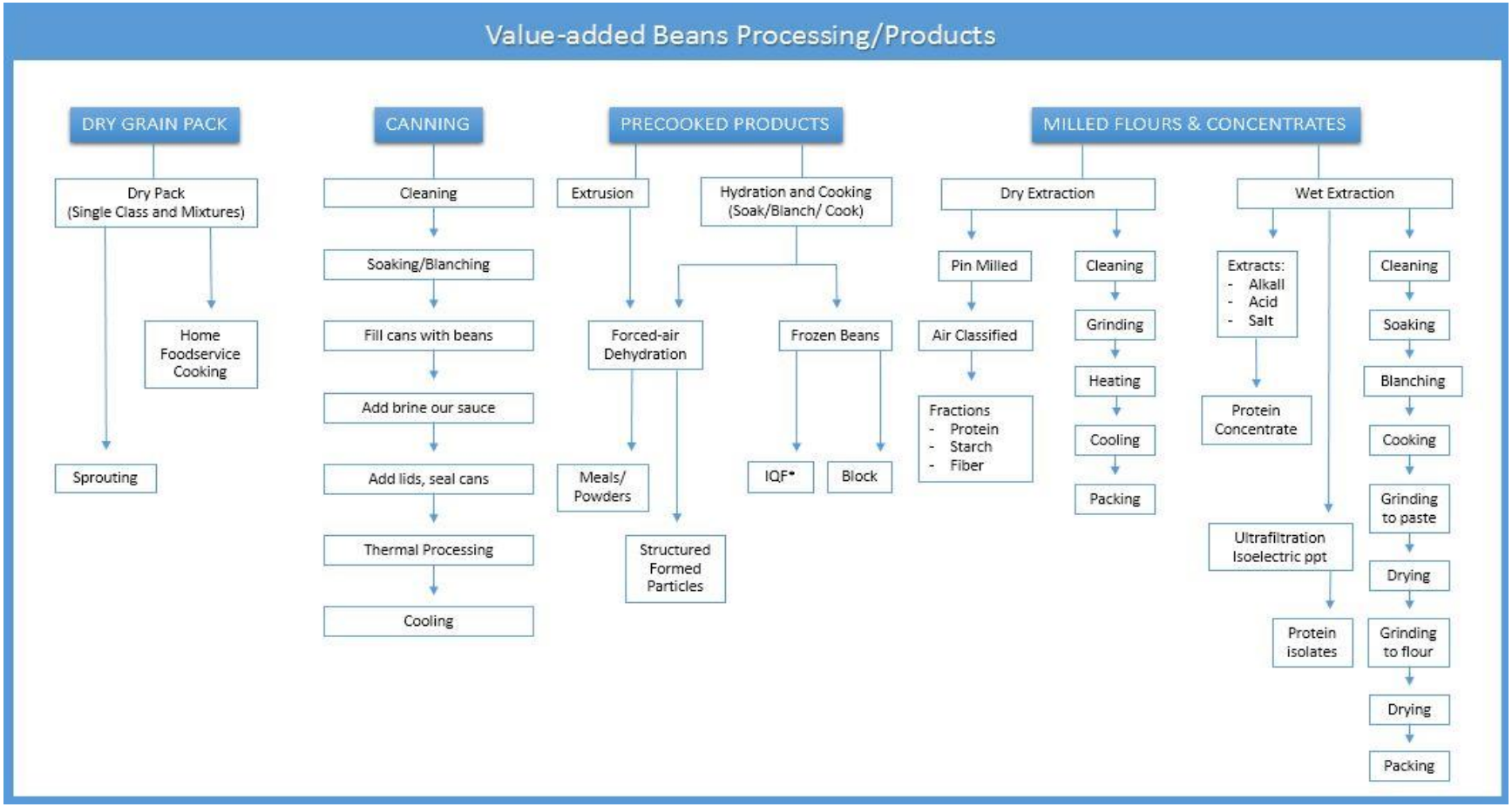
After harvest, beans are cleaned and then processed into final consumer products or ingredients. Products such as packaged dry beans, canned beans, baked beans, bean pastes, puffed snacks, texturised vegetable protein as meat analogues, cereal products, soups, frozen beans and bean flours all result from processing. The most commonly used processing methods for value-added common bean products are presented in Figure 1.4.

Canning is one of the most common forms of bean processing. Canned beans are a convenient alternative to dry beans which require long cooking times. An estimated 90% of navy beans and 45% of pinto beans (both types of common bean) consumed in the United States are sold as canned products (USDA-ERS, 2010). In developing countries, canned beans are most commonly a product for higher-income consumers (Jackson et al., 2012).

The canning process involves seven major steps (Figure 1.4):

- First, seed sorting and cleaning are performed to remove poor quality, diseased and damaged seed, stones and debris.
- Next, beans are equilibrated to 12%-16% moisture. Higher moisture values reduce the shelf life and lower values increase seed damage and splitting (Matella et al., 2012).
- A soaking and/or blanching step follows. Soaking times may vary from 30 min to 12 h at room temperature. Blanching is high heat treatment for 30 min or less prior to canning. The purpose of both treatments is to increase the water content of the seeds and uniformity of the final product.
- Beans are added to cans, followed by hot brine or sauce. Brine is a mixture of sugar, salt and calcium chloride. The calcium helps to maintain bean firmness. Sauces most commonly used in canning are tomato-based but there are many commercial products available with diverse flavour additives.
- Lids are added to the cans, which are sealed and processed in a canning retort for 52 min to 325 min at 116 to 121°C, depending on can size and brine or sauce type.
- Cans are cooled with water to an internal temperature of 38°C and are equilibrated for two weeks prior to use (Hosfield and Uebersax, 1980).

Figure 1.4. Methods of processing for value-added bean products



* Individually quick frozen.
Sources: Adapted from Siddiq and Uebersax (2012) and White and Howard (2013).

There are many ways to process the common bean into flour (Figure 1.4). One approach uses heat to inactivate the enzymes as a pre-cooking method. The steps include cleaning, soaking, blanching, cooking, grinding into a paste, drying, grinding again into flour, drying and packing. Another approach is dry milling, without pre-cooking the flour. In this case, bean seeds are ground into flour, followed by heating and packing. Both approaches generate breakfast and snack food products, as well as a texturing ingredient in tortilla chips, baked products, pasta and extruded products.

Uses

Although the major industrial food use of the common bean is canned beans, processing of the different types of bean through various treatments results in a range of ingredients for food and feed and value-added products: composite flour, extruded products, bread, cakes, pasta and tortillas and others, as presented in Table 1.2.

Table 1.2. Bean processing and products

Bean type	Pre-treatment	Product
Navy	Untreated (washed, dried, split); Treated (washed, dried, split, roasted)	Conventional bread
Great Northern	None	Pup loaf bread
Navy/Pinto	Dry roasting, air classified bean flour/protein concentrate	Straight dough bread
Black/Navy/Pinto/Small Red	Flour blends of 15%/25%/35% of hard red spring (HRS) wheat flour	Tortilla-wheat/bean
White/Red	Soaked 18 h, boiled 60 min	Corn bean tortillas
Flor de Junio Marcela	Cooked 95°C, 85 min, dried, ground	Corn bean tortillas
Black	Blended (paddle type mixer), twin-screw extruder, 20% moisture	Extruded product
White/Mexican	Counter-rotating twin-screw extruder	Extruded product
Navy/Small Red	Bean flour + corn starch (15%/30%/45%) co-rotating twin	Extruded product
Pinto/Bayo/Flor de Mayo	Soaked 18 h, 25°C, dehulled, dried 50°C	Extruded product – Single screw
Navy	Commercial navy bean flour	Extruded product
Navy/Pinto/Black	Dry roasting, dehulled, pin-milled, air classified fractions	Composite flour (10%)
Pinto/Great Northern/Small Red/Kidney	Blend 10%/20%/30% bean flour	Composite flour
Bean (unspecified)	Dehulled	Extender in beef sausage
Navy/Pinto	Isolated and purified starch	Bean starch noodle
Navy	Dried to 5%-10% moisture	Udon noodles
Navy/Pinto	Dried 24-30 h at 94-100°F to 10% moisture	Spaghetti (0%-25% bean)
Navy	Cotyledon flour precooked 12 min in boiling water, oven-dried 40°C overnight	Pasta
Bayo Victoria	Pressure cooked, blended in a food processor	Pasta
Navy	Unheated and heated 240°C/2 min, dehulled, hulls	Cake (roasted bean hulls)
Navy/Pinto/Black	Dry roasting 24°C/1 min, dehulled, pin milled, air classified	Fried doughnuts
Navy/Pinto	Milled to bean flour specifications	Pancake formulation
Navy/Pinto	Roasted (270°C/1 min)	Shortbread cookie
Navy	None	Master mix
Navy	Whole bean, hulls	Cookies
Navy	Soaked 99°C/45 min, steamed 104°C/30 min, macerated and drum dried	Cookies
Navy/Pinto	Roasted (270°C/2 min)	Pumpkin bread
Navy/Pinto	Dehulled, pin milled, air classified	Extruded snack

Source: Adapted from Maskus (2010), "Pulse processing, functionality and application".

Appropriate comparators for testing new varieties

This document suggests parameters that common bean breeders should measure when developing new modified varieties of *Phaseolus vulgaris*. The data obtained in the analysis of a new common bean variety should ideally be compared to those obtained from an appropriate near-isogenic, non-modified variety, grown and harvested under the same conditions. The comparison can also be made between values obtained from new varieties and data available in the literature or chemical analytical data generated from other commercial common bean varieties.

Components to be analysed include key nutrients, anti-nutrients and toxicants. Key nutrients are those which have a substantial impact on the overall diet of humans (food) and animals (feed). These may be major constituents (fats, proteins, and structural and non-structural carbohydrates) or minor compounds (vitamins and minerals). Similarly, the levels of known anti-nutrients and allergens should be considered. Key toxicants are those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health. Standardised analytical methods and appropriate types of material should be used, adequately adapted to the use of each product and by-product. The key components analysed are used as indicators of whether unintended effects of the genetic modification influencing plant metabolism have occurred or not.

Breeding characteristics screened by developers

The majority of common bean production occurs under low input agriculture on small-scale farms in developing countries (Miklas et al., 2006). Under such conditions, yield is mostly below its potential for the crop. Consequently, yield increase by attenuation of limiting factors is the focus of many breeding programmes (McClean et al., 2008).

Improving common bean nutritional quality, stress tolerance or resistance to pests and diseases are key objectives for various breeding programmes (Angenon et al., 1999; Suárez et al., 2008). Diseases and insects represent crucial biotic stressors that farmers have to face when growing this crop (Broughton et al., 2003). Among the fungal, bacterial and viral diseases that can affect common bean, at least five major ones are widespread: anthracnose, angular leaf spot, common bacterial blight, bean golden yellow mosaic virus and bean common mosaic virus, while several others are important locally or regionally (Broughton et al., 2003). A common bean variety that is resistant to bean golden mosaic virus (BGMV) has recently been developed (Aragão et al., 2013). Most commonly, breeders aim for resistance to one or two diseases and/or pest insects within the same variety. Since wild *Phaseolus* species present traits such as pest and pathogen resistance that are usually infrequent among cultivated common beans, they may be a potential source of novel alleles (Acosta-Gallegos et al., 2007).

The development of varieties with improved tolerance/resistant to other biotic stressors and to abiotic stressors is another important goal. Breeding programmes are developing agronomic traits such as nitrogen fixation. Other characteristics are also being explored by common bean breeding programmes, such as the increased content of specific nutrients including protein, minerals and vitamins.

Nutrients

*Composition of common bean (*Phaseolus vulgaris *L.) – General points*

This document addresses composition data relating to seeds only, not green pods (snap beans or green beans), dry shelled pods and stems.

The common bean is morphologically variable and adaptable to different environments, creating a wide range of local varieties. As a consequence, the nutritional composition of the common bean is impacted by various factors such as genotype, geographical origin, environmental and growing conditions (Broughton et al., 2003).

Constituents of common bean seed

Proximate composition

The proximate composition of raw common beans of a number of commercial varieties from Brazil, Madeira Island and the United States is shown in Table 1.3.

Carioca bean grains have a cream background with tan stripes; Pérola is the most common carioca variety in Brazil. The cooking process affects mainly the fibre content of Carioca beans (Pires et al., 2005).

Table 1.3. Proximate and total dietary fibre composition of different common bean varieties

Beans (raw mature seeds)	Protein (g/100 g DW)	Total lipid (fat) (g/100 g DW)	Ash (g/100 g DW)	Carbohydrate, by difference[1] (g/100 g DW)	Moisture (g/100 g FW)	Fibre, total dietary (g/100 g DW)
Black beans[a]	24.28	1.60	4.05	70.07	11.02	17.42
Cranberry (roman) beans[a]	26.29	1.40	3.78	68.53	12.39	28.20
Kidney beans, all varieties[a]	26.72	0.94	4.34	68.00	11.75	28.21
Navy beans[a]	25.40	1.71	3.78	69.11	12.10	17.41
Pink beans[a]	23.30	1.26	4.07	71.37	10.06	14.12
Pinto beans[a]	24.16	1.39	3.90	70.55	11.30	17.50
Small white beans[a]	23.91	1.34	4.25	70.50	11.71	28.20
Pérola, Carioca (beige)[b]	24.96	1.78	4.65	68.61	13.07	21.94
Madeira Island beans[c] (59 accessions)	18.55-29.69 mean: 23.27	0.57-2.86 mean: 1.65	3.64-5.67 mean: 4.57	63.32-75.32 mean: 70.51	6.45-16.65 mean: 10.87	..

Notes: DW = dry weight basis; FW = fresh weight basis. .. : missing value or not available.

1. Carbohydrate (by difference), DW = 100% - (crude protein% + crude fat% + ash%); this value includes total dietary fibre.

Sources: Adapted from: a. USDA-ARS (2014), *National Nutrient Database for Standard Reference, Release 27*, http://ndb.nal.usda.gov/ndb/search/list (accessed on 29 July 2014), data were converted to dry weight basis using mean moisture value; b. Delfino and Canniatti-Brazaca (2010), "Interação de polifenóis e proteínas e o efeito na digestibilidade proteica de feijão comum (*Phaseolus vulgaris* L.) (…)" http://www.scielo.br/pdf/cta/v30n2/03.pdf, data already provided on dry weight basis; c. Gouveia, et al. (2014), "Nutritional and mineral variability in 52 accessions of common bean varieties (*Phaseolus vulgaris* L.) from Madeira Island", http://dx.doi.org/10.4236/as.2014.54034, data mean and range already provided on dry weight basis.

Gouveia et al. (2014) evaluated the composition of 59 accessions of common bean varieties (52 Madeiran landraces, 5 standard and 2 commercial varieties) grown under the same field conditions in Madeira Island, to minimise the impact of the environmental factors. Regional common bean varieties exhibited great variability in the proximate parameters, presenting, on average, better nutritional performance with high protein and mineral contents compared to standard and commercial varieties.

Carbohydrates

Carbohydrates are monosaccharides and disaccharides (sugars), oligosaccharides and polysaccharides (starch, resistant starch and non-starch). Carbohydrates content in beans is mainly composed of starch, with small amounts of monosaccharides and disaccharides. Of carbohydrates, 17% to 23% has been reported to be pectin, cellulose and hemicellulose (Shiga et al., 2009). The total starch content ranges from 23.4% to 64.3% (Jacinto-Hernández and Campos, 1993; Jacinto-Hernández et al., 2002; Gouveia et al., 2014).

Beans contain a high ratio of slowly-digestible to readily-digestible starch compared with other starchy foods. Most common beans contain 27% to 40% amylose, a linear polymer of α-1-4 glucose units (Hoover et al., 2010), whereas most other starchy vegetables contain 20% to 30% amylose. Beans also contain a substantial amount of resistant starch, considered as dietary fibre. Resistant starch resists digestion by amylase in the small intestine and progresses to the large intestine for bacterial fermentation in the gut producing the short-chain fatty acids, acetic, butyric and propionic acids (Chung et al., 2010). Dry beans contain a substantial amount of carbohydrates as raw fibre in the form of cellulose and hemicellulose (Geil and Anderson, 1994).

Protein

Mean protein content shown for some common bean types in Table 1.3 varies from 23.27% to 26.72% dry matter. Madeira Island types/varieties had a protein mean content of 23.27 g/100 g with a range of 18.55 to 29.69 g/100 g (Gouveia et al., 2014). Bhatty et al. (2001) and Siddiq et al. (2010) reported a range of 20.43 to 23.62 g/100 g. Northern Portuguese beans and improved Ethiopian beans have been reported to contain total protein content ranging from 17.96 to 27.45 g/100 g (Coelho et al., 2005), and 17.96 to 22.07 g/100 g (Shimelis and Rakshit, 2005) respectively. Rodiño et al. (2001; 2003) have shown mean protein content of Portuguese beans and Iberian Peninsula beans to be 30.7 g/100 g (Rodiño et al., 2001) and 31.4 g/100 g respectively. Oliveira (2005) demonstrated that black, white and pink varieties have a protein content of 25% or more.

Table 1.4 presents the content of amino acids in common bean, based on elements collated from the USDA-ARS database (detailed by bean types, 2015), and the Feedipedia database (Heuzé et al., 2013). The amino acid profile of common bean protein is characterised by its deficiency in sulphur amino acids (methionine and cystine) and tryptophan, with methionine considered as the limiting amino acid. The amino acid most prevalent in all beans is glutamic acid (Table 1.4).

Table 1.4. Amino acid content (g/100 g, dry weight basis) of common beans

	USDA-ARS[1]							Feedipedia[2]
	Black beans[1]	Cranberry (roman) beans[1]	Kidney beans, all varieties[1]	Navy beans[1]	Pink beans[1]	Pinto beans[1]	Small white beans[1]	All common beans
Moisture content per 100 g	11.02	12.39	11.75	12.1	10.06	11.33	11.71	10.90
Alanine	1.02	1.10	1.12	1.03	0.98	0.98	1.00	0.99
Arginine	1.50	1.63	1.65	1.16	1.44	1.24	1.48	1.59
Aspartic acid	2.94	3.18	3.23	2.96	2.82	2.56	2.89	2.65
Cystine	0.26	0.29	0.29	0.21	0.25	0.21	0.26	0.27
Glutamic acid	3.70	4.01	4.07	3.52	3.55	3.41	3.64	3.67
Glycine	0.95	1.03	1.04	0.91	0.91	0.90	0.93	0.97
Histidine	0.68	0.73	0.74	0.58	0.65	0.63	0.67	0.69
Isoleucine	1.07	1.16	1.18	1.08	1.03	0.98	1.06	1.09
Leucine	1.94	2.10	2.13	1.96	1.86	1.76	1.91	1.93
Lysine	1.67	1.80	1.83	1.46	1.60	1.53	1.64	1.61
Methionine	0.37	0.39	0.40	0.31	0.35	0.29	0.36	0.27
Phenylalanine	1.31	1.42	1.44	1.32	1.26	1.23	1.29	1.34
Proline	1.03	1.11	1.13	1.27	0.99	1.21	1.01	0.87
Serine	1.32	1.43	1.45	1.34	1.27	1.32	1.30	1.36
Threonine	1.02	1.11	1.12	0.81	0.98	0.91	1.01	1.04
Tryptophan	0.29	0.31	0.32	0.28	0.28	0.27	0.28	0.32
Tyrosine	0.68	0.74	0.75	0.55	0.66	0.48	0.67	0.84
Valine	1.27	1.38	1.40	1.41	1.22	1.13	1.25	1.24

Notes: 1. Data converted from fresh weight to dry weight basis using given moisture level; 2. Data converted from percentage of protein (average) to dry weight basis using given crude protein percentage of dry matter.

Sources: 1. USDA-ARS (2015), *National Nutrient Database for Standard Reference, Release 27 (revised) – Version May 2015*, http://www.ars.usda.gov/ba/bhnrc/ndl (accessed on 15 June 2015); 2. Heuzé et al. (2013), *Common Bean (*Phaseolus vulgaris*)*, http://www.feedipedia.org/node/266 (accessed on 23 March 2015).

The protein digestibility of raw beans varies from 25% to 60% and can be increased up to 93.2%, depending on the bean variety and cooking process (Batista et al., 2010; Kiers et al., 2000; Jacinto-Hernández and Campos, 1993; Jacinto-Hernández et al., 2002). Jacinto-Hernández and Campos (1993) showed that increases in protein digestibility after cooking was very variable, with some varieties showing 8%-12% higher digestibility compared to raw beans, while others only improved digestibility by 3%-4%.

The nutritional value of beans is increased by heat processing, especially under moist heat (Gallardo et al., 1974, cited by Poel et al., 1990). This is due to denaturation of anti-nutritional factors, such as trypsin inhibitors and phytic acid (Burns, 1987, cited by Poel

et al., 1990), and improved accessibility of the bean proteins to enzymatic degradation (Romero and Ryan, 1978).

Lipids/fatty acids

Beans contain only a small amount of lipids, with the majority of fatty acids being unsaturated (Anderson et al., 1999). Total fat/lipids content in some varieties of common beans ranges from 0.57 to 1.78 g/100 g of dry matter (Table 1.3). The total saturated, monounsaturated and polyunsaturated fatty acid contents of some types of common bean are presented in Table 1.5.

Table 1.5. Fatty acid content (g/100 g, dry weight basis) in raw mature grain of common beans

Bean types	Total saturated	Total monounsaturated	Total polyunsaturated
Black	0.326	0.109	0.543
Black Turtle	0.206	0.069	0.344
Cranberry (roman)	0.277	0.093	0.462
Great Northern	0.318	0.047	0.426
Kidney, all types	0.106	0.056	0.403
Navy	0.149	0.113	0.767
Pink	0.263	0.088	0.438
Pinto	0.208	0.203	0.361
Red Kidney	0.136	0.072	0.517
Small White	0.268	0.090	0.448
White	0.194	0.066	0.323
Yellow	0.597	0.201	0.994

Note: Data were converted to dry weight basis using mean moisture value.
Source: Adapted from USDA-ARS (2014), *National Nutrient Database for Standard Reference, Release 27*, http://ndb.nal.usda.gov/ndb/search/list (accessed on 29 July 2014).

Vitamins

Common beans in particular contain water-soluble B vitamins; these include thiamine (3.9 to 11.4 mg/kg dry matter), riboflavin (1.0 to 2.9 mg/kg), niacin (3.3 to 26.8 mg/kg), vitamin B6 (0.4 to 5.7 mg/kg) and pantothenic acid (2.7 to 10.1 mg/kg) (Table 1.6). Common beans are also a prominent source of dietary folate – vitamin B9 – (0.2 to 5.8 mg/kg) (Table 1.6) (Rychlik et al., 2007). Common beans contain only small amounts of vitamin C, and little to no fat-soluble vitamins (Geil and Anderson, 1994) because of the low level of lipids in beans.

The vitamin content measured in common beans varies widely depending on commercial market classes, origin, environment and analytical methodology used for analysis (Table 1.6). Variation is greatest in folate (vitamin B9) content (Rychlik et al., 2007).

Cooking, like other food treatments, introduces another source of direct and indirect variability. Commercial methods of preparation of canned beans can cause significant loss of water-soluble vitamins, whereas home-cooked common beans seem to have less effect on nutrient retention (Augustin et al., 1981).

Table 1.6. Vitamin composition (mg/kg, dry weight basis) of common beans

Bean types	Folate	Thiamine	Riboflavin	Niacin	Pantothenic acid	Vitamin B_6
Black[1]	5	10.1	2.2	22.0	10.1	3.2
Black Turtle[2]	3.2	11.1	2.4	20.9		3.4
Black Turtle[3]	0.4-0.8	4.1-4.8	1.1	12.2-12.9	4.5-4.6	1.8-4.5
Cranberry[2]	2.1	9.7	2.7	15.7		3.6
Cranberry[3]	0.5	4.6-5.2	1.4-1.7	11.0-11.8	3.6-3.7	1.8
Dutch Brown[3]	0.2-0.4	4.7-5.2	1.4	12.3-16.1	4.1-4.4	1.8
Great Northern[1]	5.4	7.3	2.7	21.9	12.3	5.0
Great Northern[2]	1.0-1.7	9.4-9.8	2.6-2.9	14.9-19.2		4.0-5.7
Great Northern[3]	0.7-1.2	4.8-4.9	1.3	7.1-10.4	5.1-5.4	1.3-3.6
Kidney[1]	1.8-2.6	11.4	1.5-2.2	21.5		4.5-4.6
Kidney[2]	4.5	6.0-6.3	1.8-2.5	12.5-23.3	5.0-8.8	2.4-4.5
Light Red[3]	0.4	8.9-10.9	2.2-2.4	3.3	2.7-3.6	0.4-2.5
Navy[1]	1.8-2.6	9.4-9.8	1.4-2.3	24.3-26.8		4.8-5.0
Navy[2]	1.2-4.1	6.6-8.8	1.9	14.9-24.9	3.5-8.5	2.4-4.9
Navy[3]	0.7-1.5	4.2-9.1	1.1-2.0	6.0-16.6	2.7-3.6	0.4-2.5
Pink[1]	4.8-5.8	9.2	1.5	11.6-14.4		5.0-5.7
Pink[3]	0.7-1.5	5.6-6.7	1.2	9.0-9.9	4.0-4.8	1.6-2.4
Pinto[1]	4.6	8.6-9.9	1.4-2.3	17.8		4.8
Pinto[3]	0.7-1.1	6.2-7.6	1.2	9.4-12.9	3.1-4.4	1.6-2.0
Red Kidney[3]	0.6	3.9-7.3	1.6	9.1-12.9	4.1-4.8	1.7-2.5
Small Red[1]	1.8	9.6	1.6	12.5		5.3
Small Red[3]	0.7-1.0	5.0-6.4	1.1	7.3-8.4	3.8-4.3	1.5-1.9
Small White[1]	3.0	8.9	1.6	19.9		4.9
White[2]	4.4	4.9	1.6	5.4	8.3	3.6
White Kidney[3]	0.2	6.5-8.0	1.0-1.3	9.8-12.6	3.5-3.6	1.4-1.7
Overall range	**0.2-5.8**	**3.9-11.4**	**1.0-2.9**	**3.3-26.8**	**2.7-10.1**	**0.4-5.7**

Sources: 1. Augustin et al. (1981), "Variation in the vitamin and mineral content of raw and cooked commercial *Phaseolus vulgaris* classes", http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1981.tb04467.x/pdf;
2. Tiwari and Singh (2012), *Pulses Chemistry and Technology*;
3. Wang and Daun (2004) *The Chemical Composition and Nutritive Value of Canadian Pulses.*

Minerals

The bean ash is constituted by several minerals (Table 1.7). The mineral content depends on market class/variety and environmental conditions during cultivation. Regarding minerals occurring at higher quantities, ranges reported are 0.09-4.25 g/kg of the dry matter for calcium (Ca), 1.0-3.26 g/kg for magnesium (Mg), 2.30-8.42 g/kg for phosphorous (P) and 13.0-24.9 g/kg for potassium (K). A considerable variation in levels was also observed for minerals occurring at lower quantities in germplasm from different sources, as shown by Dwivedi et al. (2012).

Table 1.7. Mineral composition of common beans

Beans	Calcium	Magnesium	Phosphorous	Potassium	Iron	Zinc	Copper	Manganese
	(g/Kg, dry weight basis)				(mg/Kg, dry weight basis)			
Black[1]	1.30-1.38	1.80-2.07	3.96-5.66	14.09-17.07	51.8-56.4	0-56.6	0-12.3	0-19.4
Great Northern[1]	1.67-1.76	1.96-2.31	4.63-7.03	17.77-19.61	31.4-61.3	38.1-40.9	9.3-12.3	15.9-19.0
Kidney[1]	1.09-1.62	1.59-1.99	4.61-5.94	15.93-20.15	92.9-99.7	31.6-41.9	10.9-11.3	11.6-15.9
Navy[1]	1.67-1.76	1.96-2.31	4.63-7.03	17.77-19.61	62.5-86.5	0-32.2	0-8.4	0-19.0
White[1]	2.71-4.25	2.14-3.26	2.30-3.39	14.56-20.24	117.7-120.7	0-41.4	0-11.1	0-20.3
Dark Red (Canada)[2]	0.82	1.53	5.66	17.09	66.6	28.3	7.1	10.8
Small Red (Canada)[2]	1.34	1.68	5.73	17.31	34.1	18.9	0.4	13.2
Brown (Brazil)[3]	1.09-1.79				48.1-78.2	25.1-31.9	6.1-13.6	10.0-26.3
Red (Nicaragua)[4]	1.02-1.41		4.00-4.44		61.8-71.9	21.0-25.1		
Red (Columbia)[5]			7.44		58.3-73.0	35.5-39.5		
Cream (Columbia)[5]			6.04-8.34		63.3-90.4	30.0-52.3		
Pink (Columbia)[5]			8.42		52.3	26.7		
Purple (Columbia)[5]			7.00		80.1	39.6		
Yellow (Columbia)[5]			7.52		86.1	62.4		
Beige (Brazil)[3]					53.1-68.8	33.5-42.7		
Several Mexican varieties[6]	0.09-2.0	2.0	4.6		38.0-76.0	22.0-44.0		
Red-Mottled Beans[7] **(2 varieties)**					76-81	33-34		
Madeira Island Beans[8] **(59 accessions)**		1.0-1.8 mean: 1.5	3.0-7.5 mean: 5.0	13.0-24.9 mean: 18.9	41.0-100.0 mean: 60.1	22.0-50.0 mean: 30.1	5.0-14.0 mean: 10.1	0.009-0.021 mean: 0.015

Sources: 1. Tiwari and Singh (2012), *Pulse Chemistry and Technology*; 2. Oomah, Blanchard and Balasubramanian (2008), "Phytic acid, phytase, minerals, and antioxidant activity in Canadian dry bean (*Phaseolus vulgaris* L.) cultivars"; 3.Carvalho et al. (2012), "Iron and zinc retention in common beans (*Phaseolus vulgaris* L.) after home cooking", https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3292239/; 4. Martinez Meye et al. (2013), "Content of zinc, iron and their absorption inhibitors in Nicaraguan common beans (*Phaseolus vulgaris* L.)", http://www.sciencedirect.com/science/article/pii/S0308814612012204; 5. House et al. (2002), "Potential for increasing the amounts of bioavailable zinc in dry beans (*Phaseolus vulgaris* L.) through plant breeding", http://onlinelibrary.wiley.com/doi/10.1002/jsfa.1146/pdf; 6. Guzmán-Maldonado et al. (2002), "Calidad alimentaria y potencial nutraceutico del frijol (*Phaseolus vulgaris* L.)", http://www.redalyc.org/articulo.oa?id=60828206; 7. Blair et al. (2010), "Registration of high mineral common bean germplasm lines NUA35 and NUA56 from the red-mottled seed class"; 8. Gouveia, C.S.S. et al. (2014), "Nutritional and mineral variability in 52 accessions of common bean varieties (*Phaseolus vulgaris* L.) from Madeira Island", http://dx.doi.org/10.4236/as.2014.54034.

Common beans accumulate different proportions of iron, zinc and manganese in the seed coat, embryo and cotyledons. The highest amount of these minerals is stored in the cotyledons of mature seeds (Cvitanich et al., 2011). Iron and other constituents of the grain (phytate, tannins and fibre) are distributed differently in the hull and in the cotyledon. Food processing, such as baking and brewing, not only affect the bioavailability of iron but also factors that act as agonists or antagonists of mineral absorption (Lombardi-Boccia et al., 1995). Stripping significantly decreased the dialysability of iron, while cooking had

the same influence on a coloured variety, but not on a white variety. The tannin-protein interaction may be the main cause of the difference in iron dialysability (Lombardi-Boccia et al., 1995). The effect of reheating beans on their iron content has also been studied. In whole bean, without broth, no changes were detected during cooking. In the case of beans with broth, insoluble iron increased in grains. Both soluble and insoluble iron decreased in the broth (Amaya et al., 1991).

Anti-nutrients, toxicants and other constituents

Anti-nutrients and toxicants – General points

In spite of good nutritional quality, common beans contain some constituents having anti-nutritional effects. Thus, adverse effects may be induced by tannins, phytates, protease inhibitors and lectins. Kidney beans have also been reported to contain toxic cyanogenic compounds (Cho et al., 2013) but only at trace levels having no health implications for the consumer.

Main anti-nutrients

Tannins

Tannins are colourless polyphenolic constituents of legumes (Reed, 1995). Levels reported in common bean varieties range from 10.1 to 44.2 mg catechin-equivalents per gramme dry weight (De Mejía et al., 2003, Helbig et al., 2003; Cruz-Bravo et al., 2011). Beans differ in content of tannins, which affect quality as they are converted into pigments visible during dehydration and oxidation. Tannins also have the ability to interact with proteins, resulting in reduced protein and mineral digestibility (Junk-Knievel et al., 2008). Condensed tannins are present in the dietary fibre fraction and can be considered indigestible or poorly digestible (Bartolomé et al., 1995). Cooking does not destroy tannins but they are partially removed with the cooking broth (Bressani and Elias, 1980). According to Ziena et al. (1991), less than 10% of total tannins are broken down during cooking, while about 50% are washed away in the cooking liquid.

Phytate/phytic acid

Phytic acid (also known as inositol hexakisphosphate (IP6), inositol polyphosphate, or phytate when in salt form) chelates mineral nutrients including calcium, magnesium, potassium, iron and zinc, rendering them unavailable to non-ruminant animals (NRC, 1998; Liener, 1994). Phytates are concentrated mostly in the cotyledons and embryo axes (up to 3% of total seed weight) of common bean (Kasim and Edwards, 1998; Blair et al., 2012) (Table 1.8). The negative effect on the bioavailability of minerals is associated with inositol penta- (IP5) and hexa-phosphate (IP6). Phytates also interact with basic protein residues and can inhibit digestive enzymes such as pepsin, pancreatin and amylase (Agostini and Ida, 2006).

Phytate content in common beans varies due to genetic differences between varieties, and environmental factors such as growing conditions, agricultural practices and location. Commonly reported levels are in the range 2.6-25.1 mg/g dry weight (Stanley and Aguilera, 1985; Estévez et al., 1991; Burbano et al., 1999; Helbig et al., 2003; Díaz-Batalla et al., 2006; Oomah et al., 2008; Martin-Cabrejas et al., 2009; Martinez Meyer et al., 2013; Pedrosa et al., 2015; Carvalho et al., 2015). In beans, phytate phosphorus constitutes a major portion of the total phosphorus content and is found preferentially in the cotyledon

(Deshpande et al., 1982), accounting for 57%-81% of total phosphorus in Navy, 68%-72% in Red Kidney, 55-80% in Great Northern and 70% in California small white beans (Reddy, 2001). Low phytate bean germplasm has recently been developed (Campion et al., 2009). The proportion of phytate being IP5 and IP6, which are the most commonly detected inositol phosphate isomers, vary widely in raw beans. IP6 is the most predominant isomer, constituting from 64% (in Red Kidney beans) to 98% (in Pinto beans) of the total phytate content (Chen, 2004).

Of the various processing methods, fermentation and germination seem to be effective in decreasing the phytate concentration, while soaking and cooking can remove from 50% to more than 80% of endogenous phytate in beans (Sathe and Salunke, 1984).

Table 1.8. Phytic acid composition (mg/g) of common beans and its components

Bean types	Whole[1, 2]	Cotyledon[1]	Dehulled[2]	Hull[2]
Black	10.4-29.3	36.1	17.09	1.91
Great Northern	5.0-27.0	32.6		
Pinto	6.1-23.8	25.6	11.48	1.30
Red	8.1-20.7	30.5	8.71	2.63
Red Kidney	12.0-26.3	34.7		
White	5.5-18.0	16.3	9.83	2.30

Sources: 1. Reddy (2001), "Occurrence, distribution, content and dietary intake of phytate"; 2. Calculated from Hu et al. (2006), "Kaempferol in red and pinto bean seed (*Phaseolus vulgaris* L.) coats inhibits iron bioavailability using an *in vitro* digestion/human Caco-2 cell model".

Trypsin inhibitors

Common beans contain trypsin inhibitors which inhibit the digestive action of the trypsin enzyme. Trypsin inhibitor activity (TI) in uncooked beans have been reported to be in the range 6.3-55.2 trypsin inhibited units (TIU)/mg (Dhurandhar and Chang, 1990; Estévez et al., 1991; Jacinto-Hernández and Campos, 1993; Sotelo et al., 1995; De Mejía et al., 2003, 2005; Morales-de León et al., 2007; Olmedilla-Alonso et al., 2013; Pedrosa et al., 2015). The level of TI in the common bean is not only dependent on bean genotype but also on the environmental conditions where it was cultivated (De Mejía et al., 2003; 2005). In cooked beans, trypsin inhibitor activity is much lower than in raw beans (Jacinto-Hernández and Campos, 1993; Jacinto-Hernández et al., 2002; Morales-de León et al., 2007).

Alpha-amylase inhibitors

Common beans are the legume with the highest amount of alpha-amylase inhibitors. Alpha-amylase inhibitors inhibit the digestive enzyme α-amylase resulting in reduced digestibility of certain carbohydrates. Various types of α-amylase inhibitors have been described in the common bean (Ishimoto et al., 1995), including three different glycoprotein isoforms. Screening of 150 Brazilian bean varieties classified by colour revealed average values between 0.19 and 0.29 α-amylase inhibitor units per mg protein and a range between 0.09 and 0.40 α-amylase inhibitor units per mg protein (Table 1.9), with no correlation between inhibitory activity and seed coat colour (Lajolo et al., 1991).

Table 1.9. α-Amylase Inhibitory Activity (AIU/mg protein) of common beans classified by bean colour

Bean colour	Range	Average
Beige	0.14-0.40	0.26
Black	0.11-0.30	0.19
Brown	0.14-0.35	0.29
Dark brown	0.19-0.33	0.25
Light brown	0.09-0.32	0.20
Pale brown	0.16-0.40	0.29
Pink	0.16-0.28	0.21
Purple	0.17-0.22	0.19
Red	0.16-0.37	0.25
White	0.14-0.33	0.23

Note: α-amylase inhibitory unit (AIU) value of 10 is defined as a 50% decrease in enzyme activity at 37°C/5 min after addition of 1% starch as substrate.
Source: Lajolo, Finardi-Filho and Menezes (1991), "Amylase inhibitors in *Phaseolus vulgaris* beans".

Lectins

Lectins are proteins that bind to carbohydrate-containing molecules and are found in a variety of foods, including legumes such as the common bean (Gupta, 1987). The biological activity of lectins has been reviewed (Grant, 1991). Lectin levels reported vary with the methodology used for analysis. Several investigators reported levels between non-detectable and approximately 10 haemagglutinating units (HU) per gramme bean assayed with a method measuring haemagglutinating activity (Sotelo et al., 1995; De Mejía et al., 2003; 2005). Burbano et al. (1999), Olmedilla-Alonso et al. (2013) and Pedrosa et al. (2015) reported 0.3-165 mg/g dry weight using an indirect ELISA assay for phytohaemagglutinin quantification. Lectins have been shown to have growth inhibitory properties and result in toxicity in animals. The haemagglutinating activity of lectins can be reduced by moist-heat treatment (Gupta, 1987), making proper cooking prior to consumption an important step in the safe consumption of common beans (Ogawa and Date, 2014). Several cases of human toxicity due to ingestion of raw or under-cooked beans have been reported (Cornell University, 2014).

Other constituents

Oligosaccharides

Common bean varieties vary considerably in terms of their oligosaccharide content (Table 1.10), including the raffinose family oligosaccharides (RFOs). Thus, raffinose levels range from about non-detectable to 14.1 mg/g dry weight, stachyose from 0.9 to 63.8 mg/g and verbascose from non-detectable to a few mg/g, depending on the variety considered (Geil and Anderson, 1994; Weder et al., 1997; Burbano et al., 1999; Queiroz Kda et al., 2002; Díaz-Batalla et al., 2006; Campos-Vega et al., 2009; Cruz-Bravo et al., 2011; Kleintop et al., 2013; Olmedilla-Alonso et al., 2013; Slupski and Gebczynski, 2014; Pedrosa et al.,

2015). Díaz-Batalla et al. (2006) noted that one out of fourteen studied common bean varieties contained exceptionally high levels of verbascose (35.8 mg/g dry weight). RFOs are broken down by the enzyme α-galactosidase which is not present in the lower gastrointestinal tract. As a result, RFOs are fermented by anaerobic bacteria in the gut, resulting in flatulence (Soccol, 2012). Soaking of dry beans prior to cooking is a common practice and has been shown to reduce the content of RFOs in common bean. The amount of raffinose and stachyose removed through soaking in Mexican common bean varieties was found to range from 7% to 60%, depending on the variety considered (Table 1.10).

