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REVISED CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF LOW ERUCIC ACID RAPESEED (Canola): KEY FOOD AND FEED NUTRIENTS, ANTI-NUTRIENTS AND TOXICANTS

Series on the Safety of Novel Foods and Feeds No. 24

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Series on the Safety of Novel Foods and Feeds

No. 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

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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[No. 1, Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola) (2001) – ***WITHDRAWN****, replaced by revised Consensus Document No. 24 (2011)*]

No. 2, Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients (2001)

No. 3, Consensus Document on Compositional Considerations for New Varieties of Sugar Beet: Key Food and Feed Nutrients and Anti-nutrients (2002)

No. 4, Consensus Document on Compositional Considerations for New Varieties of Potatoes: Key Food and Feed Nutrients, Anti-nutrients and Toxicants (2002)

No. 5, Report of the OECD Workshop on the Nutritional Assessment of Novel Foods and Feeds, Ottawa, Canada, February 2001 (2002)

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No. 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)

No. 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)

No. 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)

No. 22, Consensus Document on Molecular Characterisation of Plants Derived from Modern Biotechnology (2010)

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FOREWORD

The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based ***consensus documents,*** which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents are being published on the nutrients, anti-nutrients or toxicants, information of its use as a food/feed and other relevant information.

This document updates and revises the original *Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola)* issued in 2001. The revised Consensus Document addresses compositional considerations for new varieties of low erucic acid rapeseed (canola) by identifying the key food and feed nutrients, anti-nutrients and toxicants. A general description of these components is provided. In addition, there is background material on the production, processing and uses of low erucic acid rapeseed (canola), and considerations to be taken into account when assessing new varieties of these crops. The text also suggests the constituents to be analysed related to food use and to feed use.

Canada served as the lead country in the preparation for the document, and the draft has been revised on a number of occasions based on the input from other member countries and stakeholders.

The Task Force endorsed this document, which is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD member countries and elsewhere. The need has been identified for detailed technical work aimed at establishing appropriate approaches to the safety assessment of these products.

At a Workshop held in Aussois, France (OECD, 1997), it was 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. The Task Force therefore decided to develop Consensus Documents on phenotypic characteristics and compositional data. These data are used to identify similarities and differences following a 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 member countries.

These documents are a compilation of currently available information that is important in food and feed safety assessment. They provide a technical tool for regulatory officials as a general guide and reference source, and also for industry and other interested parties and will complement those of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, OECD member countries. They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach. In assessing an individual product, additional components may be required depending on the specific case in question.

In order to ensure that scientific and technical developments are taken into account, member countries have agreed that these Consensus Documents will be reviewed periodically and updated as necessary. Users of these documents are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered. Comments and suggestions can be sent to:

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THE ROLE OF COMPARATIVE APPROACH AS PART OF A SAFETY ASSESSMENT

In 1990, a joint consultation of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (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 Organisation for Economic Co-operation and Development (OECD) further elaborated this concept and advocated the approach to 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 2000 the Task Force concluded in its report to the G8 that the concept of substantial equivalence will need to be kept under review (OECD, 2000).

The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 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.

A previous Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety (1996) 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, key toxicants and anti-nutrients for the food source in question. The comparison of key nutrients should be between the modified variety and non-modified comparators with an appropriate history of safe use. Any difference identified would then be assessed against the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO, 1996). The comparator used to detect unintended effects 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.

SECTION I - BACKGROUND

A. History of low erucic acid rapeseed

1. Oilseed rape species used to produce low erucic acid rapeseed oil and meal are derived from the *Brassica* genus of the Cruciferae (Brassicaceae) family, also known as the mustard or cabbage family. Oilseed rape was first cultivated in India about 4,000 years ago, and large-scale production was first reported in Europe in the thirteenth century. The world's supply of low erucic acid rapeseed is largely derived from two species, *B. napus* L.[1] and *B. rapa* L., and to a lesser extent from the mustard *B. juncea* (L.) Czern. Oil from low erucic acid oilseed rape (*B. napus* or *B. rapa* and now *B. juncea*) is also referred to in some countries as canola oil, canola quality mustard oil (*B. juncea*), zero erucic mustard (ZEM) oil, 0-rapeseed oil, low erucic acid oilseed rape (LEAR) oil, double-zero rapeseed oil, 00-Raps oil (in German), 00-colza oil or 'colza simple 0' (in French), and non-specifically as: rapeseed oil, huile de colza/colza oil (European French/English), turnip rape oil (oil from *B. rapa*), and mustard oil. The non-specific terms apply to rapeseed oil but are sometimes incorrectly used to describe low erucic acid oils (canola oils) from *Brassica* species.

2. Interest in rapeseed breeding intensified in Canada soon after the crop was introduced from Europe in the 1940s. The initial efforts were directed towards improving agronomic characteristics and oil content. Nutritional experiments conducted as early as 1949 indicated that consumption of large amounts of rapeseed oil with high levels of erucic acid (C22:1) could be detrimental to animals (Boulter, 1983). Concerns about the nutritional safety of rapeseed oil and its potential impact on human health stimulated plant breeders to search for "genetically controlled" low levels of erucic acid in rapeseed. After ten years of backcrossing and selection to transfer the low erucic acid trait into agronomically adapted cultivars, the first low erucic acid varieties of *B. napus* and *B. campestris* were released in 1968 and 1971 respectively (Przybylski *et al*., 2005). *B. campestris* was later changed by taxonomists to *B. rapa* to reflect its original designation (Bell, 1995). In the late 1970s, the name "canola" was adopted in North America to distinguish the new plant, low erucic acid, from other types of rapeseed. In regions of the world other than Europe, the terms "canola" and "low erucic acid rapeseed" are used interchangeably.

3. In the 1990s, low glucosinolate *B. juncea* was developed at Agriculture and Agri-Food Canada through an interspecific cross between an Indian *B. juncea* line containing only 3-butenyl-type glucosinolate, and a low-glucosinolate, zero erucic acid *B. rapa* line. The original interspecific F1 generation was then backcrossed to Indian *B. juncea* (Love *et al.,* 1990). Further breeding programs were then initiated to combine the low glucosinolate characteristics with zero erucic acid and increased oil content of *B. juncea*. In 2001, Health Canada approved the food use of low erucic acid rapeseed oil derived from three "canola-quality" *B. juncea* varieties.

4. The term "canola" has therefore been registered and adopted by many countries to describe the oil (and seeds[2] and plants) obtained from the species *B. napus*, *B. rapa* and *B. juncea*. Canola must

[1] For information on the environmental considerations for the safety assessment of oilseed rape, see the OECD Consensus Document on the Biology of *Brassica napus* L. (Oilseed rape) (OECD, 1997). *Note: An updated version of this document, expanded to address all Brassica crops, is under development; to be published in 2012*.

[2] In this document, seed refers to seed for human and animal consumption as opposed to seed for sowing.

contain less than 2% erucic acid in the oil and less than 30 µmol/g glucosinolates (anyone or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate) in the air-dried, oil-free meal. Throughout this document, the term "low erucic acid rapeseed" refers to low erucic acid-low glucosinolate rapeseed, or canola.

B. Production

5. Low erucic acid rapeseed is the oilseed with the second highest commodity production globally (after soybean), with a volume of 60.62 million metric tonnes (MMt), and the third largest source of plant based oil (after palm and soybean), with a volume of 22.35 MMt in 2009-10 (see Table 1). During the past 30 years, this crop has passed peanut, sunflower and cottonseed in worldwide plant based oil production. Canola is produced extensively in Europe, Canada, Asia, and Australia, and to a more limited extent in the United States of America (USA). By region in 2009, the European Union was the world's largest producer of low erucic acid rapeseed with a production of 21.4 MMt, followed by China at 13.5 MMt, Canada at 11.8 MMt, India at 7.2 MMt and Australia at 1.9 MMt (see Table 2).