Table 1.10. Oligosaccharide content in Mexican common bean varieties

Concentration (mg/g)

Variety	Dry grain		Soaked grain	
	Raffinose	Stachyose	Raffinose	Stachyose
Bayo Victoria	4.43	36.66	2.57	14.08
Azufrado Higuera	1.63	26.98	1.94	22.14
Flor de Durazno	2.20	31.33	1.61	16.48
Azufrado Peruano	2.06	34.27	1.73	20.41
Bayo Zacatecas	5.39	26.39	2.08	10.76
Azufrado Regional 87	1.72	23.32	1.82	20.32
Bayo Mecentral	6.16	9.43	3.36	8.03
Flor de Junio M.	5.38	28.06	3.78	18.75
Negro Otomí	3.87	26.76	2.45	16.36
Flor Mayo M38	3.94	26.50	3.74	24.48
Alubia	5.65	20.56	3.13	12.82
Negro Jamapa	7.04	35.22	6.00	24.76
Negro 8025	6.55	23.62	4.73	21.53

Source: Jacinto-Hernández et al. (2006), http://naldc.nal.usda.gov/naldc/download.xhtml?id=IND43805445&content=PDF.

Other carbohydrates in common bean include pectic substances, arabinogalactans and xyloglucans (Reddy et al., 1984; Sathe and Salunkhe, 1984). Like RFOs, these polysaccharides are subject to anaerobic fermentation (Geil and Anderson, 1994).

Saponins

Saponins are secondary plant metabolites that exist in a wide variety of edible legumes (Shi et al., 2004; Guajardo-Flores et al., 2012; Calvert et al., 1981). In common bean, they are particularly found in the seed coat. The most abundant saponin in the extracts of black bean seed coats is soyasaponin Af (Chavez-Santoscoy et al., 2013).

Phenolics

The major phenolic compounds of beans are simple phenolic acids and flavonoids. Highest phenolic content is found in the dark, highly pigmented bean varieties, in particular in their

seed coat or hulls (Oomah et al., 2005) that are rich in flavonols, flavonoids, anthocyanins and tannins. Seed coat polyphenols are partly responsible for the post-harvest seed darkening and hard-to-cook phenomenon in beans (Marles et al., 2008; Campos-Vega et al., 2012). A single gene seems to control post-harvest darkening. In Pinto beans, the slow-darkening trait is controlled by a recessive allele (Junk-Knievel et al., 2008). Total phenolic content (50-1104 mg/kg) and the spectrum of the various phenolic constituents vary widely among and within market classes of common bean, depending on genetic and environmental factors.

The most abundant simple phenolic compounds in common beans are ferulic acid, sinapic acid, vanillic acid, caffeic acid, p-coumaric acid and p-hydroxybenzoic acid, their reported amounts varying with the methodology used for analysis. Syringic acid, chlorogenic acid, gallic acid and vanillin have also been reported to be present (Espinosa-Alonso et al., 2006: Luthria and Pastor-Corrales, 2006; Xu and Chang, 2009).

Kaempferol, often occurring with O- and C-glycosidic linkages, is the most abundant flavonol in beans with red beans and pinto beans containing greater amounts (14-209 and 148 mg/kg respectively) than black or grey beans (20 mg/kg) (Díaz-Batalla et al., 2006). Quercetin, another flavonol is present in black (9.7-23.5 mg/kg), cream-red (6.7-9.4 mg/kg) and grey (7.9 mg/kg) beans (Diaz-Batella et al., 2006).

Anthocyanins occurring in beans are simple, non-acylated anthocyanidins, usually containing glucose as the only sugar; however, malvidin 3-galactoside has been detected in black beans (Xu and Chang, 2009). Six different anthocyanidins have been detected in the (coloured) common bean but their relative percentage may differ among ecotypes and commercial market classes of beans. Several investigators reported delphinidin (49%-81%) to predominate, with petunidin (4%-32%), cyanidin (1%-23%) and malvidin (4%-14%) occurring at intermediate level, and pelargonidin (0.4%-6.5%) and peonidin (0.5%-3.7%) less frequently (Choung, 2005; Espinosa-Alonso et al., 2006; Xu and Chang, 2009). However, López et al. (2013) reported cyanidin and pelargonidin as the major anthocyanidins in dark beans, where they were complemented with small amounts of acylated delphinidin and pelargonidin 3-glucosides.

Suggested constituents to be analysed related to food use

Key products consumed by humans

The common bean is a staple food typically consumed after having been soaked in water and cooked, or after being canned. While beans can be milled and used to produce processed products, the common bean is typically eaten as shelled beans (whole grain). For the purpose of compositional analysis, it is appropriate to analyse the whole grain once the shell has been removed.

Suggested analysis for food use of new varieties

In the context of the human diet, the common bean can provide nutrients such as proteins, carbohydrates, dietary fibre and folate. The common bean may also contain anti-nutrients such as phytic acid, trypsin inhibitor, α-amylase inhibitor and lectins. The suggested key nutritional and anti-nutritional parameters to be analysed are shown in Table 1.11.

Table 1.11. Suggested constituents to be analysed in common bean grain for food use

Constituent	Whole grain
Proximates	x
Dietary fibre	x
Amino acids	x
Phytic acid	x
Trypsin inhibitor	x
α-amylase inhibitor	x
Lectins	x
Vitamins[1]	x

Note: 1. B vitamins, namely thiamine (B1), riboflavin (B2) and folate (B9), are suggested.

Suggested constituents to be analysed related to feed use

Key products consumed by animals

Although less common than its use in human food, the common bean may be used in animal feed. While products from the common bean may be used as feed, this document only addresses seeds, not green pods (snap beans or green beans), dry shelled pods and stems.

The residue of packaging and processing of dried beans (including those from the genera Phaseolus) for human food may be added to animal diets. These may include broken, small and cull beans, which may comprise all or part of plant protein products for animal diets (AAFCO, 2015).

Suggested analysis for feed use of new varieties

The suggested key nutritional and anti-nutritional parameters to be analysed in the common bean for animal feed use are shown in Table 1.12.

Table 1.12. Suggested constituents to be analysed in common bean grain for feed use

Constituent	Whole grain
Proximates	x
Fibre fractions[1]	x
Amino acids	x
Phytic acid	x
Trypsin inhibitor	x
Lectins	x

Note: 1. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) should be substituted for crude fibre.

Note

[1] The FAO figures for dry beans are not limited to common bean only and aggregate data of other *Phaseolus* species, and for several countries other types of beans classified as *Vigna* species.

References

AAFCO (2015), *2015 Official Publications*, The Association of American Feed Control Officials, http://www.aafco.org/Publications (accessed on 15 June 2015).

Acosta-Gallegos, J.A., J.D. Kelly and P. Gepts (2007), "Pre breeding in common bean and use of genetic diversity from wild germplasm", *Crop Science*, Vol. 47(S3), pp. 44-59, https://www.researchgate.net/publication/242108033_Prebreeding_in_Common_Bean_and_Use_of_Genetic_Diversity_from_Wild_Germplasm.

Agostini, J.S. and E.I. Ida (2006), "Efeito das condições de germinação de girassol na redução do teor de fitato e ativação de fitase e fosfatase ácida (Effect of sunflower germination conditions for reducing phytate content and enhancing phytase and acid phosphatase activities)", *Ciências Agrárias*, Vol. 27, No. 1, pp. 61-70, in Portuguese (English abstract), http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/2398/2053.

Albala, K. (2007), *Beans – A History*, Berg Publishers, Oxford, UK.

Amaya, H., E. Acevedo and R. Bressani (1991), "Efecto del recalientamiento sobre la disponibilidad de hierro y del valor nutritivo de la proteína del frijol negro (*Phaseolus vulgaris*) cocido", *Archivos Latino Americanos de Nutricion*, Vol. 41, No. 2, Caracas, pp. 222-237.

Anderson, J.W., B.M Smith and C.S. Washnock (1999), "Cardiovascular and renal benefits of dry bean and soybean intake", *American Journal of Clinical Nutrition*, Vol. 70(suppl), pp. 464-474.

Angenon, G. et al. (1999), "Strategies for improving the nutritional quality of *Phaseolus* beans through gene engineering", *Biotechnology, Agronomy, Society and Environment*, Vol. 3(4), pp. 233-236, http://www.pressesagro.be/base/text/v3n4/233.pdf.

Angioi, S.A. et al. (2010), "Beans in Europe: Origin and structure of the European landraces of *Phaseolus vulgaris* L.", *Theoretical and Applied Genetics*, Vol. 121(5), Springer, pp. 829-843.

Aragão, F.J.L. et al. (2013), "Molecular characterization of the first commercial transgenic common bean immune to the Bean golden mosaic virus", *Journal of Biotechnology*, Vol. 166, pp. 42-50.

Augustin, J. et al. (1981), "Variation in the vitamin and mineral content of raw and cooked commercial *Phaseolus vulgaris* classes", *Journal of Food Science*, Vol. 46, Wiley, pp. 1701-1706, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1981.tb04467.x/pdf.

Bartolomé, B., L.M. Jiménez-Ramsey and L.G. Butler (1995), "Nature of the condensed tannins present in the dietary fibre fractions in foods", *Food Chemistry*, Vol. 53, No. 4, Barking, pp. 357-362.

Batista, K.A., S.H. Prudêncio and K.F. Fernandes (2010), "Changes in the functional properties and anti-nutritional factors of the extruded hard-to-cook common beans (*Phaseolus vulgaris*, L.)", *Journal of Food Science Oxford*, Vol. 75, No. 3, pp. 286-290.

Bhatty, N., A.H. Gilani and S.A. Nagra (2001), "Nutritional Improvement of Lobia (*Phaseolus vulgaris*) by supplementation with poultry, mutton and beef meat", *International Journal of Food Sciences and Nutrition*, Vol. 52, pp. 521-526.

Blair, M.W. et al. (2012), "Inheritance of seed phytate and phosphorous levels in common bean (*Phaseolus vulgaris* L.) and association with newly-mapped candidate genes", *Molecular Breeding*, Vol. 30, pp. 1265-1277.

Blair, M.W. et al. (2010), "Registration of high mineral common bean germplasm lines NUA35 and NUA56 from the red-mottled seed class", *Journal of Plant Registrations*, Vol. 4(1), pp. 55-59.

Bressani, R. and L.G. Elias (1980), "The nutritional role of polyphenols in beans", in J.H. Hulse (ed.), *Polyphenols in Cereals and Legumes*, IDRC, Ottowa, pp. 61-72.

Broughton, W.J. et al. (2003), "Beans (*Phaseolus* spp.) – Model food legumes", *Plant & Soil*, Vol. 252(1), pp. 55-128, https://www.researchgate.net/publication/226474316_Beans_Phaseolus_spp_-_Model_food_legumes.

Burbano, C. et al. (1999), "Evaluation of antinutritional factors of selected varieties of *Phaseolus vulgaris*", *Journal of the Science of Food and Agriculture*, Vol. 79, pp. 1468-1472, http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0010(199908)79:11%3C1468::AID-JSFA387%3E3.0.CO;2-G/pdf.

Calvert, G.D. et al. (1981), "A trial of the effects of soya-bean flour and soya-bean saponins on plasma lipids, faecal bile acids and neutral sterols in hypercholesterolaemic men", *British Journal of Nutrition*, Vol. 45(2), pp. 277–281.

Campion, B. et al. (2009), "Isolation and characterization of an LPA (low phytic acid) mutant in common bean (*Phaseolus vulgaris* L.)", *Theoretical and Applied Genetics*, Vol. 118, pp. 1211–1221, http://link.springer.com/article/10.1007%2Fs00122-009-0975-8.

Campos-Vega, R., H.A. Vergara-Castañeda and B.D. Oomah (2012), "Functional food sources: Beans in sight", in E. Popescu and I. Golubev (eds.), *Beans: Nutrition, Consumption and Health*, Ch.1, pp. 1-56, Nova Science Publishers Inc., New York, NY.

Campos-Vega, R. et al. (2009), "Chemical composition and *in vitro* polysaccharide fermentation of different beans (*Phaseolus vulgaris* L.)", *Journal of Food Science*, Vol. 74(7), pp. T59-T65.

Carvalho, J.L.V. et al. (2015), "Comparative analysis of nutritional compositions of transgenic RNAi-mediated virus-resistant bean (event EMB-PV051-1) with its non-transgenic counterpart", *Transgenic Research*, Vol. 24(5), pp. 813-819, http://link.springer.com/article/10.1007%2Fs11248-015-9877-5#.

Carvalho, L.M.J. et al. (2012), "Iron and zinc retention in common beans (*Phaseolus vulgaris* L.) after home cooking", *Food and Nutrition Research*, Vol. 56, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3292239/.

CGIAR (n.d.), *Common Bean Factsheet*, Consultative Group on International Agricultural Research, http://www.cgiar.org/our-strategy/crop-factsheets/beans/ (accessed on 15 June 2015).

Chacón-Sánchez, M.I., B. Pickersgill and D.G. Debouck (2005), "Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of the Mesoamerican and Andean cultivated races", *Theoretical Applied Genetics*, Vol. 110(3), pp. 432-444, http://link.springer.com/article/10.1007%2Fs00122-004-1842-2.

Chavez-Santoscoy, R.A., J.A. Gutierrez-Uribe and S.O. Serna-Saldivar (2013), "Effect of flavonoids and saponins extracted from black bean (*Phaseolus vulgaris* L.) seed coats as cholesterol micelle disruptors", *Plant Foods for Human Nutrition*, Vol. 68(4), pp. 416-423, http://link.springer.com/article/10.1007%2Fs11130-013-0384-7.

Chen, Q. (2004), "Determination of phytic acid and inositol pentakisphosphates in foods by high-performance ion chromatography", *Journal of Agricultural and Food Chemistry*, Vol. 52(15), pp. 4604-4613.

Cho, H.-J. et al. (2013), "Determination of cyanogenic compounds in edible plants by ion chromatography", *Toxicological Research*, Vol 29(2), pp. 143-147, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3834451/.

Choung, M.G. (2005), "Structural analysis of black common bean (*Phaseolus vulgaris* L.) anthocyanins", *Food Science and Biotechnology*, Vol. 14(5), pp. 672-675.

Chung, H.J., Q. Liu, and R. Hoover (2010), "Effect of single and dual hydrothermal treatments on the crystalline structure, thermal properties, and nutritional fractions of pea, lentil, and navy bean starches", *Food Research International*, Vol. 43(2), pp. 501-508, http://www.sciencedirect.com/science/article/pii/S0963996909002154.

Codex Alimentarius Commission (2003), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants – CAC/GL 45/2003*, Annexes II and III adopted in 2008, www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf.

Coelho, C.M.M., S.M. Tsai and V.A. Vitorello (2005), "Dynamics of inositol phosphate pools (tris-, tetrakis- and pentakisphosphate) in relation to the rate of phytate synthesis during seed development in common bean (*Phaseolus vulgaris*)", *Journal of Plant Physiology*, Vol. 162, pp. 1-9, http://www.sciencedirect.com/science/article/pii/S0176161704001543.

Cornell University (2014), *Plant Poisonous to Livestock – Plant Lectins*, Department of Animal Science, Cornell University, http://www.ansci.cornell.edu/plants/toxicagents/lectins.html (accessed on 20 March 2015).

Cruz-Bravo, R.K. et al. (2011), "Fermented nondigestible fraction from common bean (*Phaseolus vulgaris* L.) cultivar Negro 8025 modulates HT-29 cell behavior", *Journal of Food Science*, Vol. 76(2), pp. T41-T47, http://onlinelibrary.wiley.com/doi/10.1111/j.1750-3841.2010.02025.x/pdf.

Cvitanich, C. et al. (2011), "Micro-PIXE investigation of bean seeds to assist micronutrient biofortification", *Nuclear Instruments and Methods in Physics Research Section B*, Vol. 269(20), pp. 2297-2302, http://www.sciencedirect.com/science/article/pii/S0168583X11002308.

De Mejía, E. et al. (2005), "Tannins, trypsin inhibitors and lectin cytotoxicity in tepary (*Phaseolus acutifolius*) and common (*Phaseolus vulgaris*) beans"; *Plant Foods Human Nutr. Vol. 60*, pp. 137-145, http://link.springer.com/article/10.1007%2Fs11130-005-6842-0

De Mejía, E. et al. (2003), "Effect of cultivar and growing location on the trypsin inhibitors, tannins, and lectins of common beans (*Phaseolus vulgaris* L.) grown in the semi-arid highlands of Mexico", *Journal of Agriculture and Food Chemistry,* Vol. 51(20), pp. 5962–5966.

Delfino, R.A. and S.G. Canniatti-Brazaca (2010), "Interação de polifenóis e proteínas e o efeito na digestibilidade proteica de feijão comum (*Phaseolus vulgaris* L.) cultivar Pérola (Polyphenol and protein interaction and the effect on protein digestibility in common bean (*P. vulgaris* L.) cultivar Pérola)", *Ciência e Tecnologia de Alimentos*, Vol. 30(2), Campinas, pp. 308-312, in Portuguese, http://www.scielo.br/pdf/cta/v30n2/03.pdf.

Deshpande, S.S. et al. (1982), "Effects of dehulling on phytic acid, polyphenols and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.)", *Journal of Food Science, Chicago*, Vol. 47, No. 6, pp. 1846-1850, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1982.tb12896.x/pdf.

Dhurandhar, N.V. and K.C. Chang (1990), "Effect of cooking on firmness, trypsin inhibitors, lectins and cystine/cysteine content of navy and red kidney beans (*Phaseolus vulgaris*)", *Journal of Food Science,* Vol. 55(2), pp. 470–474, https://doi.org/10.1111/j.1365-2621.1990.tb06789.x.

Díaz-Batalla, L. et al. (2006), "Chemical components with health implications in wild and cultivated Mexican common bean seeds (*Phaseolus vulgaris* L.)", *Journal of Agricultural and Food Chemistry*, Vol. 54(6), pp.2045-2052.

Dwivedi, S.L. et al. (2012), "Nutritionally enhanced stable food crops", *Plant Breeding Reviews*, Vol. 36, pp 169-291.

Espinosa-Alonso, L.G. et al. (2006), "Polyphenols in wild and weedy Mexican common beans (*Phaseolus vulgaris* L.)", *Journal of Agricultural and Food Chemistry*, Vol. 54(12), pp. 4436-4444.

Estévez, A.M. et al. (1991), "Effect of processing on some chemical and nutritional characteristics of pre-cooked and dehydrated legumes", *Plant Foods for Human Nutrition*, Vol. 41, pp. 193-201, http://link.springer.com/article/10.1007%2FBF02196387#.

FAOSTAT (2019), "Crops – Beans, dry – Production quantity, years 1988 to 2017 – Export and import quantities, years 2012 to 2016; Food supply quantity, bean, kg/capita, year 2013", *FAO Statistics Database*, Food and Agriculture Organisation of the United Nations (FAO), http://faostat.fao.org (accessed on 10 July 2019).

Freytag, G.F. and D.G. Debouck (2002), "Taxonomy, distribution, and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America", *Sida,Botanical Miscellany*, Vol. 23, pp. 1-300.

Geil, P.B. and J.W. Anderson (1994), "Nutrition and health implications of dry beans: a review", *Journal of the American College of Nutrition*, Vol. 13, No. 6, New York, pp. 549-558.

Gepts, P. and D.G. Debouck (1991), "Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.)", in A. van Schoonhoven and O. Voysest (eds.), *Common Beans: Research for Crop Improvement*, Commonwealth Agricultural Bureaux International, Wallingford, UK, pp. 7-53.

Gepts, P. and F.A. Bliss (1988), "Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. II: Europe and Africa", *The Americas Economic Botany*, Vol. 42(1), pp. 73-85, http://link.springer.com/article/10.1007%2FBF02859038#page-2.

Gouveia, C.S.S. et al. (2014), "Nutritional and mineral variability in 52 accessions of common bean varieties (*Phaseolus vulgaris* L.) from Madeira Island", *Agricultural Sciences 2014*, Vol. 5, pp. 317-329, http://dx.doi.org/10.4236/as.2014.54034.

Grant, G. (1991), "Lectins", in J.P.F. D'Mello, C.M. Duffus and J.H. Duffus (eds.), *Toxic Substances in Crop Plants*, The Royal Society of Chemistry, Cambridge, pp. 49-67.

Guajardo-Flores, D. et al. (2012), "Characterization and quantification of saponins and flavonoids in sprouts, seed coats and cotyledons of germinated black beans", *Food Chemistry*, Vol. 134, pp. 1312-1319, http://www.sciencedirect.com/science/article/pii/S0308814613004561#.

Gupta, Y.P. (1987), "Anti-nutritional and toxic factors in food legumes: A review", *Plant Foods for Human Nutrition*, Vol. 37(3), pp. 201-228, http://link.springer.com/article/10.1007%2FBF01091786#.

Guzmán-Maldonado, H.S. et al. (2002), "Calidad alimentaria y potencial nutraceutico del frijol (*Phaseolus vulgaris* L.) (Food quality and nutraceutical potential of common bean [*Phaseolus vulgaris* L.])", *Agricultura técnica en México*, Vol. 28(2), pp. 159-173, in Spanish (English Abstract), http://www.redalyc.org/articulo.oa?id=60828206.

Helbig, E. et al. (2003), "Effect of soaking prior to cooking on the levels of phytate and tannin of the common bean (*Phaseolus vulgaris*, L.) and the protein value", *Journal of Nutritional Science and Vitaminology*, Vol. 49, pp. 81-86.

Hendry, G.W. (1918), "Bean culture in California", *Bulletin of the University of California Agricultural Experimental Station*, Vol. 294, pp. 285-347, http://babel.hathitrust.org/cgi/pt?id=uc2.ark:/13960/t2r50q843.

Heuzé, V. et al. (2013), *Common Bean (*Phaseolus vulgaris*)*, Feedipedia.org – Animal Feed Resources Information System – A programme by INRA, CIRAD, AFZ and FAO, http://www.feedipedia.org/node/266 (accessed on 23 March 2015).

Hoover, R. et al. (2010), "Composition, molecular structure, properties, and modification of pulse starches: A review", *Food Research International*, Vol. 43, pp. 399-413, http://www.sciencedirect.com/science/article/pii/S096399690900266X.

Hosfield, G.L. and M.A. Uebersax (1980), "Variability in physic-chemical properties and nutritional components of tropical and domestic dry bean germplasm", *Journal of the American Society for Horticultural Science*, Vol. 105, pp. 246-252, http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740510302/pdf.

House, W.A. et al. (2002), "Potential for increasing the amounts of bioavailable zinc in dry beans (*Phaseolus vulgaris* L.) through plant breeding", *Journal of the Science of Food and Agriculture*, Vol. 82(13), pp. 1452-1457, http://onlinelibrary.wiley.com/doi/10.1002/jsfa.1146/pdf.

Hu, Y. et al. (2006), "Kaempferol in red and pinto bean seed (*Phaseolus vulgaris* L.) coats inhibits iron bioavailability using an *in vitro* digestion/human Caco-2 cell model", *Journal of Agricultural and Food Chemistry*, Vol. 54(24), pp. 9254-9261.

Ishimoto, M. et al. (1995), "Variation of seed α-amylase inhibitors in the common bean", *Theoretical and Applied Genetics*, Vol. 90, pp. 425-429, http://link.springer.com/article/10.1007%2FBF00221985#.

Jacinto-Hernández, C. and E.A. Campos (1993), "Efecto de la cocción sobre algunas características nutricionales del frijol", *Revista Agronomía Mesoamericana*, Vol. 4, pp. 42-47, in Spanish (English abstract), http://www.mag.go.cr/rev_meso/v04n01_042.pdf.

Jacinto-Hernández, C. et al. (2006), "Effect of soaking on the oligosaccharide content in common beans", *Annual Report of the Bean Improvement Cooperative BIC, Michigan State University, East Lansing, MI 48824*, Vol. 49, pp. 159-160, http://naldc.nal.usda.gov/naldc/download.xhtml?id=IND43805445&content=PDF.

Jacinto-Hernández C. et al. (2002), "Caracterización de una población de líneas endogámicas de frijol común por su calidad de cocción y algunos componentes nutrimentales", *Agrociencia*, Vol. 36, pp. 451-459, in Spanish, http://www.redalyc.org/articulo.oa?id=30236406.

Jackson, J. et al. (2012), "Utilization of dry beans and pulses in Africa", in M. Siddiq and M.A. Uebersax (eds.), *Dry Beans and Pulses Production, Processing and Nutrition*, Blackwell Publishing Ltd., Oxford, UK, http://dx.doi.org/10.1002/9781118448298.

Junk-Knievel, D.C., A. Vandenberg and K.E. Bett (2008), "Slow darkening in pinto bean (*Phaseolus vulgaris* L.) seed coats is controlled by a single major gene", *Crop Science*, Vol. 48(1), pp. 189-193.

Kaplan, L. and T. Lynch (1999), "*Phaseolus* (Fabaceae) in archaeology: AMS radiocarbon dates and their significance for pre-Colombian Agriculture", *Economic Botany*, Vol. 53(3), pp. 261-272, http://link.springer.com/article/10.1007%2FBF02866636#.

Kasim, A.B. and W.M. Edwards (1998), "The analysis for inositol phosphate forms in feed ingredients", *Journal of the Science of Food and Agriculture*, Vol. 76, Chicago, pp.1-9, http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0010(199801)76:1%3C1::AID-JSFA922%3E3.0.CO;2-9/pdf.

Kiers, J.L., R.M.J. Nout and F.M. Rombouts (2000), "*In vitro* digestibility of processed and fermented soya bean, cowpea and maize", *Journal of the Science of Food and Agriculture*, Vol. 80, No. 9, pp. 1325–1331, http://onlinelibrary.wiley.com/doi/10.1002/1097-0010(200007)80:9%3C1325::AID-JSFA648%3E3.0.CO;2-K/pdf.

Kleintop, A.E. et al. (2013), "Adaptation of the AOAC 2011.25 integrated total dietary fiber assay to determine the dietary fiber and oligosaccharide content of dry edible beans", *Journal of Agricultural and Food Chemistry*, Vol. 61, pp. 9719-9726.

Kwak, M., J.A. Kami and P. Gepts (2009), "The putative Mesoamerican domestication center of *Phaseolus vulgaris* is located in the Lerma-Santiago basin of Mexico", *Crop Science*, Vol. 49(2), pp. 554-563.

Lackey, J.A. (1981), "Tribe 10 – Phaseoleae DC", in R.M. Polhill & P.H. Raven (eds.), *Advances in Legume Systematics*, Royal Botanic Garden, Kew, England, pp. 301-327.

Lajolo, F.M., F. Finardi-Filho and E.W. Menezes (1991), "Amylase inhibitors in *Phaseolus vulgaris* beans", *Food Technology*, Vol. 45, pp. 119-121.

Liener, I.E. (1994), "Implications of antinutritional components in soybean foods", *Critical Reviews of Food Science and Nutrition*, Vol. 34, No.1, pp. 31-67.

Lombardi-Boccia, G. et al. (1995), "Impact of processing on FE dialysability from bean (*Phaseolus vulgaris* L.)", *Food Chemistry*, Vol. 53, No. 2, London, pp. 191-195.

López, A. et al. (2013), "Effect of cooking and germination on phenolic composition and biological properties of dark beans (*Phaseolus vulgaris* L.)", *Food Chemistry*, Vol. 138(1), pp. 547-555, http://www.sciencedirect.com/science/article/pii/S0889157504001218.

Luthria, D.L. and M.A. Pastor-Corrales (2006), "Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties", *Journal of Food Composition and Analysis*, Vol. 19, pp. 205-211, http://www.sciencedirect.com/science/article/pii/S0889157505000992.

Marles, M.A.S., A. Vandenberg and K.E. Bett (2008), "Polyphenol oxidase activity and differential accumulation of polyphenolics in seed coats of pinto bean (*Phaseolus vulgaris* L.) characterize postharvest color changes", *Journal of Agricultural and Food Chemistry*, Vol. 56(16), pp. 7049-7056.

Martin-Cabrejas, M.A. et al. (2009), "The impact of dehydration process on antinutrients and protein digestibility of some legume flours", *Food Chemistry*, Vol. 114, pp. 1063-1068.

Martinez Meyer, M.R. et al. (2013), "Content of zinc, iron and their absorption inhibitors in Nicaraguan common beans (*Phaseolus vulgaris* L.)", *Food Chemistry*, Vol. 136(1), pp. 87-93, http://www.sciencedirect.com/science/article/pii/S0308814612012204.

Maskus, H (2010), "Pulse processing, functionality and application", *Literature review*, University of Winnipeg, Manitoba, Pulse Canada, pp. 1-146.

Matella, N.J., D.K. Mishra and K.D. Dolan (2012), "Hydration, blanching, and thermal processing of dry beans", in M. Siddiq and M.A. Uebersax (eds.), *Dry Beans and Pulses Production and Consumption*, Blackwell Publishing Ltd., Oxford, UK, http://dx.doi.org/10.1002/9781118448298.

McClean, P.E. et al. (2008), "*Phaseolus vulgaris*: A diploid model for soybean", in G. Stacey (ed.), *Genomics of Soybean*, Springer, New York, https://www.researchgate.net/publication/226039599_Phaseolus_vulgaris_A_Diploid_Model_for_Soybean.

Miklas, P.N. et al. (2006), "Common bean breeding for resistance against biotic and abiotic stresses from classical MAS breeding", *Euphytica*, Vol. 147, pp. 106-131, http://link.springer.com/article/10.1007%2Fs10681-006-4600-5#page-1.

Morales-de León, J.C. et al. (2007), "Preparation and characterization of protein isolate from fresh and hardened beans (*Phaseolus vulgaris*)", *Journal of Food Science*, Vol. 72, pp. C96-C102, http://onlinelibrary.wiley.com/doi/10.1111/j.1750-3841.2006.00244.x/pdf.

NRC (1998), *Nutrient Requirements of Swine, Tenth Revised Edition*, National Research Council, National Academy Press, Washington, DC, www.nap.edu/catalog/6016/nutrient-requirements-of-swine-10th-revised-edition (accessed on 15 June 2015).

OECD (2015), "Consensus Document on the Biology of Common Bean (*Phaseolus vulgaris* L.)", *Series on Harmonisation of Regulatory Oversight in Biotechnology,* No. 59, OECD, Paris, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2015)47&doclanguage=en.

Ogawa, H. and K. Date (2014), "The 'white kidney bean incident' in Japan", in J. Hirabayashi (ed.), *Lectins: Methods and Protocols, Methods of Molecular Biology*, Vol. 1200, pp. 39-45.

Oliveira, J.E.D. de (2005), "Feijão na alimentação/nutrição do brasileiro: ontem e amanhã", in *Congresso Nacional De Pesquisa De Feijão 8. 2005, Goiânia. Anais, Santo Antônio de Goiás: Embrapa Arroz e Feijão*, Vol. 2, Document no. 182, pp. 1245-1254.

Olmedilla-Alonso, B. et al. (2013), "Composition of two Spanish common dry beans (*Phaseolus vulgaris*), 'Almonga' and 'Curruquilla', and their postprandial effect in type 2 diabetics", *Journal of the Science of Food and Agriculture*, Vol. 93, pp. 1076-1082, http://onlinelibrary.wiley.com/doi/10.1002/jsfa.5852/pdf.

Oomah, B.D., C. Blanchard and P. Balasubramanian (2008), "Phytic acid, phytase, minerals, and antioxidant activity in Canadian dry bean (*Phaseolus vulgaris* L.) cultivars", *Journal of Agricultural and Food Chemistry*, Vol. 56(23), pp. 11312-11319.

Oomah, B.D., A. Cardador-Martinez and G. Loarca-Piña (2005), "Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L.)", *Journal of the Science of Food and Agriculture*, Vol. 85(6), pp. 935-942, http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2019/pdf.

Pedrosa, M.M. et al. (2015), "Effects of industrial canning on the proximate composition, bioactive compounds contents and nutritional profile of two Spanish common dry beans (*Phaseolus vulgaris* L.)", *Food Chemistry*, Vol. 166, pp. 68-75, http://www.sciencedirect.com/science/article/pii/S0308814614008887.

Piperno, D.L. (2012), "New archaeobotanical information on early cultivation and plant domestication involving microplant (phytolith and starch grain) remains", in P. Gepts et al. (eds.), *Biodiversity in Agriculture – Domestication, Evolution, and Sustainability*, Cambridge University Press, UK, pp. 136-159.

Pires, C.V. et al. (2005), "Composição físico-química de diferentes cultivares de feijão (*Phaseolus vulgaris* L.)", *Alimentos e Nutrição*, Vol. 16, No. 2, Araraquara, pp. 157-162.

Poel, T.F.B. et al. (1990), "Thermal Inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*Phaseolus vulgaris*) and effects on protein quality", *Journal of the Science of Food and Agriculture*, Vol. 53, No. 2, pp. 215-228, http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740530209/pdf.

Purseglove, J.W. (1968), *Tropical Crops – Dicotyledons 1*., John Wiley & Sons Inc., New York.

Queiroz Kda, S. et al. (2002), "Soaking the common bean in a domestic preparation reduced the contents of raffinose-type oligosaccharides but did not interfere with nutritive value", *Journal of Nutritional Science and Vitaminology*, Vol. 48(4), pp. 283-289.

Reddy, N.R. (2001), "Occurrence, distribution, content, and dietary intake of phytate", in N.R. Reddy and S.K. Sathe (eds.), *Food Phytates*, Ch.3, CRC Press, Boca Raton, FL, pp. 28.

Reddy, N.R. et al. (1984), "Chemical, nutritional and physiological aspects of dry bean carbohydrates – a review", *Food Chemistry*, Vol. 13, No. 1, London, pp. 25-68.

Reed, J.D. (1995), "Nutritional toxicology of tannins and related polyphenols in forage legumes", *Journal of Animal Science*, Vol. 73, pp. 1516-1528, https://www.ncbi.nlm.nih.gov/pubmed/7665384.

Rodiño, A.P. et al. (2006), "Novel genetic variation in common bean from the Iberian Peninsula", *Crop Science*, Vol. 46(6), pp. 2540-2546.

Rodiño, A.P. et al. (2003), "A core collection of common bean from the Iberian Peninsula", *Euphytica,* Vol. 131, pp. 165-175, http://dx.doi.org/10.1023/A:1023973309788.

Rodiño, A.P. et al. (2001), "Diversity of common bean (*Phaseolus vulgaris* L.) germplasm from Portugal", *Genetic Resources and Crop Evolution*, Vol. 48, pp. 409-417, http://dx.doi.org/10.1023/A:1012248002436.

Romero, J. and D.S. Ryan (1978), "Susceptibility of the major storage protein of the bean, *Phaseolus vulgaris* L., to *in vitro* enzymic hydrolysis", *Journal of Agricultural and Food Chemistry*, Vol. 26(4), pp. 784-788.

Rychlik, M. et al. (2007), "Folate contents of legumes determined by optimized enzyme treatment and stable isotope dilution assays", *Journal of Food Composition and Analysis*, Vol. 20(5), pp. 411-419.

Sathe, S.K. and D.K. Salunkhe (1984), "Technology of removal of unwanted components of dry beans", *CRC Critical Reviews in Food Science and Nutrition*, Vol. 21, No. 3, Cleveland, pp. 263-287.

Schrire, B.D. (2005), "Tribe Phaseoleae", in G. Lewis et al. (eds.), *Legumes of the World*, Royal Botanic Gardens, Kew, England, pp. 392-431.

Shi, J. et al. (2004), "Saponins from edible legumes: Chemistry, processing, and health benefits", *Journal of Medicinal Food*, Vol. 7(1), pp. 67-78.

Shiga, T.M., B.R. Cordenunsi and F.M. Lajolo (2009), "Effect of cooking on non-starch polysaccharides of hard-to-cook beans", *Carbohydrate Polymers*, Vol. 76, pp. 100-109, http://www.sciencedirect.com/science/article/pii/S0144861708004669.

Shimelis, E.A. and S.K. Rakshit (2005), "Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia", *Food Science and Technology International*, Vol. 38, pp. 331-338, https://www.researchgate.net/publication/222082332_Proximate_composition_and_physico-chemical_properties_of_improved_dry_bean_Phaseolus_vulgaris_L_varieties_grown_in_Ethiopia.

Siddiq, M. et al. (2010), "Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.) flours", *Journal of Food Science and Technology*, Vol. 43, pp. 232-237, http://www.sciencedirect.com/science/article/pii/S0023643809002047.

Singh, S.P., P. Gepts and D.G. Debouck (1991), "Races of common bean (*Phaseolus vulgaris*, Fabaceae)", *Economic Botany*, Vol. 45(3), pp. 379-396, http://link.springer.com/article/10.1007%2FBF02887079#.

Slupski J. and P. Gębczyński (2014), "Changes due to cooking and sterilization in low molecular weight carbohydrates in immature seeds of five cultivars of common bean", *International Journal of Food Sciences and Nutrition*, Vol. 65(4), http://www.tandfonline.com/doi/pdf/10.3109/09637486.2013.869794.

Soccol, C.R. (2012), "Chapter 6: Advances and applications of galactosidases in food industry", in A.K. Haghi (ed.), *Food Science: Research and Technology*, p.58.

Sotelo, A., H. Sousa and M. Sánchez (1995), "Comparative study of the chemical composition of wild and cultivated beans (*Phaseolus vulgaris*)", *Plant Foods for Human Nutrition*, Vol. 47, pp. 93-100, http://link.springer.com/article/10.1007%2FBF01089257#.

Stanley, D.W. and J.M. Aguilera (1985), "A review of textural defects in cooked reconstituted legumes – The influence of structure and composition", *Journal of Food Biochemistry*, Vol. 9, No. 4, Westport, pp. 277-323, http://onlinelibrary.wiley.com/doi/10.1111/j.1745-4514.1985.tb00355.x/pdf.

Suárez, R. et al. (2008), "Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia", *Molecular plant-microbe interaction*, Vol. 21, No. 7, pp. 958-966, http://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI-21-7-0958.

Tiwari, B. and N. Singh (2012), *Pulse Chemistry and Technology*, The Royal Society of Chemistry Publishing, Cambridge, UK.

Tohme, J. et al. (1995), "Variability in Andean Nuña common bean (*Phaseolus vulgaris*, Fabaceae)", *Economic Botany*, Vol. 49(1), pp. 78-95, http://www.jstor.org/stable/4255694?seq=1#page_scan_tab_contents.

USDA-ARS (2015), *National Nutrient Database for Standard Reference, Release 27 (revised) – Version May 2015*, United States Department of Agriculture, Agricultural Research Service, http://www.ars.usda.gov/ba/bhnrc/ndl (accessed on 15 June 2015).

USDA-ARS (2014), *National Nutrient Database for Standard Reference, Release 27*, United States Department of Agriculture, Agricultural Research Service, http://ndb.nal.usda.gov/ndb/search/list (accessed on 29 July 2014).

USDA-ESR (2010), *Dry Edible Beans, Historic Data*, United States Department of Agriculture, Economic Research Service webpage (accessed on 20 December 2013, no longer available).

Voysest, O. (1983), *Variedades de Frijol en América Latin y su Origen*, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

Voysest, O. and M. Dessert (1991), "Bean cultivars: Classes and commercial seed types", in A. van Schoonhoven and O. Voysest (eds.), *Common Beans: Research for Crop Improvement*, Commonwealth Agricultural Bureaux International, Wallingford, UK, pp. 119-162.

Wang, N. and J.K. Daun (2004), *The Chemical Composition and Nutritive Value of Canadian Pulses*, Canadian Grain Commission, Grain Research Laboratory, pp. 1-85.

Weder, J.K.P. et al. (1997), "Antinutritional factors in Anasazi and other pinto beans (*Phaseolus vulgaris* L.)", *Plant Foods for Human Nutrition*, Vol. 51, pp. 85-98, http://link.springer.com/article/10.1023%2FA%3A1007924931856#.

Westphal, E. (1974), "Pulses in Ethiopia, their taxonomy and agricultural significance", *Centre for Agricultural Publishing and Documentation*, Vol. 815, Wageningen, The Netherlands, pp. 159-176, https://edepot.wur.nl/197905.

Xu, B. and S.K.C. Chang (2009), "Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (*Phaseolus vulgaris* L.) as affected by thermal processing", *Journal of Agricultural and Food Chemistry*, Vol. 57(11), pp. 4754-4764.

Zhang, X., M.W. Blair and S. Wang (2008), "Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers", *Theor. Appl. Genet*, Vol. 117(4), pp. 629-640, http://link.springer.com/article/10.1007%2Fs00122-008-0807-2.

Ziena, H.M., M. Youssef and A.R. El-Mahdy (1991), "Amino acid composition and some anti-nutritional factors of cooked faba beans (Medamnins): Effects of cooking temperature and time", *Journal of Food Science*, Vol. 56, No. 5, Chicago, pp. 1347-1349, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1991.tb04769.x/pdf.

Chapter 2. Rice (*Oryza sativa*)

This chapter deals with the composition of rice (Oryza sativa). It contains elements that can be used in a comparative approach as part of a safety assessment of foods and feeds derived from new varieties. Background is given on rice industry terminology, cultivated species, production and consumption worldwide, processing from paddy rice to brown, milled or parboiled rice products for human consumption, and feed use of by-products. Appropriate varietal comparators and characteristics screened by rice breeders are presented. Nutrients in paddy rice, brown rice, milling fractions, whole plant and straw, as well as main anti-nutrients, toxicants and putative allergens are then detailed. The final sections suggest key constituents for analysis in rice matrices of new varieties, for food use and for feed use.

This chapter was prepared by the OECD Working Group for the Safety of Novel Foods and Feeds, with Japan as the lead country and expertise from other stakeholders including the International Rice Research Institute (IRRI), Philippines. It updates and replaces the original publication on rice composition considerations issued in 2004 (contained in Volume 1) and was initially issued in August 2016. FAOSTAT data on cereals production, including Figure 2.2, and IRRI World Rice Statistics data on rice production, trade and consumption, including Tables 2.2 and 2.4, have been updated.

Terminology

A number of technical and scientific terms that are specific to the rice industry are used in this document. In order to facilitate common understanding, these terms and their definitions are listed in Table 2.1.

Table 2.1. Definitions in this document

Term	Synonym(s)	Definition
Bran		Germ and several histologically identifiable soft outer layers (pericarp, seed coat, nucellus and aleurone layer)
Broken rice		Milled broken rice grains, subdivided into second heads (½ - ¾), screenings (¼ - ½) and brewer's rice (< ¼) by the grain length, compared with that of the whole rice
Brown rice	caryopsis, cargo rice, hulled rice, husked rice, dehulled rice, dehusked rice, unpolished rice	Paddy rice from which the hull only has been removed; the process of hulling and handling may result in some loss of bran
Endosperm		Starchy tissue covered by the aleurone layer; divided into two regions, the subaleurone layer and the central core region containing mainly starch
Germ	embryo	The part consisting of scutellum, plumule, radicle and epiblast
Glutinous rice	waxy rice, sticky rice	Rice of which amylose content is less than 5%
Head rice	head yield	Milled whole rice kernels, exclusive of broken rice that is smaller than ¾ of the grain length of the whole rice
Hull	husk, shell, chaff	Outermost layer of paddy rice
Hulling	dehulling, husking, dehusking, shelling	Removal of the hull from paddy rice
Milled rice	white rice	Rice grain with removed germ and outer layer such as pericarp, seed coat and a part of aleurone layer by milling
Milling	scouring, whitening	Removal of all or most of the bran to produce the milled rice that is white
Paddy rice	rice grain, rough rice	Rice grain after threshing and winnowing; retains its hull
Parboiled rice		Hulled or milled rice processed from paddy or hulled rice which has been soaked in water and subjected to a heat treatment so that the starch is fully gelatinized, followed by a drying process
Polished rice		Rice grain with removed outer layer by polishing of milled rice
Polishing		Abrasive removal of traces of bran on the surface of milled rice to give a smoother finish
Polishings	polish	The by-product from polishing rice, consisting of the inner bran layers of the kernel with part of the germ and a small portion of the starchy interior

Figure 2.1. Rice plants

Source: Courtesy of the IRRI, licenced under CC BY-SA.

Background

Cultivated rice species

Most of the rice varieties grown in the world belong to the species *Oryza sativa* which has its origin in Asia. Another species grown in western Africa, *Oryza glaberrima*, is considered to have been domesticated in the Niger river delta. Varieties of the species *Oryza glaberrima* are cultivated in limited regions and detailed production data are scarcely available. For these reasons, this document deals only with *Oryza sativa* that occupies the great majority of the rice production and consumption in the world.

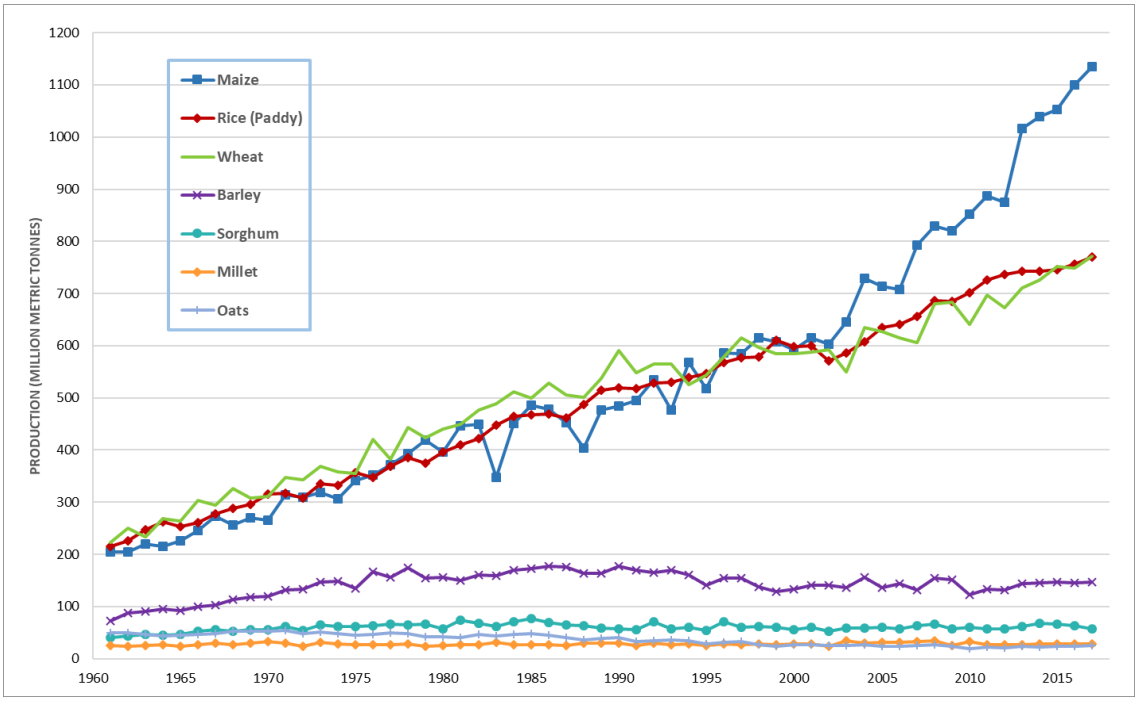
Oryza sativa has two types, indica and japonica, which account for almost all global rice production. Indica is the dominant type, estimated to account for more than 80% of global rice production. It is mostly grown in the tropics and subtropics. Indica rice cooks fluffy, dry and separate, and the grain is usually more slender than that of japonica rice. Japonica rice is typically grown in more temperate areas such as Australia, northern China, Japan and Europe. It cooks moist and clingy. It accounts for 15% of global rice production and typically achieves higher yields than indica. Aromatic rice varieties, primarily, basmati and jasmine, account for 1% of total world rice production. These varieties are noted for their fragrant taste and smell, contributed primarily by the presence of 2-acetyl-1-pyrroline. Glutinous rice varieties of both indica and japonica types account for most of the remainder of world rice production.

Further description on the rice taxonomy, centre of origin and diversity, identification among rice species and groups, reproductive biology, intraspecific and interspecific crosses, and ecology can be found in the Consensus Document on the Biology of *Oryza sativa* (Rice) (OECD, 1999).

Production and consumption

Rice is cultivated in more than 100 countries around the world, being one of three major staple crops after maize and with a total production similar to wheat (Figure 2.2). Rice is a basic food for about half of the world's population. In 2017, its global cropping area covered about 161 million hectares and the production of paddy rice exceeded 729 million metric tonnes (Table 2.2). Asia is the main rice-producing region by far, totalling more than 93% of paddy rice harvested globally (IRRI World Rice Statistics, 2019). The country with the highest production is the People's Republic of China, representing 29% of the total share in 2017, followed by India (23%). Yield (tonnes/hectare) has rapidly increased since the second half of the 1960s as the semi-short (short-stem) and high-yield varieties became widespread. Rice is mostly consumed in each producing country. The world trade amount of rice was approximately 49 million metric tonnes in 2017 (Table 2.3), which represented 10% of the world production of milled rice.

Figure 2.2. Production of major staple cereal crops in the world, 1961-2017



Note: Aggregate may include official, semi-official, estimated or calculated data.
Source: FAOSTAT (2019), *Online database: Production/ Crops: Barley, Maize, Millet, Oats, Rice (paddy), Sorghum, Years 1961 to 2017*, http://www.fao.org/faostat/ (accessed on 10 July 2019).

Table 2.2. World production and main producing countries of paddy rice in 2017

Rank	Country	Production (million metric tonnes)
1	China (People's Republic of)	208.6
2	India	165.0
3	Indonesia	58.3
4	Bangladesh	49.0
5	Viet Nam	45.7
6	Thailand	30.9

Rank	Country	Production (million metric tonnes)
7	Myanmar	20.6
8	Philippines	19.5
9	Brazil	11.9
10	Pakistan	11.3
11	Japan	10.4
12	Cambodia	8.9
13	United States	8.1
14	Egypt	6.2
15	Nigeria	6.0
	World	**729.1**

Source: IRRI World Rice Statistics (2019), *Online database: Paddy Rice Production in 2017,* http://ricestat.irri.org:8080/wrsv3/entrypoint.htm (accessed on 10 July 2019).

Table 2.3. World rice exports and imports in 2017

In million metric tonnes

Rank	Exporting country	Exports	Importing country	Imports
1	India	12.8	China (People's Republic of)	5.5
2	Thailand	10.5	Nigeria	2.6
3	Viet Nam	6.8	Indonesia	2.0
4	Pakistan	4.0	Côte d'Ivoire	1.5
5	Myanmar	3.5	Philippines	1.4
6	United States	3.2	Iran	1.3
7	China (People's Republic of)	1.6	Saudi Arabia	1.3
8	Cambodia	1.3	Bangladesh	1.2
9	Uruguay	0.9	Senegal	1.2
10	Brazil	0.9	Iraq	1.1
	World (Total)	**48.7**	**World (Total)**	**46.0**

Source: IRRI World Rice Statistics (2019), *Online database: Rice Export and Import Quantities in 2017*; http://ricestat.irri.org:8080/wrsv3/entrypoint.htm (accessed on 10 July 2019).

Rice consumption worldwide is shown in Table 2.4, with the highest per capita consumption being reported for Asia. Rice accounts for 19% of global caloric intake and the values are even higher in Asia (IRRI World Rice Statistics, 2019).

Table 2.4. Production and consumption of milled rice by continent/region

Region	Production* (million metric tonnes)	Consumption** (kg/capita/year)
Asia	439.6	77.8
Africa	20.6	23.9
South America	16.7	28.7
North and Central America	7.6	10.7
Europe	3.3	4.6
Oceania	0.6	13.4
World	**488.3**	**54.0**

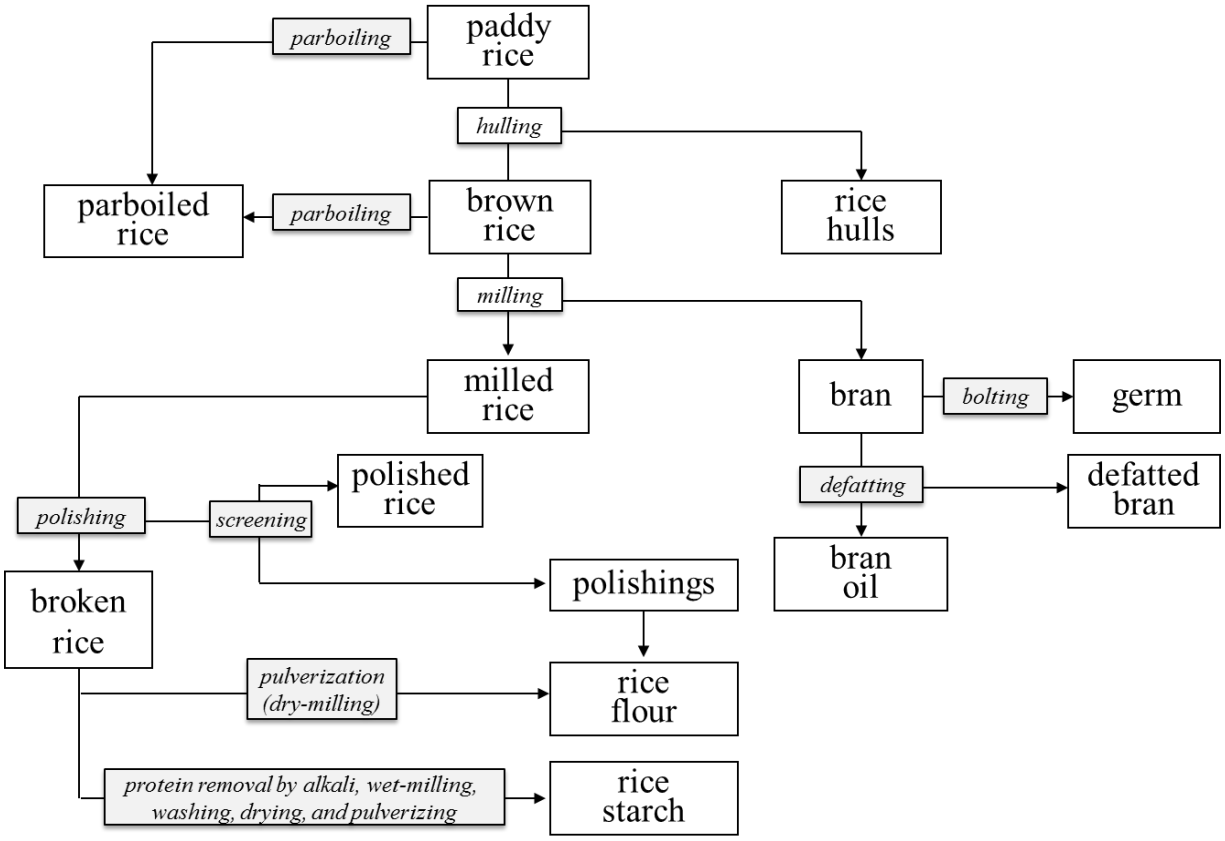
Notes: * 2017 data.
** 2013 data.

Source: IRRI World Rice Statistics (2019), *Paddy Rice Production in 2017*; *Rice Consumption Per Capita in 2013,* http://ricestat.irri.org:8080/wrsv3/entrypoint.htm (accessed on 10 July 2019).

Processing

Paddy rice is processed as shown in Figure 2.3. Parboiled rice is prepared by soaking in water, draining, heating (most often steaming; sometimes under pressure), then drying, followed by hulling and milling. Brown rice is produced from paddy rice by removing the hulls (hulling). Milled rice is derived from brown rice by milling to remove all or most of the bran which primarily consists of a seed coat, aleurone layer and germ. Germ seed is separated through bolting/sieving of the by-products of milling. Milled rice is processed by polishing to remove residual bran on the surface to give a smoother finish and may further be polished to obtain the inner part of rice grain containing less protein for further processing. Most of the rice used for food is milled rice. Rice flour is a pulverised product of the outer part or the whole milled rice. Rice bran oil which is used as cooking oil is made from rice bran by squeezing and, as necessary, successive refining.

Figure 2.3. Rice processing and the resulting products



Source: Satake (1990), *Modern Rice Milling Technology*.

Table 2.5 provides weight ratios for the main rice milling fractions.

Table 2.5. Rice fractions by hulling and milling

Fraction	Ratio (%, on a weight basis)
Hull	16-28 (average 20) of paddy rice
Brown rice	72-84 (average 80) of paddy rice
Milled rice	90 of brown rice
Bran + polishings	10 of brown rice

Source: Adapted from Juliano and Bechtel (1985), "The rice grain and its gross composition".

Uses

Rice is consumed as brown rice, milled rice or parboiled rice after being cooked in the grain form. There are many recipes for cooked brown or milled rice in which rice is boiled, steamed, boiled into porridge or mixed with other grain flours. Boiled or steamed rice can be further baked or fried.

It is estimated that a fifth of the world's consumed rice is parboiled (Bhattacharya, 2004). Use of parboiled rice seems to have increased in recent years due to its numerous advantages: easy hulling, reduced grain breakage during milling, reduced loss of nutrients during washing, maintaining grain integrity after cooking, reduced loss of solids in cooking water, reduced insect infestation and loss of nutrients during storage, high content of bran oil which becomes stable to free fatty acid formation due to inactivation of triacylglycerol lipase by parboiling, and suitability for the production of canned, expanded and flaked rice. A disadvantage to parboiling is the destruction of antioxidants and some B vitamins. Parboiled brown rice as a whole shows lower content of B vitamins but the content depends on its fraction. For example, the content of B vitamins in the parboiled milled rice fraction is higher than in raw milled rice, while that in parboiled bran fraction is lower than in raw rice bran (Padua and Juliano, 1974).

Only a relatively small amount of rice is consumed as prepared rice products worldwide. However, prepared rice products are widely found and consumed in Asia as noodle, cake, cracker, sweets and alcoholic beverages. For example, rice noodles are found in different shapes and given local names in Asian countries such as the People's Republic of China and Thailand. Rice sweets and cakes are also common in Asia. Glutinous rice is used in desserts, rice cakes and ceremonial dishes (Childs, 2004). As for alcoholic beverages, there are rice wines and distilled rice wines in Japan, Korea, and the People's Republic of China. Alcohol from the fermentation of rice flour is partly used for increasing alcohol degree of rice wine.

Poor grade paddy rice and by-products of food processing such as broken rice, hulls, bran, rice flour and hulls/polishings of parboiled rice are used for feed. Defatted bran (cake of rice bran) can be further utilised for feed and as fertiliser.

Appropriate comparators for testing new varieties

This document suggests parameters that rice breeders should measure when developing new modified varieties. The data obtained in the analysis of a new *O. sativa* variety should ideally be compared to those obtained from an appropriate near-isogenic non-modified variety, grown and harvested under the same conditions.[1] The comparison can also be made between values obtained from new varieties and data available in the literature or chemical analytical data generated from other commercial rice varieties.

Components to be analysed include key nutrients and other constituents. Key nutrients are those which have a substantial impact on the overall diet of humans (food) and animals (feed). These may be major constituents (fats, proteins, and structural and non-structural carbohydrates) or minor compounds (vitamins and minerals). Similarly, the levels of other constituents such as anti-nutrients, toxicants and allergens should be considered. Toxicants are those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health. Standardised analytical methods and appropriate types of material should be used, adequately adapted to the use of each product and by-product. The key components analysed are used as indicators of whether unintended effects of the genetic modification influencing plant metabolism have occurred or not.

Breeding characteristics screened by developers

Phenotype characteristics provide important information related to the suitability of new varieties for commercial distribution. Selecting new varieties is based on data from parental lines. Plant breeders developing new varieties of rice evaluate many parameters at different stages in the developmental process (OECD, 1999). In the early stages of growth, breeders evaluate stand count, seedling vigour, and tillering, and as plants mature, insect-resistance and resistance to disease such as blast disease are evaluated. At near maturity or maturity, heading, maturation, lodging, blanking, shattering, shedding and pre-harvest sprouting (for hybrids) are evaluated. The matured plant is measured for plant height (ground to tip of panicle on the tallest tiller), panicle length, number of panicles, and yield of crop. The harvested grain is measured for yield of grain, moisture, test weight, shape, size, visual quality, dormancy, components content, milling quality and palatability.

Natural variation for agronomic characteristics such as resistance to insect pests and diseases are also considered in the breeding process. More information can be found in the Consensus Document on the Biology of *Oryza sativa* (Rice) (OECD, 1999).

Figure 2.4. Planting in a paddy field

Source: Courtesy of the IRRI, licenced under CC BY-SA.

Conventional breeding of rice, as well as those based on modern biotechnology, can include considerations of nutritive improvements with increased content (biofortification) of elements such as pro-vitamin A, iron, or zinc. In these cases, the amounts of these components are specifically evaluated for those objectives.

Figure 2.5. Growing rice

Source: Courtesy of the IRRI, licenced under CC BY-SA.

Nutrients

Key nutrients in rice products for food use

Key nutrients in rice products for food use are listed in Tables 2.7 and 2.8. Compositional data to compare between indica and japonica varieties are rarely available.

Carbohydrates

Most of the digestible carbohydrates as energy sources are found in the endosperm of rice grain. Milled rice mainly consists of starch with a few other carbohydrates including free sugars and non-starch polysaccharides. The hull is comprised of mostly non-starch polysaccharides such as cellulose and hemicellulose, and it may contain a small amount of starch. The bran and germ are comprised mainly of non-starch polysaccharides such as cellulose and hemicellulose and partly of free sugars as well as a small amount of starch.

Starch

Starch, the principal component of rice, consists of amylose (linear fraction) and amylopectin (branched fraction). Starch in non-glutinous rice is, in general, composed of 10% to 30% amylose and 70% to 90% amylopectin. Starch in glutinous rice contains less than 5% of amylose and consists mostly of amylopectin (Juliano and Villareal, 1993).

Amylose content shows a high positive correlation with the hardness of cooked rice, and it may be used to roughly distinguish between indica and japonica varieties (OECD, 1999).

Amylose content may range depending on the variety: waxy rice (0%-2.0%); very-low-amylose rice (2.1%-10.0%); low-amylose rice (10.1%-17.0%); intermediate-amylose rice (17.1%-22.0%); high-amylose rice (> 22.0%) (Juliano et al., 2012). As amylose content varies depending on the method of analysis: iodine-amylose complex (Juliano et al., 2012), size exclusion (gel permeation) chromatography (Horibata et al., 2004; Nakaura et al., 2011), differential-scanning calorimetry (Mestres et al., 1996), this factor should be considered when comparing the levels among varieties.

Amylose content for a particular variety may show seasonal and regional variations of 1% to 4%, and it does not reach the range observed for varietal differences (Juliano and Villareal, 1993).

Dietary fibre

Although dietary fibre and resistant starch are important nutrients, they are low in cooked rice such as cooked milled rice and milled rice porridge. Dietary fibre is lost by hulling, milling and polishing as shown in Tables 2.7 and 2.8.

Protein

Total protein content in rice is calculated by multiplying total nitrogen content by the rice-specific Kjeldahl conversion factor of 5.95, which is based on the nitrogen content of glutelin, the major protein in rice (Juliano, 1985a). The protein content fluctuates according to the variety grown and can also be affected by growing conditions such as early or late maturing, soil fertility and water stress. The protein content in brown rice ranges from 5% to 17% on a dry matter basis based on the analysis of about 8 000 samples ranging (Juliano, 1968).

Rice proteins are classified based on solubility as albumin (water-soluble), globulin (salt-water-soluble), prolamin (alcohol-soluble) or glutelin (soluble in aqueous alkaline solution) (Hoseney, 1986). The percentage of each protein with respect to the total protein content is shown in Table 2.6. Albumin and globulin have a balanced composition of amino acids. They are found mostly in the outer layer of brown rice, and less in the inner layer of milled rice. Prolamin and glutelin are considesred to be the storage proteins of rice, and the proteins exist in the outer layer and the inside of milled rice. Thus, the protein composition of bran and germ differ greatly from that of milled rice. However, it should be noted that the ratios and the range for each fraction vary widely, depending on the rice variety and the extraction conditions (Shih, 2004).

Table 2.6. Typical proportions of milled rice protein fractions

Protein fraction	Percentage of total protein
Albumin (soluble in water)	2-5
Globulin (soluble in saltwater)	2-10
Prolamin (soluble in alcohol)	20-25
Glutelin (soluble in aqueous alkaline solution)	60-65

Note: Proteins were fractionated by the method of Osborne (Hoseney, 1986).
Source: Ogawa et al (1989), "Mutants for rice storage protein (…)".

Table 2.7. Proximate, carbohydrate components (% of dry matter) and energy content of paddy rice and brown rice

Nutrient	Paddy rice						Brown rice			
	Juliano and Bechtel (1985)[a]	ILSI-CCDB (2014)[b]		Heuzé, Tran and Hassoun in Feedipedia (2015)		NRC (1982)	Juliano and Bechtel (1985)[a]	USDA (2014)[c]	NARO (2011)[d]	
	range	mean	range	mean	range	mean	range	mean	mean	range
Water (% of fresh weight)	*14*	*16.85*	*9.05-28.35*	*12.0*	*7.6-16.4*	*11.0*	*14*	*11.37*	*13.8*	*12.1-16.4*
Crude protein[e]	6.7-9.0	8.55	7.41-10.00 [f]	8.3	5.9-11.8	8.9 f	8.3-9.7	8.71	7.7	6.5-10.0
Crude fat	1.7-2.7	2.76	2.52-3.47	2.1	1.7-2.6	1.9	1.9-3.3	3.16	3.3	2.8-3.9
Crude ash	3.4-6.0	4.77	3.61-6.54	5.9	3.9-8.6	5.3	1.2-1.7	1.58	1.5	1.2-1.7
Carbohydrates (calculated)[g]		83.91	79.98-85.53					86.55	87.5[h]	85.2-88.9[h]
Digestible carbohydrates	74.0-85.1						84.8-88.2			
Starch	62.1			64.2	61.9-67.2		77.2			
Free sugars	0.6-1.4						0.8-1.5			
Neutral detergent fibre	19.1	18.49	16.15-21.47	21.5	15.0-32.2		4.5			
Acid detergent fibre		15.06	11.79-16.75	13.3	10.8-18.2					
Dietary fibre/ insoluble		18.98	18.84-19.12							
Dietary fibre/ soluble		1.26	-							
Total dietary fibre		19.15	16.73-22.97					3.9		
Crude fibre	8.4-12.1	14.51	10.89-18.13	11.1	8.6-14.8	10.0	0.7-1.2			
Cellulose										
Hemicelluloses										
Pentosans	4.3-6.2						1.4-2.4			
Lignin	4.0			5.4	4.9-5.8					
Energy (kJ/g)	*18.4*			*17.6*	*17.1-22.3*		*17.6-18.7*	*17.3*	*17.4*	*17.2-17.5*

Notes: a. Data from Juliano and Bechtel are presented on a fresh weight basis; values at 14% moisture in the literature were converted to those at percentage of dry matter; b. The data are measured using an indica rice variety; c. Average data for long and medium grains; d. n=138 (data obtained in Japan between 1999 and 2009); the values for each sample were converted to those in dry matter basis by using each moisture content; e. Crude protein = Protein (N x 5.95); f. The conversion factor for ILSI-CCDB and NRC data is not confirmed to be 5.95; g. Carbohydrate (calculated) = 100 – Protein – Crude Fat – Ash – Moisture; h. n=123 (data obtained in Japan between 1999 and 2009); the values for each component reported were converted to dry matter by using moisture content.

Table 2.8. Proximate, carbohydrate components (% of dry matter) and energy content of rice fractions

Nutrient	Milled rice			Bran		Germ	Polishings
	Resources Council STA Japan (2000)	Juliano and Bechtel (1985)[a]	USDA (2014)	Juliano and Bechtel (1985)[a]	USDA (2014)	Juliano and Bechtel (1985)[a]	Juliano and Bechtel (1985)[a]
	mean	range	mean	range	mean	range	range
Water (% of fresh weight)	*15.5*	*14*	*12.31*	*14*	*6.13*	*14*	*14*
Protein (N x 5.95)[b]		7.3-8.3	7.65	13.1-17.3	14.22	16.4-24.0	13.0-14.4
Crude fat		0.3-0.6	0.65	17.4-22.9	22.21	19.3-23.8	11.7-14.4
Crude ash		0.3-0.9	0.64	7.7-11.5	10.63	5.6-10.1	6.0-8.5
Carbohydrates (calculated)[c]			91.07		52.93		
Digestible carbohydrates[d]		89.1-91.2		39.7-60.8		39.8-48.1	59.4-64.0
Starch		90.2		16.0		2.4	48.3-55.3
Free sugars		0.3-0.5		6.4-8.0		9.3-14.0	
Sugar (calculated)[e]			0.14		0.96		
Neutral detergent fibre		0.8-2.7		27.6-33.3		15.2	
Acid detergent fibre							
Dietary fibre/ insoluble	0.5						
Dietary fibre/ soluble	trace						
Total dietary fibre	0.5		2.8		22.4		
Crude fibre		0.2-0.6		8.1-13.3		2.8-4.1	2.7-3.7
Cellulose				6.9-10.5		3.1	
Hemicelluloses		0.1		11.0-19.7		11.3	
Pentosans		0.6-1.6		8.1-9.7		5.7; 7.4	4.2-5.5
Lignin		0.1		33-4.5		0.8-4.7	3.3
Energy (kJ/g)		*17.0-18.1*	*17.3*	*19.4-23.1*	*14.1*		*20.8*

Notes: a. Data from Juliano and Bechtel are presented on a fresh weight basis; values at 14% moisture in the literature were converted to those at percentage of dry matter; b. Crude protein = Protein (N x 5.95); c. Carbohydrate (calculated) = 100 – Protein – Crude Fat – Ash – Moisture ; d. Digestible carbohydrates = Carbohydrates (calculated) – Crude fibre ; e. Sugar (calculated) = Carbohydrates (calculated) – Fibre.

Amino acid composition

The key protein in rice is glutelin (oryzenin) and the most limiting amino acid is lysine. To evaluate the nutritional value of each protein as food, the amino acid score is calculated as follows: 100 x (milligram (mg) of essential amino acid in the protein)/(mg of the essential amino acid in the reference protein ideal for human consumption) (WHO, 1985; 2007). Rice, with an Amino Acid Score (AAS) of 68, offers a more complete and balanced amino acid composition than those of other major cereals such as wheat (medium flour: AAS of 43) and maize (corn grits: AAS of 35), due to its higher contents of lysine and sulphur-containing amino acids (WHO, 1985; 2007). Protein content and amino acid composition vary in paddy and brown rice (Table 2.9).

Table 2.9. Amino acid composition (% of dry matter) of paddy rice and brown rice

Amino acid	Paddy rice			Brown rice		
	Juliano (1985a)[a]	ILSI-CCDB (2014)[b]		Juliano (1985a)[a]	NARO (2011)	
	range	mean	range	range/value(s)	mean	range
Alanine	0.39-0.57	0.44	0.38-0.50	0.54	0.45	0.37-0.59
Arginine	0.61-0.85	0.57	0.53-0.65	0.79-0.98	0.63	0.52-0.88
Aspartic acid	0.61-0.94	0.76	0.68-0.85	0.84-0.88	0.71	0.59-0.96
Cystine	0.10-0.26	0.18	0.15-0.20	0.20-0.22	0.20	0.15-0.28
Glutamic acid	1.31-1.74	1.24	1.10-1.37	1.57-1.64	1.32	1.06-1.88
Glycine	0.35-0.48	0.37	0.34-0.42	0.44-0.45	0.37	0.32-0.48
Histidine	0.14-0.25	0.22	0.20-0.25	0.22-0.24	0.20	0.16-0.27
Isoleucine	0.27-0.43	0.30	0.27-0.34	0.33-0.43	0.29	0.22-0.40
Leucine	0.61-0.78	0.62	0.55-0.71	0.77-0.83	0.62	0.51-0.85
Lysine	0.29-0.42	0.29	0.28-0.32	0.36-0.40	0.30	0.26-0.40
Methionine	0.14-0.31	0.19	0.17-0.21	0.21-0.23	0.22	0.14-0.34
Phenylalanine	0.28-0.52	0.40	0.36-0.44	0.47-0.49	0.40	0.32-055
Proline	0.33-0.54	0.35	0.29-0.42	0.45-0.47	0.34	0.25-0.46
Serine	0.36-0.51	0.40	0.36-0.47	0.45-0.54	0.39	0.30-0.53
Threonine	0.27-0.40	0.30	0.27-0.33	0.36-0.37	0.28	0.23-0.38
Tryptophan	0.11-0.18	0.10	0.09-0.12	0.12-0.14	0.09	0.05-0.13
Tyrosine	0.34-0.48	0.14	0.13-0.18	0.35-0.43	0.32	0.21-0.51
Valine	0.41-0.63	0.43	0.39-0.49	0.47-0.61	0.45	0.37-0.59
Protein (% N x 5.95 dry weight)	*8.5*	*8.55*	*7.41-10.00*	*9.3*	*6.6*	*5.6-8.5*

Notes: a. Data from Juliano presented as g/16.8g N in the literature were converted to percentage of dm based on the protein contents in (%N x 5.95 dry matter).
b. The data are obtained from measurements using an indica rice variety.
c. n=138 (data obtained in Japan between 1999 and 2009).

Lipids

Rice grain lipid is contained mainly in the germ, aleurone layer and sub-aleurone layer. Most of the rice lipids are neutral. They are triglycerides in which glycerol is esterified with three fatty acids, primarily oleic, linoleic, and palmitic acid. Besides triglycerides, free fatty acids, sterol and diglycerides are also found in rice grain. Rice grain also contains lipid-conjugates such as acylsterolglycoside and sterolglycoside, glycolipids such as cerebroside, and phospholipids such as phosphatidylcholine and phosphatidylethanolamine.

Lipids in a starch-lipid complex are not extracted by an organic solvent such as ether, but by water-saturated butanol and others for analyses. The percentage of these lipids contained in non-glutinous brown rice is 0.5%-0.7% and in glutinous brown rice approximately 0.2% respectively. The major lipid components are phospholipids, neutral lipids and glycolipids. Among fatty acids, palmitic and linoleic acids make up a large proportion, and oleic acid makes up a lesser amount (Choudhury and Juliano, 1980a; 1980b).

Fatty acid composition is dependent on the growing season and the varieties adapted to specific eco-geographical conditions. Cultivated rice is eco-geographically classified into four groups of varieties: Indian, Chinese, Japanese and Javanese. The level of palmitic acid is in the order of Indian > Chinese > Japanese > Javanese (Taira, Nakagahra and Nagamine, 1988). In early season crops in Japan, oleic acid content is high due to high temperatures during ripening: similarly, the linoleic acid content is high in late season crops (Kitta et al., 2005). The fatty acid composition of paddy rice and brown rice are given in Table 2.10.

Table 2.10. Fatty acid composition (% of total fatty acids) in paddy rice and brown rice

Fatty acid component	Paddy rice		Brown rice		
	ILSI-CCDB (2014)[a]		Juliano (1985a)	NARO (2011)[b]	
	mean	range	value	mean[3]	range[c]
Myristic (14.0)	0.38	0.32-0.48		0.7	0.5-1.1
Pentadecanoic (15:0)				0.1	0.1-0.3
Palmitic (16.0)	15.44	14.90-16.94	23	21.9	18.2-31.2
Palmitoleic (16:1)	0.41	0.26-0.93		0.2	0.1-0.2
Heptadecanoic (17:0)				0.1	0.1-0.6
Stearic (18:0)	1.88	1.68-2.09		2.0	1.5-2.8
Oleic (18:1)	39.59	37.49-40.49	35	36.9	30.9-42.0
Linoleic (18:2)	37.84	37.51-38.49	38	34.7	26.1-39.0
Linolenic (18:3)	1.15	1.12-1.21		1.2	0.9-1.6
Arachidic (20:0)	0.72	0.66-0.79		0.6	0.4-0.7
Eicosenoic (20:1)	0.56	0.54-0.58		0.5	0.4-0.6
Behenic (22:0)	0.62	0.48-0.82		0.3	0.2-0.6
Docosenoic/Erucic (22:1)	0.20	0.11-0.24		0.1	0.1-0.2
Lignoceric (24:0)	1.18	1.06-1.34		0.6	0.4-0.9
Tetracosenoic (24:1)	0.15	0.12-0.21		0.2	0.1-0.3
Others			4[d]		

Notes: a. The data are obtained from measurements using an indica rice variety.
b. n = 138 (of only market varieties).
c. Fatty acid profile for fatty acids which are not involved in starch-lipid complexes.
d. Trace to 3% myristic acid; 2%-4% stearic acid; and 1%-2% linolenic acid.

Fatty acid composition appears to be influenced by temperature during the ripening stages. Especially, the amount of polyunsaturated fatty acids decreases with increasing temperature during the ripening stages. However, in some varieties, fatty acid composition does not seem to be influenced by temperature but by genetic factors (Kitta et al., 2005). Rice bran oil contains 4%-8% unsaponifiable matter, rich in *gamma*-oryzanol, tocopherols and tocotrienols.

The content of rice antioxidants, phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols and gamma-oryzanol has been reviewed (Goufo and Trindade, 2014).

Minerals

Mineral content is greatly influenced by cultivation conditions including fertilisation and soil conditions. Among the inorganic elements contained in rice, silicon is dominant in paddy rice. The mineral content of paddy rice is detailed in Table 2.11. In brown and milled rice, phosphorus is principal but comparable amounts of potassium, magnesium and silicon are also found (Table 2.12). Phosphorus is primarily found as phytic phosphorus, especially in bran.

Table 2.11. Mineral content in paddy rice

Mineral	Paddy rice					
	Juliano and Bechtel (1985)[a]	ILSI-CCDB (2014)[b]		Heuzé, Tran and Hassoun in Feedipedia (2015)		NRC (1982)
	range	mean	range	mean	range	mean
Macro-minerals (mg/g dry matter)						
Calcium	0.1-0.9	0.32	0.25-0.43	0.6	0.2-1.5	0.7
Magnesium	0.7-1.7			1.0	0.3-1.4	1.5
Phosphorus	2.0-4.5	2.89	2.49-3.35	2.9	1.9-4.7	3.2
Potassium	1.7-4.3			2.8	1.9-3.5	3.6
Silicon	12.6					
Sulphur	0.5-1.9					0.5
Micro-minerals (µg/g dry matter)						
Copper	2-13			3		3.0
Iron	16-70	56.4	36.3-74.2	53		57.0
Manganese	20-109			82	46-117	20.0
Sodium	62-942			300	0-1 000	600
Zinc	2.0-36			14		17.0

Notes: a. Data from Juliano and Bechtel are presented on a fresh weight basis; values at 14% moisture in the literature were converted to those at percentage of dry matter.
b. The data are obtained from measurements using an indica rice variety.

Table 2.12. Mineral content in brown rice and other rice milling fractions

Mineral	Brown rice		Milled rice		Hull	Bran		Germ	Polishings
	Juliano and Bechtel (1985)[a]	USDA (2014)	Juliano and Bechtel (1985)[a]	USDA (2014)	Juliano and Bechtel (1985)[a]	Juliano and Bechtel (1985)[a]	USDA (2014)	Juliano and Bechtel (1985)[a]	Juliano and Bechtel (1985)[a]
	range	mean	range	mean	range	range	mean	range	range
Macro-minerals (mg/g dry matter)									
Calcium	0.1-0.6	0.32	0.1-0.3	0.12	0.7-1.5	0.3-1.4	0.61	0.2-1.2	0.6-0.8
Magnesium	0.2-1.7	1.61	0.2-0.6	0.29	0.3	5.8-15.1	8.32	5-15	7-8
Phosphorus	2.0-5.0	3.36	0.9-1.7	1.11	0.3-0.8	13-29	17.87	12-24	12-26
Potassium	0.7-3.2	2.77	0.8-1.5	0.98	1.7-8.7	12-23	15.82	13-17	8; 13
Silicon	0.7-1.6		0.1-0.5		74-110	3-6		0.5-1.0	1.3; 1.9
Sulphur	0.3-2.2		0.9		0.5	2.0			1.9
Micro-minerals (µg/g dry matter)									
Copper	1-7	3.13	2-3	2.10	35-45	10-40	7.76	10-40	6-30
Iron	2-60	18.5	2-33	18.8	45-110	100-500	197.5	70-209	50-180
Manganese	2-42	42.24	7-20	11.95	116-337	110-267	151.4	106-140	
Sodium	20-395	60	6-100	30	78-960	83-390	50	162-740	trace-160
Zinc	7-33	22.8	7-27	12.9	10-47	50-300	64.3	66-300	20; 70

Note: a. Data from Juliano and Bechtel are presented on a fresh weight basis; values at 14% moisture in the literature were converted to those at percentage of dry matter.

Minerals are unevenly distributed in a brown rice grain. By milling stepwise from the outer layers towards the endosperm of a brown rice grain with an abrasive rice mill, mineral contents in each layer fraction can be measured. Mineral contents in a brown rice grain tend to decrease towards the endosperm. The endosperm contains lesser amounts of minerals than the germ and the outer bran layer fractions (Kubo, 1960; Ohtsubo and Ishitani, 1995).

Vitamins

Rice grain contains water-soluble vitamins such as thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), cyanocobalamin (B12) and fat-soluble vitamin E, tocopherols. It does not contain significant amounts of other fat-soluble vitamins, like vitamin A, D and K. Vitamins are mainly present in the endosperm and bran layers; thus, milled rice contains fewer vitamins as compared with brown rice (Table 2.13).