6. By country, Canada is the largest exporter of low erucic acid rapeseed seed and oil, accounting for 41.8% and 29.8% respectively of world exports. The USA is the largest single importing country of low erucic acid rapeseed oil, estimated at 1.0 MMt for 2008. The USA is Canada's largest export market for low erucic acid rapeseed oil however its market share is still only about 5%, or 500,000 tonnes of the over 10 million tonnes of all oil sources consumed annually (Agriculture and Agri-Food Canada, 2006). By country, Japan is the world's largest importer of rapeseed seed, estimated at 2.3 MMt for 2008 (Table 2).

Table 1. Commodity view of major oilseed and plant based oil production

Crop	Oilseed production 2009-2010 (MMt)	Plant based oil production 2009-2010 (MMt)
Copra	5.88	---
Coconut	---	3.62
Cottonseed	39.22	4.66
Olive	---	2.91
Palm		45.86
Palm kernel	12.22	5.50
Peanut	32.98	4.67
Rapeseed	60.62	22.35
Soybean	211.96	38.76
Sunflower	30.39	11.66

Source: USDA, Foreign Agricultural Service

Table 2. World production, imports and exports

	Rapeseed Production 2009 (MMt)	Exports Rapeseed 2008 (MMt)	Exports Rapeseed oil 2008 (MMt)	Imports Rapeseed 2008 (MMt)	Imports Rapeseed oil 2008 (MMt)
Australia	1.9	0.5	0.1	-	-
Canada	11.8	6.7	1.3	0.1	-
China	13.5	-	-	1.3	0.3
European Union	21.4	8.2	2.7	8.4	2.7
India	7.2	-	-	-	-
Japan	-	-	-	2.3	-
USA	0.7	0.5	0.2	1.0	1.0
World	61.6	15.9	4.3	16.0	4.4

Source: FAOSTAT

7. The majority of low erucic acid rapeseed production in China is crushed for domestic oil and meal use, although small amounts of exports do occur. Low erucic acid rapeseed oil is second to soybean oil in China and represents approximately 30% of the domestic market (Agriculture and Agri-Food Canada, 2006).

8. Globally, transgenic low erucic acid rapeseed varieties were grown on 5.9 million hectares in 2008 compared to 5.5 million hectares in 2007. Cultivation areas are found predominantly in Canada and the USA. In Canada, transgenic varieties represented 87% of its total low erucic acid rapeseed crop in 2007. Australia cultivated transgenic rapeseed for the first time in 2008 (GMO Compass). Transgenic varieties are also cultivated in Chile (James, 2011).

9. The *B. napus* varieties are produced in areas with longer growing seasons, while *B. rapa* are grown in short season areas. The *B. juncea* varieties have been shown to mature early, and to be more heat and drought tolerant, as well as higher yielding and more resistant to blackleg (a fungal disease), than *B. napus* and *B. rapa*. These characteristics make *B. juncea* well adapted to the semi-arid growing conditions of the Canadian prairies (Potts *et al.*, 1999).

C. Processing

10. Canola seed is traditionally crushed and solvent extracted in order to separate the oil from the meal. The process usually includes seed cleaning, seed pre-conditioning and flaking, seed cooking/ conditioning, pressing the flake to mechanically remove a portion of the oil, solvent extraction of the press-cake to remove the remainder of the oil, oil and meal desolventizing, degumming and refining of the oil, and toasting of the meal. Canola seed can also be subject to cold-press extraction (*i.e.* no heat or solvent). The main steps of the solvent extraction process are schematised in Figure 1.

Seed cleaning

11. The seed is cleaned to remove plant stalks, grains from other plant species and other materials from the bulk of the seed. Aspiration, indent cleaning, sieving, or some combination of these is used in the cleaning process. Dehulling of the seed is, at present, not a commercial process.

Seed pre-conditioning and flaking

12. Many crushing plants in colder climates preheat the seed to approximately 35°C through grain dryers in order to prevent shattering which may occur when cold seed from storage enters the flaking unit (Unger, 1990). The cleaned seed is first flaked by roller mills set for a narrow clearance to physically rupture the seed coat. The objective here is to rupture as many cell walls as possible without damaging the quality of the oil. The thickness of the flake is important, with an optimum of between 0.3 and 0.4 mm. Flakes thinner than 0.2 mm are very fragile while flakes thicker than 0.4 mm result in lower oil yield.

Seed cooking/conditioning

13. Flakes are cooked/conditioned by passing them through a series of steam-heated drum or stack-type cookers. Cooking serves to thermally rupture oil cells which have survived flaking, reduce oil viscosity and thereby promote coalescing of oil droplets, increase the diffusion rate of prepared oil cake, and denature hydrolytic enzymes. Cooking also adjusts the moisture of the flakes, which is important in the success of subsequent pre-pressing operations. At the start of cooking, the temperature is rapidly increased to 80–90°C. The rapid heating serves to inactivate the myrosinase enzyme present in canola. This enzyme can hydrolyze the small amounts of glucosinolates present in canola and will produce undesirable breakdown products which affect both oil and meal quality.

14. The cooking cycle usually lasts 15 to 20 minutes and the temperatures usually range between 80 and 105°C, with an optimum of about 88°C. In some countries, especially China, cooking temperatures of up to 120°C have been traditionally used when processing high glucosinolate rapeseed to volatize some of the sulphur compounds which can cause odours in the oil. However, these high temperatures can negatively affect meal protein quality.

Pressing

15. The cooked canola seed flakes are then pressed in a series of low pressure continuous screw presses or expellers. This action removes most of the oil while avoiding excessive pressure and temperature. The objective of pressing is to reduce the oil content of the seed from about 42% to 14–20%, making the solvent extraction process more economical and efficient, while producing acceptable quality presscake.

Solvent extraction

16. Since the pressing is not able to remove all of the oil from the canola seed, the presscake is solvent extracted to remove the remaining oil. The cake from the expellers, containing between 14 and 20% oil, is sometimes broken into uniform pieces prior to solvent extraction. In solvent extraction, hexane specially refined for use in the vegetable oil industry is used. After a series of extractions, the marc (hexane saturated meal) that leaves the solvent extractor contains less than 1% oil.

Desolventizing of oil and meal

17. The micella and meal are "stripped" of solvent, to recover solvent-free oil and meal. The micella containing the oil is desolventized using evaporator equipment. The solvent is removed from the marc in a desolventizer-toaster. This is done in a series of compartments or kettles within the desolventizer, often by injection of live steam, followed by final stripping and drying at a temperature of 103–107°C. The final, solvent-free meal contains about 1% oil and 8 to 10% moisture.

Degumming of oil

18. The "crude" oil from the two extraction stages (physical and chemical) is usually blended and then degummed before being stored for sale or further processing. Degumming removes phosphatides co-extracted with the oil, which tend to separate from the oil as sludge during storage. The phosphatide content of crude oil varies, but is usually in the order of 1.25% (or 500 ppm if measured as phosphorus). Two degumming methods are in use: (a) using water to precipitate phosphatides and; (b) using an acid such as citric, malic, or phosphoric and water (super-degumming).