Table 2.13. Vitamin content (µg/g dry matter) in paddy rice, brown rice and milling fractions

Vitamin	Paddy rice	Brown rice			Milled rice	Hull	Bran		Germ	Polishings
	Juliano and Bechtel (1985)[a]	Juliano and Bechtel (1985)[a]	NARO (2011)		USDA (2014)[b]	Juliano and Bechtel (1985)[a]	Juliano and Bechtel (1985)[a]	Juliano and Bechtel (1985)[a]	USDA (2015)	Juliano and Bechtel (1985)[a]	Juliano and Bechtel (1985)[a]
	range	range	mean	range	mean	range	range	range	mean	range	range
Retinol (A)	0-0.09	0-0.13	..	..	0	0-trace	0	0-4.2	0	0-1.2	0-1.1
Thiamine (B1)	3.0-3.8	3.4-7.1	5.1	3.6-8.1	4.6	0.2-1.3	1.0-2.4	14-28	28	20-69	4-22
Riboflavin (B2)	0.7-1.3	0.5-1.6	0.5	0.2-0.7	0.8	0.2-0.7	0.6-0.8	2.1-5.0	2.8	2.0-5.0	2.0-2.8
Niacin (B3)	34-65	41-62	79.0	50.4-134.7	53	15-28	19-49	310-580	340	33-97	260-452
Pantothenic acid (B5)	8-14	11-17			16.8	4-8		23-71		13-33	30-65
Pyridoxine (B6)	5-8	6-11	4.4	1.8-6.5	5.7	0.5-1.4		11-33	41	15-17	11-31
Biotin (B7)	0.05-0.09	0.05-0.12				0.01-0.07		0.2-0.6		0.4-0.6	0.1-0.7
Choline, total	880-1 140	1 100				450-1 020		1 070-1 700		1 980; 3 000	1 000-1 450
Folic acid (B9)	0.2-0.5	0.1-0.6			0.2	0.03-0.16		0.5-1.6	0.6	0.9-4.8	1.1-2.1
Cyanocobalamin (B12)	0-0.003	0-0.005			0	0-0.0016		0-0.005		0-0.01	0-0.004
alpha-Tocopherol (E)	10-23	10-29	14.9	8.9-21		trace-3		30-151	49	88	63-100
beta-Tocopherol			0.5	trace-1.4							
gamma-Tocopherol			2.2	trace-4.8							
delta-Tocopherol			0.1	0-0.6							

Notes: .. : missing value or not available.
a. Data from Juliano and Bechtel are presented on a fresh weight basis; values at 14% moisture in the literature were converted to those at percentage of dry matter.
b. Mean of medium- and long-grain brown rice.

Key nutrients in rice products for feed use

According to the OECD guidance document on residues in livestock (OECD, 2013), rice straw is used to prepare feed for cattle and sheep in Australia, Japan and Europe. Whole crop silage is only used in Japan as cattle feed. Rice grain is fed to a wide range of livestock (i.e. cattle, sheep, swine, and poultry) in Australia and the United States. Rice hulls are fed to cattle, sheep, swine and turkeys in Australia. Rice bran and polishings are included in all kind of livestock feed in Australia, Japan, the United States and Europe. Some rice products for feed are common with those for food and the key nutrients for these rice products can be found in the above section "Key nutrients in rice products for food use".

The whole rice plant is sometimes used for feed, in particular in Japan (Kato, 2008). Table 2.14 provides nutrient values for the whole rice plant at different growth stages. Nutritional composition of the whole rice plant is dependent on its growth stage. Starch content increases as the rice kernel ripens. However, the nutritional value may decrease, if the harvest is delayed until its mature stage. Therefore, rice for feed use is generally harvested at its yellow ripe stage. Crude protein content of whole rice plant at that stage is low (about 7%). The mineral content of rice plant is high; however, the contents of calcium and phosphorus are low as is the case with rice straw. Data on silage (processed whole rice plant) are not listed in the table, since the data are dependent on the process. Silage composition data are available in the following literature: Horiguchi et al. (1992); Nakui et al. (1988); Quinitio, Taji and Kumai (1990); Taji et al. (1991); Taji and Quinitio (1992).

As most of the valuable nutrients are transferred from the leaves and stems to the ripening seeds and stored therein, the straw which consists of the mature stems and leaves contains a relatively small amount of protein, starch, and fat. Rice straw is low in calcium, phosphorus and most vitamins, but high in manganese. The high content of fibre, lignin, and silica are responsible for the low digestibility (Juliano, 1985b).

Table 2.14. Protein, ash, carbohydrate, and fibre content (% of dry matter) of the whole rice plant

	Ripening stage										
	Late vegetative		Early bloom		Milk stage		Dough stage		Yellow ripe		Mature
Nutrient	NARO (2009)[a]	NARO (2009)[b]	NARO (2009)[a]	NARO (2009)[b]	NARO (2009)[a]	NARO (2009)[b]	NARO (2009)[a]	NARO (2009)[b]	NARO (2009)[a]	Enishi & Shiji-maya (1998)[c]	NARO (2009)[b]
Protein	9.8	14.5	8.4	10.0	8.5	7.4	7.0	6.3	6.5	4.9, 5.0	5.3
NDF	56.2	48.4	58.7	53.0	60.7	52.5	51.0	47.6	48.3	43.4, 56.8	44.1
ADF	30.4	31.2	33.4	33.3	34.5	33.1	31.2	30.7	28.8	26.5, 35.0	28.7
NFE	45.5	41.2	46.50	45.00	47.1	47.9	50.3	51.7	53.5	-	57.3
Ash	15.7	14.5	14.5	13.7	14.0	13.4	13.9	13.2	13.6	-	11.8

Notes: NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; NFE: Nitrogen Free Extract.
a. Data from a rice variety (not specified), which is typically used as forage for animals.
b. Data from a rice variety (not specified), which is typically used as food for humans.
c. Data from high-yielding rice varieties: Hokuriku 147 and Hokuriku 153.

Tables 2.15 and 2.16 show the nutrient content of rice products used as feed from broken rice and for rice straw respectively. For other fractions used as feed components, proximate and other compounds are provided in Tables 2.7 and 2.8 of the section "Key nutrients in rice products for food use" and may provide useful information.

Most animal nutritionists prefer that fibre be measured as neutral detergent fibre (NDF) and acid detergent fibre (ADF) instead of crude fibre. Crude fibre, nitrogen-free extractives (NFE) and ether extract in feed evaluation systems do not sufficiently separate digestible from non-digestible fractions. The determination of NDF and ADF are now widely used for forage and other feed evaluation as they provide useful measurements for nutritionally important parameters, such as structural carbohydrates (Mueller-Harvey, 2004). Both of these measures are used to calculate feed energy values.

Table 2.15. Proximate, fibre, major minerals and amino acid contents (% of dry matter) of rice products used as feed – Broken rice

Component	Broken rice			
	Farrell and Hutton (1990)	NRC (1982)	NRC (1994)	NRC (2012)
		mean	mean	mean
Moisture (% fw)[a]	*12.35*	*11*	*11*	*11*
Dry matter (% fw)[a]		*89*	*89*	*89*
Protein (N x 6.25)[b]	8.1	8.6	9.78	8.88
Crude fat	1.0			
Neutral detergent fibre				9.74
Acid detergent fibre				5.11
Crude fibre	0.3		11.01	
Ash	0.6			
Starch				60.00
Calcium		0.03	0.09	
Phosphorus		0.3	0.09	
Arginine	0.63, 0.75	0.56	0.83	0.58
Glycine		0.38	0.56	
Histidine	0.18, 0.22	0.2	0.29	0.20
Isoleucine	0.34, 0.40	0.37	0.41	0.38
Leucine	0.65, 0.76	0.77	0.83	0.75
Lysine	0.30, 0.36	0.3	0.48	0.34
Methionine	0.21, 0.26	0.14	0.25	0.20
Cystine		0.09	0.24	0.12
Phenylalanine	0.43, 0.50	0.44	0.54	0.44
Serine		0.46	0.49	
Threonine	0.27, 0.32	0.27	0.4	0.29
Tryptophan		0.11	0.11	0.11
Tyrosine	0.29, 0.37	0.46	0.37	0.43
Valine	0.46, 0.85	0.53	0.61	0.55

Notes: For paddy rice, brown rice or other rice fractions used as feed, refer to Tables 2.7 and 2.8 in the Section on key nutrients.
a. % fw: data on fresh weight basis.
b. Animal scientists commonly use a conversion factor of N x 6.25 for crude protein (AOAC, 2002).

Table 2.16. Proximate, fibre, major minerals and amino acid contents (% of dry matter) of rice products used as feed – Rice straw

Component	Rice straw												
	Drake et al. (2002)	Enishi, Shijimaya and Ohta (1995)	Itoh et al. (1975)	Rahal, Singh & Singh (1997)	Wanapat et al. (1996)	Nour (2003)	Juliano (1985b)[a]	ILSI-CCDB (2014)[b]		Jin and Chen (2007)		Heuzé and Tran in Feedipedia (2015)	
								mean	range	mean	range	mean	range
Moisture (% fw)[c]								*55.15*	*41.71-73.69*				
Moisture (% adw)[d]			*9.5*							*6.9*	*4.2 – 9.8*		
Dry matter (% fw)[c]	*90*				*93*	*90.93*						*92.8*	*89.3-96.5*
Protein (N x 6.25)[e]	2.9-7.5	3.0-5.4	4.8	5.4-8.3	4.25	4.62	6.0	5.99	4.02-8.33			4.2	2.4-6.8
Crude fat			1.6	1.3-4.2[f]		1.14[f]		2.46	1.92-3.52			1.4	0.9-2.1
Neutral detergent fibre			73.6	67.9-73.8	78.6			61.97	51.89-70.32			69.1	61.7-78.6
Acid detergent fibre	41.4-56.7	38.3-45.2	44.6	45.3-52.4	47.2			43.27	36.12-55.29			42.4	36.7-52.0
Crude fibre			32.6			35.39						35.1	29.8-41.5
Ash			13.7	12.2-20.8	14.6	20.32		14.25	10.75-18.88	11.8	7.8-15.6	18.1	12.0-24.0
Carbohydrates								77.17	71.04-81.64				
Starch													
Lignin			7.3							10.2	7.2 -12.8	4.8	2.9-7.1
Energy (kJ/g DM)												15.5	15.1-16.8
Calcium	0.21-0.71											0.29	0.17-0.44
Phosphorus	0.07-0.16											0.09	0.05-0.17
Arginine							0.31						
Glycine							0.31						

Component	Rice straw											
	Drake et al. (2002)	Enishi, Shijimaya and Ohta (1995)	Itoh et al. (1975)	Rahal, Singh & Singh (1997)	Wanapat et al. (1996)	Nour (2003)	Juliano (1985b)[a]	ILSI-CCDB (2014)[b]		Jin and Chen (2007)		Heuzé and Tran in Feedipedia (2015)	
								mean	range	mean	range	mean	range
Histidine							0.13						
Isoleucine							0.27						
Leucine							0.45						
Lysine							0.33						
Methionine							0.16						
Cystine							0.11						
Phenylalanine							0.32						
Threonine							0.33						
Tryptophan							0.05						
Tyrosine							0.2						
Valine							0.38						

Notes: For paddy rice, brown rice or other rice fractions used as feed, refer to Tables 2.7 and 2.8 in the Section on key nutrients.
a. n = 2 varieties.
b. The data are obtained from measurements using an indica rice variety.
c. % fw: data on fresh weight basis.
d. % adw: data on air-dried weight basis.
e. Animal scientists commonly use a conversion factor of N x 6.25 for crude protein (AOAC, 2002).
f. Crude fat determined as ether extract.

Other constituents

Anti-nutrients and toxicants

Generally, rice is considered to be a safe source of food. There are a few compounds in rice which are not favourable for human or animal nutrition, but these compounds have not historically been present in rice-based foods at levels that would cause the food to be unsafe. These anti-nutritional factors, most of which are concentrated in the bran, are phytic acid, trypsin inhibitors and hemagglutinin-lectins, oryzacystatin and alpha-amylase/ subtilisin inhibitor. With the exception of phytic acid, the other anti-nutritional factors are proteinaceous in nature and can be subjected to denaturation by heat.

Phytic acid

In most plant materials, large portions of phosphorus are present in the form of phytic acid. Phytic acid is regarded as the primary storage form of phosphorus and inositol in almost all seeds. Phytin is the calcium-magnesium salt of phytic acid. During germination, phytin is hydrolysed by the enzyme phytase, also present in seeds, and serves as a source of inorganic phosphorus and cations for the emerging seedling (Cheryan and Rackis, 1980).

Free phytic acid binds metal ions such as zinc, iron and magnesium in the digestive tract and reduces their availability for absorption, although binding of calcium to phytic acid is pH-dependent (Thompson and Weber, 1981). The phytate-mineral complexes formed are generally insoluble at physiological pH, making the minerals biologically unavailable to mono-gastric animals and humans. Ruminants utilise considerably more phosphorus since rumen microbes produce phytase that breaks down phytate and releases phosphorus. It is common for feed formulators to add phytase to swine and poultry diets to improve the utilisation of phosphorus. Phytic acid may also form complexes with proteins and has been found to inhibit polyphenol oxidase, alpha-amylase, alcohol dehydrogenase, trypsin and other enzymes (Cheryan and Rackis, 1980).

Maga (1982) reported that brown rice contained 0.89% phytic acid whereas the germ had 3.48%, and the pericarp had 3.37% with the endosperm having 0.01%, based on dry weight. Ravindran, Ravindran and Sivalogan (1994) reported phytic acid contents of 0.99 g/100 g dm, 0.60 g/100 g dm, and 3.65 g/100 g dm in brown rice, milled rice and rice bran respectively. Phytic acid contents in brown rice vary between 0.9% to 1.2% dm, whereas those in milled rice are from 0.1% to 0.3% dm (Fretzdorff, 1992). Oberdoerfer et al. (2005) reported phytic acid contents in paddy rice, milled rice and rice bran were determined as 0.83% dm, 0.29% dm, and 5.14% dm respectively.

Trypsin inhibitors

Trypsin inhibitors are proteins known to inhibit biologically active trypsin, interfere with digestion and ultimately absorption of food material, and thus act as anti-nutrients. They are typical anti-nutritional components in soybeans, cereals and potatoes. Proteinase inhibitors are of particular significance in animal nutrition causing growth depression and pancreatic hypertrophy (Liener, 1953).

A trypsin inhibitor was isolated from rice bran and characterised by Tashiro and Maki (1979). These investigators reported a specific activity of 0.011-0.045 units per mg protein in defatted rice bran (Tashiro and Maki, 1979; Maki and Tashiro, 1983). Rice bran trypsin inhibitor (RBTI) is a powerful inhibitor of bovine, swine and rat trypsins, and a partial inhibitor of human trypsin (Tashiro and Maki, 1979).

Trypsin inhibitors are susceptible to heat. No trypsin inhibitor activity was found in paddy rice and milled rice (<1.0 trypsin inhibitor units [TIU]/mg dm). Rice bran samples had an activity of 2.27 TIU/mg dm (Oberdoerfer et al., 2005).

Lectins

Lectins are carbohydrate-binding proteins and may agglutinate cells and precipitate glycoconjugates or polysaccharides (Goldstein et al., 1980). The toxicity of lectins is due to their ability to bind to specific carbohydrate receptor sites on the intestinal mucosal cells and interference with the absorption of nutrients across the intestinal wall (Liener, 1986).

Hemagglutinin activity is confined to the germ or primary axis of the rice grain (Peumans, Stinissen and Carlier, 1983). Whole rice grain and white rice did not show any hemagglutinating activity against red blood cells of rat, rabbit, monkey and human erythrocytes (A, B, and 0) (Ayyagari, Rao and Roy, 1989; Amann, 1998). The rice bran lectin has been found to be associated with agglutination of human A, B and O group receptors with specific binding to 2-acetamido-2-deoxy-D-glucose (Poola, 1989). Rea, Thompson and Jenkins (1985) reported lectin activity of white rice to be below the limit of detection (less than 1.3 HU/mg). Rice bran lectin is heat-labile at temperatures above 80°C (Ory, Bog-Hansen and Mod, 1981; Poola, 1989). Mannose-binding rice lectin is distributed in all parts of the rice plant and it has a potential ability to agglutinate bacterial cells of *Xanthomonas campestris* pv. *oryzae*, the pathogen causing bacterial leaf blight in rice, and also spores and protoplasts of *Magnaporthe grisea*, the rice blast fungus (Hirano et al., 2000). Haemagglutinating activity was found to be below the limit of quantification (<0.1 HU/mg dm) in paddy rice and milled rice (Oberdoerfer et al., 2005).

Oryzacystatin

Oryzacystatin is a proteinaceous (globulin) cysteine proteinase inhibitor (cystatin) from rice grain and is probably the first well-defined cystatin superfamily member of plant origin (Abe et al. al.,; 1987; 1991). Oryzacystatin has been isolated from rice bran. Oryzacystatins I and II are synthesised in rice seeds during maturation. They occur in the cytosol and are decomposed as soon as germination starts (Abe et al. al., 1987; Kondo et al. al. 1990). Oryzacystatin is inactivated by heat above 120°C (FAO, 1993), where retort (pre-cooked) rice is processed. It effectively inhibited cysteine proteinases such as papain, ficin, chymopapain and cathepsin C and had no effect on serine proteinases (trypsin, chymotrypsin, and subtilisin) or carboxyl proteinase (pepsin) (FAO, 1993).

Rice alpha-amylase/subtilisin inhibitor (RASI)

The amino acid sequence of the bifunctional rice alpha-amylase/subtilisin inhibitor (RASI) is known, and it has been cloned and expressed in bacteria (Ohtsubo and Richardson, 1992; Yamagata et al. al., 1998). It is a 21 kDa protein which is expressed only in seed (Yamasaki et al, 2006). The bifunctional RASI inhibits rice alpha-amylase more than barley alpha-amylase (Yamagata et al. al., 1998). These inhibitors have been proposed to be associated with the defensive function of the seed against insect pests and pathogenic microorganisms (Franco et al. al., 2002).

Allergens

Rice is not considered by allergists to be a common allergenic food. However, rice allergy has been reported in Asian countries including Indonesia, Japan, Malaysia and Thailand, as well as some European countries like Denmark, Estonia, Finland, France, Lithuania,

Spain Sweden, as well as the Russian Federation (Besler, Tanabe and Urisu, 2001; Kumar et al. al., 2007). Rice allergy is more common in East Asian countries than in Europe and the United States where it is considered rare. The prevalence of IgE-mediated rice allergy is about 10% in atopic subjects in Japan. Rice allergy is more prominent in adults than in children. Symptoms frequently associated with rice allergy are atopic dermatitis, eczema and asthma. Anaphylactic reactions have been reported in severe cases (Besler, Tanabe and Urisu, 2001).

While rice is not considered to be a common allergic food, allergic reactions have been documented and proteins in rice grain have been shown to be IgE-binding proteins. The first demonstration of a rice protein binding to human sera from patients allergic to cereal grain was demonstrated in 1975 (Hoffman, 1975). Allergenicity from the rice protein fractions containing albumin, globulin and glutelin was first reported in Japan in 1979 (Shibasaki et al. al., 1979). A group of rice allergens including 14-16, and 33 kDa proteins of rice seeds have been identified and shown to be IgE-binding proteins (Alvarez et al., 1995; Nakamura and Matsuda, 1996; Tada et al. al., 1996; Trcka et al., 2012; Limas et al. al., 1990; Kumar et al. al., 2007). These rice food allergens, Oryza glyoxalase I (33 kDa) and Oryza trypsin alpha-amylase inhibitors (14-16 kDa), are listed in a database of the Food Allergy Research and Resource Program (FARRP, 2014). In addition, certain proteins with molecular weights of 9, 14, and 31 kDa appear to be rice allergens in children (Jeon et al. al., 2011). However, clinical correlations have not been fully established.

There are two putative rice food allergens, Oryza trypsin alpha-amylase inhibitors (14-16 kDa) and Oryza glyoxalase I (33 kDa), which are listed in a database of the Food Allergy Research and Resource Program (FARRP, 2014).

14-16 kDa proteins

The first reported rice allergens were 14-16 kDa proteins (also called the RAG2 proteins), which were detected using sera from patients allergic to rice (Matsuda et al. al., 1988; Alvarez et al. al., 1995; Tada et al., 1996). The 14-16 kDa protein family was isolated and characterised to be the alpha-amylase/trypsin inhibitor family, constituting multigene families which are immunologically cross-reactive proteins (Alvarez et al. al., 1995). It was confirmed that the 16 kDa rice protein was a relevant rice allergen among atopic patients in Japan (Urisu et al., 1991). The 16 kDa protein has significant amino acid homology to barley trypsin inhibitor and wheat alpha-amylase inhibitor which have been shown to be allergens (Izumi et al., 1992).

33-kDa protein

The 33-kDa allergen was identified to be a novel type of plant glyoxalase I that was expressed in various plant tissues, including maturing seeds, stem, and leaf (Usui et al., 2001) and was initially designated as Glb33.

Suggested constituents to be analysed related to food use

Key rice products for food

Brown, milled, polished and parboiled rice are the major rice products consumed by humans in the form of grain after being cooked. Rice is also consumed as food ingredients which are part of food products. For example, rice flour is used in cereals, baby food, and snacks. The primary nutrients provided by rice are carbohydrates and proteins. Rice bran

also provides some vitamins, fat and fibre. Rice oil extracted from bran is valued as high-quality cooking oil.

As compared with the consumption of cooked milled or brown rice, a relatively small amount of rice is consumed as prepared products; a variety of such products is available in the market, in particular in Asia.

More detailed information on the uses of rice and rice products as food is given in above Background section.

Recommendation of key components to be analysed related to food use

Table 2.17 shows suggested nutritional and compositional parameters to be analysed in rice matrices for food use.

Table 2.17. Suggested nutritional and compositional parameters to be analysed in rice matrices for food use

Parameter	Paddy rice or Brown rice
Proximates[1]	x
Total dietary fibre	x
Vitamins[2]	x
Amino acids	x
Fatty acids	x

Notes: 1. Proximates includes moisture, protein, fat, ash and carbohydrate (calculated).
2. B vitamins, namely thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5) and Pyridoxine (B6), and E vitamin alpha-tocopherol are suggested.

Suggested constituents to be analysed related to feed use

Key rice products for feed

Animals are fed paddy rice and its by-products such as rice straw, rice hulls and rice bran. Whole rice plants can be fed as whole crop silage. Rice and rice products are used as feed in some countries like Japan.

Paddy rice

The use of paddy rice and brown rice is limited as animal feeds because of the cost. Paddy rice is mostly consumed by humans, and fed to livestock only when the quality is poor or off-grade. Because of the hull, paddy rice is higher in crude fibre content and lower in caloric content than brown rice.

Paddy rice can replace other grains in animal feeding. For dairy and beef cattle diets, paddy rice can replace maize at the maximum rates of 40% (hereafter, in weight percentages) and 65% respectively (JSFA, 1979a; 1979b). For poultry and swine, paddy rice can replace maize up to 60-65% (JSFA, 1979a). As rice endosperm is hard and enclosed in hard rice hull, paddy rice should be ground for efficient feed use.

Brown rice is an excellent animal feed, but is usually too expensive for such use. For swine and poultry feeds, brown rice can replace maize at a rate of 40% (JSFA, 1970). Brown rice

should be ground before used as animal feed except in the case of poultry. It is also an excellent poultry feed because of its high energy and low fibre content. As paddy rice is lacking carotene, the colour of egg yolks will become paler as rice content of poultry feed increases (JSFA, 1970). Broken rice is commonly used particularly in pet foods in the United States.

Rice provides a number of other by-products that are valuable feedstuffs through harvest and processing: straw, hull, bran, and whole rice plant.

Straw

As rice straw is high in fibre, it can be fed to ruminants as roughage. In the tropical zone of monsoon Asia, rice straw is used as roughage especially in the dry season.

Ruminants cannot subsist only on rice straw because of its low protein content (Table 2.16). Thus, an adequate protein balance should be achieved by supplementing the straw. Rice straw can only partly replace forage because of the low protein content and low digestibility. The straw contains oxalates that chelate calcium and decrease its absorption. Rice is coated with prickly hairs to which cattle need some time to adapt. Rice straw containing less than 50% acid detergent fibre (ADF) could be good forage.

Others

The hull is not a very good feed, as it is very low in protein and high in fibre. The sharp edges of the hull that may irritate the digestive tract of cattle should be broken by sufficiently grinding the hull. Digestibility can be improved by specific processes which remove silica. Monocalcium phosphate is added to the hull, and the mixture is ammoniated under heat and pressure to make an acceptable sheep feed. The hull is commonly used as a carrier for mineral and animal drug premixes.

Rice bran is a good source of protein and vitamins. The quality of rice bran feed is dependent on the hull content. Fresh bran is fairly palatable. However, it often turns rancid during storage unless treated with heat, because of the high oil content and the release of enzymes during processing. Heating and drying at milling can improve storage life (Morimoto et al., 1985).

Rice bran is a good feed component for dairy cows unless the bran amount exceeds 20% of the concentrate feed mixture. In Japan, rice bran has been used as one of the most important feed ingredients for Japanese Black cattle (known as Wagyu in Japanese). Ricebran can be blended up to 20% of swine feed (OECD, 2013). When too much rice bran is fed to juvenile pigs, it may lead to serious scouring. Due to the fatty acid composition in bran, swine and dairy cattle fed with bran in excess may lead both body fat and butter fat to undesirable soft characteristics (Morimoto et al., 1985).

Rice bran can replace wheat bran or wheat middling in poultry feed. The bran contains a high amount of phytate (3% to 5%) which reduces the availability of minerals, and particularly phosphorus (NRC, 2012). Compared with rice bran, defatted rice bran has a long storage life and a high content in crude protein, crude fibre and ash.

Rice polishings also find their way into animal diets because they are an excellent source of nutritionally important vitamins such as thiamine (vitamin B1) and niacin (vitamin B3). Like rice bran, rice polishings easily become rancid during storage and should be fed as fresh as possible. Polishings can be used as a part of the concentrate feed mixture for dairy and beef cattle, and are good feed for swine.

Rice screenings, a mixture of small and broken rice seeds, can be used for feed. However, the nutrient content of screenings is highly variable.

In Japan, whole rice plants can be fed to dairy and beef cattle after *ensilaging*. Its nutritional value is almost equivalent to that of barley whole crop silages (Horiguchi et al., 1992). Rice whole crop silage is low in crude protein and calcium, which should be supplemented (Table 2.14). Rice whole crop silage is palatable for cows (Goto et al., 1991) and dry matter intake by dairy cows ranges from 6.3 to 9.5 kg per day (Ishida et al., 2000). There is only limited compositional information on the whole rice plant.

Recommendation of key components to be analysed related to feed use

The components in the by-products as feed may change during their processing and storage, and the analysis of components must be carried out after storage of the harvested materials under proper conditions.

The suggested nutritional and compositional parameters to be analysed in rice matrices for animal feed use are shown in Table 2.18. In addition to proximate analysis, calcium and phosphorus need to be analysed in rice straw or whole rice plant which is fed to ruminants. Moreover, when using rice grain and its by-products as feed for swine or poultry, amino acids and phytic acid should also be analysed.

Table 2.18. Suggested nutritional and compositional parameters to be analysed in rice matrices for feed use

Parameter	Paddy rice	Straw or Whole plant
Proximates[1]	x	x
Acid detergent fibre		x
Neutral detergent fibre		x
Amino acids	x	

Note: 1. Proximates includes moisture, protein, fat, ash and carbohydrate (calculated).

Note

[1] For additional discussion of appropriate comparators, see the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants CAC/GL 45/2003 of the Codex Alimentarius Commission (paragraphs 44 and 45).

References

Abe, K. et al. (1987), "Molecular cloning of a cysteine proteinase inhibitor of rice (oryzacystatin)", *Journal of Biological Chemistry*, Vol. 102, pp. 16793-16797.

Abe, K. et al. (1991), "Oryzacystatins as the first well-defined cystatins of plant origin and their target proteinases in rice seeds", *Biomedica Biochimica Acta*, Vol. 50(4-6), pp. 637-641.

Alvarez, A.M. et al. (1995), "Classification of rice allergenic protein cDNAs belonging to the alpha-amylase/trypsin inhibitor gene family", *Biochimica Biophysica Acta*, Vol. 1251(2), pp. 201-204.

Amann, M.M. (1998), *Recommended Compositional and Nutritional Parameters to Test in Rice*, TAS-Environ, Arlington, Virginia, US.

AOAC (2002), *Official Methods of Analysis of AOAC International (17th ed.)*, Chapter 4, Association of Official Analytical Chemists, Gaithersburg, MD, US, pp. 20-27.

Ayyagari, R., B.S.N. Rao and D.N. Roy (1989), "Lectins, trypsin inhibitors, BOAA and tannins in legumes and cereals and effects of processing", *Food Chemistry*, Vol. 34, pp. 229-238.

Besler, M., S. Tanabe and A. Urisu (2001), "Rice (*Oryza sativa*)", *Internet Symposium on Food Allergens*, Vol. 3 (Suppl. 2), http://www.food-allergens.de (accessed 15 October 2014).

Bhattacharya, K.R. (2004), "Parboiled rice", in E.T. Champagne, (ed.), *Rice: Chemistry and Technology*, *3rd ed*., *Am. Assoc. Cereal Chem.*, St. Paul, MN, US, pp.329-404.

Cheryan, M. and J.J. Rackis (1980), "Phytic acid interactions in food systems", *Critical Reviews in Food Science and Nutrition*, Vol. 13(4), pp. 297-335.

Childs, N.W. (2004), "Production and utilization of rice", in E.T. Champagne, (ed.), *Rice: Chemistry and Technology*, *3rd ed*., *Am. Assoc. Cereal Chem.*, St. Paul, MN, US, pp. 1-23.

Choudhury, N.H. and B.O. Juliano (1980a), "Lipids in developing and mature rice grain", *Phytochemistry, Vol. 19,* pp. 1063-1069.

Choudhury, N.H. and B.O. Juliano (1980b), "Effect of amylose content on the lipids of mature rice grain", *Phytochemistry*, Vol. 19, pp. 1385-1389.

Codex Alimentarius Commission (2003), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants* - CAC/GL 45/2003, Annexes II and III adopted in 2008, www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf.

Drake, D.J., G. Nader and L. Forero (2002), *Feeding Rice Straw to Cattle*, Publication no. 8079, Division of Agriculture and Natural Resources, University of California, http://anrcatalog.ucanr.edu/Details.aspx?itemNo=8079.

Enishi, O. and K. Shijimaya (1998), "The fermentative patterns of rice whole crop silage", *Journal of Japanese Society of Grassland Science*, Vol. 44, pp. 179-181 (in Japanese).

Enishi, O., K. Shijimaya and H. Ohta (1995), "The effect of varieties and strains on chemical composition and *in vitro* dry matter digestibility of sodium hydroxide and ammonia treated rice (*Oryza sativa* L.) straw", *Journal of Japanese Society of Grassland Science*, Vol. 41, in Japanese with English summary, pp. 160-163 .

FAO (1993), *Rice in Human Nutrition*, Prepared by B.O. Juliano, International Rice Research Institute, Food and Agriculture Organization of the United Nation, http://www.fao.org/docrep/t0567e/t0567e00.htm.

FAOSTAT (2019), "Production/crops: Barley, maize, millet, oats, rice (paddy), sorghum, years 1961 to 2017", FAO statistics database, Food and Agriculture Organization of the United Nations, http://www.fao.org/faostat/ (accessed on 10 July 2019).

Farrell, D.J. and K. Hutton (1990), "Nontraditional feed sources for use in swine production", in P.A. Thacker and R.N. Kirkwood (eds.), *Rice and Rice Milling By-Products*, Chapter 24, Butterworths Publishers, Stoneham, MA, US.

FARRP (2014), *AllergenOnline (database)*, Food Allergy Research and Resource Program, Department of Food Science and Technology at the University of Nebraska, Lincoln, NE, http://allergenonline.org/ (accessed on 11 February 2016).

Franco, O.L. et al. (2002), "Plant alpha-amylase inhibitors and their interaction with insect alpha-amylases", *European Journal of Biochemistry*, Vol. 269(2), pp. 397-412.

Fretzdorff, B. (1992), "Phytinsäure in Getreidenährmitteln", *Getreide, Mehl und Brot*, Vol. 46, pp. 180-185.

Goldstein, I.J. et al. (1980), "What should be called a lectin?", *Nature*, Vol. 285(5760), pp. 66-66.

Goto, M. et al. (1991), "Feeding value of rice whole crop silage as compared to those of various summer forage crop silages", *Animal Science and Technology*, Vol. 62, pp. 54-57.

Goufo, P. and H. Trindade (2014), "Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocynidins, tocopherols, tocotrienols, gamma-oryzanol, and phytic acid", *Food Science and Nutrition*, Vol. 2(2), pp. 75-104.

Heuzé, V. and G. Tran (2015), *Rice Straw,* Feedipedia: A programme by INRA, CIRA, AFZ and FAO, http://www.feedipedia.org/node/557 (accessed on 9 June 2016).

Heuzé, V., G. Tran and P. Hassoun (2015), *Rough Rice (Paddy Rice),* Feedipedia: A programme by INRA, CIRA, AFZ and FAO, http://www.feedipedia.org/node/226 (accessed 9 June 2016).

Hirano K. et al. (2000), "Novel mannose-binding rice lectin composed of some isolectins and its relation to a stress-inducible *salT* gene", *Plant Cell and Physiology*, Vol. 41(3), pp. 258-267.

Hoffman, D.R. (1975), "The specificities of human IgE antibodies combining with cereal grains", *Immunochemistry*, Vol. 12(6-7), pp. 535-538.

Horibata, T. et al. (2004), "Structural and physicochemical characteristics of endosperm starches of rice cultivars recently bred in Japan", *Journal of Applied Glycoscience*, Vol. 51(4), pp. 303-313.

Horiguchi, K. et al. (1992), "Comparisons of nutritive value among whole crop silage of rice plant with V-type leaves and other forages", *Journal of Japanese Society of Grassland Science*, Vol. 38, pp. 242-245 (in Japanese with English summary).

Hoseney, R.C. (1986), "Proteins", in *Principles of Cereal Science and Technology*, American Association of Cereal Chemists, St. Paul, MN, pp. 69-88.

ILSI-CCDB (2014), *ILSI Crop Composition Database Ver. 5.1*, International Life Sciences Institute, https://www.cropcomposition.org/query/index.html (accessed on 11 February 2016).

IRRI World Rice Statistics (2019), *Online Database: Rice Harvested Area, Paddy Rice and Milled Rice Production, Export and Import Quantities in 2017*; *Rice Consumption Per Capita in 2013,* International Rice Research Institute, http://ricestat.irri.org:8080/wrsv3/entrypoint.htm (accessed on 10 July 2019).

Ishida, M. et al. (2000), "Preliminary observation on milk yield and nutrients utilization by Holstein cows fed the round baled silage of the newly developed variety of whole crop rice, 'Kanto-shi-206'", *Kanto Journal of Animal Science*, Vol. 50(1), pp. 14-21 (in Japanese, with English summary).

Itoh, H. et al. (1975), "Improving the nutritive values of rice straw and rice hulls by ammonia treatment", *Japanese Journal of Zootechnological Science*, Vol. 46(2), pp. 87-93.

Izumi, H. et al. (1992), "Nucleotide sequence of a cDNA clone encoding a major allergenic protein in rice seeds. Homology of the deduced amino acid sequence with members of alpha-amylase/trypsin inhibitor family", *FEBS Letters*, Vol. 302(3), pp. 213-216.

Jeon, Y.H. et al. (2011), "Identification of major rice allergen and their clinical significance in children", *Korean Journal of Pediatrics*, Vol. 54(10), pp. 414-421.

Jin, S. and H. Chen (2007), "Near-infrared analysis of the chemical composition of rice straw", *Industrial Crops and Products*, Vol. 26, pp. 207-211, http://www.sciencedirect.com/science/article/pii/S0926669007000416.

JSFA (1970), *Report on Feed Processing Test of Rice and Related Issues*, Japan Scientific Feed Association (in Japanese).

JSFA (1979a), *FY1978 Report on Feed Processing Test of Rough Rice: Feeding Tests Using Broiler, Layer and Swine and Lactation Test using Dairy Cows*, Japan Scientific Feed Association (in Japanese).

JSFA (1979b), *FY1978 Report on Feed Processing Test of Tough Rice: Feeding Test Using Beef Cattle*, Japan Scientific Feed Association (in Japanese).

Juliano, B.O. (1968), "Screening for high protein rice varieties", *Cereal Science Today*, Vol. 13, pp. 299-301; 313.

Juliano, B.O. (1985a), "Polysaccharides, proteins, and lipid of rice", in B.O. Juliano (ed.), *Rice: Chemistry and Technology*, *2nd ed*., American Association of Cereal Chemists, St. Paul, MN, pp 59-174.

Juliano, B.O. (1985b), "Rice hull and rice straw", American Association of Cereal Chemists, pp 689-755.

Juliano, B.O. and Bechtel, D.B. (1985), "The rice grain and its gross composition", B.O. Juliano (ed.), *Rice: Chemistry and Technology*, *2nd ed*., American Association of Cereal Chemists, St. Paul, MN, pp 17-57.

Juliano, B.O. and Villareal, C.P. (1993), *Grain Quality Evaluation of World Rices*, International Rice Research Institute (IRRI).

Juliano, B.O. et al. (2012), "Replacement of acetate with ammonium buffer to determine apparent amylose content of milled rice", *Cereal Foods World*, Vol. 57(1), pp. 14-19.

Kato, H. (2008), "Development of rice varieties for whole crop silage (WCS) in Japan", *JARQ*, Vol. 42(4), pp. 231-236.

Kitta, K. et al. (2005), "Variations in lipid content and fatty acid composition of major non-glutinous rice cultivars in Japan", *Journal of Food Composition and Analysis*, Vol. 18, pp. 269-278.

Kondo, H. et al. (1990), "Two distinct cystatin species in rice seeds with different specificities against cysteine proteinases. Molecular cloning, expression, and biochemical studies on oryzacystatin-II", *Journal of Biological Chemistry*, Vol. 265(26), pp. 15832-15837.

Kubo, S. (1960), "Transmigration of chlorine in a rice grain. V. transmigration of several elements other than chlorine", *Nippon Nogeikagaku Kaishi*, Vol. 34, pp. 689-694 (in Japanese).

Kumar, R. P. et al. (2007), "Rice (*Oryza sativa*) allergy in rhinitis and asthma patients: A clinico-immunological study", *Immunobiology*, Vol. 212(2), pp. 141-147.

Liener, I. E. (1953), "Soyin, a toxic protein from the soybean, I. Inhibition of the rat growth", *Journal of Nutrition*, Vol. 49, pp. 527-539.

Liener, I.E. (1986), in I.E. Liener, N. Sharon and I.J. Goldstein (eds.), *The Lectins: Properties, Functions and Applications in Biology and Medicine*, Academic Press, New York, pp. 527-552.

Limas, G.G. et al. (1990), "Purification and characterization of ten new rice NaCl-soluble proteins: Identification of four protein-synthesis inhibitors and two immunoglobulin-binding proteins", *Planta,* Vol. 181(1), pp. 1-9.

Maga, J. (1982), "Phytate: Its chemistry, occurrence, food interactions, nutritional significance and methods of analysis", *Journal of Agricultural and Food Chemistry*, Vol. 30(1), pp. 1-9.

Maki, Z. and M. Tashiro (1983), "Nutritional significance of a rice brand concentrate with trypsin inhibitor activity", *Journal of Nutritional Science and Vitaminology*, Vol. 29(3), Tokyo, pp. 293-302.

Matsuda, T. et al. (1988), "Purification and properties of an allergenic protein in rice grain", *Agricultural and Biological Chemistry*, Vol. 52(6), pp. 1465-1470.

Mestres, C. et al. (1996), "A rapid method for the determination of amylose content by using differential-scanning calorimetry", *Starch/Staerke*, Vol. 48(1), pp. 2-6.

Morimoto, H., M. Yoshida and Y. Ohyama (1985), *Shiryoh-gaku (Feed Science), 2nd ed.*, Yoken-Do, Tokyo, Japan, pp. 152-156 (in Japanese).

Mueller-Harvey, I. (2004), "Modern techniques for feed analysis", in *Assessing Quality and Safety of Animal Feeds, FAO Animal production and Health Paper No. 160*, Food and Agriculture Organization, Rome.

Nakamura, R. and T. Matsuda (1996), "Rice allergenic protein and molecular-genetic approach for hypoallergenic rice", *Bioscience, Biotechnology and Biochemistry*, Vol. 60(8), pp. 1215-1221.

Nakaura, Y. et al. (2011), "Properties of endosperm starches and physical properties of cooked rice from Japanese upland rice cultivars containing M-type amylopectin", *Journal of Applied Glycoscience*, Vol. 59(3), pp. 111-117.

Nakui, T. et al. (1988), "The making of rice whole crop silage and an evaluation of its value as forage for ruminants. Dietary fiber content and composition in six cereals at different extraction rates (wheat, rye, barley, sorghum, rice, and corn)", *Bulletin of Tohoku National Agricultural Experimental Station*, Vol. 78, pp. 161-174 (in Japanese, with English summary).

NARO (2009), *Standard Tables of Feed Composition in Japan,* Japan Livestock Industry Association.

NARO (2011), *Food Composition Database for Safety Assessment of Genetically Modified Crops as Foods and Feeds*, National Agricultural Research Organization, Japan.

Nour, A.M. (2003), *Rice Straw and Rice Hulls in Feeding Ruminants in Egypt*, Dept. Animal Production, Alexandria University, Alexandria, Egypt.

NRC (1982), *United States–Canadian Tables of Feed Composition (3rd Revision)*, National Academy Press, Washington, DC, pp 76-125.

NRC (1994), *Nutrient Requirements of Poultry (9th Revised Ed.),* National Academy Press, Washington, DC, pp. 64-67.

NRC (2012), *Nutrient Requirements of Swine (11th Revised Ed.)*, National Academy Press, Washington, DC, pp.320-320

Oberdoerfer, R.B. et al. (2005), "Rice (*Oryza sativa* L.) containing the bar gene is compositionally equivalent to the nontransgenic counterpart", *Journal of Agricultural and Food Chemistry*, Vol. 53(5), pp. 1457-1465.

OECD (1999), "Consensus Document on the Biology of *Oryza sativa* (Rice)", *Series on Harmonisation of Regulatory Oversight in Biotechnology*, No. 14, OECD, Paris, http://www.oecd.org/env/ehs/biotrack/46815658.pdf.

OECD (2013), "Guidance document on residues in livestock", *OECD Environment, Health and Safety Publications, Series on Pesticides*, No. 73, Inter-Organization Programme for the Sound Management of Chemicals (IOMC), OECD, Paris, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)8&doclanguage=en.

Ogawa, M. et al. (1989), "Mutants for rice storage proteins. 2. Isolation and characterization of protein bodies from rice mutants", *Theoretical Applied Genetics, Vol. 78,* pp. 305-310.

Ohtsubo, K. and M. Richardson (1992), "The amino acid sequence of a 20kDa bifunctional subtilisin/α-amylase inhibitor from bran of rice (*Oryza sativa* L.) seeds", *FEBS Letters*, Vol. 309, pp. 68-72.

Ohtsubo, K. and T. Ishitani (1995), *Kome no Kagaku*, Asakurashoten, Tokyo, Japan, pp.18-48 (in Japanese).

Ory, R.L., T.C Bog-Hansen and R.R. Mod (1981), "Properties of hemagglutinins in rice and other cereal grains", in R.L. Ory (ed.), *Antinutrients and Natural Toxicants in Foods*, Westport, CT, US, Food & Nutrition Press.

Padua, A.B. and B.O. Juliano (1974), "Effect on parboiling on thiamin, protein and fat of rice", *Journal of the Science of Food and Agriculture*, Vol. 25, pp. 897-701.

Peumans, W.J., H.M. Stinissen and A.R. Carlier (1983), "The rice lectin and its relationship to cereal lectins", *Biochemie und Physiologie der Pflanzen*, Vol. 178(6–7), pp. 423-431.

Poola, I. (1989), "Rice lectin: Physicochemical and carbohydrate-binding properties", *Carbohydrate Polymers*, Vol. 10, pp. 281-288.

Quinitio, L.F., K. Taji and S. Kumai (1990), "Feeding value and starch digestibility in paddy rice silage at the milk and the dough stages", *Japanese Journal of Zootechnological Science*, Vol. 61, pp. 663-665.

Rahal, A., A. Singh and M. Singh (1997), "Effect of urea treatment and diet composition on, and prediction of nutritive value of rice straw of different cultivars", *Animal Feed Science and Technology*, Vol. 68, pp. 165-182.

Ravindran, V., G. Ravindran and S. Sivalogan (1994), "Total and phytate phosphorus contents of various foods and feedstuffs of plant origin", *Food Chemistry*, Vol. 50(2), pp. 133-136.

Rea R.L., L.U. Thompson and D.J.A. Jenkins (1985), "Lectins in foods and their relationship to starch digestibility", *Nutrition Research*, Vol. 5, pp. 919-929.

Resources Council, Science and Technology Agency of Japan (2000), *Standard Tables of Food Composition in Japan,* Fifth Revised Edition.

Satake, T. (1990), *Modern Rice Milling Technology*, University of Tokyo Press, Tokyo, Japan, pp. 295.

Shibasaki, M. et al. (1979), "Allergenicity and lymphocyte-stimulating property of rice protein", *Journal of Allergy and Clinical Immunology*, Vol. 64(4), pp. 259-265.

Shih, F.F. (2004), "Rice proteins", in E.T. Champagne (ed.), *Rice: Chemistry and Technology, 3rd ed.*, American Association of Cereal Chemists, St. Paul, MN, pp. 143-162.

Tada, Y. et al. (1996), "Reduction of 14-16 kDa allergenic proteins in transgenic rice plants by antisense gene", *FEBS Letters*, Vol. 391(3), pp. 341-345.

Taira, H., M. Nakagahra and T. Nagamine (1988), "Fatty acid composition of Indica, Sinica, Javanica, and Japonica groups of nonglutinous brown rice", *Journal of Agricultural and Food Chemistry*, Vol. 36, pp. 45-47.

Taji, K. and L.F. Quinitio (1992), "Feeding value, digestibility and effect of combining hay cube in whole paddy rice silage and ammoniated whole crop paddy rice at the flowering stage", *Journal of Japanese Society of Grassland Science*, Vol. 38, pp. 16-25.

Taji, K. et al. (1991), "Feeding value of paddy rice silage at the ripe stage in sheep and beef cattle", *Memoirs of the College of Agriculture, Ehime University*, Vol. 35, pp. 171-177.

Tashiro, M. and Z. Maki (1979), "Purification and characterization of a trypsin inhibitor from rice bran", *Journal of Nutritional Science and Vitaminology*, Vol. 25, pp. 255-264.

Thompson, S.A. and C.W. Weber (1981), "Effect of dietary fiber sources on tissue mineral levels in chickens", *Poultry Science*, Vol. 60, pp. 840-845.

Trcka, J. et al. (2012), "Rice-induced anaphylaxis: IgE-mediated allergy against a 56-kDa glycoprotein", *International Archives of Allergy and Immunology*, Vol. 158(1), pp. 9-17.

Urisu, A. et al. (1991), "Rice protein 16KD – A major allergen in rice grain extract", *Arerug*, Vol. 40(11), pp. 1370-1376 (in Japanese, with English abstract).

USDA (2014), *USDA National Nutrient Database for Standard Reference, Release 27, Nutrient Data,* U.S. Department of Agriculture, Agricultural Research Service, Laboratory Home Page, http://www.ars.usda.gov/Services/docs.htm?docid=25706 (accessed on 15 October 2014).

USDA (2015), *USDA National Nutrient Database for Standard Reference, Release 28, Nutrient Data,* U.S. Department of Agriculture, Agricultural Research Service, Laboratory Home Page, http://www.ars.usda.gov/Services/docs.htm?docid=8964 (accessed on 15 May 2016).

Usui, Y. et al. (2001), "A 33-kD allergen from rice (*Oryza sativa* L. *Japonica*). cDNA cloning, expression, and identification as a novel glyoxalase 1", *Journal of Biological Chemistry*, Vol. 276(14), pp. 11376-11381.

Wanapat, M., K. Sommart and K. Saardrak (1996), "Cottonseed meal supplementation of dairy cattle fed rice straw", *Livestock Research for Rural Development*, Vol. 8(3), pp. 23-26.

WHO (1985), "Energy and protein requirements", in *Report of a Joint FAO/ WHO/ UNU Expert Consultation, WHO Technical Report Series,* No. 724.

WHO (2007), "Energy and protein requirements", in *Joint FAO/ WHO/ UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition, WHO Technical Report Series*, No. 935.

Yamagata, H. et al. (1998), "Rice bifunctional alpha-amylase/subtilisin inhibitor: Characterization, localization, and changes in developing and germinating seeds", *Bioscience, Biotechnology and Biochemistry*, Vol. 62(5), pp. 978-985.

Yamasaki, T. et al. (2006), "Rice bifunctional alpha-amylase/subtilisin inhibitor: Cloning and characterization of the recombinant inhibitor expressed in Escherichia coli", *Bioscience, Biotechnology and Biochemistry*, Vol. 70(5), pp. 1200-1209.

Chapter 3. Cowpea (*Vigna unguiculata*)

*This chapter deals with the composition of cowpea (*Vigna unguiculata*). It contains elements that can be used in a comparative approach as part of a safety assessment of foods and feeds derived from new varieties. Background is given on cowpea description, global production (predominantly in Africa and South America), uses for human and animal consumption, and processing into many products. Appropriate varietal comparators and characteristics screened by breeders are presented. Nutrients in whole grain, leaves and aerial parts of the cowpea plant, as well as main anti-nutrients and other constituents, are then detailed. The final sections suggest key products and constituents for analysis of new cowpea varieties for food use and for feed use.*

This chapter was prepared by the OECD Working Group for the Safety of Novel Foods and Feeds, with Australia as the lead country. It was initially issued in December 2018. FAOSTAT data on production, including in Table 3.1 and Figure 3.2, have been updated.

Background

General description of cowpea Vigna unguiculata *L.*

The cowpea (*Vigna unguiculata* (L.) Walp.) is an annual herbaceous legume (family Fabaceae) grown predominantly in Africa and is an important staple crop providing an affordable source of protein (Muranaka et al., 2016). The cowpea has a number of common names, including Black-eye pea, Black-eye bean, Crowder pea and Southern pea, frijol caupí and feijão-caupí. Yardlong bean or asparagus bean are common names for the related subspecies, *sesquipedalis*, the pods of which are a popular green vegetable in the People's Republic of China, South and South-East Asia.

Cowpeas are classified into five cultivar-groups: *biflora*, *melanophthalmus*, *sesquipedalis*, *textilis* and *unguiculata* (Pasquet, 2000).

Among the cultivated crop plants, the cowpea is one of the most variable species in terms of its plant growth, morphology, maturity and grain[1] types (Singh, 2014). The cowpea has a long taproot and adaptation mechanisms such as turning the leaves upwards to prevent them from becoming too hot and closing the stomata that help give it drought tolerance. As a legume crop, the cowpea fixes atmospheric nitrogen through symbiotic interactions with soil rhizobia (Sarr, Fujimoto and Yamakawa, 2015).

The cowpea corolla is yellowish-white to violet-white (Figure 3.1, Panel A), the pods occur in pairs and the leaves are trifoliate with oval leaflets (Figure 3.1, Panel B). Cultivated cowpeas are mostly indeterminate and some have the potential to produce multiple flushes of flowers (Gwathmey, Hall and Madore, 1992). Cowpeas are also diverse in their grain appearance, including the colour of the seed coat, seed size and eye colour (Figure 3.1, Panel C) (Carnovale, Lugaro and Marconi, 1991; Farinu and Ingrao, 1991; Kochhar, Walker and Pike, 1988; Gerrano, Jansen van Rensburg and Adebola, 2017a).

The cowpea was first domesticated in Africa between 1700 to 1500 before the Current Era (Singh, 2014) and all cultivated varieties grown in the world today originated from East and West Africa (Xiong et al., 2016). Despite the considerable morphological diversity, limited genetic diversity occurs among cultivated cowpea varieties owing to a single domestication event that has given rise to all cultivated varieties (Fang et al., 2007; Pasquet, 2000; 1999).

The present-day importance of the cowpea as an agricultural plant stems largely from its use as a short season protein-rich grain crop for human or animal consumption. In the African marketplace, harvested cowpea grain provides a cost-effective substitute for the less affordable foods from livestock and fish. Cowpea leaves can be harvested for direct use as needed during times of food scarcity while end of season collection of above-ground biomass after harvest provides valuable feedstock as fodder hay either for direct use or as a transportable commodity for sale or barter (Kristjanson et al, 2001; Hollinger and Staatz, 2015).

Further description on the cowpea taxonomy, plant, geographic distributions, habitats, crop production, centres of origin and diversity, reproductive biology, genetics and genome mapping, species/sub-species hybridisation and introgression, ecology, common pests and pathogens, and biotechnological developments can be found in the OECD Consensus Document on the Biology of cowpea (OECD, 2015).

Figure 3.1. Some key organs from the cowpea

A) flower; B) green pods and leaves; C) display of seed variety from different cultivars

Source: Courtesy of Carl Davies, CSIRO and Jeff Ehlers, University of California.

Production

Cowpeas are cultivated predominantly in Africa (Table 3.1) and are grown for food, fodder and green manure. Cowpea production has expanded in the world over the past decades (Figure 3.2). In 2017, over 87% of the crop was produced in Africa (Table 3.1). In South America, Brazil showed a recent increase in cowpea cultivation, placing the country in third place in terms of global area and production. According to FAOSTAT (2019) and the Brazilian National Supply Company (CONAB, 2018), the ten top producers of dry cowpeas in 2017 were Nigeria (3 410 thousand tonnes (kt)), Niger (1 959 kt), Brazil (749 kt), Burkina Faso (604 kt), the United Republic of Tanzania (201 kt), Cameroon (198 kt), Myanmar (179 kt), Kenya (146 kt), Mali (145 kt) and Sudan (130 kt).

Table 3.1. Global and regional production of the cowpea in 2017

Dry, thousand tonnes

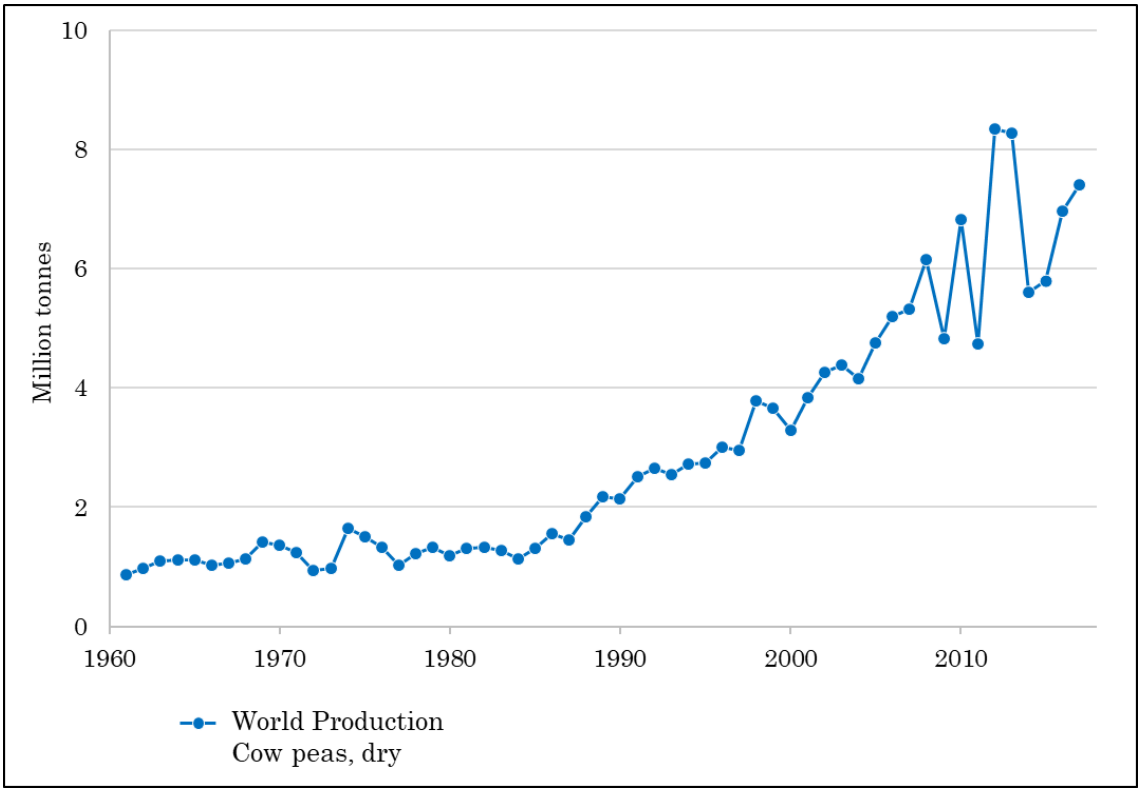
Region	Production
Africa	7 107
Americas	819*
Asia	204
Europe	27
Oceania	(-)
World	**8 157***

Notes: * FAOSTAT (2019) with the addition of Brazil production data, 749 kt in 2017/18 reported by the National Supply Company CONAB (2018).

Sources: FAOSTAT (2019), "Production – Crops – Production quantity – Cow peas, dry – 2017", http://www.fao.org/faostat/en/ (accessed on 10 July 2019), Aggregate may include official, semi-official, estimated or calculated data;
CONAB (2018), *Observatório Agrícola – Acompanhamento da safra brasileira de grãos,* http://www.conab.gov.br/OlalaCMS/uploads/arquivos/18_03_13_14_15_33_grao_marco_2018.pdf (accessed on 21 March 2018).

Figure 3.2. Increasing worldwide production of the cowpea, 1961–2017

Dry, million tonnes



Notes: This figure highlights the increasing trend in the cowpea's world production; the amounts for recent years, however, might be underestimated (e.g. Brazil data missing from the totals).
Source: FAOSTAT (2019), "World Production – Cow peas, dry – Years 1960-2017", http://www.fao.org/faostat/en/ (accessed on 10 July 2019), Aggregate may include official, semi-official, estimated or calculated data.

The cowpea is the most economically important indigenous African legume crop (Langyintuo et al., 2003). The majority of cowpea exports and imports occur within Africa for human consumption. It is actively traded from West to Central Africa because of the comparative advantage that drier areas of West Africa have in growing cowpea. Niger, Burkina Faso, Benin, Mali, Cameroon, Chad and Senegal are net exporters; Nigeria, Ghana, Togo, Côte d'Ivoire, Gabon, and Mauritania are net importers (Langyintuo et al., 2003). Since 2008, Brazil has exported the brown-eyed white commercial type to countries such as India, Israel, Pakistan, Turkey, the United Arab Emirates, Singapore, Indonesia, Nepal, Viet Nam, Portugal, and Italy (Aguiar, 2016; Freire Filho et al., 2017).

Uses

For human consumption, the cowpea is mainly grown for grain (dry and fresh) and sometimes for fresh pods in West Africa, India, and South America, while also grown for leaves in East Africa. It is an underused legume crop with a high potential for food and nutritional security in South Africa and produced for grain, immature green pods and fresh leaves due to its nutritional composition (Gerrano et al., 2015a; 2017a). The cowpea can be used to produce a large range of dishes and snacks (Uzogara and Ofuya, 1992; Asif et al., 2013) (Table 3.2).

Table 3.2. Examples of food uses of cowpea

Cowpea food	Description	Uses
Akara	Fried cowpea ball	Breakfast foods and snacks
Moin-moin	Steamed cowpea paste	Lunch and dinner foods
Ewa-ibji	Boiled whole cowpea	Lunch and dinner foods
Danwake	Boiled dehulled cowpea	Lunch and dinner foods
Gbegiri	Cowpea soup	Appetizers
Adayi	Cowpea purée	Pureed baby foods
Cowpea spread	Boiled mashed cowpeas with fat and seasoning	Spread on bread and yam
Roasted cowpea	Flavoured roasted cowpea	Snack food
Cowpea bread	Local bread made with cereal flour and cowpea flour	Breakfast, lunch and snack food
Cowpea cake	Cowpea used as an ingredient in cakes and pies	Breakfast and snack food
Rice and beans jollof	Boiled rice and boiled cowpeas	Food for adults
Akidi-na-oka	Dish of maize, cowpea	Food for adults
Cowpea sorghum dish	Boiled sorghum and cowpea	Food for adult
Cowpea plantain potage	Boiled cowpea and plantain	Food for adult
Cowpea yam potage	Boiled cowpea and yam	Food for adult
Cowpea weaning food	Dehulled, boiled cowpea supplemented to cereal-based infant foods	Infants, children food

Source: Asif, M. et al. (2013), "Application and opportunities of pulses in food system: A review", http://www.sciencedirect.com/science/article/pii/0308814690900456/pdf?md5=079b319a1346fef268dee5b0ccf323a2&pid=1-s2.0-0308814690900456-main.pdf.

The consumption of the cowpea as a dietary staple in West Africa over millennia has produced extensive and varied culinary practices and many individual foods and dishes. Cowpea consumption in West Africa has led to a culinary practice that requires seed coat removal (also called decortication or dehulling). For example, the popular West African cowpea-based foods, such as *Akara* and *Moin-moin*, are decorticated (Phillips, 2012). Four popular dishes in Brazil include "*Baião de dois*", a mix of cowpea and rice, cooked together (Figure 3.3, Panel A); *Akara* or "*Acaraje*", fried cowpea ball (Figure 3.3, Panel B); *Abará*, fried cowpea and shrimp ball rolled in banana leaves (Figure 3.3, Panel C), and "*Mugunzá*", a mix of cowpea, corn and pork meat (Figure 3.3, Panel D). In the United States, cowpeas are available to consumers as dry, canned or frozen grain (Phillips, 2012).

Consumer preferences for seed coat and eye colours vary from place to place and the cowpea variety can also affect food use (Table 3.3). For example, Ghanaian consumers pay a premium for black-eye whereas those in Cameroon discount black-eye. The most common preference for seed coat colour is white but, in some areas, consumers prefer red, brown or mottled grains. Up to nine different varieties may be on sale in a single domestic market (Langyintuo et al., 2003). In Brazil, the commercial varieties include Smooth White, Rough White, Smooth Brown, Evergreen, and Crowder (Freire Filho et al., 2017).

The cowpea is also utilised as fodder, fertiliser and as a quick-growing cover-crop and plays a particularly critical role in feeding animals during the dry season in many parts of West Africa (Uzogara and Ofuya, 1992; Singh and Tarawali, 1997). The haulms (stems) are a tradable commodity in fodder markets and the economic value of haulms has prompted cowpea breeders and livestock nutritionists to explore haulm fodder traits as additional selection and breeding criteria (Samireddypalle et al., 2017).

Short-duration spreading varieties are preferred for grain production and long-duration spreading varieties are preferred for fodder, the International Intitute of Tropical Agriculture (IITA) in collaboration with the International Livestock Research Institute (ILRI) have developed medium-maturing, semi-erect, dual-purpose varieties with higher grain and fodder yields and with enhanced fodder quality (Singh et al., 2003; Kristjanson et al., 2005; Samireddypalle et al., 2017). Similarly, Gerrano et al. (2015b) identified different cowpea genotypes that possess good vegetative traits and are also recommended for use as suitable parent lines when breeding for leaf or fodder production.

Table 3.3. Cowpea cultivars in Nigerian markets

Cultivar	Description	Food use
Black-eye variety	White seed coat and black hilum with tight-fitting seed coat	Boiled; Moin-moin and Akara after dehulling for paste production
Brown variety e.g. Ife brown	Brown seed coat and white hilum	Combination dishes with cereals, tubers, plantains and other legumes; not suitable for Akara and Moin-moin because of the brown colouration
White variety	White seed coat and white hilum	Paste products, e.g. Moin-moin and Akara

Source: Adapted from Uzogara and Ofuya (1992), "Processing and utilization of cowpeas in developing countries".

Figure 3.3. Examples of Brazilian (A-D) and Nigerian (E-H) cowpea dishes

Notes: F. Fried cowpea dough (called "*Akara*" in Igbo and Yoruba, "*Kosei*" in Hausa) in a bread roll.
G. "*Moin-moin*", called cowpea or bean pudding in English, "*Olele*" in Yoruba, "*Alele*" in Hausa.
H. Bean (cowpea) soup, called "*Mian Wake*" in Hausa, "*Gbegiri*" in Yoruba.
Sources: A. to D. Courtesy of Maurisrael de Moura Rocha, Embrapa; E. Courtesy of Mohammed Ishiyaku, IAR, Zaria; F. to H. Courtesy Umaru Abu, AATF.

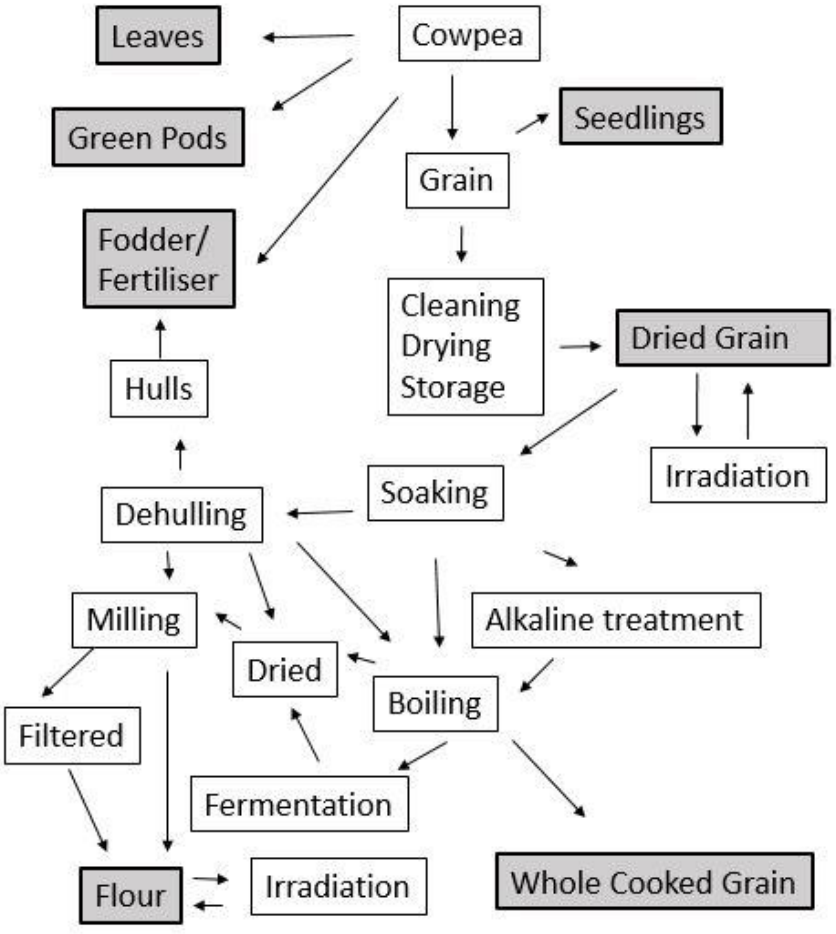
Processing

Processing of cowpeas and legumes, in general, is essential to make them nutritious, nontoxic, palatable and acceptable. The cowpea is utilised either whole or decorticated or dehulled. It is decorticated by soaking in water (at room temperature) for about 30-60 min,

and the seed coat removed by squeezing between the palms or by gentle abrasion using grinding stones. The seed coat is separated by subsequent filtration (Adebooye and Singh, 2007).

The constraints to maximum utilisation of cowpeas can be overcome by appropriate processing technology. For example, these techniques include dehulling, grinding, soaking, germination, fermentation, addition of salts, wet and dry heat treatments, cooking and roasting (Uzogara and Ofuya, 1992; Adebooye and Singh, 2007). Irradiation by gamma rays can also be used to sterilise cowpea flours and pastes but high levels of irradiation can reduce food quality (Abu et al., 2005). The most commonly used processing methods for cowpea products are presented in Figure 3.4.

Figure 3.4. Methods of processing for cowpea value-added products



Note: Shaded boxes represent end-use.

Sources: Adapted from Madode et al. (2013), "Enhancing the digestibility of cowpea (*Vigna unguiculata*) by traditional processing and fermentation" and Prinyawiwatkul et al. (1997), "Functional characteristics of cowpea (*Vigna unguiculata*) flour and starch as affected by soaking, boiling, and (…)" http://www.sciencedirect.com/science/article/pii/S0308814696002592/pdf?md5=072d9a708b842e2276c8b576a49b544c&pid=1-s2.0-S0308814696002592-main.pdf..

Soaking cowpeas prior to cooking softens the cotyledons and reduces the cooking time by over 30% (Uzogara and Ofuya, 1992). Reduced cooking time is needed for cowpea varieties with small grain size and a rough seed coat (Nielsen, Brandt and Singh, 1993). Seed coat removal results in faster cooking times, increased digestibility, better texture and appearance (Uzogara and Ofuya, 1992; Phillips, 2012). In Ghana and Nigeria, the cooking time of cowpeas is traditionally reduced by cooking them with a naturally-occurring alkaline rock-salt known as "kanwa" (Uzogara, Morton and Daniel, 1988).

Soaking and boiling of cowpeas is required to improve texture and reduce oligosaccharide levels to lessen the incidence of flatulence (Akinyele and Akinlosotu, 1991; Akpapunam and Achinewhu, 1985; Egounlety and Aworh, 2003; Madode et al., 2013; 2011; Onyenekwe, Njoku and Ameh, 2000; Phillips and McWatters, 1991; Prinyawiwatkul et al., 1996; Singh, 2014). Fermentation has also been used as a process to further reduce oligosaccharide levels (Akinyele and Akinlosotu, 1991; Akpapunam and Achinewhu, 1985; Madode et al., 2013; Prinyawiwatkul et al., 1997; Uzogara and Ofuya, 1992; Egounlety and Aworh, 2003).

The eating quality of milled cowpea products, particularly their texture, depends on the flour's composition, degree of grinding fineness and relative proportions of particles with different mesh grades, and cooking conditions (Uzogara and Ofuya, 1992; Yeung et al., 2009).

Appropriate comparators for testing new varieties

This document suggests parameters that cowpea breeders should measure when new cowpea varieties are produced. Measurement data from the new variety should preferably be compared to those obtained from the near-isogenic non-modified variety (or other existing varieties), where both have been grown and harvested under similar conditions.[2] The comparison can also be made between values obtained from other varieties described in the literature.

Critical components include key nutrients and anti-nutrients. Key nutrients are those components in cowpea that may have a substantial impact on the overall diet, including major constituents (proteins, fats and carbohydrates) and minor components (vitamins and minerals). Similarly, the levels of known anti-nutrients should be considered. As part of the comparative approach, selected plant metabolites, for which characteristic levels in the species are known, can be analysed as further indicators of the absence of unintended effects of the breeding strategy on metabolism.

Traditional characteristics screened by developers

The majority of cowpea production occurs under low input agriculture on small-scale farms in developing countries, and under such conditions, yield is mostly below its potential for the crop (Singh, 2014). Improving cowpea yields, nutritional quality, stress tolerance or resistance to pests and diseases are key objectives for various national and international breeding programmes[3] (OECD, 2015). The cowpea plant is attacked by pests during every stage of its life cycle, including storage. Pests include viruses, bacteria, fungi, aphids, flower thrips, pod borers, weevils, parasitic weeds and nematodes (Singh, 2014; IITA, Nigeria).

Breeders have developed varieties that are high yielding, early or medium maturing, have large seeds, altered seed coat texture/colour, enhanced cooking and nutritional aspects,[4] dual feed/fodder use and pest resistance. Due to the demand for cultivars that are suitable

for fully mechanised cultivation, the cowpea plant architecture has been targeted for improvement, primarily to obtain erect plants and insertion of pods above the leaves (Figure 3.5) (Rocha, Damasceno-Silva and Menezes-Júnior, 2017).

Figure 3.5. Modern cowpea breeding to obtain erect plants with pods inserted above the leaves

Source: Courtesy of Maurisrael de Moura Rocha, Embrapa.

Nutrients

Composition of the cowpea – General points

Most of the nutrient composition data is based on cowpea whole grain, although there is a limited amount of data for dehulled grains, sprouted grains and leaves. Whole grains include the seed coat which represents 6% of grain dry matter (Aremu, 1990).

The cowpea is morphologically variable and adapted to different environments, resulting in a wide range of local varieties (OECD, 2015). The nutritional composition of cowpea is impacted by genetic characteristics, agro-climatic conditions, biotic stresses and postharvest management (Goncalves et al., 2016; Murdock et al., 2003; Oluwatosin, 1998; Silveira et al., 2001).

The cowpea is highly nutritious and has potential health benefits because of its high protein, high fibre and low glycaemic index, (Aguilera et al., 2013; Carnovale, Lugaro and Marconi, 1991; Siddhuraju and Becker, 2007; Sreerama, Sashikala and Pratape, 2012; Xiong, Yao and Li, 2013; Xu and Chang, 2012).

Constituents of the cowpea

Proximate composition, fibre, amino acids and fatty acids

The proximate composition of a large number of cowpea varieties is listed in Tables 3.4 and 3.5.

Carbohydrates and fibres

The cowpea contains a high proportion of carbohydrates, representing the majority of the dry weight of the grain, leaves, and sprouts (Tables 3.4 and 3.5). Eight sugars (simple carbohydrates) have been reported in the cowpea, namely, sucrose (11-19 g/kg), glucose (4-5 g/kg), fructose (1-2 g/kg), galactose (≤ 15 g/kg), maltose (≤ 11 g/kg); and three carbohydrates considered to be anti-nutrients, stachyose (17-60 g/kg), verbascose (6-13 g/kg), and raffinose (5-10 g/kg) (Goncalves et al., 2016).

The crude fibre (complex carbohydrates) content of whole cowpeas ranges from 2.5% to 32% of total dry matter (Table 3.4). The crude fibre content decreases when the seed coat is removed.

The means for total, insoluble and soluble dietary fibre of dehulled cowpeas reported by Khan et al. (2007) are 18.2%, 14.8%, and 3.3% of dry matter respectively. Total dietary fibre includes cellulose (6%), hemicellulose (3.9%), lignin (2%), and pectin (1.8%) (Khan et al., 2007).

Protein

The cowpea provides a source of protein (Boukar, Massawe and Muranaka, 2011) with the whole grain containing levels ranging from 16% to 31% (Table 3.4). The seed coat contains 12% protein (Aremu, 1990). Most of the cowpea grain proteins consist of globulins with lower levels of albumins, glutelins, and prolamins (Goncalves et al., 2016; Vasconcelos et al., 2010).

The amino acid composition of the cowpea is rich in lysine, leucine, arginine and other essential amino acids and can largely fulfil the essential amino acid requirements of a human diet. However, cowpeas are low in the sulphur amino acids (methionine and cysteine) compared to cereals and animal products and thus, for a balanced diet, cowpeas need to be supplemented with cereals or vegetables, meat and/or dairy products (Iqbal et al., 2006; Uzogara and Ofuya, 1992; Hussain and Basahy, 1998; FAO, 2004) (Tables 3.6 and 3.7).

Lipids/fatty acids

The lipid content of cowpea whole grain ranges from 0.5% to 3.9% (Table 3.4). The lipid profile of cowpea indicates a predominance of triglycerides (41.2% of total fat), followed by phospholipids (25.1% of total fat), monoglycerides (10.6% of total fat), free fatty acids (7.9% of total fat), diglycerides (7.8% of total fat), sterols (5.5% of total fat) and hydrocarbons and sterol esters (2.6% of total fat) (Goncalves et al., 2016). With respect to fatty acids, linoleic acid and palmitic acid predominate followed by oleic acid, stearic acid and linolenic acid (Thangadurai, 2005; Goncalves et al., 2016).

Minerals

Cowpeas are a source of essential minerals, calcium, magnesium, potassium, iron, zinc and phosphorus (Tables 3.8 and 3.9). Low availability of soil phosphorus is a primary constraint to cowpea production in developing countries (Burridge et al., 2016). Levels of grain phosphorous, potassium and manganese vary widely due to environmental conditions (Adebooye and Singh, 2007).

Most minerals are at higher concentrations in leaves (Gerrano et al., 2015a) and immature green pods (Gerrano, Jansen van Rensburg and Adebola, 2017b) compared to grain (Belane and Dakora, 2012; Madode et al., 2011). Some minerals are lost when the seed coats are removed (Table 3.8 vs. Table 3.9) (Mamiro et al., 2011).

Vitamins

Cowpeas are a source of thiamin and niacin, and also contain reasonable amounts of other water-soluble vitamins such as riboflavin (Table 3.10). Vegetative tissues including germinated grain tend to have higher levels of niacin, thiamin and riboflavin than grain (Nnanna and Phillips, 1989; Goncalves et al., 2016). Seed coat removal results in up to 30% loss in niacin content, while thiamin is reduced 41% by cooking (Nnanna and Phillips, 1989). Vitamin C values are higher in leaves than grains and increased by 4 to 38-fold after grains sprout (Devi, Kushwaha and Kumar, 2015; Goncalves et al., 2016). Cooking in an alkaline solution containing "kanwa" (naturally-occurring rock-salt) decreases thiamin, niacin and riboflavin levels compared to cooking without "kanwa" (Uzogara, Morton and Daniel, 1991). Fermentation results in a significant increase in the levels of thiamin and niacin (Akinyele and Akinlosotu, 1991).

Table 3.4. Proximate and fibre composition of cowpea whole grain

Percentage of dry matter

	Hussain and Basahy (1998)[a]	Maia et al. (2000)[a]		Rivas-Vega et al. (2006)[b]	Carvalho et al. (2012)		Devi, Kushwaha and Kumar (2015)[c]		Heuzé and Tran (2015)[a]		Yewande and Thomas (2015)		USDA-ARS (2016)
	mean	*mean*	*range*	*mean*	*mean*	*range*	*mean*	*range*	*mean*	*range*	*mean*	*range*	*mean*
Ash	3.6	3.6	3.2-4.1	3.9	3.7	3.0-4.1	4	3.8-3.9	4.1	3.1-5.8	3.7	3.7-3.7	3.39
Carbohydrate*	58.8	71	68-73	74.8	40.6	30-52	66	62-68			53.6	53.4-54.7	59.6
Crude fibre				2.6	24.2	18-32	4.57	4.3-5.0	5.6	2.5-10.5	4.4	4.3-4.5	10.7[d]
Crude protein	23	22.7	20-26	26.1	20.3	16-25	27.7	25-31	25.2	18.2-30.4	23.4	22.8-23.9	23.9
Crude fat	3.4	2.4	1.2-3.6	1.05	1.2	1.2-1.4	2.2	2-2.5	1.6	0.5-3.9	2	1.9-2.1	2.1
Water (% of fresh weight)	11.2	13	12-14	7.9			7.8	6.9-9.8	10.1	5.2-14.2	12.9	12.2-13.7	11.1

Notes: * Unless otherwise indicated, carbohydrate is measured by difference.
a. Carbohydrate values include fibre.
b. Anthrone method used to measure carbohydrates.
c. Carbohydrate measured as a nitrogen-free extract.
d. This value is for total dietary fibre and not crude fibre.

Table 3.5. Proximate and fibre composition of cowpea decorticated grain (DecGrain), leaves and aerial parts

Percentage of dry matter

	Rivas-Vega et al. (2006)		Devi, Kushwaha and Kumar (2015)[a]		Heuzé et al. (2015)[b]		Yewande and Thomas (2015)	
	DecGrain	**Sprouts[b]**	**Sprouts[c]**		**Leaves/aerial**		**DecGrain**	
	mean	*mean*	*mean*	*range*	*mean*	*range*	*mean*	*range*
Ash	3.75	4.23	4.2	3.9-4.5	11.3	8.1-14.4	2	2.0-2.0
Carbohydrate*	78.9	85.9	62.3	59.7-65.2			57.9	57.8-57.9
Crude fibre	0.8	2.12	6	5.1-6.5	24.1	11.5-35.9	1.4	1.4-1.4
Crude protein	25.6	29.5	30.6	28.1-33.6	18.1	13.5-24.3	21.3	20.8-21.8
Crude fat	1.29	1.4	2.2	2.0-2.5	2.8	1.3-4.1	1.6	1.6-1.6
Water (% of fresh weight)	7.85	6.36	9.2	8.5-10-6	79.1	88.9-73.6	15.9	15.3-16.4

Notes: * Unless otherwise indicated, carbohydrate is measured by difference.
a. Carbohydrate measured as a nitrogen-free extract.
b. Sprouts germinated for 3 days.
c. Sprouts germinated to be ¼ - ½ inches in length.