Alkali and physical refining of oil

19. Degummed oil is further purified in a process of refining. One of two methods are used, namely, alkali refining, especially with water degummed oil, and physical refining with acid-water degummed oil. Alkali refining is the most common process used, even with acid-water degummed oil. Physical refining is a relatively new development. While it is very economical, physical refining requires well-degummed oil of moderate chlorophyll and free fatty acid content. Alkali refining reduces soap, free fatty acid, and phosphorus levels. The further removal of free fatty acids is done by steam distillation in a deodorizer. This simultaneously deodorizes the oil. Because deodorization is the last process normally carried out on edible oils, this step may be delayed until other processes, such as hydrogenation of the oil, have been done. Alkali-refined oil contains chlorophylloid compounds which give the oil a green colour, and catalyze oil oxidation. These compounds are removed by adsorptive bleaching with acid-activated clays.

Effects of processing on meal quality

20. The quality of the meal can be enhanced or diminished by altering the processing conditions in the crushing plant. Minimum processing temperatures (see paragraph 22) are needed in order to deactivate myrosinase enzyme, which, if not destroyed, will break down glucosinolates into their toxic metabolites in the animal's digestive tract. The canola crushing process can also cause thermal degradation of 30 to 70% of glucosinolates in the meal (Daun and Adolphe, 1997). However, if temperatures are too high for too long a period, then the protein quality of the meal can decrease. There can be considerable variation in temperatures used during canola processing. In these cases, it is important for canola meal users to consider the protein quality of the meal used for animal feed.

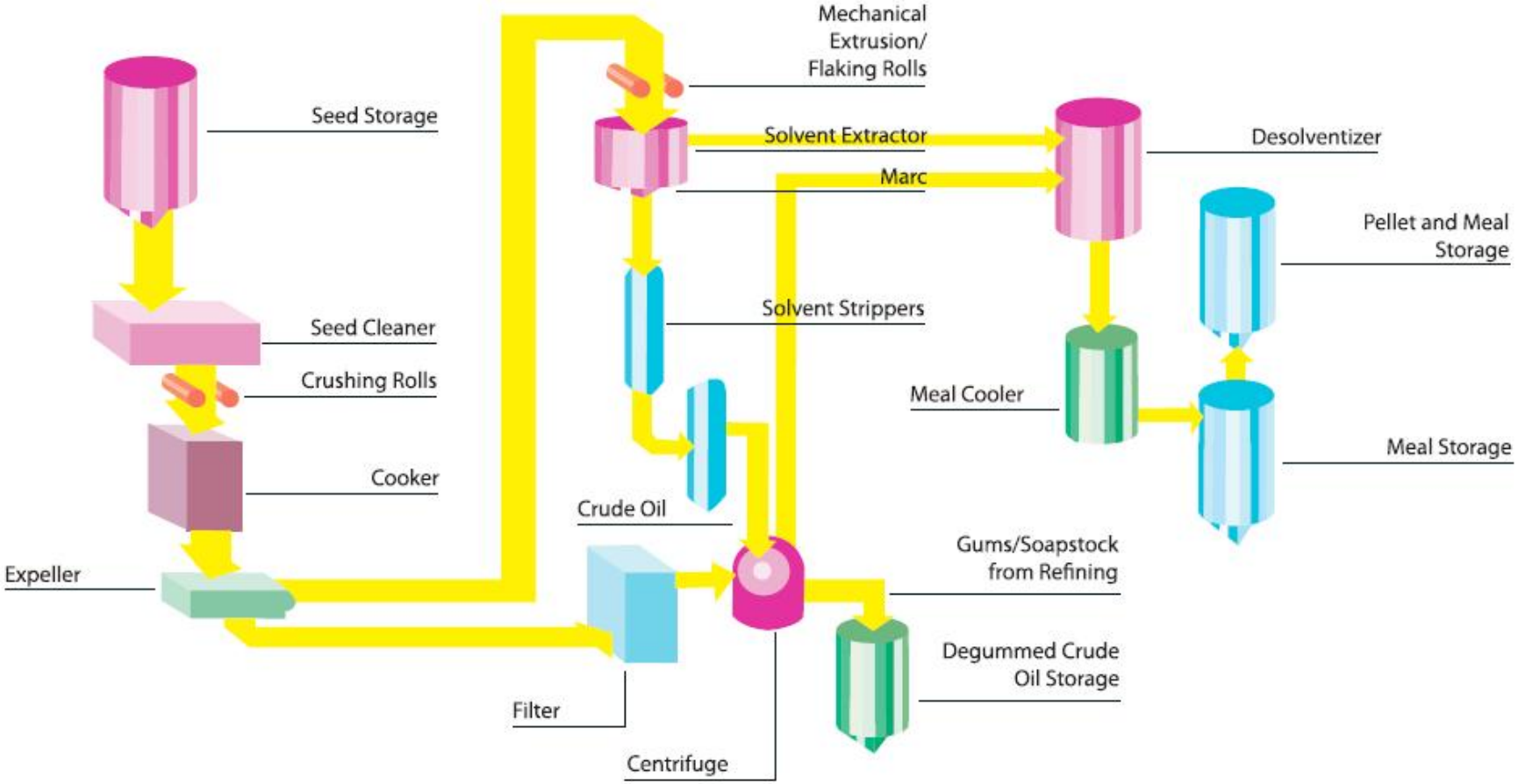
21. Some of the by-products of canola processing are sometimes added back into the canola meal. In the case of added gums and soap stocks, these oil rich components will increase the energy content of the meal. In the case of added screenings and foreign material, the meal quality will decrease as the fibre content increases. These differences in processing practices may be identified as part of quality control programs.

Temperature

22. Deactivation of myrosinase enzyme is best accomplished during the canola seed cooking stage. The early research of Youngs and Wetter (1969) regarding steps to minimize glucosinolate hydrolysis by myrosinase has become the operating practice for processors around the world. Moisture content of the seed during processing should be between 6 and 10%. Above 10% moisture, glucosinolate hydrolysis will proceed rapidly, and below 6% moisture the myrosinase enzyme is only slowly inactivated by heat. In addition, the temperature must be raised to 80 to 90°C as rapidly as possible during seed cooking. Myrosinase catalyzed hydrolysis of glucosinolates will proceed with increasing temperature until the enzyme is deactivated so that a slow rate of heating favours glucosinolate hydrolysis.

23. Excessive heating during processing can result in reduced animal digestibility of some amino acids, particularly lysine. Processors must exercise strict process control to ensure amino acid damage is minimized by not overheating the meal in the desolventizer-toaster. Examination of meal quality at various processing stages in several Canadian crushing plants revealed that canola meal is a uniform and high-quality product until it enters the desolventizer-toaster phase (Newkirk and Classen, 2000). During this stage crude protein and lysine digestibility and lysine content were significantly reduced and the apparent metabolisable energy was numerically lower. This research by Newkirk and Classen suggests that the commonly used temperatures in the desolventizer-toaster stage of 105°C cause some protein damage. They found that processing with a maximum temperature of 95°C in the desolventizer-toaster significantly increases lysine digestibility, to similar levels found in soybean meal. Also, traditional toasting causes the meal to become much darker in colour. This is a quality concern for some feed manufacturers, whose customers prefer using light coloured ingredients.

Figure 1. Prepress solvent extraction process



Source: Canola Council of Canada (CCC) website

D. Use

24. Low erucic acid rapeseed seeds are processed into two major products: oil and meal. The oil and meal are then further manufactured into a wide variety of products for human and agricultural use as well as industrial use. Human food use of whole seeds and flour of low erucic acid rapeseed have been reported anecdotally, and a sensory evaluation of canola greens has been reported (Miller-Cebert *et al.,* 2009).