Table 3.6. Amino acid composition of cowpea whole grain

Percentage of total protein

Amino acid	Iqbal et al. (2006)	Adebooye and Singh (2007)		Khattab, Arntfield and Nyachoti (2009)		Vasconcelos et al. (2010)		Carvalho et al. (2012)[b]		Heuzé and Tran (2015)[b]		USDA-ARS (2016)[c]	Goncalves et al. (2016)
	mean	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
Alanine	4.2			4.6	4.6-4.5			4.8	4.5-5.0	4.2	3.4-5.1	4.6	4.2-4.5
Arginine	7.5			7.2	6.7-7.7	7.6	6.4-9.9	7.6	7.0-8.5	6.7	5.0-8.7	7	6.8-10.8
Aspartic acid	10.8			11.3	11-11.4			10.8	6.0-11.5	10.4	9.2-12.7	12.2	11-13
Cysteine	0.5			0.3	0.3-0.3					1.1	0.6-1.4	1.1	0.6-2.4
Glutamic acid	17.2			18.3	18-18.5			17.8	8.5-18.6	15.8	14.1-18.7	19.1	17-19
Glycine	3.8			4.3	4.1-4.5			4.1	3.2-4.3	3.9	3.1-4.8	4.2	4.1-4.4
Histidine	3.1	3.5	3.4-3.6	3.1	3.1-3.2	3.8	2.0-4.5	3.7	2.2-4.0	3.1	2.4-4.1	3.1	2.7-3.4
Isoleucine	4.5	4.8	4.7-4.9	3.8	3.8-3.8	4.4	3.8-5.4	3.8	3.0-4.7	4	2.8-5.2	4.1	3.9-4.5
Leucine	7.7	8.5	8.3-8.7	7.7	7.7-7.7	7.3	5.7-8.2	8.3	7.9-9.8	7.4	5.8-11.3	7.7	7.5-7.8
Lysine	7.5	7.2	7.1-7.2	5.8	5.7-5.9	6.1	3.9-8.1	8.0	7.6-8.3	6.5	5.2-7.1	6.8	3.5-7.9
Methionine	2.2	1.6	1.5-1.6	1.8	1.5-2.1			1.7	1.6-1.8	1.4	0.9-1.6	1.4	1.1-3.5
Phenylalanine	7.5	5.9	5.8-6.0	5.6	5.5-5.8			10.3	9.9-10.6	5.5	4.4-6.4	5.9	
Proline	4			5.7	5.6-5.9			8.1	7.6-8.9	4.6	3.8-5.7	4.5	3.1-6.2
Serine	3			5.5	5.4-5.6			5.2	4.5-5.8	4.9	3.8-5.6	5.1	4.0-5.2
Threonine	3.8	3.7		4.1	4.0-4.1	4.4	3.2-5.9	4.0	4.0-4.1	3.8	3.0-5.3	3.8	3.4-4.0
Tryptophan	0.7			1.1	1.0-1.1			1.3	1.1-1.5	1.1	0.9-1.3	1.2	1.1-1.3
Tyrosine	3			3.5	2.9-4.0					3	2.6-3.6	3.2	3.4-4.5
Valine	5	5.8	5.7-5.9	4.9	4.7-5.1	4.7	4.0-6.3	4.6	3.6-5.9	4.7	3.4-5.5	4.8	4.5-6.2

Notes: a. Total protein was chosen instead of dry weight because protein content is influenced by environmental factors and between seasons.
b. Cysteine values included in methionine data. Tyrosine values included in phenylalanine data.
c. Recalculated from g/100 g edible portions of grain.

Table 3.7. Amino acid composition of cowpea decorticated grain (DecGrain), leaves and aerial parts

Percentage of total protein[a]

Amino acid	Iqbal et al. (2006)	Adebooye and Singh (2007)		Heuzé et al. (2015)		Goncalves et al. (2016)
	DecGrain	**DecGrain**		**Leaves/aerial**		**Leaves**
	mean	mean	range	mean	range	range
Alanine	4.2			4.6		5.8-9.8
Arginine	7.5					16.1-17.3
Aspartic acid	10.8					17.0-26.7
Cysteine	0.5			0.9	0.9-0.9	1.0-2.9
Glutamic acid	17.2					24.3-45.3
Glycine	3.8			4.8		8.5-12.6
Histidine	3.1	3.2	3.2	1.8		6.6-8.6
Isoleucine	4.5	4.2	4.1-4.2	4.3		9.8-11.1
Leucine	7.7	8.2	7.9-8.4	7.4		17.9-19.6
Lysine	7.5	7	6.9-7.0	3.3	3-3.5	10.3-16.3
Methionine	2.2	1.4	1.3-1.5	1.4	1-1.8	2.9-4.5
Phenylalanine	7.5	5.7	5.6-5.7	4.6		12.6-14.4
Proline	4					10.4-15.9
Serine	3					11.4-11.6
Threonine	3.8	3.4	3.2-3.5	4	3.4-4.6	7.8-10.8
Tryptophan	0.7			1.3	1.3-1.4	2.4-4.1
Tyrosine	3			3.2		6.5-9.3
Valine	5	5.5		5.3		11.5-12.8

Note: a. Total protein was chosen instead of dry weight because protein content is influenced by environmental factors and between seasons.

Table 3.8. Levels of minerals in cowpea whole grain

Mineral	Akinyele and Akinlosotu (1991)	Boukar, Massawe and Muranaka (2011)		Belane and Dakora (2012)		Carvalho et al. (2012)		Heuzé and Tran (2015)[a]		USDA-ARS (2016)[a]
	mean	mean	range	mean	range	mean	range	mean	range	mean
Macro-minerals (mg/g dry matter)										
Calcium	0.446	0.826	0.31-1.395	0.6	0.37-1.13	0.37	0.29-0.51	1.1	0.3-2.7	0.95
Phosphorus		5.06	3.45-6.73	4.7	3.8-4.7			4.2	2.1-5.4	4.92
Potassium	12.36	14.89	11.40-18.45	13.3	11.4-16.4	11.07	9.57-12.51	15	12.8-21.5	15.44
Magnesium	0.905	1.92	1.52-2.50	1.7	1.3-2.4	1.46	1.30-1.69	2.2	1.6-2.8	3.74
Micro-minerals (mg/100 g dry matter)										
Copper				0.6	0.5-0.8	2.1	2.0-2.2	0.9	0.6-1.4	1.2
Iron	16.9	5.3	3.4-8.0	6.1	4.8-9.7	6.9	6.0-8.1	42.2	9.6-135.6	11.2
Manganese				3.3	2.1-4.3	2	1.7-2.9	2	1.4-3.2	1.7
Sodium						12.5	8.4-17.7	10	10-20	65
Zinc	4.5	3.8	2.2-5.8	4.3	3.3-6.5	3.3	2.7-4.4	3.8	2.4-4.6	6.9

Note: a. Recalculated from wet weight data where the water content was 11.05 g/100 g wet weight.

Table 3.9. Levels of minerals in cowpea decorticated grain (DecGrain) and leaves

Mineral	Akinyele and Akinlosotu (1991)	Iqbal et al. (2006)	Adebooye and Singh (2007)		Belane and Dakora (2012)		Heuzé et al. (2015)	
	DecGrain	**DecGrain**	**DecGrain**		**Leaves**		**Leaves/aerial parts**	
	mean	mean	mean	range	mean	range	mean	range
Macro-minerals (mg/g dry matter)								
Calcium	0.43	1.76	7.64	7.53-7.75	24.5	15.20-46.20	12.5	6.8-20.6
Phosphorus		3.03			4	2.30-6.10	2.4	1.1-5.2
Potassium	11.31	12.8	7.4	6.90-7.87	21.6	9.30-35.60	19.1	10.9-31.6
Magnesium	0.86	0.05	3.46	3.02-3.90	5.6	4.30-8.40	3.1	1.9-5.0
Micro-minerals (mg/100 g dry matter)								
Copper		9.7	0.95	0.9-1.0	1.3	0.9-2.2	3.0	
Iron	11.5	2.6	4.6	4.4-4.8	38	17-216	169	
Manganese		1.7	1.5	1.1-1.9	96	37-204		
Sodium		102						
Zinc	4.3	5.1	9	7.4-9.8	8.3	3.8-22.3	4.6	

Table 3.10. Vitamin levels in cowpea whole grain

mg/100 g dry matter

Vitamin	Elias, Bressani and Colindre (1964)		Uzogara, Morton and Daniel (1991)	Goncalves et al. (2016)	USDA-ARS (2016)[a]
	mean	range	mean	range	mean
Vitamin A				0.07	0.02
Vitamin B1 (thiamine)	0.74	0.41-0.99	0.77	0.2-1.7	0.76
Vitamin B2 (riboflavin)	0.42	0.29-0.76	0.25	0.1-0.3	0.19
Vitamin B3 (niacin)	2.81	2.51-3.23	3.45	0.7-4.0	3.14
Vitamin B5 (pantothenic acid)				1.7-2.2	
Vitamin B6 (pyridoxine)				0.2-0.4	0.41
Vitamin B7 (biotin)				0.02-0.03	
Vitamin B9 (folic acid)				0.1-0.4	
Vitamin B12 (cobalamin)				Trace	0
Vitamin C					1.69
Vitamin D (D2+D3)					0
Vitamin E				2-20	

Note: a. Recalculated from wet weight data where the water content was 11.05 g/100 g wet weight.

Anti-nutrients and other constituents

Anti-nutrients

Cowpeas contain some constituents that have anti-nutritional effects. These include oligosaccharides, phytic acid, polyphenols, protease inhibitors and lectins.

Oligosaccharides

For some humans, flatulence is a constraint to the consumption of cowpeas and other legumes. This response to legumes, which may vary according to gender, age, composition of colonic microflora and other factors, is attributed mainly to oligosaccharides that include stachyose, raffinose and verbascose. These oligosaccharides escape breakdown and absorption in the stomach and small intestine and are fermented by microorganisms present in the colon resulting in the production of flatus and other attendant discomfort (Onyenekwe, Njoku and Ameh, 2000; Phillips and Abbey, 1989). The concentration of oligosaccharides in cowpeas varies between varieties (Table 3.11).

Dehulling, soaking, germination and cooking can reduce oligosaccharide content (Aguilera et al., 2013; Akinyele and Akinlosotu, 1991; Akpapunam and Achinewhu, 1985; Egounlety and Aworh, 2003; Goncalves et al., 2016; Onyenekwe, Njoku and Ameh, 2000; Phillips, 2012; Singh, 2014; Somiari and Balogh, 1993; Uzogara and Ofuya, 1992).

Phytic acid

In legumes, the major portion of the phosphorus is present in the form of phytic acid (Reddy, Sathe and Salunkhe, 1982). Phytic acid can reduce the bioavailability of minerals and the digestibility of protein and starch by inhibiting proteases and amylases (Goncalves et al., 2016; Thompson and Yoon, 1984; Reddy, Sathe and Salunkhe, 1982). Phytic acid levels vary between varieties (Table 3.12) and may be altered with drying, storage, dehulling, soaking, germination, fermentation, cooking or roasting (Goncalves et al., 2016; Egounlety and Aworh, 2003; Adebooye and Singh, 2007). For example, phytic acid decreased 4 to 16 fold in sprouted grains (Devi, Kushwaha and Kumar, 2015).

Polyphenols

Polyphenols are included as anti-nutrients as they play a role in the reduction of protein and starch digestibility (Thompson and Yoon, 1984), and range in concentration among cowpea varieties (Table 3.12). Significant genetic variability was found for total flavonoid content and antioxidant activity in cowpea grains (Nassourou et al., 2016).

Polyphenols are mainly present in the seed coat. Cultivars with a coloured seed coat contain more polyphenols than white-seeded cultivars which have no detectable tannin, a polyphenol (Kachare, Chavan and Kadam, 1988). Cooking and dehulling reduce total phenolic content (Adebooye and Singh, 2007). Germinating cowpea seedlings have slightly higher polyphenol concentrations than raw cowpea grains (Aguilera et al., 2013).

Table 3.11. Oligosaccharide content in cowpea whole grain and decorticated grain (DecGrain)

mg/g dry weight

	Akpapunam and Markakis (1979)		Onigbinde and Akinyele (1983)				Phillips and Abbey (1989)		Akinyele and Akinlosotu (1991)		Somiari and Balogh (1993)		Muranaka et al. (2016)	
	Grain		**Grain**		**DecGrain**		**Grain**		**Grain**	**DecGrain**	**Grain**		**Grain**	
	mean	range	mean	range	mean	range	mean	range	mean	mean	mean	range	mean	range
Raffinose	12	11-12	26	13-42	17.8	5.8-33.9	3.8	2.9-4.7	20	8.5	25	22-28	3.4	1.7-4.5
Stachyose	34	29-41	33	12-50	24	8.9-37.5	20	17-22	36	30	42	33-48	31	24-43
Verbascose	9	6-10					5	3.8-6.0	40	9.5				

Table 3.12. Phytic acid and polyphenol composition in cowpea whole grain, decorticated grain (DecGrain) and sprouts

mg/g dry weight

	Preet and Punia (2000)		Madode et al. (2011)		Afiukwa et al. (2012)		Devi, Kushwaha and Kumar (2015)				Muranaka et al. (2016)	
	Grain		**Grain**		**DecGrain**		**Grain**		**Sprouts**		**Grains**	
	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
Phytic acid	9.1	8.2-9.5	3.3	0.8-5.0	3.1	2.6-3.9	3.4	3.1-3.8	0.46	0.2 0.7	28.3	22-37
Polyphenols	8.5	7.8-9.3	5.4	0.7-9.1							4.3	0.1-49

Protease inhibitors and lectins

Protease inhibitors and lectins are heat-labile and inactivated by cooking (Boukar et al., 2015) but are important to the plant as they have a role in protecting the plant from certain pests and diseases (Bell et al., 2001; Xu et al., 1996; Zhu et al., 1994; Machuka et al., 2000; Marconi, Ruggeri and Carnovale, 1997). Trypsin inhibitors are regarded as one of the most important anti-nutritional factors in cowpeas (Kochhar, Walker and Pike, 1988) and their levels vary considerably across cowpea varieties (Table 3.13). Germinating cowpea seedlings had reduced trypsin inhibitors but similar levels of chymotrypsin inhibitors compared to raw cowpea grains (Aguilera et al., 2013; Devi, Kushwaha and Kumar, 2015).

Lectins are found in most plants and are glycoproteins that selectively and reversibly bind carbohydrates, resulting in reduced nutrient absorption (Zhang et al., 2009). Lectin levels also vary widely among cowpea varieties (Table 3.13).

Table 3.13. Protease inhibitor activity (trypsin and chymotrypsin inhibitors) and lectin (measured by haemagglutination activity) in dry cowpea grain and decorticated cowpea grain (DecGrain)

	Units	Marconi, Ng and Carnovale (1993)[a,c]		Carvalho et al. (2012)[b,c]		Afiukwa et al. (2012)[a,d]	
		Grain		Grain		DecGrain	
		mean	range	mean	range	mean	range
Trypsin inhibitor	TIU/mg	19	9-47	2.8	2.2-4.2	21	15-28
Chymotrypsin inhibitor	CIU/mg	18	7-56	2.9	2.3-3.8		
Haemagglutination activity	HU	286	13-1173	220	40-640	64	5-83

TIU = trypsin inhibitor units; CIU = chymotrypsin inhibitor units; HU = haemagglutination units.

Notes:
a. Trypsin and chymotrypsin inhibitor expressed as units/mg flour.
b. Trypsin and chymotrypsin inhibitor expressed as units/mg protein.
c. Haemagglutination activity expressed as the reciprocal of the highest dilution (g/mL) resulting in positive agglutination.
d. Haemagglutination activity expressed as activity per g of flour (as per Liener and Hill, 1953).

Allergens

Allergic reactions to legumes, including peanuts and soybeans, are relatively common (Verma et al., 2013) but are rare for cowpeas. However, Rao et al. (2000) reported that serum from six individual patients that were allergic to cowpeas identified 41 kDa and 55 kDa proteins to be the major allergens of cowpea.

Suggested constituents to be analysed related to food use

Key products consumed by humans

The cowpea is a staple food and provides a major source of protein, and very likely other nutrients, to many people in Africa and elsewhere. Typically, the cowpea is consumed after having been soaked in water and cooked. Cowpeas are also consumed as roasted dried grain, flour, seedlings, leaves and green pods.

Suggested analysis for food use of new varieties

The cowpea can provide protein, carbohydrates, vitamins and dietary fibre. It also contains anti-nutrients such as lectins, oligosaccharides, phytic acid and trypsin inhibitor. These constituents are recommended for analysis of new cowpea varieties for food use (Table 3.14).

Table 3.14. Suggested nutritional and compositional parameters to be analysed in the cowpea for food use

Constituent	Grain
Proximates*	x
Amino acids	x
Fibre	x
Niacin	x
Riboflavin	x
Thiamine	x
Lectins	x
Raffinose	x
Stachyose	x
Phytic acid	x
Trypsin inhibitor	x

Note: * Proximates are Crude protein, Total lipid (fat), Ash, Carbohydrate (by difference) and Moisture.

Suggested constituents to be analysed related to feed use

Key products consumed by animals

The majority of cowpea grain is used for human consumption. Plant parts not used by humans are often used as fertiliser, grazed by livestock or harvested for fodder.

Suggested analysis for feed use of new varieties

The cowpea is an important animal feed that is able to provide good levels of protein, carbohydrates, vitamins and minerals for a range of animal species and these constituents are suggested for analyses for feed use (Table 3.15). A number of anti-nutrients are also relevant for feed use. An anti-nutrient effect is not an intrinsic property of a compound but also depends on the physiology of the ingesting animal. For example, trypsin inhibitors do not exert any anti-nutrient effects on ruminants as they are degraded in the rumen (Akande and Fabiyi, 2010).

Table 3.15. Suggested nutritional and compositional parameters to be analysed in the cowpea for feed use

Constituent	Grains	Leaves
Amino acids	x	
Neutral detergent fibre (NDF)	x	x
Acid detergent fibre (ADF)	x	x
Lectins	x	
Trypsin inhibitor	x	
Phytic acid	x	
Calcium	x	x
Proximates*	x	x

Note: * Proximates are Crude protein, Total lipid (fat), Ash, Carbohydrate (by difference) and Moisture.

Notes

[1] The terms "seed" and "grain" are often used in literature with equivalent meaning. This is also the case in this document where the use of these terms were harmonised as far as possible along the following principles: the term "seed" refers to a grain intended for sowing, or is used in specific botanical descriptions of the grain as being a distinct part of the plant (e.g. "seed coat"). The term "grain" is used in all other cases, more directly referring to the harvested product intended for food and feed. In addition, for legume crops, grain is sometimes referred to as "grain legume" or "legume".

[2] For additional discussion of appropriate comparators, see the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants CAC/GL 45/2003 of the Codex Alimentarius Commission (paragraphs 44 and 45).

[3] These include breeding programmes at the International Institute of Tropical Agriculture (IITA) in Nigeria, the USAID Bean/Cowpea Collaborative Research Support Program (CRSP), the University of California (UCR), the Texas A&M University and the Brazilian Agricultural Research Corporation (Embrapa).

[4] E.g. biofortication for higher levels of iron and zinc (Rocha, 2015).

References

Abu, J.O. et al. (2005), "Functional properties of cowpea (*Vigna unguiculata* L. Walp) flours and pastes as affected by gamma-irradiation", *Food Chemistry*, Vol. 93, pp. 103-111.

Adebooye, O.C. and V. Singh (2007), "Effect of cooking on the profile of phenolics, tannins, phytate, amino acid, fatty acid and mineral nutrients of whole-grain and decorticated vegetable cowpea (*Vigna unguiculata* L. Walp)", *Journal of Food Quality*, Vol. 30, pp. 1101-1120, http://onlinelibrary.wiley.com/doi/10.1111/j.1745-4557.2007.00155.x/epdf.

Afiukwa, C.A. et al. (2012), "Characterization of cowpea cultivars for variations in seed contents of some antinutritional factors (ANFs)", *Continental Journal of Food Science and Technology*, Vol. 6, pp. 25-34. https://www.cabdirect.org/cabdirect/abstract/20123407711.

Aguiar, P.M.R. (2016), "O nosso feijão-caupi no mundo", *Congresso Nacional de Feijão-caupi. Feijão-caupi: avanços e desafios tecnológicos e de mercados: resumos. Sorriso, MT, 2016*, Embrapa, Brasília, DF, pp. 262-263.

Aguilera, Y. et al. (2013), "Changes in Nonnutritional Factors and Antioxidant Activity during Germination of Nonconventional Legumes", *Journal of Agricultural and Food Chemistry*, Vol. 61, pp. 8120-8125. http://pubs.acs.org/doi/pdf/10.1021/jf4022652

Akande, K.E. and E.F. Fabiyi (2010), "Effect of processing methods on some antinutritional factors in legume seeds for poultry feeding", *International Journal of Poultry Science*, Vol. 9, pp. 996-1001.

Akinyele, I.O. and A. Akinlosotu (1991), "Effect of soaking, dehulling and fermentation on the oligosaccharides and nutrient content of cowpeas (*Vigna unguiculata*)", *Food Chemistry*, Vol. 41, pp. 43-53, http://www.sciencedirect.com/science/article/pii/030881469190130G/pdf?md5=a0b6ac6a100e4acf26f75f2d5039a097&pid=1-s2.0-030881469190130G-main.pdf.

Akpapunam, M.A. and S.C. Achinewhu (1985), "Effects of cooking, germination and fermentation on the chemical-composition of nigerian cowpea (*Vigna unguiculata*)", *Qualitas Plantarum-Plant Foods for Human Nutrition*, Vol. 35, pp. 353-358, https://link.springer.com/content/pdf/10.1007%2FBF01091780.pdf.

Akpapunam, M.A. and P. Markakis (1979), "Oligosaccharides of 13 American cultivars of cowpeas (*Vigna sinensis*)", *Journal of Food Science*, Vol. 44, pp. 1317-1319, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1979.tb06428.x/epdf.

Aremu, C.Y. (1990), "Proximate and amino-acid-composition of cowpea (*Vigna unguiculata,* Walp) protein-concentrate prepared by isoelectric point precipitation", *Food Chemistry*, Vol. 37, pp. 61-68, http://www.sciencedirect.com/science/article/pii/0308814690900456/pdf?md5=079b319a1346fef268dee5b0ccf323a2&pid=1-s2.0-0308814690900456-main.pdf.

Asif, M. et al. (2013), "Application and opportunities of pulses in food system: A review", *Critical Reviews in Food Science and Nutrition*, Vol. 53, pp. 1168-1179, http://www.sciencedirect.com/science/article/pii/0308814690900456/pdf?md5=079b319a1346fef268dee5b0ccf323a2&pid=1-s2.0-0308814690900456-main.pdf.

Belane, A.K. and F.D. Dakora (2012), "Elevated concentrations of dietarily-important trace elements and macronutrients in edible leaves and grain of 27 cowpea (*Vigna unguiculata* L. Walp.) genotypes: Implications for human nutrition and health", *Food and Nutrition Sciences*, Vol. 3, pp. 377-386, http://file.scirp.org/pdf/FNS20120300013_33040138.pdf.

Bell, H.A. et al. (2001), "Effect of dietary cowpea trypsin inhibitor (CpTI) on the growth and development of the tomato moth *Lacanobia oleracea* (Lepidoptera: Noctuidae) and on the success of the gregarious ectoparasitoid *Eulophus pennicornis* (Hymenoptera: Eulophidae)", *Pest Management Science,* Vol. 57, pp. 57-65, http://onlinelibrary.wiley.com/doi/10.1002/1526-4998(200101)57:1%3C57::AID-PS273%3E3.0.CO;2-4/epdf.

Boukar, O. et al. (2015), "Cowpea", in A.M. De Ron (ed.), *Grain Legumes*, Springer New York, pp. 219-250, https://link.springer.com/content/pdf/10.1007%2F978-1-4939-2797-5_7.pdf.

Boukar, O., F. Massawe and S. Muranaka (2011), "Evaluation of cowpea germplasm lines for protein and mineral concentrations in grains", *Plant Genetic Resources-Characterization and Utilization*, Vol. 9, pp. 515-522, https://www.cambridge.org/core/services/aop-cambridge-core/content/view/0F959F3C28CCAA3166AEFBA2F057FD7F/S1479262111000815a.pdf/evaluation_of_cowpea_germplasm_lines_for_protein_and_mineral_concentrations_in_grains.pdf.

Burridge, J. et al. (2016), "Legume shovelomics: High-throughput phenotyping of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* subsp. *unguiculata*) root architecture in the field", *Field Crops Research*, Vol. 192, pp. 21-32.

Carnovale, E., E. Lugaro and E. Marconi (1991), "Protein-quality and antinutritional factors in wild and cultivated species of *vigna* spp.", *Plant Foods for Human Nutrition*, Vol. 41, pp. 11-20, https://link.springer.com/content/pdf/10.1007%2FBF02196377.pdf.

Carvalho, A.F.U. et al. (2012), "Nutritional ranking of 30 Brazilian genotypes of cowpeas including determination of antioxidant capacity and vitamins", *Journal of Food Composition and Analysis,* Vol. 26, pp. 81-88.

Codex Alimentarius Commission (2003), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants -CAC/GL 45/2003*, Annexes II and III adopted in 2008, www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf.

CONAB (2018), *Observatório Agrícola – Acompanhamento da safra brasileira de grãos*, Vol. 5 -SAFRA 2017/18 – No. 6 -Sexto levantamento Março 2018, Companhia Nacional de Abastecimento, Brasília, http://www.conab.gov.br/OlalaCMS/uploads/arquivos/18_03_13_14_15_33_grao_marco_2018.pdf (accessed on 21 March 2018).

Devi, C.B., A. Kushwaha and A. Kumar (2015), "Sprouting characteristics and associated changes in nutritional composition of cowpea (*Vigna unguiculata*)", *Journal of Food Science and Technology*, Vol. 52, pp. 6821-6827. https://link.springer.com/content/pdf/10.1007%2Fs13197-015-1832-1.pdf.

Egounlety, M. and O.C. Aworh (2003), "Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms)", *Journal of Food Engineering,* Vol. 56, pp. 249-254, . https://www.sciencedirect.com/science/article/pii/S0260877402002625.

Elias, L.G., R. Bressani and R. Colindre (1964), "Nutritive value of 8 varieties of cowpea (*Vigna sinensis*)", *Journal of Food Science*, Vol. 29, pp. 118, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1964.tb01705.x/epdf.

Fang, J. et al. (2007), "Genetic diversity of cowpea *Vigna unguiculata* (L.) Walp. in four West African and USA breeding programs as determined by AFLP analysis", *Genetic Resources and Crop Evolution*, Vol. 54, pp. 1197-1209, https://link.springer.com/content/pdf/10.1007%2Fs10722-006-9101-9.pdf.

FAO (2004), *Cowpea: Post-Harvest Operations*, Food and Agriculture Organization of the United Nations, Rome, http://www.fao.org/3/a-au994e.pdf.

FAOSTAT (2019), "Production – Crops – Production quantity – Cow peas, dry – 2017", *FAO Statistics online database*, Food and Agriculture Organization of the United Nations, Statistics Division, Rome, http://www.fao.org/faostat/en/ (accessed on 10 July 2019).

Farinu, G.O. and G. Ingrao (1991), "Gross composition, amino-acid, phytic acid and trace-element contents of 13 cowpea cultivars and their nutritional significance", *Journal of the Science of Food and Agriculture,* Vol. 55, pp. 401-410, http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740550308/epdf.

Freire Filho, F.R. et al. (2017), "A cultura: Aspectos sócio-econômicos", in J.C. Vale, C. Bertini and A. Borém, *Feijão-caupi: do plantio à colheita*, Editora UFV, Viçosa, pp. 9-34.

Gerrano, A.S., W.S. Jansen van Rensburg and P.O. Adebola (2017a), "Preliminary evaluation of seed and germination traits in cowpea *Vigna unguiculata* (L.) Walp. genotypes", *South African Journal of Plant and Soil,* Vol. 34(5), pp. 399-402, https://doi.org/10.1080/02571862.2017.1317849.

Gerrano, A.S., W.S. Jansen van Rensburg and P.O. Adebola (2017b), "Nutritional composition of immature pods in selected cowpea *Vigna unguiculata* (L.) Walp. genotypes in South Africa", *Australian Journal of Crop Science*, Vol. 11 (02), pp. 134-141, https://doi.org/10.21475/ajcs.17.11.02.p72.

Gerrano, A.S. et al. (2015a), "Genetic variability and heritability estimates of nutritional composition in the leaves of selected cowpea genotypes *Vigna unguiculata* (L.) Walp.", *HortScience*, Vol. 50(10), pp. 1435-1440.

Gerrano, A.S. et al. (2015b), "Genetic variability in cowpea *Vigna unguiculata* (L.) Walp. genotypes", *South African Journal of Soil and Plant*, Vol. 32(3), pp. 165-174.

Goncalves, A. et al. (2016), "Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints", *Journal of the Science of Food and Agriculture,* Vol. 96, pp. 2941-2951.

Gwathmey, C.O., A.E. Hall and M.A. Madore (1992), "Adaptive attributes of cowpea genotypes with delayed monocarpic leaf senescence", *Crop Science,* Vol. 32, pp. 765-772.

Heuzé, V. and G. Tran (2015), "Cowpea (*Vigna unguiculata)* seeds", Feedipedia: A Programme by INRA, CIRAD, AFZ and FAO, http://www.feedipedia.org/node/232, (accessed on 2 June 2017).

Heuzé, V. et al. (2015), "Cowpea (*Vigna unguiculata*) forage", Feedipedia: A Programme by INRA, CIRAD, AFZ and FAO, http://www.feedipedia.org/node/233, (accessed 2 June 2017).

Hollinger, F. and J.M. Staatz (eds.) (2015), *Agricultural Growth in West Africa. Market and Policy Drivers*, Food and Agriculture Organization of the United Nations (FAO) and African Development Bank, Rome.

Hussain, M.A. and A.Y. Basahy (1998), "Nutrient-composition and amino acid pattern of cowpea (*Vigna unguiculata* (L.) Walp, Fabaceae) grown in the Gizan area of Saudi Arabia", *International Journal of Food Sciences and Nutrition*, Vol. 49, pp. 117-124, http://www.tandfonline.com/doi/pdf/10.3109/09637489809089391.

Iqbal, A. et al. (2006), "Nutritional quality of important food legumes", *Food Chemistry*, Vol. 97, pp. 331-335.

Kachare, D.P., J.K. Chavan and S.S. Kadam (1988), "Nutritional quality of some improved cultivars of cowpea", *Plant Foods for Human Nutrition*, Vol. 38, pp. 155-162, https://link.springer.com/content/pdf/10.1007%2FBF01091720.pdf.

Khan, A.R. et al. (2007), "Dietary fiber profile of food legumes", *Sarhad Journal of Agriculture*, Vol. 23, pp. 763-766, https://www.aup.edu.pk/sj_pdf/DIETARY%20FIBER%20PROFILE%20OF%20FOOD%20LEGUMES.pdf.

Khattab, R.Y., S.D. Arntfield and C.M. Nyachoti (2009), "Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation", *Lwt-Food Science and Technology*, Vol. 42, pp. 1107-1112.

Kochhar, N., A.F. Walker and D.J. Pike (1988), "Effect of variety on protein-content, amino-acid composition and trypsin-inhibitor activity of cowpeas", *Food Chemistry*, Vol. 29, pp. 65-78.

Kristjanson, P. et al. (2005), "Farmers' perceptions of benefits and factors affecting the adoption of improved dual-purpose cowpea in the dry savannas of Nigeria", *Agricultural Economics*, Vol. 32, pp. 195-210, https://cgspace.cgiar.org/bitstream/handle/10568/738/Farmers%27%20perceptions.pdf?sequence=2&isAllowed=y.

Kristjanson, P. et al. (2001), "Genetically improved dual-purpose cowpea: Assessment of adoption and impact in the dry savanna region of West Africa", *ILRI Impact Assessment Series 9*, International Livestock Research Institute, Nairobi, Kenya.

Langyintuo, A.S. et al. (2003), "Cowpea supply and demand in West and Central Africa", *Field Crops Research*, Vol. 82, pp. 215-231, http://www.tropicalsoybean.com/sites/default/files/Cowpea%20Supply%20And%20Demand%20In%20West%20And%20Central%20Africa_Langyintuo,%20et%20al._2003.pdf.

Liener, I.E. and E.G. Hill (1953), "The effect of heat treatment on the nutritive value and haemagglutinating activity of soybean oil meal", *Journal of Nutrition*, Vol. 49, pp. 609-619.

Machuka, J.S. et al. (2000), "The African yam bean seed lectin affects the development of the cowpea weevil but does not affect the development of larvae of the legume pod borer", *Phytochemistry*, Vol. 53, pp. 667-674, http://www.sciencedirect.com/science/article/pii/S0031942299005749/pdfft?md5=875b3826b8a7aa1c4466b42cd94711fc&pid=1-s2.0-S0031942299005749-main.pdf.

Madode, Y.E. et al. (2013), "Enhancing the digestibility of cowpea (*Vigna unguiculata*) by traditional processing and fermentation", *Lwt-Food Science and Technology*, Vol. 54, pp. 186-193.

Madode, Y.E. et al. (2011), "Preparation, consumption, and nutritional composition of West African cowpea dishes", *Ecology of Food and Nutrition,* Vol. 50, pp. 115-136, http://www.tandfonline.com/doi/pdf/10.1080/03670244.2011.552371?needAccess=true.

Maia, F.M.M. et al. (2000), "Proximate composition, amino acid content and haemagglutinating and trypsin-inhibiting activities of some Brazilian *Vigna unguiculata* (L) Walp cultivars", *Journal of the Science of Food and Agriculture*, Vol. 80, pp. 453-458.

Mamiro, P.S. et al. (2011), "Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania", *African Journal of Food, Agriculture, Nutrition and Development*, Vol. 11, pp. 4490-4506, https://www.ajol.info/index.php/ajfand/article/view/65876.

Marconi, E., N.Q. Ng and E. Carnovale (1993), "Protease inhibitors and lectins in cowpea", *Food Chemistry*, Vol. 47, pp. 37-40.

Marconi, E., S. Ruggeri and E. Carnovale (1997), "Chemical evaluation of wild under-exploited *Vigna* spp. seeds", *Food Chemistry*, Vol. 59, pp. 203-212.

Muranaka, S. et al. (2016), "Genetic diversity of physical, nutritional and functional properties of cowpea grain and relationships among the traits", *Plant Genetic Resources,* Vol. 14, pp. 67-76, https://www.cambridge.org/core/services/aop-cambridge-core/content/view/2D2223ABE62156CF3D9C115B9A2CCFF5/S147926211500009Xa.pdf/genetic_diversity_of_physical_nutritional_and_functional_properties_of_cowpea_grain_and_relationships_among_the_traits.pdf.

Murdock, L.L. et al. (2003), "Preservation of cowpea grain in sub-Saharan Africa-Bean/Cowpea CRSP contributions", *Field Crops Research*, Vol. 82, pp. 169-178.

Nassourou, M.A. et al. (2016), "Genetics of seed flavonoid content and antioxidant activity in cowpea (*Vigna unguiculata* L. Walp.)", *Crop Journal,* Vol. 4, pp. 391-397, http://www.sciencedirect.com/science/article/pii/S2214514116300551/pdfft?md5=0e4de6d2fd915401d1836903be1da8b4&pid=1-s2.0-S2214514116300551-main.pdf.

Nielsen, S.S., W.E. Brandt and B.B. Singh (1993), "Genetic-variability for nutritional composition and cooking time of improved cowpea lines", *Crop Science*, Vol. 33, pp. 469-472.

Nnanna, I.A. and R.D. Phillips (1989), "Amino-acid composition protein-quality and water-soluble vitamin content of germinated cowpeas (*Vigna unguiculata*)", *Plant Foods for Human Nutrition*, Vol. 39, pp. 187-200, https://link.springer.com/content/pdf/10.1007%2FBF01091899.pdf.

OECD (2015), "Consensus Document of the Biology of Cowpea (*Vigna unguiculata* (L.) Walp.), *Series on Harmonisation of Regulatory Oversight in Biotechnology No. 60*, Environment Directorate, OECD, Paris, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2015)48&doclanguage=en.

Oluwatosin, O.B. (1998), "Genetic and environmental variability in starch, fatty acids and mineral nutrients composition in cowpea (*Vigna unguiculata* (L) Walp)", *Journal of the Science of Food and Agriculture*, Vol. 78, pp. 1-11, http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0010(199809)78:1%3C1::AID-JSFA47%3E3.0.CO;2-H/epdf.

Onigbinde, A.O. and I.O. Akinyele (1983), "Oligosaccharide content of 20 varieties of cowpeas in Nigeria", *Journal of Food Science*, Vol. 48, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1983.tb09203.x/epdf.

Onyenekwe, P.C., G.C. Njoku and D.A. Ameh (2000), "Effect of cowpea (*Vigna unguiculata)* processing methods on flatus causing oligosaccharides", *Nutrition Research,* Vol. 20, pp. 349-358, http://www.sciencedirect.com/science/article/pii/S0271531700001287/pdf?md5=b3c17c08446ca7aaeeba3dce57f1274a&pid=1-s2.0-S0271531700001287-main.pdf.

Pasquet, R.S. (2000), "Allozyme diversity of cultivated cowpea *Vigna unguiculata* (L.) Walp", *Theoretical and Applied Genetics*, Vol. 101, pp. 211-219, https://link.springer.com/content/pdf/10.1007%2Fs001220051471.pdf.

Pasquet, R.S. (1999), "Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation", *Theoretical and Applied Genetics,* Vol. 98, pp. 1104-1119, https://link.springer.com/content/pdf/10.1007%2Fs001220051174.pdf.

Phillips, R.D. (2012), "Cowpea Processing and Products", in *Dry Beans and Pulses Production, Processing and Nutrition*, Blackwell Publishing Ltd., pp. 235-259, http://onlinelibrary.wiley.com/doi/10.1002/9781118448298.ch10/pdf.

Phillips, R.D. and B.W. Abbey (1989), "Composition and flatulence-producing potential of commonly eaten Nigerian and American legumes", *Food Chemistry,* Vol. 33, pp. 271-280, http://www.sciencedirect.com/science/article/pii/030881468990037X/pdf?md5=576f6a2528b4bdc3f100bc54b963ac73&pid=1-s2.0-030881468990037X-main.pdf.

Phillips, R.D. and K.H. McWatters (1991), "Contribution of cowpeas to nutrition and health", *Food Technology,* Vol. 45, pp. 127-130, http://agris.fao.org/agris-search/search.do?recordID=US9143047.

Preet, K. and D. Punia (2000), "Proximate composition, phytic acid, polyphenols and digestibility (*in vitro*) of four brown cowpea varieties", *International Journal of Food Sciences and Nutrition*, Vol. 51, pp. 189-193, http://www.tandfonline.com/doi/pdf/10.1080/09637480050029692?needAccess=true.

Prinyawiwatkul, W. et al. (1997), "Functional characteristics of cowpea (*Vigna unguiculata*) flour and starch as affected by soaking, boiling, and fungal fermentation before milling", *Food Chemistry,* Vol. 58, pp. 361-372, http://www.sciencedirect.com/science/article/pii/S0308814696002592/pdf?md5=072d9a708b842e2276c8b576a49b544c&pid=1-s2.0-S0308814696002592-main.pdf.

Prinyawiwatkul, W. et al. (1996), "Cowpea flour: A potential ingredient in food products", *Critical Reviews in Food Science and Nutrition,* Vol. 36, pp. 413-436, http://www.tandfonline.com/doi/pdf/10.1080/10408399609527734?needAccess=true.

Rao, T.R. et al. (2000), "Isolation and characterization of allergens from the seeds of *Vigna sinensis*", *Asian Pacific Journal of Allergy and Immunology*, Vol. 18, pp. 9-14, https://www.researchgate.net/publication/10935019_Isolation_and_characterization_of_allergens_from_the_seeds_of_Vigna_sinensis.

Reddy, N.R., S.K. Sathe and D.K Salunkhe (1982), "Phytates in legumes and cereals", *Advances in Food Research*, Vol. 28, pp. 1-92.

Rivas-Vega, M.E. et al. (2006), "Nutritional value of cowpea (*Vigna unguiculata* L. Walp) meals as ingredients in diets for Pacific white shrimp (*Litopenaeus vannamei* Boone)", *Food Chemistry*, Vol. 97, pp. 41-49.

Rocha, M.M. (2015), "Resultados das ações de melhoramento de feijão-caupi nos projetos HarvestPlus e BioFort", in *Reunião de Biofortificação no Brasil, 5., São Paulo. Apresentações*. Embrapa Agroindústria de Alimentos, Rio de Janeiro, https://docplayer.com.br/11883483-Data-v-reuniao-de-biofortificacao-no-brasil-13-a-15-de-outubro-de-2015-sao-paulo-sp.html.

Rocha, M.M., K.J. Damasceno-Silva and Ja.N. Menezes-Júnior (2017), "Cultivares", *Feijão-caupi: do plantio à colheita*, Editora UFV, Viçosa, pp. 113-142.

Samireddypalle, A. et al. (2017), "Cowpea and groundnut haulms fodder trading and its lessons for multidimensional cowpea improvement for mixed crop livestock systems in West Africa", *Frontiers in Plant Science*, Vol. 8, p. 9, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5282470/pdf/fpls-08-00030.pdf.

Sarr, P.S., S. Fujimoto and T. Yamakawa (2015), "Nodulation, nitrogen fixation and growth of rhizobia-inoculated cowpea (*Vigna unguiculata* L. Walp) in relation with external nitrogen and light intensity", *International Journal of Plant Biology and Research*, Vol. 3(1), p. 1025.

Siddhuraju, P. and K. Becker (2007), "The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts", *Food Chemistry*, Vol. 101, pp. 10-19, http://www.sciencedirect.com/science/article/pii/S0308814606000367/pdfft?md5=a57b722d06c56295e11009e51531a2a8&pid=1-s2.0-S0308814606000367-main.pdf.