25. The oil is used in food processing as well as for home cooking and baking. Refined low erucic acid rapeseed oil is widely used in both salad and cooking oil products, and is also acceptable in hydrogenated products such as margarine and shortenings (Przybylski *et al*., 2005; Malcolmson and Vaisey-Genser, 2001). In Canada, low erucic acid rapeseed oil represents about 68% of the edible plant based oil consumed. It is widely used in both salad and cooking oil products (representing nearly 90% of these products), as well as in hydrogenated products such as margarine (representing 45% of these products) and shortenings (representing 50% of these products) (Malcolmson and Vaisey-Genser, 2001). In the USA, low erucic acid rapeseed oil represents 7 to 8% of total oil consumption, and is used in all food products requiring an oil source. The oil is also used in a wide variety of non-food products such as dust de-pressants, de-icer for airplanes, suntan oils, biodiesel and bioplastics (MCGA, 2008). By-products such as soap stock are also manufactured from the oil.

26. Food use of protein fractions from low erucic acid rapeseed meal has not been reported to any great extent (Tan *et al*., 2011). However, patents have recently been granted in Canada (*e.g*. Canadian patent CA 2553640) (Canadian Patent Database, 2011)), and a firm has notified the U.S. Food and Drug Administration (FDA) of certain uses of particular canola protein isolates that the firm has concluded are generally recognized as safe (GRN No. 327) (GRAS Notice Inventory, FDA, 2010).

27. The meal left after extraction of oil from the seed is used as a high (36-44%) protein feed source for all classes of livestock, poultry and fish. Prior to the late 1970s, the use of this oilseed processing by-product as an animal feed was limited by the presence of glucosinolates in the seed. Glucosinolates themselves are generally considered to be innocuous; however, the hydrolysis products have negative effects on animal production. The low palatability and the adverse effects of glucosinolates metabolites due to their antithyroid activity, led to the development of varieties of rapeseed which have combined low levels of both glucosinolates and erucic acid (also known as “double zero” varieties). On a unit weight basis, canola meal has 55-65% of the value of 47% protein soybean meal for feeding broiler growers, 65-75% for feeding growing swine, and 75-85 % for dairy cattle (CCC, 2009).

28. Low erucic acid rapeseed meal is typically balanced with other protein ingredients (*e.g.* soybean meal, field peas). Because low erucic acid rapeseed meal contains 30% hulls, it has a high fibre content, which limits its use in monogastric diets (to approximately 15% of the total diet). Higher inclusion rates are practical in ruminant rations, especially for dairy cows. Low erucic acid rapeseed meal can be used as the sole protein supplement for ruminants. De-hulled low erucic acid rapeseed meal has the potential to compete with soybean meal in swine and poultry diets. Meals derived from *B. juncea* have been shown to contain more crude protein and less total dietary fibre on a dry basis than either *B. napus* or *B. rapa* (Simbaya *et al*., 1995; Newkirk *et al*., 1997).

29. Because the oil is highly unsaturated, the amount that can be added to a ration may limit the use of meal from low erucic acid rapeseed meal high in residual oil (*i.e.* that has been cold-pressed) (Downey, 2007). Excessive levels of supplementation may also be undesirable as the protein requirements of the animal would be exceeded and nitrogen excretion would be increased. Typical rates of inclusion of seed, oil and meal from low erucic acid rapeseed into feed (for different animals) are shown in Table 3.

Table 3. Recommended maximum rates of inclusion of low erucic acid rapeseed in feeds

Animal	Ingredient			
	Low erucic acid rapeseed Seed	Low erucic acid rapeseed Meal	Low erucic acid rapeseed Cold pressed meal	Low erucic acid rapeseed Oil
Beef [1]				
• Cow	6–10	30	15	3
• Feedlot	6	30	15	3
Dairy [1]				
• Lactating	3	25	10	3
• Dry	3	25	10	3
• Calves	Not determined	20	15	3
Swine [2]				
• Nursery	Not determined	5		3
• Grower	12–14	15	15	3
• Finisher	12–14	15	15	3
• Sow	12	15		3
Poultry [2]				
• Starter	Not determined	5		4
• Grower	10	15		4
• Finisher	10	20		4
• Layers	10	10		3
Fish [2]				
• Trout/salmon	20	20		10
• Catfish	30	30		10
• Tilapia	15	15		10

Sources: Hickling, 2005; McAllister *et al.*, 1999; Racz and Christensen, 2004; Van Barneveld and King, 2002

[1] % of concentrates on dry matter basis
[2] % of complete feed on dry matter basis

30. With the increase in market demand for low erucic acid rapeseed oil for the biodiesel market, a significant increase in the supply of low erucic acid rapeseed meal is expected. Properties of the meal arising from biodiesel production are also likely to be different if the oil is derived using cold-press extraction procedures.

E. Appropriate comparators for testing new varieties

31. This document suggests parameters that breeders of low erucic acid rapeseed should measure when developing new modified varieties. The data obtained in the analysis of a new variety of low erucic acid rapeseed should ideally be compared to those obtained from an appropriate near isogenic non-modified variety, grown and harvested under the same conditions[3]. 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 varieties of low erucic acid rapeseed.

[3] 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).

32. Components to be analysed include key nutrients, anti-nutrients and toxicants. Key nutrients are those which have a substantial impact in 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. Standardized 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.

F. Breeding characteristics screened by developers

33. Phenotypic characteristics provide important information related to the suitability of new varieties for commercial distribution. Selecting new varieties is initially based on parental data. Plant breeders developing new varieties of low erucic acid rapeseed evaluate many parameters at different stages in the developmental process. Typical goals include increasing agronomic flexibility and productivity, capturing niche markets and/or offering end-users more options. Included in this list might be features such as improved yields and yield stability, maturity, winter-hardiness, disease and pest resistance, lodging resistance and specific product attributes. New varieties must meet minimum criteria for yield, oil content, protein content, fatty acid profile, glucosinolate content and disease-resistance. In response to concerns about trans fat in partially hydrogenated vegetable oils, low erucic acid rapeseed breeders continue work to develop lines that produce oils with a high oleic and low linolenic acid content.

34. Herbicide-resistant transgenic low erucic acid rapeseed was first introduced to Canada in 1995. In 2006, over 80% of the acreage of low erucic acid rapeseed in Canada was sown with transgenic varieties. The early stages of transgenic development in low erucic acid rapeseed in Canada focused mainly on herbicide tolerance and the evaluation of transgenic pollination control. The focus of development has shifted to hybrids over the past few years and now the major traits of interest include stress tolerance, metabolic pathway enhancement, biotic stress resistance as well as fatty acid composition modifications.

SECTION II - NUTRIENTS

A. Composition of low erucic acid rapeseed

35. Low erucic acid rapeseed consists mainly of lipids, proteins and fibre. Lipids and protein are quantitatively the most important fractions and account for more than 60% of the seed weight. The average composition of low erucic acid rapeseed is presented in Table 4. The data are taken from 2006 to 2009 quality reports from Canada and Australia.