Silveira, Ja.G. et al. (2001), "Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants", *Environmental and Experimental Botany*, Vol. 46, pp. 171-179.

Singh, B.B. (2014), "Cowpea: The food legume of the 21st century", in *Cowpea: The Food Legume of the 21st Century*, Crop Science Society of America ed., ACSESS Publications.

Singh, B.B. and S.A. Tarawali (1997), *Cowpea and Its Improvement: Key to Sustainable Mixed Crop/Livestock Farming Systems in West Africa*, http://oar.icrisat.org/8758/1/Cowpea%20as%20a%20key%20factor%20for%20a%20new%20approach.pdf.

Singh, B.B. et al. (2003), "Improving the production and utilization of cowpea as food and fodder", *Field Crops Research*, Vol. 84, pp. 169-177, https://www.sciencedirect.com/science/article/pii/S0378429003001485?via%3Dihub.

Somiari, R.I. and E. Balogh (1993), "Effect of soaking, cooking and crude alpha-galactosidase treatment on the oligosaccharide content of cowpea flours", *Journal of the Science of Food and Agriculture*, Vol. 61, pp. 339-343, http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740610308/epdf.

Sreerama, Y.N., V.B. Sashikala and V.M. Pratape (2012), "Phenolic compounds in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia and hypertension", *Food Chemistry*, Vol. 133, pp. 156-162, https://www.researchgate.net/publication/257164100.

Thangadurai, D. (2005), "Chemical composition and nutritional potential of *Vigna unguiculata* ssp *cylindrica* (Fabaceae)", *Journal of Food Biochemistry*, Vol. 29, pp. 88-98, http://onlinelibrary.wiley.com/doi/10.1111/j.1745-4514.2005.00014.x/pdf.

Thompson, L.U. and J.H. Yoon (1984), "Starch digestibility as affected by polyphenols and phytic acid", *Journal of Food Science*, Vol. 49, pp. 1228-1229, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1984.tb10443.x/pdf.

USDA-ARS (2016), "Nutritional data for Cowpeas, catjang, mature seeds, raw NDB No. 16060", in *National Nutrient Database for Stand Reference, Release 28 (revised) – Version 2016*, http://www.ars.usda.gov/ba/bhnrc/ndl.

Uzogara, S.G. and Z.M. Ofuya (1992), "Processing and utilization of cowpeas in developing-countries – A review", *Journal of Food Processing and Preservation*, Vol. 16, pp. 105-147.

Uzogara, S.G., I.D. Morton and J.W. Daniel (1991), "Thiamin, riboflavin and niacin retention in cooked cowpeas as affected by kanwa treatment", *Journal of Food Science*, Vol. 56, pp. 592-593, http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1991.tb05335.x/epdf.

Uzogara, S.G., I.D. Morton and J.W. Daniel (1988), "Quality changes and mineral-content of cowpea (*Vigna unguiculata*) seeds processed with kanwa alkaline salt", *Food Chemistry*, Vol. 30, pp. 1-18, http://www.sciencedirect.com/science/article/pii/0308814688900192/pdf?md5=b6fa8e2f4e9bcfb6c448e679e7dc4c01&pid=1-s2.0-0308814688900192-main.pdf.

Vasconcelos, I.M. et al. (2010), "Protein fractions, amino acid composition and antinutritional constituents of high-yielding cowpea cultivars", *Journal of Food Composition and Analysis*, Vol. 23, pp. 54-60.

Verma, A.K. et al. (2013), "A comprehensive review of legume allergy", *Clinical Reviews in Allergy & Immunology*, Vol. 45, pp. 30-46, https://link.springer.com/content/pdf/10.1007%2Fs12016-012-8310-6.pdf.

Xiong, H. et al. (2016), "Genetic diversity and population structure of cowpea (*Vigna unguiculata* L. Walp)", *Plos One*, Vol. 11, e0160941, http://dx.doi.org/10.1371%2Fjournal.pone.0160941.

Xiong, S.L., W.L. Yao and A.L. Li (2013), "Antioxidant properties of peptide from cowpea seed", *International Journal of Food Properties*, Vol. 16, pp. 1245-1256, http://www.tandfonline.com/doi/pdf/10.1080/10942912.2011.582976?needAccess=true.

Xu, B.J. and S.K.C. Chang (2012), "Comparative study on antiproliferation properties and cellular antioxidant activities of commonly consumed food legumes against nine human cancer cell lines", *Food Chemistry*, Vol. 134, pp. 1287-1296.

Xu, D.P. et al. (1996), "Constitutive expression of a cowpea trypsin inhibitor gene, CpTi, in transgenic rice plants confers resistance to two major rice insect pests", *Molecular Breeding*, Vol. 2, pp. 167-173, https://link.springer.com/content/pdf/10.1007/BF00441431.pdf.

Yeung, H. et al. (2009), "Rapid screening methods to evaluate cowpea cooking characteristics", *Field Crops Research*, Vol. 112, pp. 245-252, http://www.sciencedirect.com/science/article/pii/S0378429009000872/pdfft?md5=1e27256efcb135f0c99a90ff9a13eec3&pid=1-s2.0-S0378429009000872-main.pdf.

Yewande, B.A. and A.O. Thomas (2015), "Effects of processing methods on nutritive values of ekuru from two cultivars of beans (*Vigna unguiculata* and *Vigna angustifoliata*)", *African Journal of Biotechnology*, Vol. 14, pp. 1790-1795, https://www.ajol.info/index.php/ajb/article/viewFile/119806/109264.

Zhang, J.S. et al. (2009), "Biological properties and characterization of lectin from red kidney bean (*Phaseolus vulgaris*)", *Food Reviews International*, Vol. 25, pp. 12-27, http://www.tandfonline.com/doi/pdf/10.1080/87559120802458115?needAccess=true.

Zhu, K.Y. et al. (1994), "Cowpea trypsin-inhibitor and resistance to cowpea weevil (Coleoptera, Bruchidae) in cowpea variety Tvu-2027", *Environmental Entomology,* Vol 23, pp. 987-991, https://academic.oup.com/ee/article-abstract/23/4/987/411970/Cowpea-Trypsin-Inhibitor-and-Resistance-to-Cowpea?redirectedFrom=PDF.

Chapter 4. Apple (*Malus* × *domestica*)

*This chapter deals with the composition of apple fruit (*Malus × domestica*). It contains elements that can be used in a comparative approach as part of a safety assessment of foods and feeds derived from new varieties. Background is given on apple production worldwide, main cultivars, apple uses and processing for human consumption, and feed use of by-products. Appropriate varietal comparators and characteristics screened by breeders are presented. Nutrients in apple fruits, juice and pomace, chemical composition during storage, as well as main allergens, toxicants and other metabolites are then detailed. The final sections suggest key products and constituents for analysis of new apple cultivars for food use and for feed use.*

This chapter was prepared by the OECD Working Group for the Safety of Novel Foods and Feeds, with Canada and Germany as the lead countries. It was initially issued in July 2019.

Background

Introduction

The apple *Malus* x *domestica* Borkh. is a widely distributed, temperate zone fruit crop (Figure 4.1) that has been cultivated for millennia.

Figure 4.1. Apple fruit and seed

Source: Maks Narodenko/Shutterstock.com.

The apple is a member of the *Rosaceae* family, *Amygdaloideae* subfamily, *Maleae* tribe, *Malinae* subtribe and *Malus* genus (Potter et al., 2007). The *Rosaceae* family is distributed worldwide and includes a range of economically important fruit crop species such as the pome fruit species (for example: apple, pear and quince), the stone fruit species (for example: sweet and sour cherry, plum, prune, apricot and peach) and the berry fruit species (for example: strawberry, blackberry and raspberry). The genus *Malus* consists of six sections with 27 primary species (Forsline et al., 2003). Most of the species belonging to the *Malus* genus are cross-compatible, hence natural and artificial hybridisation techniques have resulted in numerous interspecific hybrids and secondary species.

The domestication of apple took place around 4 000 to 10 000 years ago in the Tien Shan Mountains of Central Asia. The origin of the *Malus* genus is said to be southeast of the People's Republic of China and the species of the genus were distributed from there in all directions.

The presumed main ancestor of the cultivated apple is *Malus sieversii* (Ledeb.) M. Roem., which grew wild in the forests of Central Asia from Tajikistan to Western People's Republic of China (Luby, 2003; Hancock et al., 2008). Based on genomic studies performed in the last century, other species belonging to the *Malus* genus have contributed to the genome of the cultivated apple. These were mainly *Malus sylvestris* (L.) Mill. distributed from Western Asia to Europe, and *Malus orientalis* Uglitzk which grew in the forests of the Caucasus region (Hancock et al., 2008).

The cultivated apple belongs to the genus *Malus*. The binomial denomination of the cultivated apple *Malus* × *domestica* Borkh. reflects its interspecific origin and replaces the former name *M. pumila* Mill. as well as other names like *Pyrus malus* L. *Malus* × *domestica* is allopolyploid (2n = 2x = 34), gametophytic incompatible, mostly self-unfruitful and requiring pollination. Most of the cultivars are diploid; however, a number of tri- and tetraploid cultivars also exist.

Further description on the apple taxonomy, geographic distribution, centres of origin and diversity, reproductive biology, genetics, hybridisation and introgression, interaction with other organisms (ecology), common pests and pathogens, and biotechnological developments can be found in the OECD Consensus Document on the Biology of Apple (OECD, 2019).

Production of apples

World production

Among fruit crops, apple is only exceeded in global production by total citrus fruits and banana and is comparable to grapes. In 2016, the world apple production was around 85 million tonnes (FAOSTAT, 2019). As shown in Table 4.1, the main producers are the People's Republic of China and the United States, followed by Poland, Turkey, India, the Islamic Republic of Iran and Italy.

Table 4.1. Production, exports and imports of apples in 2016

Kilotonnes

Rank	Country	Production	Exports	Imports
1	China (People's Republic of)	40 393	1 322	67
2	United States	5 161	777	193
3	Poland	3 604	1 093	12
4	Turkey	2 926	140	1
5	India	2 521	13	247
6	Iran	2 470	56	1
7	Italy	2 456	1 049	63
8	Russia	1 844	14	677
9	France	1 820	573	177
10	Chile	1 743	765	2
11	Ukraine	1 099	13	42
12	Brazil	1 049	31	155
13	Uzbekistan	1 034	4	0
14	Germany	1 033	89	611
15	Argentina	968	91	3
16	South Africa	913	511	0
17	Democratic People's Republic of Korea	779	-	52
18	Japan	765	32	2
19	Egypt	755	1	230
20	Mexico	717	2	213
	World	**85 204**	**9 044**	**8 896**

Notes: The countries are listed in order of production.
Aggregate may include official, semi-official, estimated or calculated data.
Source: FAOSTAT (2019), "Production/Export/Import Crops–apple, Year 2016", http://www.fao.org/faostat/ (accessed 10 July 2019).

Cultivars

It is estimated that 40 cultivars account for the bulk of commercial production worldwide. Some cultivars have given rise to many mutants, which have been selected for growth habit, fruit colour (Figure 4.2), ripening time and other characteristics. Economically important mutants are especially known among the cultivars "Delicious", "Jonagold" and "Gala".

Figure 4.2. Fruit colour/shape diversity of some apple cultivars

Source: cynoclub/Shutterstock.com.

Regional differences in the relative importance of apple cultivars are evident and the choice of cultivars varies from country to country. For instance, southern Europe produces many "Golden Delicious" whereas "Elstar" and "Jonagold" are popular in northern Europe. Australia and New Zealand are major apple exporters based on "Gala", "Granny Smith" and "Braeburn". The leading cultivar in the People's Republic of China is "Fuji". In many regions of North America, "McIntosh" and "Delicious" are important cultivars; however, many others such as "Fuji", "Pink Lady", "Gala", "Braeburn" and "Jonagold" are also popular.

Uses and processing

Apples for human consumption

The apple industry encompasses growers, packers, shippers and processors. Apples destined for the fresh market (primary market) are shipped from the orchard to a packer. The packers then distribute the product to retailers and exporters. Improvements in shipping and techniques for delaying fruit ripening allow many apple cultivars to be offered all year round in many countries. Apples may also be sold directly to consumers at the orchard or at farmers markets.

Apples are mainly cultivated for the fresh fruit market with the rest being processed into apple juice, apple cider, applesauce, apple butter, cider vinegar, dried apples and canned apples. Due to their proposed use apple cultivars can be referred to as eating apples, cider apples, and cooking apples.

Fresh apples (eating apples)

The term "fresh apples" as used in this document refers to "eating apples" cultivars, which are consumed in their natural form ("out of hand"). Fresh apples vary in flavour, ranging from sour to sweet, and texture, from dry and mealy to crisp and juicy. Fresh apples are sold in categories, classes or grades, which reflect their perceived quality. The quality criteria are specific to individual cultivars. Consumers often find products such as pre-washed, pre-sliced or bagged apple slices appealing and convenient (AAFC, 2010).

Apple juice

Apple juice is the liquid extracted from ripe apples. Generally, the apples are ground, pressed and filtered to remove skins and pulp. The juice may or may not be pasteurised and can be sold in unconcentrated or concentrated forms. Apple juice is widely used in fruit juice blends. The apple solids remaining after juice extraction can be used for the production of pectin (a carbohydrate used as a gelling agent in the production of jams and jellies) and as animal feed.

Apple cider and cider vinegar

Cider is the fermented juice of the apple. It can be unfiltered, unsweetened, alcoholic or non-alcoholic. Non-alcoholic cider is unfiltered, usually unpasteurised juice from apples. In making alcoholic apple cider, the juice is inoculated with specific yeast strains that ferment sugar in the juice into ethanol and produce flavours characteristic to the apple cider. Apple cider can be further processed by inoculating it with bacterial cultures that will oxidise ethanol to acetic acid to produce apple cider vinegar.

Applesauce

Applesauce is a purée made of apples that are cleaned, sorted, peeled and cooked with or without sugar. It can include a variety of spices such as cinnamon and allspice. The cooked apples can be passed through a screen to remove any undesirables and for sizing. Applesauce used as baby food goes through a screen to create a fine texture. The applesauce can then be either canned or bottled.

Apple butter

Apple butter is a highly concentrated form of applesauce produced by slow cooking apples with apple cider or water to a point where the sugar in the apple caramelises, turning the applesauce to a deep brown colour. The sugar concentration in this product allows it to have a longer shelf life than applesauce.

Apple pectin

Apples and apple pomace contain pectin at 1%-1.5% and 15%-20% respectively. Pectin is a mixture of complex polysaccharides and is used as a gelling agent, thickener, stabiliser and emulsifier in food products like jams and fruit jellies. Pectin is obtained via hot acidified water extraction and further processing of apple pomace.

Other products

Apples are also used to produce dehydrated apple slices, fruit leather, apple-filled snack bars, apple jelly and appleseed oil. Leftover by-products of apple processing are used as

food ingredients in, for example, baked goods, for extraction of ester flavours and other components (e.g. essences for use in food and non-food products), or for animal feed.

Apples for animal feed

Leftover by-products of apple processing, such as pomace containing peel, seeds, core and stem tissues may be fed to livestock (NRC, 1983). In large scale apple juice processing industries, two types of waste are generated. The first is the unprocessed discarded apple fruit (culls), and the second is the pomace (pulp, peels, seeds, and cores) which is left after juice extraction. About 250 to 350 kg of wet pomace can be obtained from a tonne of apples processed for juice (Dairy Farm Guide, 2015). Apple pomace from juice extraction often contains rice hulls or husks that are added by commercial juice manufacturers to aid filtration and recovery of the juice. The residual material from canning, drying and freezing of apples is also known as pomace and consists of the peels, cores and culled apples or pieces.

Apple pomace is an acceptable feedstuff, given the high level of carbohydrates, pectin and fibre. However, due to the high moisture content of fresh apple pomace, it spoils rapidly and therefore must be used quickly or be preserved by drying or ensiling. Drying to about 10% moisture content prevents spoilage and spontaneous combustion (Dairy Farm Guide, 2015). Drying often takes place in direct-fired, rotary-drum driers after which the pomace is ground in hammer mills (NRC, 1983). Apple pomace ensiled alone results in a very high moisture product leading to loss of nutrients by drainage; therefore, it is often mixed with alfalfa or corn prior to ensiling. Cull apples may also be preserved as silage by mixing them with about 20% alfalfa hay (NRC, 1983).

Pectin pulp, the residue remaining after extraction of pectin from pomace (Shalini and Gupta, 2010) may be used fresh, dried or ensiled as feed for livestock (Dairy Farm Guide, 2015).

Wet, dried, or ensiled apple pomace and pectin pulp are used as energy feeds typically for ruminant animals. Apple pomace is palatable to cattle and sheep, while pectin pulp is less palatable to dairy cows. The addition of molasses was suggested to increase the palatability of pectin pulp for dairy cows (Smock and Neubert, 1950). Tiwari, Narang and Dubey (2008) showed that the inclusion of apple pomace at 12% of the ration had no adverse effects on milk yield or milk constituents of crossbred dairy cows. Rust and Buskirk (2008) indicated that about 18-27 kg of apple pomace can be fed to beef cows daily. Smith (1950) reported that cattle can be fed up to 16 kg of apple pomace silage daily, mature pigs up to 1.8 kg and sheep up to 1 kg daily. Givens and Barber (1987) reported feeding sheep with apple pomace at 579-760 g dry matter per day, in addition to basal hay to meet the animal's metabolisable energy requirements for maintenance. Inclusion of apple pomace to up to 20% of swine rations was found to have no significant effects on daily weight gain, feed efficiency and carcass characteristics (Bowden and Berry, 1958). Matoo et al. (2001), however, reported better performance of broiler chicken fed apple pomace diets supplemented with enzymes, due to the high fibre content of apple pomace.

Solid-state fermentation processes of apple pomace using microorganisms (e.g. *Saccharomyces cerevisiae*, *Aspergillus niger*, *Phanerochaete chrysosporium*, etc.) to obtain value-added products such as higher soluble protein-enriched pomace for livestock, have also been investigated (Joshi and Attri, 2006; Ajila et al., 2015). Ajila et al. (2015) observed that the addition of 5% weight by weight (w/w) protein-enriched apple pomace increased the protein content of pig diets by 36%. This increase in protein content

resulted in corresponding improvement in weight gain and performance when compared to control diets. The high organic acids, carbohydrates and soluble fibres (pectin) in apple pomace make it a good substrate to produce a value-added product such as fermented apple pomace (high in protein) for livestock feed, as well as for the production of pectinases, ethanol and citric acid.

Appropriate comparators for testing new cultivars

This document suggests parameters that apple breeders should measure when developing new cultivars.

The data obtained in the analysis of a new apple cultivar should ideally be compared to those obtained from an appropriate near-isogenic non-modified variety, grown and harvested under the same conditions.[1,2] The comparison can also be made between values obtained from new varieties and data available in the literature or chemical analytical data generated from commercial apple cultivars.

Components to be analysed include key nutrients, anti-nutrients, toxicants and allergens. Key nutrients are those which have a substantial impact on the overall diet of humans (food) and animals (feed). These may be major (fats, proteins, and structural and non-structural carbohydrates) or minor constituents (vitamins and minerals). Similarly, the levels of known metabolites and allergens should be considered. Key toxicants are those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health. Standardised analytical methods and appropriate types of material should be used, adequately adapted to each product and by-product. The key components analysed are used as indicators of whether unintended effects of the genetic modification influencing plant metabolism have occurred or not.

Breeding characteristics screened by developers

Prior to 1900, apple improvement was based on finding chance seedlings with good fruit quality. Scientific breeding work began in the early 20th century as leading horticultural/agricultural experiment stations and institutes were just being established at that time worldwide. Apple breeding started to be based on controlled crosses combining the best characteristics of cultivars chosen as mother or pollen parents.

Apple cultivars have been developed by selection of desired fruit phenotypes (appearance, uniformity, size, firmness, juiciness, crispiness, taste), as well as for agronomic characteristics (yield, stability of yield, tree growth, pruning effort), resistance to diseases and tolerance to abiotic stress. Apple cultivars are generally propagated vegetatively on rootstocks. The rootstock can impact characteristics that are important for commercial production, for example, vigour of vegetative growth and fruit size. The choice of the rootstock is also important for the purpose of the cultivar, e.g. commercial fruit production or landscape growing.

The traits of major interest for modern breeding programmes include better quality or increased marketability of the fruit, improved storability, reduced production costs, as well as improved disease and pest resistance. Molecular techniques have been developed to facilitate and accelerate apple breeding. Molecular markers have been developed for several disease resistance genes, as well as for some quality traits (Costa et al., 2005; Peil et al., 2011). Marker-assisted seedling selection is applied already by some breeders (Baumgartner et al., 2015). The genome of the apple has been published (Velasco et al.,

2010), which will aid in developing more markers. Genomic selection, a statistical approach for estimating breeding potential, has been demonstrated as a tool that could be useful for selecting fruit quality traits (Kumar et al., 2012). Furthermore, there are continuing efforts to address some of the breeding bottlenecks using innovative breeding technologies, like cisgenesis and fast-track breeding approaches (Flachowsky et al., 2007; CFIA, 2014).

Nutrients

Constituents of apple fruits

The composition of apples varies greatly among cultivars. Environmental factors such as climate, soil condition, site of cultivation and storage conditions after harvest have an influence on the overall composition of the fruit. Sugars, organic acids and polyphenol compounds are responsible for the apple's main sensory attributes of sweetness, acidity and bitterness. The ripening process alters apple composition, which affects the consistency as well as the taste.

Proximate nutrient content

Proximate composition (including moisture, protein, fat, ash, crude fibre and calculated carbohydrates) of fresh apples with peel is given in Table 4.2. The moisture content of apples generally varies between 82.5%-86.2% but for some cultivars, values of as high as 88.1% have been reported (Rop et al., 2011). Carbohydrates make up the major fraction (greater than 90%) of apple dry matter. Protein ranges from 1.42% to 4.35%, total fat ranges between 0.28% and 3.62% and ash ranges between 1.32% and 2.08% on a dry matter basis.

Carbohydrates

Carbohydrate content of apples can be divided into soluble sugars, fibre (non-starch polysaccharides) and starch. The most abundant compounds within the carbohydrates are the soluble sugars at up to 83%. Fructose is the major sugar among them. In general, the concentration of sugars increases during ripening (Zhang, Li and Cheng, 2010).

Apples are a source of dietary fibre. The amount of dietary fibre in apple skin is about 30% higher than in the pulp (Gorinstein et al., 2001). The major non-starch polysaccharides accounting for most of the dietary fibre in apples are pectins. Values of 8 to 24 g pectin/100 g dry matter have been reported (Rop et al., 2011). Pectins are major components of the cell wall and are associated with the firmness of the fruit. As pectins undergo significant structural variations during ripening the apple texture changes and usually softens (Mangas et al., 1992).

Minerals

Potassium and phosphorus are the main minerals found in apples (Table 4.3) Potassium ranges between 676.91 and 843.96 mg/100 g dry matter. Phosphorus ranges from 57.97 to 120.83 mg/100 g dry matter.

Fatty acids

Linoleic acid and palmitic acid are the major fatty acids in apples (Table 4.4).

Table 4.2. Proximate and carbohydrate composition of apple fruit (% dry matter, edible portion)

Nutrient	USDA Database (2015)	German Nutrient Database (2014)	Danish Food Compo. Database (2019)	Public Health England (2015)	Swiss Food Compo. Database (2015)	China Food Compo. Database (2009)
			Mean value, g per 100 g fresh weight			
Moisture	85.56	82.47	84.9	86.20	85.00	85.90
			Mean value, g per 100 g dry matter[a]			
Protein[b]	1.80	1.93	2.0	4.35	2.00	1.42
Fat[b]	1.18	0.28	1.3	3.62	2.00	1.42
Ash	1.32	1.82	2.0			1.42
Carbohydrate total[c]	95.63		94.7			95.74
Carbohydrate available[d]		81.63	80.1	83.04[e]	77.99[e]	
Fibre, total dietary	16.62	11.43	14.6	9.42	14.00	8.52
Sugars, total	71.95	58.65	72.2	83.04	77.33	
Sucrose	*14.35*	*14.48*	*20.5*	*19.27*		
Glucose (dextrose)	*16.83*	*11.54*	*11.7*	*15.22*		
Fructose	*40.86*	*32.63*	*40.1*	*48.55*		
Starch	0.35	3.41	0.00	0.00	0.67	

Notes: a. Mean values based on dry matter were calculated from a fresh weight basis (wet weight) using the mean moisture level reported from each source.
b. Specifications given for fat and protein reflect the wording of the original source. As no additional information about the analytical method used for determination is available, no further differentiation in respect of crude vs. true (protein/fat) is made.
c. Carbohydrates total calculated by difference = 100 - protein - fat - ash - moisture
d. Carbohydrates available = Carbohydrates total - dietary fibre
e. Carbohydrates available = total sugars + starch.

Sources: Sources use different terminology in regards to apple data. The terms "fresh" and "raw" are not clearly defined. While it is assumed that they are used to describe the same trait, the following information on the sources is given in order to facilitate comprehension**: USDA Database**, Release 28, September 2015, accessed online 7/2016. 09003: Apples, raw, with skin, based on analytical data for Red Delicious, Golden Delicious, Gala, Granny Smith and Fuji varieties; **German Nutrient Database**, version 3.02, 2014, accessed online 7/2016. F110100: Apples, raw with skin, edible portion; **Danish Food Composition Database**, version April 2019, FoodID 2, Apple, raw, all varieties; **Public Health England** - McCance and Widdowson Dataset 2015, accessed online 2/2016. Food Code 14-319: Apples, eating, raw, flesh and skin, UK grown and imported apples including Gala, Braeburn, Golden Delicious, Pink Lady, Cox and Granny Smith; **Swiss Food Composition Database**, Version 5.2, accessed online 7/2016. Food ID 378: Apples, fresh; **China Food Composition Database**, printed version 2009, Food ID 06-1-101: apple average.

Table 4.3. Mineral composition of apple fruit (per 100 g dry matter, edible portion)

Minerals	Unit	USDA Database (2015)	German Nutrient Database (2014)	Danish Food Compo. Database (2019)[a]	Public Health England (2015)	Swiss Food Compo. Database (2015)	China Food Compo. Database (2009)
Calcium, Ca	mg	42	28.44	27.4	36.23	33.33	28.37
Iron, Fe	mg	0.83	1.41	0.80	0.65	1.33	4.26
Magnesium, Mg	mg	35	28.44	29.7	28.98	26.66	28.37
Phosphorus, P	mg	76	62.57	63.0	57.97	59.99	85.11
Potassium, K	mg	742	676.91	781.5	724.60	799.92	843.96
Sodium, Na	mg	7	5.69	4.0	7.25	26.66	11.35
Zinc, Zn	mg	0.28	0.22	0.16	trace	0.67	1.35
Copper, Cu	mg	0.187	0.30	0.21	0.22		0.43
Manganese, Mn	mg	0.243	0.24	0.40	0.29		0.21
Selenium, Se	µg				trace		0.85
Fluoride, F	µg	22.9	51.19				
Iodide, I	µg		4.55	0.65	28.98	5.33	

Note: a. Mean values based on dry matter were calculated from a fresh weight basis (wet weight) using the mean moisture level reported from each source.

Sources: Data from different databases refer to: **USDA Database**, Release 28, September 2015, accessed online 7/2016. 09003: Apples, raw, with skin, based on analytical data for Red Delicious, Golden Delicious, Gala, Granny Smith and Fuji varieties; **German Nutrient Database**, version 3.02, 2014, accessed online 7/2016. F110100: Apples, raw with skin, edible portion; **Danish Food Composition Database**, version April 2019, FoodID 2, Apple, raw, all varieties; **Public Health England** - McCance and Widdowson Dataset 2015, accessed online 2/2016. Food Code 14-319: Apples, eating, raw, flesh and skin, UK grown and imported apples including Gala, Braeburn, Golden Delicious, Pink Lady, Cox and Granny Smith; **Swiss Food Composition Database**, Version 5.2, accessed online 7/2016. Food ID 378: Apples, fresh; **China Food Composition Database**, printed version 2009, Food ID 06-1-101: apple average.

Table 4.4. Fatty acid composition of apple fruit (mg per 100 g dry matter, edible portion)

Fatty Acids	USDA Database (2015)	German Nutrient Database (2014)	Danish Food Compo. Database (2019)[a]	Public Health England (2015)	Swiss Food Compo. Database (2015)
Fatty acids, total saturated	194	1 143	298	870	670
Palmitic - 16:0	*166*	*711*	*251*		
Stearic - 18:0	*21*	*216*	*46*		
Fatty acids, total monounsaturated	49	125	46	290	133
Palmitoleic - 16:1 undifferentiated	*0*	*17*			
Oleic - 18:1 undifferentiated	*49*	*102*	*46*		
Fatty acids, total polyunsaturated	353	1 519	867	1 449	667
Linoleic - 18:2 undifferentiated	*298*	*1 143*	*682*		
Linolenic - 18:3 undifferentiated	*62*	*250*	*185*		

Note: a. Mean values based on dry matter were calculated from a fresh weight basis (wet weight) using the mean moisture level reported from each source.

Sources: Data from different databases refer to: **USDA Database**, Release 28, September 2015, accessed online 7/2016. 09003: Apples, raw, with skin, based on analytical data for Red Delicious, Golden Delicious, Gala, Granny Smith and Fuji varieties; **German Nutrient Database**, version 3.02, 2014, accessed online 7/2016. F110100: Apples, raw with skin, edible portion; **Danish Food Composition Database**, version April 2019, FoodID 2, Apple, raw, all varieties; **Public Health England** - McCance and Widdowson Dataset 2015, accessed online 2/2016. Food Code 14-319: Apples, eating, raw, flesh and skin, UK grown and imported apples including Gala, Braeburn, Golden Delicious, Pink Lady, Cox and Granny Smith; **Swiss Food Composition Database**, Version 5.2, accessed online 7/2016. Food ID 378: Apples, fresh.

Amino acids

Aspartic acid is the most abundant amino acid in fresh apple fruits (Table 4.5).

Table 4.5. Amino acid composition of apple fruit (mg per 100 g dry matter, edible portion)

Amino acids	USDA Database (2015)	German Nutrient Database (2014)	Danish Food Compo. Database (2019)[a]	China Food Compo. Database (2009)
Tryptophan	7	11	20	50
Threonine	42	46	53	50
Isoleucine	42	57	60	64
Leucine	90	91	93	85
Lysine	83	85	86	71
Methionine	7	17	20	21
Cystine	7	6	7	57
Phenylalanine	42	51	46	78
Tyrosine	7	28	26	71
Valine	83	68	66	99
Arginine	42	46	40	43
Histidine	35	34	26	21
Alanine	76	85	73	64
Aspartic acid	485	575	517	319
Glutamic acid	173	142	146	142
Glycine	62	51	53	57
Proline	42	57	53	50
Serine	69	68	73	64

Note: a. Mean values based on dry matter were calculated from a fresh weight basis (wet weight) using the mean moisture level reported from each source.

Sources: Data from different databases refer to: **USDA Database**, Release 28, September 2015, accessed online 7/2016. 09003: Apples, raw, with skin, based on analytical data for Red Delicious, Golden Delicious, Gala, Granny Smith and Fuji varieties; **German Nutrient Database**, version 3.02, 2014, accessed online 7/2016. F110100: Apples, raw with skin, edible portion; **Danish Food Composition Database**, version April 2019, FoodID 2, Apple, raw, all varieties; **China Food Composition Database**, printed version 2009, Food ID 06-1-101: apple average.

Vitamins

The total vitamin C levels in fresh apples range between 31.9 and 69.44 mg/100 g dry matter. Vitamin C is sensitive to processing and degrades easily. Consequently, vitamin C intake via apples is highest in unprocessed fruits (Varming, Petersen and Toldam-Andersen, 2013). Apples do not contain significant amounts of fat-soluble vitamins like vitamin A, D and E. Vitamin composition of apple fruit is given in Table 4.6.

Table 4.6. Vitamin composition of apple fruit (per 100 g dry matter, edible portion)

Vitamins	Unit	USDA Database (2015)	German Nutrient Database (2014)	Danish Food Compo. Database (2019)	Public Health England (2015)	Swiss Food Compo. Database (2015)	China Food Compo. Database (2009)
Vitamin C, total ascorbic acid	mg	31.9	68.26	54.70	43.48	33.33	28.37
Thiamine (Vitamin B1)	mg	0.118	0.06	0.09	0.29	0.20	0.43
Riboflavin (B2)	mg	0.18	0.05	0.05	0.29	0.13	0.14
Niacin	mg	0.631	1.71	0.81	0.72	0.67	1.42
Pantothenic acid	mg	0.423	0.57	0.48		0.67	
Pyridoxin (B6)	mg	0.284	0.24	0.30	0.51	0.33	
Biotin	µg		28.44	6.60	7.97		
Folate, total	µg	21	28.40	59.6		86.66	
Vitamin A, RE	µg		28.44		14.49		21.28
Vitamin A, RAE	µg	21		13.77		13.33	
Carotene, beta	µg	187	164.96	165.6	101.44	133.32	141.84
Cryptoxanthin, beta	µg	76					
Lutein + zeaxanthin	µg	201					
Vitamin E (alpha-tocopherol)	mg	1.25	2.78	1.68	3.81[a]	0.65[a]	10.85
Vitamin K (phylloquinone)	µg	15.2	34.13	19.9	36.13	40.58	

Note: a. Total vitamin E calculated as α-Tocopherol Equivalents (α-TE).

Sources: Data from different databases refer to: **USDA Database**, Release 28, September 2015, accessed online 7/2016. 09003: Apples, raw, with skin, based on analytical data for Red Delicious, Golden Delicious, Gala, Granny Smith and Fuji varieties; **German Nutrient Database**, version 3.02, 2014, accessed online 7/2016. F110100: Apples, raw with skin, edible portion; **Danish Food Composition Database**, version April 2019, FoodID 2, Apple, raw, all varieties; **Public Health England** - McCance and Widdowson Dataset 2015, accessed online 2/2016. Food Code 14-319: Apples, eating, raw, flesh and skin, UK grown and imported apples including Gala, Braeburn, Golden Delicious, Pink Lady, Cox and Granny Smith; **Swiss Food Composition Database**, Version 5.2, accessed online 7/2016. Food ID 378: Apples, fresh; **China Food Composition Database**, printed version 2009, Food ID 06-1-101: apple average.

Constituents of products and by-products from apple processing

Nutrient composition of apple juice

Apple juice is produced by squeezing or crushing the apple fruit (see also previous Section 'Uses and processing'). Subsequent processing can include filtration and pasteurisation. Fortification with vitamin C is possible and needs to be considered when comparing data in Table 4.7 (see sources).

Table 4.7. Nutrient composition of apple juice (per 100 g juice)

	Unit	USDA Database (2015)	German Nutrient Database (2014)	Danish Food Compo. Database (2019)	Public Health England (2015)	Swiss Food Compo. Database (2015)
Moisture	g	88.2	87.9	87.9	86.6	87.7
Ash	g	0.2	0.2	0.2		
Protein	g	0.1	0.1	0.1	0.1	0.1
Total fat	g	0.13	0.04	0.10	trace value	0.10
Carbohydrate	g	11.3	11.1	11.7	9.6	10.8
Total dietary fibre	g	0.2	0.0	0	trace value	0.0
Total sugars	g	9.6	10.5	10.2	9.6	10.3
Calcium, Ca	mg	8	7	9	6	7
Iron, Fe	mg	0.12	0.26	0.3	0.06	0.20
Magnesium, Mg	mg	5.0	4.0	4.5	4.0	5.5
Phosphorus, P	mg	7.0	7.0	6	6.0	8.0
Potassium, K	mg	101	116	80	89	120
Sodium, Na	mg	4.0	2.0	10	3.0	2.3
Zinc, Zn	mg	0.02	0.12	0.04	trace value	0.1
Copper, Cu	mg	0.012	0.059	0.006	0.010	
Manganese, Mn	mg	0.074	0.120	0.056	0.030	
Selenium, Se	µg	0.10		0.03	trace value	
Iodide, I	µg		1.0	0.7	trace value	2.0
Vitamin C	mg	0.9	1.4	0.9	26.0	7.4
Thiamine (Vitamin B1)	mg	0.021	0.020	0.021	0.050	0.020
Riboflavin (Vitamin B2)	mg	0.017	0.025	0.017	0.020	0.020
Niacin	mg	0.07	0.30	0.1	0.20	0.16
Pantothenic acid	mg	0.05	0.06	0.06	0.05	0.07
Pyridoxin (B6)	mg	0.02	0.10	0.03	0.05	0.04
Vitamin A, RAE	µg	0.00	8	0	0.00	0.00
Carotene, beta	µg	0.00	45	0	trace value	0.00
Vitamin E (a-Tocopherol)	mg	0.01	0.05	0.01	trace value	0.51

Note: a. Total vitamin E calculated as α-Tocopherol Equivalents (α-TE).

Sources: Data from different databases refer to: **USDA Database**, Release 28, September 2015 (accessed online 1/2016). 09016: Apple juice, canned or bottled, unsweetened, without added ascorbic acid. Other apple juices in the database are available with added ascorbic, calcium, potassium and fortified with Vitamin C; **German Nutrient Database**, version 3.02 (2014), accessed online 1/2016. Lebensmittel F115600 Apfel Fruchtsaft (apple fruit juice, without added sugar). According to database manager, no additives in juices used for this data; **Danish Food Composition Database**, version April 2019 Food ID 194, Apple juice, canned or bottled. No information concerning fortification in database available. No other apple juice option available; **Public Health England** - McCance and Widdowson Dataset 2015 (accessed online spreadsheet 1/2016). Food Code 14-331: Apple juice, clear, ambient and chilled. No other apple juice option available. No information about fortification; **Swiss Food Composition Database**, V5.2 (accessed online 1/2016). ID Food 568: Apfelsaft (apple juice). No data concerning fortification available.

Nutrient composition of apple pomace

Apple pomace is a high fibre, low protein feed material with small amounts of minerals such as potassium, phosphorus and calcium (Table 4.8). Fresh apple pomace contains mean values between 20%-35.9% dry matter, 1.82%-5% ash, 4.45%-7.7% crude protein, 2.7%-5.2% crude fat, 4.7%-48.72% crude fibre (36%-52.5% neutral detergent fibre [NDF], 27%-43.2% acid detergent fibre [ADF]), and up to 0.23% calcium and 0.14% phosphorus. Alibes, Munoz and Rodriguez (1984) showed that ensiling the pomace did not change the dry matter and ash content, however, there was an increase in the crude protein, crude fibre, NDF and ADF. Apple pomace also has a high degree of acidity (pH 3.5) and high levels of lactic acid, acetic acid and ethanol which are increased when ensiled (Alibes, Munoz and Rodriguez, 1984).

Apple pomace contains a substantial amount of carbohydrates and soluble dietary fibre such as pectin, which makes it useful as an energy source in ruminant diets. It is however high in lignin (7.2%-12%) with low digestibility, which contributes to its lower nutritional value as an animal feed. Ensiled apple pomace was shown to contain higher crude protein, crude fibre, NDF, ADF and lignin compared to fresh apple pomace (Alibes, Munoz and Rodriguez, 1984).

Rust and Buskirk (2008) reported that unprocessed apples (culls) have an energy value (total digestible nutrients [TDN] 69.7%) similar to corn silage (TDN 72%), while apple pomace has less energy content (TDN 63.4%) than corn silage and serves as an energy replacement for poor to average quality hay. They indicated that apple pomace works better in diets of beef cows with low energy demand, such as during the second trimester of pregnancy; however, the total diets should be evaluated periodically to provide adequate protein. Apple pomace in animal diets, therefore, requires considerable protein supplementation (Givens and Barber, 1987; Rust and Buskirk, 2008). Fontenot et al. (1977) found that protein nitrogen supplementation was more acceptable than non-protein nitrogen (urea), which reduced intake of the pomace.

Apple pomace is rich in bioactive compounds, such as polyphenols, organic acids and other natural antioxidants (Shalini and Gupta, 2010; Parmar and Rupasinghe, 2012).