Table 4. Canadian and Australian average composition of low erucic rapeseed seed, oil and meal (2006-2009)

Component	2006		2007		2008		2009	
	CA [4]	AU [7]	CA [4]	AU [8]	CA [5]	AU [9]	CA [6]	AU
Oil content in seed, %	44.6[1]	42.2[2]	43.4[1]	44.0[2]	44.3[1]	41.8[2]	44.5[1]	NA
Protein content in oil free meal, % [1]	41.0[1]	40.1[3]	41.2[1]	40.0[3]	40.3[1]	41.0[3]	38.7[1]	NA
Total glucosinolates in seed, µmol/g [1]	10.0[1]	4.0[2]	10.0[1]	8.0[2]	10.6[1]	10.0[2]	9.6[1]	NA
Erucic acid in oil, %	0.05	0.1	0.04	0	0.01	<0.1	0.01	NA
Linoleic acid in oil, %	NA	20.2	19.3	20.4	18.4	20.3	18.8	NA
Linolenic acid in oil, %	9.9	11.1	9.8	11.0	9.1	10.7	10	NA
Oleic acid in oil, %	62.0	60.0	61.5	59.7	63.2	60.0	62.2	NA
Total saturated fatty acids in oil, %	7.0	7.2	7.0	7.4	7.1	7.6	6.8	NA
Iodine value (calculated)	113.0	116.8	113.0	116.6	111.0	115.7	114	NA

CA = Canada, mean values from samples taken from 3 Canadian provinces;
AU = Australia, mean values from samples taken from 4 Australian states;
NA = Not available

[1] 8.5% moisture basis
[2] 6% moisture basis
[3] 10% moisture basis

Sources: Agriculture and Agri-Food Canada [4] (2008); [5] (2009); [6] (2010)
Seberry, D.E., R.J. Mailer and P.A. Parker [7] (2007); [8] (2008); [9] (2009)

Fatty acids

36. Dietary fat serves several important nutritional functions. It is an important source of energy as well as the source of essential fatty acids that are important constituents of cell membranes. Fat serves as a precursor for many biologically active compounds and as a carrier for the fat-soluble vitamins (Przybylski *et al*., 2005).

37. Low erucic acid rapeseed oil consists of 91.8-99.0% triglycerides, up to 3.5% phospholipids, 0.5-1.8% free fatty acids, 0.5-1.2% non-saponifiable matter including 700-1000 mg/kg total tocopherols and 5-35 mg/kg pigments and 5-25 mg/kg sulphur (Przybylski *et al*., 2005).

38. Low erucic acid rapeseed oil has the lowest content of saturated fatty acids (*ca.* 7%) of the vegetable oils (Gunstone, 2005) and it is also characterized by a relatively high level of

monounsaturated fatty acids and an appreciable amount of alpha linolenic acid (alpha C18:3) (Przybylski *et al*., 2005). Fatty acid profiles and levels for low erucic acid rapeseed oil have been defined in the Codex Standard for Named Vegetable Oils (Codex Alimentarius Commission, 2005). Samples falling within the appropriate ranges specified in Table 5 are in compliance with this Standard. Fatty acid profiles for rapeseed oil and low erucic acid rapeseed oil from the Codex Standard are presented in Table 5.

39. Minor fatty acids occur in low erucic acid rapeseed oil at a range of about 0.01-0.1%, except for palmitoleic acid (C16:1) which is around 0.6%. Conjugated linoleic acid (C18:2) may also be found in the oil often as artefacts of refining and deodorization. The refining process is also a source of *trans*-isomers of fatty acids that occur as artefacts caused by the isomerization of one or more of the double bonds of *cis* linolenic acid (*cis* C18:3). Such *trans*-isomers can be found in any oil containing linolenic acid (C18:3) and may account for 1% or more of the parent fatty acid.

Table 5. Codex Standard for fatty acid composition of rapeseed oil and low erucic acid rapeseed oil (% of total fatty acids)

Fatty acid	Common name	Rapeseed	Low erucic acid rapeseed
C6:0	Caproic	ND	ND
C8:0	Caprylic	ND	ND
C10:0	Capric	ND	ND
C12:0	Lauric	ND	ND
C14:0	Myristic	ND–0.2	ND–0.2
C16:0	Palmitic	1.5–6.0	2.5–7.0
C16:1	Palmitoleic	ND–3.0	ND–0.6
C17:0	Heptadecanoic	ND–0.1	ND–0.3
C17:1	Heptadecenoic	ND–0.1	ND–0.3
C18:0	Stearic	0.5–3.1	0.8–3.0
C18:1	Octadecenoic (oleic)	8.0–60.0	51.0–70.0
C18:2	Linoleic	11.0–23.0	15.0–30.0
C18:3	Linolenic	5.0–13.0	5.0–14.0
C20:0	Arachidic	ND–3.0	0.2–1.2
C20:1	Gadoleic (eicosenoic)	3.0–15.0	0.1–4.3
C20:2	Ecosadienoic	ND–1.0	ND–0.1
C22:0	Behenic	ND–2.0	ND–0.6
C22:1	Erucic	> 2.0–60.0	ND–2.0
C22:2	Docosadienoic	ND–2.0	ND–0.1
C24:0	Lignoceric	ND–2.0	ND–0.3
C24:1	Nervonic (tetracosenoic)	ND–3.0	ND–0.4

ND: Non-detectable, defined as ≤ 0.05%
Source: Codex Alimentarius Commission, 2005

Vitamin K

40. Low erucic acid rapeseed oil is a source of Vitamin K_1 (phylloquinone) and the vitamin K_1 content of the oil has been described in several publications (Table 6). Rapeseed, soybean, and olive oils are good sources of phylloquinone, and contain 50-200 µg vitamin K_1/100g oil. These vegetable oils are categorized as the second most substantial contributors of vitamin K_1 to the human diet after green leafy vegetables (FAO/WHO, 2002). The vitamin K_1 content of low erucic acid rapeseed oil has been shown to be significantly affected by processing and storage conditions (temperature, exposure to light, etc.) (Ferland and Sadowski, 1992; Gao and Ackman, 1995). Therefore when considering the vitamin K_1 content of low erucic acid rapeseed oil, it may be useful to take into account the state of processing and the storage conditions.

Table 6. Vitamin K_1 levels in low erucic acid rapeseed oil (per 100 g of oil)

Reference	Vitamin K_1 (Phylloquinone) (micrograms/100 g of oil)
Ferland and Sadowski (1992)	141
Gao and Ackman (1995)	125
Shearer *et al.* (1996)	123
Piironen *et al*. (1997)	150 130 [1]
Cook *et al.* (1999)	108 [2] 97 [3]
Bolton-Smith *et al.* (2000)	112.5
Kamao *et al.* (2007)	92
USDA-ARS (2011)	71.4

These measurements were obtained by various types of HPLC-based analytical methodologies. These data were obtained from analysis of oil available for retail sale.

1 Cold-pressed oil
2 Sample prepared by enzymatic digestion and extraction
3. Sample prepared by direct extraction

Tocopherols and sterols

41. The main non-saponifiable components of vegetable oils are tocopherols and sterols. Tocopherols, which include Vitamin E, are natural antioxidants and their level in plants is governed by the level of unsaturated fatty acids. A simple increase in unsaturation will result in the formation of higher levels of antioxidants to protect the oil (Przybylski *et al*., 2005). The distribution of natural tocopherols varies with the different vegetable oils both quantitatively and in the amount of different isomers (Table 7). Low erucic acid rapeseed contains mostly alpha and gamma-tocopherols usually at a 1:2 ratio.

Table 7. Codex standard for levels of tocopherols in low erucic acid rapeseed oil (mg/kg)

Tocopherol (mg/kg)	Low erucic acid rapeseed oil
Alpha-tocopherol	100–386
Beta-tocopherol	ND–140
Gamma-tocopherol	189–753
Delta-tocopherol	ND–22
Total	430–2680

ND: Non-detectable, defined as ≤ 0.05%.
Source: Codex Alimentarius Commission, 2005

42. Besides the tocopherols, the sterols are the other non-saponifiable components of vegetable oils. Sterols are found in low erucic acid rapeseed in two forms in equal amounts, free and esterified sterols. The amount of total sterols present in the oil is approximately twice that found in soybean oil and slightly lower than the amount found in corn oil. Total sterols range from 450 to 1130 mg/100 g of oil. The proportions of major sterols are presented in Table 8. Although the refining, bleaching and deodorization of the oil reduces the levels of both tocopherols and sterols (Przybylski *et al.,* 2005), low erucic acid rapeseed oil is still a source of these compounds.

Table 8. Codex Standard of major sterols in low erucic acid rapeseed oil (% of total sterols)

Sterol (% of total sterols)	**Low erucic acid rapeseed oil**
Cholesterol	ND–1.3
Brassicasterol	5.0–13.0
Campesterol	24.7–38.6
Stigmasterol	0.2–1.0
Beta-sitosterol	45.1–57.9
Delta-5-avenasterol	2.5–6.6
Delta-7-stigmastenol	ND–1.3
Delta-7-avenasterol	ND–0.8
Others	ND–4.2

ND: Non-detectable, defined as ≤ 0.05%.
Source: Codex Alimentarius Commission, 2005

Pigments

43. Pigments in oilseeds impart undesirable colour to the oil and can promote oxidation in the presence of light as well as inhibit catalysts used for hydrogenation (Przybylski *et al*., 2005). Chlorophylls without phytol such as chlorophyllides and pheophorbides may present a nutritional effect because of their phototoxicity, which may be followed by photosensitive dermatitis (Endo *et al*., 1992). A bleaching step in the processing of low erucic acid rapeseed oil removes chlorophyll-related pigments and other colour bodies. In order to mitigate the "poisoning" effect of catalysts during hydrogenation, grading standards for low erucic acid rapeseed seed specify tolerance levels for the number of "green seeds" permitted. Lots which exceed the maximum tolerance level are rejected.

Trace elements

44. Maximum permitted levels for iron, copper, lead and arsenic for low erucic acid rapeseed oil are provided in the Codex Standard for Named Vegetable Oils (Codex Alimentarius Commission, 2005). These are generally removed to trace levels during processing. Divalent sulphur components, which are decomposition products of glucosinolates, are found in crude low erucic acid rapeseed oil in ranges of 15 to 35 mg/kg. Refining, bleaching and deodorizing steps reduce these levels to 9 mg/kg or lower (Przybylski *et al*., 2005).

Other identity characteristics of oil

45. Non-specific measurements such as Saponification Values, Unsaponifiable Matter, Iodine Values, and Crismer Values are not considered to be necessary in the context of a comparative safety assessment. These measurements are required to compare with the Codex Standard for Named Vegetable Oils (Codex Alimentarius Commission, 2005).

B. Composition of low erucic acid rapeseed seed and meal

46. Low erucic acid rapeseed meal is the by-product that remains after lipid extraction. Unlike other oilseeds, the hull is usually not separated from the seed. Table 9 provides typical nutritional profiles for low erucic acid rapeseed seed and meal.

Table 9. Range in proximate and fibre composition of low erucic acid rapeseed seed and meal (DM basis, unless otherwise noted)

Component	Low erucic acid rapeseed seed [1]			Low erucic acid rapeseed meal [2]		
	Samples	Mean	Range	Samples	Mean	Range
Moisture % fw	91	5.6	3.2–8.1	1584	9.3	7.1–11.5
Crude Protein %	91	24.7	21.3–28.1	1560	39.9	35.6–44.3
Fat %	77	40.3	35.6–44.9	644	7.4	0.3–14.5
Ash %	10	5.0	4.1–5.9	285	7.4	6.1–8.7
Crude fibre %	1	9.1	-	89	9.5	7.7–11.2
Acid detergent fibre %	15	19.4	11.9–26.8	890	20.8	17.6–23.9
Neutral detergent fibre %	15	26.7	18.7–34.7	949	30.1	25.6–34.6

Source: Dairy One Cooperative Inc.

[1] Canola Seed Accumulated crop years: 05/01/2000 through 04/30/2010
[2] Canola Meal, Dry Accumulated crop years: 05/01/2000 through 04/30/2010

47. As can be seen from Table 9, there is a considerable range in the proximate composition of the seed and meal, some of which can be traced to the regional variability in the seed (Racz and Christensen, 2004) as well as to the method used to extract oil (Bonnardeaux, 2007). Regional and environmental variability in the composition of the seed is demonstrated in data presented by Pritchard *et al.* (2000), where a substantially lower range (17.4 – 23.0 % DM) of crude protein content is reported.

48. Levels of vitamins and minerals are given in Tables 10 and 11.

Table 10. Vitamin composition of low erucic acid rapeseed meal (DM basis)

Vitamin (mg/kg)	Low erucic acid rapeseed meal
Biotin	0.98–1.1
Choline	6700.0
Folic Acid	0.8–2.3
Niacin	160.0
Pantothenic acid	9.5
Pyridoxine	7.2
Riboflavin	5.8
Thiamin	5.2
Vitamin E	13.0–14.0

Sources: Hickling, 2001; Bell, 1995

Table 11. Range in mineral composition of low erucic acid rapeseed meal (DM basis)

Mineral	Low erucic acid rapeseed meal [1]		
	Samples	**Mean**	***Range***
Calcium, %	589	0.74	0.49–0.99
Phosphorus, %	597	1.12	0.94–1.29
Magnesium, %	556	0.53	0.39–0.68
Potassium, %	557	1.28	1.11–1.46
Sodium, %	557	0.06	0.00–0.31
Sulfur, %	379	0.71	0.54–0.89
Chloride, %	137	0.12	0–0.27
Iron, ppm	553	243.02	56.85–429.19
Zinc, ppm	553	61.25	10.53–111.96
Copper, ppm	553	5.92	0–24.24
Manganese, ppm	553	64.06	15.25-112.86
Molybdenum, ppm	553	0.93	0.31-1.55

Source: Dairy One Cooperative Inc.

[1] Canola Meal, Dry Accumulated crop years: 05/01/2000 through 04/30/2010

49. The amino acid composition and ranges over all geographic locations of low erucic acid rapeseed seed and meal are given in Table 12. The amino acid composition of low erucic acid rapeseed meal compares generally very well with that of soybean meal. Soybean meal has higher lysine content and low erucic acid rapeseed meal contains more of the sulphur containing amino acids, methionine and cystine.