Table 4.8. Nutrient composition of apple pomace (% dry matter)

	Unit	Preston (2014)	Givens and Barber (1987)	Joshi and Attri (2006)	NRC		Heuzé et al.in Feedipedia (2016)	
					(2000)	(2001)	range	mean
Dry matter (on fresh basis)	g	20	23.3		22	35.9	13.9-28.6	20.8
Crude protein	g	5	6.7	4.45-5.67	5.4	7.7	4.4-16.0	6.8
Crude fat	g	5.2	2.7	3.49-3.90	4.7	5.0	2.3-7.0	4.2
Ash	g	3	2.3	1.82	5	2.6	1.7-2.5	2.5
Crude fibre	g	18	38.2	4.7-48.72			14.2-32.0	20.7
Carbohydrates	g			48-62				
Total digestible nutrients (TDN)	g	68			68.9	57.1		
Neutral detergent fibre (NDF)	g	36	50.3		41	52.5	30.1-56.4	45.1
Acid detergent fibre (ADF)	g	27	37.8			43.2	24.5-45.6	34.2
Calcium	g	0.13	0.16	0.06	0.23	0.20	0.09-0.24	0.17
Phosphorus	g	0.12	0.14		0.11	0.14	0.01-0.16	0.11
Potassium	g	0.5	0.68	0.95	0.53	0.73	0.60-0.74	0.68
Magnesium	g		0.06	0.02	0	0.09	0.04-0.10	0.07
Sulphur	g	0.04			0.11	0.07		
Sodium	g		0.02	0.2	0	0.04	0.00-0.04	0.02

Changes in chemical composition during storage

Apples are still living organisms after harvest and have an active metabolism. Respiration and metabolic activity lead to degradation and transformation of apple metabolites like sugars, acids and vitamins. For example, malic acid is the major substrate for respiration and, therefore, the concentration decreases during storage (Vandendriessche et al., 2013).

To reduce the loss of nutrients, it is important to store apples at temperatures from 0-2°C, if the cultivars are not sensitive to chilling injuries. Low temperatures decelerate respiration and metabolic activity, resulting in slower ripening and senescence of the apples; however, it is difficult to maintain acceptable fruit quality beyond 6 to 8 months of storage. A combination of low temperature and controlled atmosphere (CA, defined as oxygen concentration held at 1%-3% and carbon dioxide at 1%-5%, adjusted according to cultivar) can preserve apple quality over longer storage times. Apples stored under CA at 1°C were shown to be firmer and contain higher levels of acid and vitamin C compared to apples stored at the same temperature under air (Schirmer and Trierweiler, 2005). Apple firmness is strongly correlated with the pectin content of the cell wall. The expression of endogenous enzymes that modify pectin is controlled by ethylene. Apples stored under CA produce less ethylene, which results in less pectin modification over time (Gwanpua et al., 2014; Storch et al., 2015). The pectin metabolising enzymes exhibit activity even at a storage temperature of 4°C, so maintaining the appropriate temperature is important (Gwanpua et al., 2014).

Maintenance of vitamin levels, especially vitamin C, during long-term storage under controlled atmosphere also varies with cultivar. Vitamin C concentration stayed more or less stable in the cultivars “Topaz” and “Braeburn” during seven months of CA-storage

at 1°C. In contrast, other cultivars like "Jonagold" and "Fuji" lost about 50% of their vitamin C after several months of storage (Trierweiler, Krieg and Tauschen, 2004). CA storage also results in reduced levels of the volatile esters and other compounds that impart "Gala" apples with their aroma, due to the inhibition of precursor biosynthesis at low oxygen concentrations (Both et al., 2014). The combination of lower concentrations of volatiles and ethylene results in slower ripening of the fruit, however, amino acid and polyphenol levels were unchanged with CA storage (Amarowicz et al., 2009; Both et al., 2014).

Cultivars that are highly sensitive to CA storage include "Braeburn" and "Empire", which are very susceptible to internal browning at elevated carbon dioxide concentrations (Lee et al., 2011; Hatoum et al., 2016). In "Braeburn" apples, this quality degradation is marked by biochemical changes, including higher levels of aspartate, acetaldehyde, ethanol and ethyl esters, decreased levels of glutamate, and at very high carbon dioxide levels, an increase of cellobiose, which might indicate a cell wall breakdown (Hatoum et al., 2016). Flesh browning can potentially be reduced by treating the apples with antioxidants before storage (Lee et al., 2012).

Other investigations to maintain the nutrient quality of apples have been carried out with 1-methylcyclopropene (1-MCP; Smartfresh®). 1-MCP is an ethylene inhibitor which slows down or inhibits the ripening of the apples by binding to the ethylene receptor and therefore influencing the metabolite profile, for example, amino acids such as threonine, glutamate, ethanol, methanol and volatiles (Lee et al., 2011; Hatoum et al., 2016). Treatment with 1-MCP improved storability by slowing down ripening and reduced flesh browning in certain apple cultivars like "Braeburn" and "Empire" (Fawbush, Nock and Watkins, 2008).

In summary, storage of apples at low temperatures and controlled atmosphere can effectively maintain nutrient quality of many apple cultivars over several months; however, other cultivars would require special treatment or conditions. Further investigation would be necessary to determine the optimal storage conditions for sensitive cultivars, both existing and new.

Other constituents

Allergens

Apple oral allergy is one of the most common fruit allergies. The prevalence of sensitisation (assessed by skin prick test) was around 4.2% in the general population (both children and adults) in Germany (Zuberbier et al., 2004) and 0.1% in children (2-14 years) in France (Rancé et al., 2005). The prevalence of a perceived allergy to apple varied from 0.9% to 8.5% in European children and was estimated to be 0.5% in adults in a study including patients from Europe, the United States, Australia and New Zealand (Woods et al., 2001).

Four main classes of apple allergens have been identified so far. Two major allergens, Mal d 1 and Mal d 3, are responsible for most apple allergies in the general population. The Mal d 1 protein is the main apple allergen observed in northern Europe. It cross-reacts with Bet v1, the main allergen in birch pollen, due to its similar structure. The Mal d 3 protein is the main allergen in the Mediterranean area (Schmitz-Eiberger and Matthes, 2011). Symptoms of an allergy caused by Mal d 3 can be more severe (for example, generalised urticaria, vomiting and abdominal pain) and in rare cases are even life-threatening. The Mal d 3 protein cross-reacts with the peach allergen Pru p 3. The Mal d 1 protein is unstable so that people with an allergy often tolerate processed apple products like stewed fruit, cakes and pasteurised juices. The process of pasteurisation most likely

eliminates allergenicity. In contrast, Mal d 3 is very stable and resistant to heating. In this case, even processed apple products can cause an allergic reaction. The last two proteins (Mal d 2 and Mal d 4) are considered minor allergens and are also involved in the birch-apple syndrome. Even if apple allergens seem to be mainly restricted to these four families of proteins, several additional proteins located in fruit tissues have been reported as potential allergens (Savazzini, Ricci and Tartarini, 2015).

Different apple cultivars cause a difference in intensity of symptoms. Scientific studies showed that apple cultivars differ in the content of internal factors involved in apple allergy (Matthes et al., 2009).

Toxicants

Apple seeds contain small amounts of amygdalin (D-mandelonitrile-β-D-gentiobioside), a cyanogenic glycoside. Cyanogenic glycosides are naturally occurring plant toxins and are stored in the vacuoles within plant cells to serve as important chemical defence compounds against herbivores (Bolarinwa, Orfila and Morgan, 2015). Total cyanogen content was determined to be 1.08 mg CN^-/g apple seeds (Surleva and Drochioiu, 2013). The amygdalin levels were measured in desiccated apple seeds for 15 apple varieties and ranged from 0.95 ± 0.22 to 3.91 ± 0.49 mg/g (Bolarinwa, Orfila and Morgan, 2015). The lethal dose for cyanide is reported to be 0.5-3.5 mg/kg body weight (bw). An acute reference dose (ARfD) was estimated to be 20 µg/kg bw (EFSA CONTAM Panel, 2016).

Degradation of amygdalin by enzymes can lead to the production of cyanide (prussic acid) when the seeds are macerated or crushed. Apple seeds are generally not consumed by humans but apple juice is produced from whole apples including the seeds. Among the commercially-available apple products, one variety of pressed apple juice had an amygdalin content of 0.09 mg/g, apple purée had 0.02 mg/g and a fruit smoothie had 0.01 mg/g. Amygdalin was not detected in the cider of two brands (Bolarinwa, Orfila and Morgan, 2014). The amygdalin content of 10 commercially-available apple juices ranged from 0.010 to 0.039 mg/mL (Bolarinwa, Orfila and Morgan, 2015). Although the level of amygdalin is considered low in juice in relation to the toxic level, the concentration might be higher in pomace and closer to a level with toxic effect in animals. However, due to the fact that a high level of amygdalin would only occur when the seeds are crushed, and this is not expected to be the normal situation in pomace, the level of amygdalin is expected to be well below any toxic effect level. For humans, the small amounts of amygdalin present in apple seeds and, in turn, apple juice are unlikely to present any health problems to consumers.

Other metabolites: Organic acids, phenolic compounds

Apples are a source of phenolic compounds because of their widespread consumption in many countries and their year-round availability. Phenolic compounds and organic acids are responsible for the characteristic acidic taste and astringency of the fruit (Campo et al., 2006). The most abundant organic acid in apples is malic acid (up to 90%) (Kyzlink, 1990) while citric and quinic acids are also present in substantial quantities (Fuleki, Pelayo and Palabay, 1995).

During ripening, there is a general tendency for a decrease in acidity (Campo et al., 2006). However, there is some disagreement concerning changes in phenolics during maturation and storage. Whether there is an increase or decrease in the amount of phenolic compounds seems to depend on the apple cultivar (Burda, Oleszek and Lee, 1990).

In apples, the content of phenolic compounds is represented mainly by epicatechin and procyanidin (Burda, Oleszek and Lee, 1990; Wojdylo, Oszmiański and Laskowski, 2008). Chlorogenic acid is also found in considerable amounts. It is important to note that phenolic compounds are present in the skin at a relatively higher level than in the flesh of the apples. This is especially true for the apple anthocyanins; apple cultivars with red or partially darkred peels are generally the richest sources of anthocyanins. It is also noteworthy that quercetin glycosides are essentially only located in the apple skin (Burda, Oleszek and Lee, 1990; Gorinstein et al., 2001; Veberic et al., 2005). Table 4.9 provides a list of "other metabolite" levels present in apple fruits. Additional information regarding concentration levels of phenolic compounds in apples based on their fresh weight can be found in Ceyman et al. (2012), Jakobek and Barron (2016) and Stracke et al. (2009).

Table 4.9. Concentration of other metabolites in apple fruit (mg per 100 g dry matter)

Other metabolites	Wojdylo, Oszmiański and Laskowski (2008)	Alonso-Salces et al. (2005)	Liaudanskas et al. (2015)	USDA database (2015)
	range	range	range	mean
Hydroxycinnamic acids	5-350	122-304		
Chlorogenic acid	*1.5-296*	*108-293*	*76.2-293.4*	
p-Coumaroylquinic acid	*0.4-26*	*10-14*		
Flavan-3-ol/procyanidins	462-2 548	64-280	87.5-154.8	
Procyanidins B2	*6.9-200*	*51-253*	*81.1-146.6*	
Procyanidins C1	*5.8-97*			
(-)-Epicatechin	*6.6-276*	*24-177*		*52.2*
(-)-Epigallocatechin				*1.8*
(-)-Epicatechin 3-gallate				*0.1*
(-)-Epigallocatechin 3-gallate				*1.3*
(+) Catechin	*1-72*			*8.9*
Oligomeric procyanidins	*137.4-1 985*			
Flavanols	8-166[b]	0-4[e]	29.2-58.4[f]	
Quercetin				*27.7*
Kaempferol				*0.9*
Dihydrochalcones	4.9-43.4[a]	13-70[d]	7.5-15.2[g]	
Anthocyanins	1-55.1[c]			11
Total polyphenols	523-2724			

Notes: a. Sum of ploretin 2'-xyloglucose and phloretin-2'-glucoside.
b. Sum of quercetin glycosides (quecetin 3-rutinoside; quercetin 3-galactoside; quercetin 3-glucoside; quercetin 3-arabinoside; quercetin 3-xyloside; quercetin 3-rhamnoside).
c. Sum of cyanidin 3-galactoside and cyanidin 3-glucoside.
d. Sum of hydroxyphloretin diglycoside, hydroxyphloretin monoglycoside, phloridzin; phloretin-2-O -xyloglucoside.
e. Sum of quercetin 3-rhamnoside and an unknown quercetin glycoside.
f. Phloridzin.
g. Sum of quercetin glycosides (hyperoside, isoquercitrin, rutin, avicularin, quercitrin).

Sources: Data from different sources refer to: **Wojdylo, Oszmiański and Laskowski** (2008): 67 cultivars, whole apple; **Alonso-Salces et al.** (2005): 6 cultivars, apples peeled and cored; **Liaudanskas et al.** (2015): 4 cultivars, apple slices with skin; **USDA Database**, Release 28, September 2015, accessed online 7/2016. 09003: 5 varieties, apples, raw, with skin.

Suggested constituents to be analysed related to food use

Key products consumed by humans

The majority of apples are consumed as fresh apples for their flavour and nutritional qualities. Apples are a source of potassium and soluble fibre, including pectin and other complex carbohydrates, and phenolic antioxidants.

Suggested analysis for food use of new cultivars

The suggested key nutritional parameters to be analysed in apples for human food use are shown in Table 4.10. Demonstration that composition of a novel apple variety is as expected, i.e. similar to control and/or within reference ranges would be sufficient to extrapolate to juice and other processed apple products for food.

Table 4.10. Suggested nutritional and compositional parameters to be analysed in apple fruit with peel for food use

Parameter	Fruit (with peel)
Moisture	x
Protein	x
Fat	x
Ash	x
Carbohydrate[1]	x
Total dietary fibre	x
Potassium	x
Vitamin C	x

Note: 1 Carbohydrate by calculation or by suitable analytical method.

Suggested constituents to be analysed related to feed use

Key products consumed by animals

As reported above, most of the use of apple processing waste for animal feed is apple pomace, and most of the apple pomace is fed to ruminants. The nutrients of major concern are crude protein, crude fat, carbohydrate and ash, and, for ruminants, ADF and NDF. While calcium and phosphorus are very important minerals in animal feeds, measuring these nutrients in apple is not warranted, due to their very low concentrations relative to the dietary requirements for livestock. Total phenolics may be of importance due to their effects on protein digestibility. They are present mainly in the skin of the apple, which is concentrated in apple by-products fed to livestock.

Suggested analysis for feed use of new cultivars

Table 4.11 below shows the suggested nutritional and compositional parameters to be analysed in unprocessed apples and apple pomace for feed use. For comparative purpose, it is suggested that analysing either the apple fruit (unprocessed) or apple pomace would suffice. The nutrient content of the pomace would not be expected to change if the nutrient content of the apple fruit does not change.

Table 4.11. Suggested nutritional and compositional parameters to be analysed in unprocessed apple or apple pomace for feed use

Parameter	Unprocessed apple	Apple pomace
Moisture	x	x
Protein[1]	x	x
Fat[2]	x	x
Carbohydrate[3]	x	x
Ash	x	x
Neutral detergent fibre (NDF)	x	x
Acid detergent fibre (ADF)	x	x
Total phenolics	x	x

Notes: 1. Derived from proximate analysis (e.g. crude protein).
2. Derived from proximate analysis (e.g. crude fat).
3. Carbohydrate by calculation or by suitable analytical method.

Notes

[1] Like many fruit trees, apple trees do not reproduce true-to-type from seed. Consequently, in production orchards, cultivars are propagated vegetatively. This is done by taking vegetative buds from a young shoot (scion) of the desired cultivar or seedling and grafting those buds onto a rootstock. All vegetatively-propagated seedlings or cultivars are genetically identical. Desirable cultivars are clonally propagated by grafting onto rootstocks. It is possible that the rootstock onto which the scion is grafted may change the characteristics of the scion. A special consideration for apples and other fruits, as opposed to many other types of crops, is that new cultivars should preferably be compared to the non-modified cultivar grown on the same rootstock and harvested and stored under the same conditions. Exceptions might be where the new variety is fitted to special environments.

[2] For additional discussion of appropriate comparators, see the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants CAC/GL 45-2003 of the Codex Alimentarius Commission (paragraphs 44 and 45).

References

AAFC (2010), A *Snapshot of the Canadian Apple Industry - 2010*, Market Analysis and Information, Horticulture and Cross-Sectoral Division, Agriculture and Agri-Food Canada, http://publications.gc.ca/collections/collection_2012/agr/A118-45-2012-eng.pdf (accessed on 26 February 2016).

Ajila, M.C. et al. (2015), "Fermented apple pomace as a feed additive to enhance growth performance of growing pigs and its effects on emissions", *Agriculture*, Vol. 5, pp. 313-329.

Alibes, X., F. Munoz and J. Rodriguez (1984), "Feeding value of apple pomace silage for sheep", *Animal Feed Science and Technology*, Vol. 11, pp. 189-197.

Alonso-Salces, R.M. et al. (2005), "A validated solid-liquid extraction method for the HPLC determination of polyphenols in apple tissues: Comparison with pressurised liquid extraction", *Talanta*, Vol. 65, pp. 654-662.

Amarowicz, R. et al. (2009), "Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods", *Molecular Nutrition & Food Research*, Vol. 53, pp. 151-183.

Baumgartner, I. et al. (2015), "Breeding of elite apple parents carrying pyramided homozygous resistance genes against apple scab and resistance against powdery mildew and fire blight", *Plant Molecular Biology Reporter*, Vol. 121, pp. 647-656.

BLS (2014), *German Nutrient Database, Version 3.02*, Nutritional value table on the basis of electronic data process, Bundeslebensmittelschlüssel (BLS), Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Germany, https://www.blsdb.de/ (accessed on 15 January 2016).

Bolarinwa, I.F., C. Orfila and M.R. Morgan (2015), "Determination of amygdalin in apple seeds, fresh apples and processed apple juices", *Food Chemistry*, Vol. 170, pp. 437-442.

Bolarinwa, I.F., C. Orfila and M.R. Morgan (2014), "Amygdalin content of seeds, kernels and food products commercially-available in the UK", *Food Chemistry*, Vol. 152, pp. 133-139.

Both, V. et al (2014), "Effect of storage under extremely low oxygen on the volatile composition of "Royal Gala" apples", *Food Chemistry*, Vol. 156, pp. 50-57.

Bowden, D.M. and J.C. Berry (1958), "Effects of level of dried apple pomace in swine ration on growth rate, feed efficiency, carcass quality and size of certain organs", *Canadian Journal of Animal Science*, Vol. 39, pp. 26-33.

Burda, S., W. Oleszek and C.Y. Lee (1990), "Phenolic-compounds and their changes in apples during maturation and cold-storage", *Journal of Agricultural and Food Chemistry*, Vol. 38, pp. 945-948.

Campo, G. et al. (2006), "Ripening changes in chemical composition of seven cider apple varieties", *Food Science and Technology International*, Vol. 2, pp. 477-487.

CDC (2009), *China Food Composition Book 1, 2nd Edition,* Printed Version, National Institute of Nutrition and Food Safety, China's Center for Disease Control and Prevention (CDC), edited by Yang Yuexin, Wang Guangya, Pan Xingchang, Beijing Medical University Press, People's Republic of China.

Ceymann, M. et al. (2012), "Identification of apples rich in health-promoting flavan-3-ols and phenolic acids by measuring the polyphenol profile", *Journal of Food Composition and Analysis*, Vol. 26, pp. 128-135.

CFIA (2014), *The Biology of* Malus domestica *Borkh. (Apple), Biology Document BIO2014-01*: *A Companion Document to Directive 94-08 (Dir94-08), Assessment Criteria for Determining Environmental Safety of Plant with Novel Traits*, Plant and Biotechnology Risk Assessment Unit, Plant Health Science Division, Canadian Food Inspection Agency, http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/malus-domestica/eng/1404417088821/1404417158789.

Codex Alimentarius Commission (2003), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants - CAC/GL 45-2003,* Annexes II and III adopted in 2008, http://www.fao.org/fao-who-codexalimentarius/codex-texts/all-standards/en/.

Costa, F. et al. (2005), "Role of the genes Md-ACO1 and Md-ACS1 in ethylene production and shelf life of apple (*Malus domestica* Borkh.)", *Euphytica*, Vol. 141, pp. 181-190.

Dairy Farm Guide (2015), *Fruits and vegetable Processing Residues*, http://www.dairyfarmguide.com/fruit-and-vegetable-processing-0170.html (accessed on 15 December 2015).

Danish Food Composition Database (2019), *Danish Food Composition Database, Revision 7*, *Apple Data (Saxholt et al., 2009),* Technical University of Denmark (DTU), DTU Food, Department of Nutrition, National Food Institute, http://www.foodcomp.dk/v7/fvdb_default.asp (accessed 11 April 2019).

EFSA CONTAM Panel (2016), "Scientific opinion on the acute health risks related to the presence of cyanogenic glycosides in raw apricot kernels and products derived from raw apricot kernels", *EFSA Journal 2016*, Vol. 14(4), Panel on Contaminants in the Food Chain (CONTAM), European Food Safety Authority, p. 4424, http://dx.doi.org/10.2903/j.efsa.2016.4424.

FAOSTAT (2019), "Production/Crops - apple, Year 2016", FAO statistics online database, Food and Agriculture Organization of the United Nations, http://www.fao.org/faostat/ (accessed 10 July 2019).

Fawbush, F., J.F. Nock and C.B. Watkins (2008), "External carbon dioxide injury and 1-methylcyclopropene (1-MCP) in the 'Empire' apple", *Postharvest Biology and Technology*, Vol. 48, pp. 92-98.

Flachowsky, H. et al. (2007) "Overexpression of BpMADS4 from silver birch (*Betula pendula* Roth.) induces early-flowering in apple (*Malus × domestica* Borkh.)", *Plant Breeding*, Vol. 126(2), pp.137-145.

Fontenot, J.P. et al. (1977), "Supplementation of apple pomace with non-protein nitrogen for gestation beef cows. I. Feed intake and performance", *Journal of Animal Science*, Vol. 46, pp. 513-522.

Forsline, P.L. et al. (2003), "Collection, maintenance, characterization, and utilization of wild apples of Central Asia", *Horticultural Reviews*, Vol. 29, pp. 1-61.

FSVO (2015), *Swiss Food Composition Database, Version 5.2,* Federal Food Safety and Veterinary Office (FSVO), Switzerland, http://naehrwertdaten.ch/ (accessed on 15 November 2016).

Fuleki, T., E. Pelayo and R.B. Palabay (1995), "Carboxylic-acid composition of varietal juices produced from fresh and stored apples", *Journal of Agricultural and Food Chemistry*, Vol. 43, pp. 598-607.

German Nutrient Database: see BLS (2014) above.

Givens, D. I. and W.P. Barber (1987), "Nutritive value of apple pomace for ruminants", *Animal Feed Science and Technology*, Vol. 16, pp. 311-315.

Gorinstein, S. et al. (2001), "Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples", *Journal of Agricultural and Food Chemistry*, Vol. 49, pp. 952-957.

Gwanpua, S.G. et al. (2014), "Pectin modifications and the role of pectin-degrading enzymes during postharvest softening of Jonagold apples", *Food Chemistry*, Vol. 158, pp. 283-91.

Hancock, J.F. et al. (2008), "Apples", in J.F. Hancock (ed.), *Temperate Fruit Crop Breeding: Germplasm to Genomics,* Springer Science+Business Media B.V., New York, NY, pp. 1-37.

Hatoum, D. et al. (2016), "Effect of browning related pre- and postharvest factors on the "Braeburn" apple metabolome during CA storage", *Postharvest Biology and Technology*, Vol. 111, pp. 106-116.

Heuzé, V. et al. (2016), *Apple Pomace and Culled Apples*, Feedipedia: A programme by INRA, CIRAD, AFZ and FAO, http://www.feedipedia.org/node/20703 (accessed on 24 November 2016).

Jakobek, L. and A.R. Barron (2016), "Ancient apple varieties from Croatia as a source of bioactive polyphenolic compounds", *Journal of Food Composition and Analysis*, Vol. 45, pp. 9-15.

Joshi, V.K. and D. Attri (2006), "Solid state fermentation of apple pomace for the production of value added products", *Natural Product Radiance*, Vol. 5, pp. 289-296.

Kumar, S. et al. (2012), "Genomic selection for fruit quality traits in apple (*Malus × domestica* Borkh.)", *PLoS ONE*, Vol. 7(5), e36674, http://doi.org/10.1371/journal.pone.0036674.

Kyzlink, V. (1990), "Principles of food preservation", in *Development in Food Science*, Vol. 22, Elsevier Science Publishers BV, Amsterdam, The Netherlands.

Lee, J. et al. (2012), "Antioxidant treatment alters metabolism associated with internal browning in 'Braeburn' apples during controlled atmosphere storage", *Postharvest Biology and Technology*, Vol. 68, pp. 32-42.

Lee, J. et al. (2011), "Metabolic changes in 1-methylcyclopropene (1-MCP)-treated 'Empire' apple fruit during storage", *Metabolomics*, Vol. 8, pp. 742-753.

Liaudanskas, M. et al. (2015), "A comparative study of phenolic content in apple fruits", *International Journal of Food Properties*, Vol. 18, pp. 945-953.

Luby, J.J. (2003), "Taxonomic classification and brief history", in D.C. Ferree and I.J. Warrington (eds.), *Apples: Botany, Production and Uses*, CABI International, Cambridge.

Mangas, J.J. et al. (1992), "Changes in pectic fractions during ripening of cider apples", *Hortscience*, Vol. 27, pp. 328-330.

Matoo, F. et al. (2001), "Performance of broilers fed an apple pomace diet supplements with enzymes", *Indian Journal of Animal Nutrition*, Vol. 18, pp. 349-352.

Matthes, A. et al. (2009), "Effect of variety, cultivation and storage conditions on Mal d 1 protein of apple fruits", *Acta Horticulturae*, Vol. 841, pp. 563-565.

NRC (2001), *Nutrient Requirements of Dairy Cattle (7th edition*), National Academy Press Washington, DC.

NRC (2000), *Nutrient Requirements of Beef Cattle (updated 2000),* National Academy Press, Washington, DC.

NRC (1983), *Underutilized Resources as Animal Feedstuff*s, National Academy Press, Washington, DC, pp. 27-29.

OECD (2019), "Consensus Document of the Biology of Apple (*Malus domestica* Borkh.), *Series on Harmonisation of Regulatory Oversight in Biotechnology No. 66*, Environment Directorate, OECD, Paris, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env-jm-mono(2019)30%20&doclanguage=en

Parmar, I. and H.P.V. Rupasinghe (2012), "Optimization of dilute acid-based pretreatment and application of laccase on apple pomace", *Bioresource Technology*, Vol. 124, pp. 433-439.

Peil, A. et al. (2011), "Apple breeding – From the origin to genetic engineering", *Fruit, Vegetable and Cereal Science and Biotechnology*, Vol. 5 (Special Issue 1), pp. 118-138.

Potter, D. et al. (2007), "Phylogeny and classification of Rosaceae", *Plant Systematics and Evolution*, Vol. 266, pp. 5-43.

Preston, R.L (ed.) (2014), *Feed Composition Table*, http://beefmagazine.com/datasheet/2014-feed-composition-table (accessed on 15 November 2014).

Public Health England (2015), *McCance and Widdowson's The Composition of Foods Integrated Dataset 2015,* Public Health England, London, UK, https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid.

Rancé, F. et al. (2005), "Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France", *Clinical & Experimental Allergy*, Vol. 35, pp. 167-72.

Rop, O. et al. (2011), "Antioxidant capacity, scavenging radical activity and selected chemical composition of native apple cultivars from central Europe", *Journal of Food Quality*, Vol. 34, pp. 187-194.

Rust, S. and D. Buskirk (2008), "Feeding apples and apple pomace in cattle diets", *Cattle Call*, Vol. 13(4), Beef Brief, Michigan State University Beef Team.

Savazzini, F., G. Ricci and S. Tartarini (2015), "Apple allergens genomics and biotechnology: Unravelling the determinants of apple allergenicity", in *Applied Plant Genomics and Biotechnology*, Elsevier, pp. 35-54.

Schirmer, H. and B. Trierweiler (2005), "Lagerung der Apfelsorte/Clubsorte Cameo", *Obstbau*, Vol. 10, pp. 510-512.

Schmitz-Eiberger, M. and A. Matthes (2011), "Effect of harvest maturity, duration of storage and shelf life of apples on the allergen Mal d 1, polyphenoloxidase activity and polyphenol content", *Food Chemistry*, Vol. 127, pp. 1459-1464.

Shalini, R. and D.K. Gupta (2010), "Utilization of pomace from apple processing industries", *Review Journal of Food Science and Technology*, Vol. 47, pp. 365-371.

Smith, E.L. (1950), "Apple pomace silage", *Journal of the Ministry of Agriculture*, Vol. 57, pp. 328-332.

Smock, R.M. and A.M. Neubert (1950), "*Apples and Apple Products*", Interscience, New York.

Storch, T. et al. (2015), "Ethylene-dependent regulation of an alpha-L-arabinofuranosidase is associated to firmness loss in 'Gala' apples under long term cold storage", *Food Chemistry*, Vol. 182, pp. 111-119.

Stracke, B.A. et al. (2009), "Three-year comparison of the polyphenol contents and antioxidant capacities in organically and conventionally produced apples (*Malus domestica* Bork. Cultivar 'Golden Delicious')", *Journal of Agricultural and Food Chemistry*, Vol. 57, pp. 4598-4605.

Surleva, A. and G. Drochioiu (2013), "A modified ninhydrin micro-assay for determination of total cyanogens in plants". *Food Chemistry*, Vol. 141, pp. 2788-2794.

Swiss Food Composition Database: see FSVO (2015) above.

Tiwari, S P., M.P. Narang and M. Dubey (2008), "Effect of feeding apple pomace on milk yield and milk composition in crossbred (Red Sindhi x Jersey) cow", *Livestock Research for Rural Development*, Vol. 20(4), Article 62, India, http://www.lrrd.org/lrrd20/4/tiwa20062.htm.

Trierweiler, B., M. Krieg and B. Tauscher (2004), "Antioxidative capacity of different apple cultivars after long-time storage", *Journal of Applied Botany – Angewandte Botanik*, Vol. 78, pp. 117-119.

USDA Database: see USDA (2015) below.

USDA (2015), *USDA National Nutrient Database for Standard Reference, Release 28*, United States Department of Agriculture, Agriculture Research Service, https://ndb.nal.usda.gov/ndb/ (accessed on 15 November 2016).

Vandendriessche, T. et al. (2013), "High-throughput NMR based metabolic profiling of Braeburn apple in relation to internal browning", *Postharvest Biology and Technology*, Vol. 80, pp. 18-24.

Varming, C., M.A. Petersen, and T.B. Toldam-Andersen (2013), "Ascorbic acid contents in Danish apple cultivars and commercial apple juices", *Lwt-Food Science and Technology*, Vol. 54, pp. 597-599.

Veberic, R. et al. (2005), "Phenolic compounds in some apple (*Malus domestica* Borkh) cultivars of organic and integrated production", *Journal of the Science of Food and Agriculture*, Vol. 85, pp. 1687-1694.

Velasco, R. et al. (2010), "The genome of the domesticated apple (*Malus × domestica* Borkh.)", *Nature Genetics*, Vol. 42, pp.833-839.

Wojdylo, A., J. Oszmiański and P. Laskowski (2008), "Polyphenolic compounds and antioxidant activity of new and old apple varieties", *Journal of Agricultural and Food Chemistry*, Vol. 56, pp. 6520-6530.

Woods, R.K. et al. (2001), "International prevalence of reported food allergies and intolerances: Comparisons arising from the European Community Respiratory Health Survey (ECRHS) 1991-94", *European Journal of Clinical Nutrition*, Vol. 55, pp. 298-304.

Zhang, Y.Z., P.M. Li and L.L. Cheng (2010), "Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp' apple flesh", *Food Chemistry*, Vol. 123, pp. 1013-1018.

Zuberbier, T. et al (2004), "Prevalence to adverse reactions to food in Germany – A population study", *Allergy*, Vol. 59, pp. 338-345.

Annex A. List of OECD consensus documents on the safety of novel foods and feeds, 2002-19

Table A A.1. Published in the Series on the safety of novel foods and feeds from 2002 to 2019, lead country(ies), year of issue and Volume

Consensus document	Lead country(ies)	Year issued	Volume
PLANT CROPS COMPOSITION			
Alfalfa (*Medicago sativa*) and other temperate forage legumes	Canada and the United Kingdom	2005	Vol. 1
Barley (*Hordeum vulgare*)	Finland, Germany and the United States	2004	Vol. 1
Cassava (*Manihot esculenta*)	South Africa	2009	Vol. 2
Common bean (*Phaseolus vulgaris*)	Brazil	2015	Vol. 3
Cotton (*Gossypium hirsutum* and *G. barbadense*)	United States	2009	Vol. 2
Cowpea (*Vigna unguiculata*)	Australia	2018	Vol. 3
Grain sorghum (*Sorghum bicolor*)	United States and South Africa	2009	Vol. 2
Low erucic acid rapeseed (Canola)	Canada	2011	Vol. 2
Maize* (*Zea mays*)	Netherlands and the United States	2002	Vol. 1
Potato* (*Solanum tuberosum* ssp. *tuberosum*)	Germany	2002	Vol. 1
Rice** (*Oryza sativa*)	Japan	2016	Vol. 3**
Sugar beet (*Beta vulgaris*)	Germany	2002	Vol. 1
Sugarcane (*Saccharum* ssp. hybrids)	Australia	2011	Vol. 2
Soybean (*Glycine max*)	United States	2012	Vol. 2
Sunflower (*Helianthus annuus*)	Canada, France, Germany and the United States	2007	Vol. 1
Sweet potato (*Ipomea batatas*)	South Africa and Japan	2010	Vol. 2
Tomato (*Lycopersicon esculentum*)	Greece	2008	Vol. 1
Wheat (*Triticum aestivum*)	Australia	2003	Vol. 1
FRUIT CROPS COMPOSITION			
Apple (*Malus* × *domestica*)	Germany and Canada	2019	Vol. 3
Papaya (*Carica papaya*)	Thailand and the United States	2010	Vol. 2
MUSHROOMS COMPOSITION			
Cultivated mushroom (*Agaricus bisporus*)	Sweden	2007	Vol. 1
Oyster mushroom (*Pleurotus ostreatus*)	Sweden	2013	Vol. 2
FACILITATING HARMONISATION			
Animal feedstuffs derived from genetically modified plants	Canada and the United Kingdom	2003	Vol. 1
Unique Identifier for transgenic plants (revised version) (guidance document)	Working Group on Harmonisation of Regulatory Oversight in Biotechnology	2006	Vol. 1
Molecular characterisation of plants derived from modern biotechnology	Canada, joint publication of the Biosafety and the Food/Feed Safety Working Groups	2010	Vol. 2

* Document under revision.
** Rice composition document updating and replacing the original 2004 issue contained in Volume 1.

Table A A.2. Published in the Series on the Safety of Novel Foods and Feeds, by number

1	Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola) (2001) – ***REPLACED with revised Consensus Doc. No. 24 (2011)***
2	Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-Nutrients (2001) – ***REPLACED with revised Consensus Doc. No. 25 (2012)***
3	Consensus Document on Compositional Considerations for New Varieties of Sugar Beet: Key Food and Feed Nutrients and Anti-Nutrients (2002)
4	Consensus Document on Compositional Considerations for New Varieties of Potatoes: Key Food and Feed Nutrients, Anti-Nutrients and Toxicants (2002)
5	Report of the OECD Workshop on the Nutritional Assessment of Novel Foods and Feeds, Ottawa, Canada, February 2001 (2002)
6	Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites (2002)
7	Consensus Document on Compositional Considerations for New Varieties of Bread Wheat (*Triticum aestivum*): Key Food and Feed Nutrients, Anti-Nutrients and Toxicants (2003)
8	Report on the Questionnaire on Biomarkers, Research on the Safety of Novel Foods and Feasibility of Post-Market Monitoring (2003)
9	Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants (2003)
10	Consensus Document on Compositional Considerations for New Varieties of Rice (*Oryza sativa*): Key Food and Feed Nutrients and Anti-Nutrients (2004) – ***REPLACED with revised Consensus Doc. No. 28 (2016)***
11	Consensus Document on Compositional Considerations for New Varieties of Cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key Food and Feed Nutrients and Anti-Nutrients (2004)
12	Consensus Document on Compositional Considerations for New Varieties of Barley (*Hordeum vulgare* L.): Key Food and Feed Nutrients and Anti-Nutrients (2004)
13	Consensus Document on Compositional Considerations for New Varieties of Alfalfa (*Medicago sativa*) and Other Temperate Forage Legumes: Key Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites (2005)
14	An Introduction to the Food/Feed Safety Consensus Documents of the Task Force for the Safety of Novel Foods and Feeds (2006)
15	Consensus Document on Compositional Considerations for New Varieties of the Cultivated Mushroom *Agaricus Bisporus*: Key Food and Feed Nutrients, Anti-Nutrients and Toxicants (2007)
16	Consensus Document on Compositional Considerations for New Varieties of Sunflower: Key Food and Feed Nutrients, Anti-Nutrients and Toxicants (2007)
17	Consensus Document on Compositional Considerations for New Varieties of Tomato: Key Food and Feed Nutrients, Anti-Nutrients, Toxicants and Allergens (2008)
18	Consensus Document on Compositional Considerations for New Varieties of Cassava (*Manihot esculenta* Crantz): Key Food and Feed Nutrients, Anti-Nutrients, Toxicants and Allergens (2009)
19	Consensus Document on Compositional Considerations for New Varieties of Grain Sorghum [*Sorghum bicolor* (L.) Moench]: Key Food and Feed Nutrients and Anti-Nutrients (2010)
20	Consensus Document on Compositional Considerations for New Varieties of Sweet Potato [*Ipomoea batatas* (L.) Lam.]: Key Food and Feed Nutrients, Anti-Nutrients, Toxicants and Allergens (2010)
21	Consensus Document on Compositional Considerations for New Varieties of Papaya (*Carica papaya* L.): Key Food and Feed Nutrients, Anti-Nutrients, Toxicants and Allergens (2010)
22	Consensus Document on Molecular Characterisation of Plants Derived from Modern Biotechnology (2010) *- joint publication of the Biosafety Working Group and the Food/Feed Safety Working Group*
23	Consensus Document on Compositional Considerations for New Varieties of Sugarcane (*Saccharum* spp. hybrids.): Key Food and Feed Nutrients, Anti-Nutrients and Toxicants (2011)
24	Revised Consensus Document on Compositional Considerations for New Varieties of Low Erucic Acid Rapeseed (Canola): Key Food and Feed Nutrients Anti-Nutrients and Toxicants (2011)
25	Revised Consensus Document on Compositional Considerations for New Varieties of Soybean [*Glycine max* (L.) Merr.]: Key Food and Feed Nutrients, Anti-Nutrients, Toxicants and Allergens (2012)
26	Consensus Document on Compositional Considerations for New Varieties of Oyster Mushroom (*Pleurotus ostreatus*): Key Food and Feed Nutrients, Anti-Nutrients and Toxicants (2013)
27	Consensus Document on Compositional Considerations for New Varieties of Common Bean (*Phaseolus vulgaris* L.): Key Food and Feed Nutrients, Anti-Nutrients and Other Constituents (2015)

28	Revised Consensus Document on Compositional Considerations for New Varieties of Rice (*Oryza sativa*): Key Food and Feed Nutrients, Anti-Nutrients and Other Constituents (2016)
29	High-throughput DNA Sequencing in the Safety Assessment of Genetically Engineered Plants: Proceedings of the OECD Workshop held in April 2016 (2016)
30	Consensus Document on Compositional Considerations for New Varieties of Cowpea (*Vigna unguiculata*): Key Food and Feed Nutrients Anti-Nutrients and Other Constituents (2018)
31	Consensus Document on Compositional Considerations for New Cultivars of Apple (*Malus* × *domestica* Borkh.): Key Food and Feed Nutrients, Anti-Nutrients, Allergens, Toxicants and Other Metabolites (2019)

The individual documents composing the Safety of Novel Foods and Feeds Series, latest version, are available online at the OECD BIOTRACK website: www.oecd.org/biotrack.

The Series of Biosafety Consensus Documents (environmental safety), issued by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, as well as the OECD *Biotech Product Database*, are also available at the same address.

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ISBN 978-92-64-79779-6 – 2019

Novel Food and Feed Safety

Safety Assessment of Foods and Feeds Derived from Transgenic Crops, Volume 3

COMMON BEAN, RICE, COWPEA AND APPLE COMPOSITIONAL CONSIDERATIONS

This volume compiles the consensus documents developed by the OECD Working Group for the Safety of Novel Foods and Feeds from 2015 to 2019. It deals with the composition of common bean, rice, cowpea and apple, four important crops for agriculture and food consumption worldwide. The science-based information collated here is intended for use during the regulatory assessment of food/feed products derived from modern biotechnology, i.e. issued from transgenic plants. Compositional considerations are provided for each species, including tables detailing the key nutrients, anti-nutrients, possible toxicants, allergens and other metabolites contained in the products. This essential information and solid data can be used in the comparative approach as part of the novel food/feed safety assessment. It should be of value to crop breeders and applicants for commercial uses of novel foods and feeds, to regulators and risk assessors in national authorities, as well as the wider scientific community. More information can be found at BioTrack Online.

Consult this publication on line at ***https://doi.org/10.1787/f04f3c98-en***.

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ISBN 978-92-64-79779-6

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