Table 12. Mean and/or range of amino acid composition of low erucic acid rapeseed seed and meal (% of DM basis)

Amino Acid	Fickler 2005				Bell *et al.* 1998			Newkirk *et al.* 2003		CCC 2009
	Low erucic acid rapeseed seed		Low erucic acid rapeseed meal		*B. napus* meal	*B. rapa* meal	*B. juncea* meal	NTCM [1]	TCM [2]	Low erucic acid rapeseed meal
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*
Alanine	0.86	*0.71–1.09*	1.54	*1.19–1.81*	1.70	1.75	1.88	1.74	1.71	1.57
Arginine	1.19	*0.93–1.55*	2.07	*1.37–2.65*	2.15	2.13	2.53	2.34	2.59	2.08
Aspartate + asparagine	-	-	-	-				2.90	2.83	2.61
Aspartic acid	1.48	*1.20–2.03*	2.50	*1.96–3.47*	2.68	2.73	3.02	-	-	-
Cystine	0.46	*0.32–0.52*	0.85	*0.58–1.13*	0.97	0.83	0.90	0.92	0.93	0.86
Glutamate + glutamine	-	-	-	-				6.45	7.13	6.53
Glutamic acid	3.23	*3.23–4.35*	6.11	*4.22–7.60*	5.92	5.60	6.02	-	-	-
Glycine	0.99	*0.82–1.29*	1.78	*1.36–2.07*	1.92	1.87	2.00	1.95	1.92	1.77
Histidine	0.53	*0.41–0.68*	0.96	*0.65–1.25*	1.03	1.01	1.12	1.24	1.21	1.12
Isoleucine	0.76	*0.62–1.02*	1.38	*1.02–1.62*	1.03	1.18	1.28	1.73	1.69	1.56
Leucine	1.34	*1.07–1.77*	2.46	*1.80–2.84*	2.47	2.50	2.69	2.80	2.76	2.54
Lysine	1.14	*0.96–1.50*	1.76	*1.13–2.36*	2.03	2.05	2.08	2.35	2.16	2.00
Methionine	0.38	*0.27–0.52*	0.69	*0.50–0.84*	0.79	0.76	0.75	0.77	0.81	0.74
Methionine + cystine	0.84	*0.64–1.19*	1.56	*1.11–1.97*				-	-	1.60
Phenylalanine	0.79	*0.64–1.07*	1.42	*1.06–1.70*	1.72	1.66	1.77	1.53	1.50	1.38
Proline	1.13	*0.85–1.53*	2.16	*1.43–3.19*	2.59	2.43	2.66	2.39	2.34	2.15
Serine	0.83	*0.69–1.12*	1.49	*1.16–1.87*	1.99	1.95	2.05	1.59	1.57	1.44
Threonine	0.86	*0.74–1.17*	1.51	*1.12–1.67*	1.40	1.49	1.54	1.74	1.71	1.58
Tryptophan	0.27	*0.20-0.37*	0.48	*0.35–0.58*	0.29	0.41	0.23	-	-	0.48
Tyrosine	-	-	-	-	1.14	1.07	1.14	-	-	1.16
Valine	0.99	*0.80–1.33*	1.77	*1.33–2.09*	1.33	1.49	1.57	2.18	2.1*b*	1.97

[1] NTCM = Non-toasted canola meal
[2] TCM = Toasted canola meal

SECTION III - OTHER CONSTITUENTS

A. Anti-nutrients and toxicants

50. Glucosinolates are considered anti-nutritional factors in low erucic acid rapeseed meal. On their own they are innocuous, but when cells of the seed are ruptured glucosinolates come in contact with myrosinase. The myrosinase enzyme hydrolyzes the glucosinolates releasing sulphur, glucose and isothiocyanates. The isothiocyanates are goitrogenic, reducing the ability of the thyroid to absorb iodine (Downey, 2007). These metabolites of glucosinolates can affect animal performance and can be toxic to the liver and kidneys (Tripathi and Mishra, 2007). Heating during processing of the meal eliminates most of the myrosinase, but is not completely effective in eliminating the effects of glucosinolates because some intestinal microflora also produces myrosinase (Tripathi and Mishra, 2007). Isothiocyanates are bitter compounds, and can also reduce palatability. Mean levels of glucosinolates in seed and meal are presented in Table 13.

Table 13. Mean levels of glucosinolates of low erucic acid rapeseed seed and meal (µmol/g)

Toxicant	Newkirk *et al.* 2003		Bell 1995		Bell *et al.* 1998		
	NTCM [1]	TCM [2]	seed	meal	*B. napus* meal	*B. rapa* meal	*B. juncea* meal
Total Glucosinolates	26.0	31.0	38.42	21.06			
3-Butenyl	3.40	1.94	7.44	4.97	3.2	3.4	22.6
4-Pentenyl	0.67	0.38	2.55	1.67	0.4	2.6	1.7
2- Hydroxy-3-butenyl	6.28	3.64	13.44	8.82	7.4	6.7	3.5
2-Hydroxy-4-pentenyl	0.2	0.2	0.99	0.74	0.1	1.0	0.1
3-Indolylmethyl	0.58	0.22	0.63	0.38	1.1	0.2	0.1
4-Hydroxy-3-indoylmethyl	4.20	0.78	13.37	4.48	9.2	4.2	4.0
Contaminant Glucosinolates							
2-propenyl (allyl)	0.52	0.37	1.41	1.05	-	0.2	0.3
4-Hydroybenzyl	-	-	2.31	2.25	-	-	-

[1] NTCM = Non-toasted canola meal
[2] TCM = Toasted canola meal

51. Low erucic acid rapeseed contains several phenolic compounds. Sinapine is the choline ester of sinapic acid and is the principle phenolic compound found in low erucic acid rapeseed. Levels in the meal have been reported to be in the range of 0.7-1.1% for North American and European plant varieties (Kowslowska *et al.*, 1990), and 1.5% in Australian varieties (Bonnardeaux, 2007). Sinapine is converted into trimethylamine by intestinal microflora that is then absorbed. Most animals have the ability to convert the trimethylamine to trimethylamine oxide, a compound easily excreted. However, some animals, in particular laying hens, cannot readily catabolise trimethylamine, resulting in higher than normal levels in tissues and eggs, imparting a fishy odour and flavour.

52. Tannins are more complex phenolic compounds that can bind proteins and some complex carbohydrates and can reduce digestibility. Levels in low erucic acid rapeseed are typically 1-3% (Kozlowska *et al.*, 1990). Some analytical methods include the simpler phenols, such as sinapine, and may therefore overestimate the amounts of tannins (Kozlowska *et al*., 1990).

53. Phytic acid (known as inositol hexakisphosphate (IP6), or phytate when in salt form) is the principal storage form of phosphorus in many plant tissues. Because of phytic acid binding capabilities, bio-availability of phosphorus from low erucic acid rapeseed is less available for monogastric animals because they lack the digestive enzyme, phytase, required to separate phosphorus from the phytate molecule. Phytic acid has also strong binding affinity to important minerals such as calcium, magnesium, iron, and zinc and thus reducing the absorption of these minerals.

54. Anti-nutrient levels in low erucic acid rapeseed meal as a percent of oil-free meal are shown in Table 14.

Table 14. Anti-nutrients of low erucic acid rapeseed meal (% of oil-free meal)

Anti-nutrient	Bell 1995	CCC 2009	Kozlowska *et al.*, 1990	Bonnadeaux 2007
Tannins	1.5	1.5	1-3	
Sinapine	0.7–3.0	1.0	0.7-1.1	1.5
Phytic acid	2.0–5.0	3.3		

B. Allergens

55. There are several published studies reporting sensitivity and allergenicity of adults to *Brassica* species, however, most describe rare cases of respiratory symptoms due to occupational exposure (Suh, 1998; Alvarez, 2001), or residence in proximity to areas of intense canola cultivation (Trinidade, 2010). Discussion of occupational exposure is outside the scope of this document. There are also published studies investigating the potential for *B. rapa* and *B. napus* to be food allergens in children. In one report, 1887 children presenting primarily with atopic dermatitis (a symptom frequently associated with food allergy) were screened for *Brassica* sensitivity in a skin prick test, of which 206 (10.9%) tested positive (Poikonen *et al.*, 2006). Allergic reaction was confirmed in 89% of these cases by oral challenge (lip swab and ingestion) with crushed seeds of *B. rapa* (*ibid.*). It was also observed that sensitization to canola in children is associated with multiple allergies to other foods and pollens (Poikonen *et al.*, 2008), and monosensitive patients are very rare. Parallel studies identified the likely major IgE-reactive antigens in seeds (Puumalainen *et al.*, 2006) and characterized potential cross-reactivity with related mustard plants, which are known food allergens (Poikonen *et al.*, 2009). Because protein is either at very low levels or absent in low erucic acid rapeseed oil, the significance of the results of these allergenicity studies in determining the safety of consumption of low erucic acid rapeseed oil by the general population is likely low (Gylling, 2006). Food allergy to low erucic acid rapeseed oil has not been reported in the scientific literature.

SECTION IV – SUGGESTED CONSTITUENTS TO BE ANALYSED RELATED TO FOOD USE

A. Low erucic acid rapeseed oil

56. Globally, low erucic acid rapeseed oil has the potential to help consumers achieve dietary goals because it has the lowest concentration of saturated fatty acids (7% of total fatty acids) of all oils commonly consumed globally.

57. The successful reduction in erucic acid content has led to continued interest in compositional modifications to low erucic acid rapeseed oil. Subsequent mutagenesis of low erucic acid rapeseed led to the development of low erucic acid rapeseed oil with the linolenic acid content reduced from approximately 10% to less than 3%. Although high levels of linolenic acid are desirable from a nutritional point of view, they are undesirable in terms of chemical stability. High levels of polyunsaturated fatty acids lead to oxidative rancidity, a reduction in shelf life of the oil, and the development of off-flavours and odours after prolonged storage or repeated frying use (Przybylski *et al*., 2005). Reducing the level of linolenic acid also reduces the need for partial hydrogenation of edible oils used in the liquid form.

58. Other recent developments in low erucic acid rapeseed oil include the application of mutagenesis to produce high levels of oleic acid (*i.e.* from 60% to 75% total fatty acid content). The resulting high oleic acid producing cultivar was then crossed to low-linolenic cultivars to create high oleic/low linolenic lines. High oleic oils resemble the fatty acid composition of olive oil more closely than that of traditional low erucic acid rapeseed. Recombinant DNA technology has been applied to increase the levels of lauric (39%) and myristic acids (14%) in low erucic acid rapeseed oil. These oils have been developed for use in confectionery coatings, coffee whiteners, whipped toppings, and centre filling fats. Low erucic acid rapeseed oil with stearic acid levels as high as 40% are being developed as replacements for hydrogenated fats in baked products. Oils with approximately 10% palmitic acid levels that result in improved crystallization in margarine products have also been developed and are being marketed in North America, Europe and Asia. These oils have also been developed through the use of recombinant DNA technology (Przybylski *et al*., 2005).

B. Recommendation of key components to be analysed

59. For human nutrition, it is important to assess the fatty acid composition, vitamin E and vitamin K_1 content of the oil. Constituents to be analysed are suggested in Table 15. Because low erucic acid rapeseed meal may be used in the production of protein isolates, key nutrients in the protein fraction would include protein and amino acid composition, both of which could be analysed in either seed or meal. Because there are several different processes that may be used to produce canola protein isolate (Tan *et al.*, 2011), compositional analysis of the seed or meal may be of greater utility than compositional analysis of specific individual protein isolates.

60. The complete fatty acid profile (including C6:0 to C24:0) should be quantified in low erucic acid rapeseed oil for the purpose of compositional comparison between a modified low erucic acid rapeseed and appropriate comparators (*e.g.* commercial low erucic acid rapeseed varieties).

Table 15. Suggested constituents to be analysed in low erucic acid rapeseed for human food

Constituent	Seed or meal	Oil
Crude protein [1]	X	
Crude fat [1]	X	
Ash [1]	X	
Amino acids	X	
Fatty acids [2]	X	X
Vitamin K_1 [2]	X	X
Vitamin E [2]	X	X
Glucosinolates	X	
Tannins	X	
Sinapine	X	
Phytic acid	X	

[1] These components should be measured using a method suitable for the measurement of proximates.
[2] Measurement of this component can be conducted in seed and/or oil.

SECTION V – SUGGESTED CONSTITUENTS TO BE ANALYSED RELATED TO FEED USE

A. Low erucic acid rapeseed for feed

61. Low erucic acid rapeseed is used as a protein source for all classes of livestock, poultry and fish. The protein content of the meal is lower than that found in the meal from other oilseeds such as sunflower or soybean, because the hull of the low erucic acid rapeseed is typically not removed. Consequently, the fibre content is higher than in other oilseed meals. Low erucic acid rapeseed oil is frequently used to increase the energy density of diets, and to improve palatability by reducing dust. Low erucic acid rapeseed oil would be used at 3-10% of the total ration, depending on the animal species.

62. Low erucic acid rapeseed meal is often blended with other sources of protein in feed ration balancing schemes. The meal is recognized as an excellent source of methionine and cystine, but contains less lysine than soybean meal. The digestibility of amino acids from low erucic acid rapeseed meal by pigs and poultry tends to be in the 75–85% range, about 10% lower than soybean meal (Hickling, 2001).

63. Processing methods in countries like Canada are reasonably standard (Hickling, 2005), and there is little variation in the amount of oil in low erucic acid rapeseed meal. However, this can be more variable in some parts of the world (Van Barneveld and Ed-King, 2002) and higher oil levels dilute the amounts of other nutrients in the final product. There may also be varietal and environment-influenced differences in the protein content of seeds. It is therefore advisable to routinely analyse low erucic acid rapeseed meal for fat and crude protein.

64. In most countries, a maximum fibre level in the form of acid detergent fibre (ADF) and neutral detergent fibre (NDF) is stated for finished feed products. Low erucic acid rapeseed meal can make a significant contribution to the fibrousness of feeds, particularly for non-ruminants, and can be the limiting factor regarding rate of inclusion in diets. Fibre analyses may be required if levels must meet a guarantee.

65. The mineral and vitamin composition of low erucic acid rapeseed meal is comparable to the mineral composition of other oilseeds. Minerals and vitamins are often added to livestock diets in stock quantities as premixes or base mixes, which de-emphasizes the minerals and vitamins in the meal. One exception is phosphorus. The phosphorus in low erucic acid rapeseed meal is only about 30–50% available, due to the presence of phytic acid.

B. Recommendation of key nutrients and anti-nutrients to be analysed

66. Proximate and fibre (acid detergent fibre and neutral detergent fibre) analyses are generally used by animal nutritionists to evaluate feed ingredients and to formulate least cost rations for livestock, poultry and fish. Protein, fat and fibre are the key indicators of livestock feed quality. Amino acids and digestibility must also be considered when formulating rations based on low erucic acid rapeseed meal. The amino acid profile is a key indicator of protein quality. It is additionally advisable to provide analytical results for calcium and phosphorus, as shown in Table 16.

Table 16. Suggested constituents to be analysed in low erucic acid rapeseed for feed use

Constituent	Seed or meal	Oil
Crude protein [1]	X	
Crude fat [1]	X	
Ash [1]	X	
Amino acids	X	
Fatty acids [2]	X	X
Acid Detergent Fibre	X	
Neutral Detergent Fibre	X	
Calcium	X	
Phosphorus	X	
Tannins	X	
Glucosinolates	X	
Sinapine	X	
Phytic acid	X	

[1] These components should be measured using a method suitable for the measurement of proximates.
[2] Measurement of this component can be conducted in seed and/or oil.

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