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.*

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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

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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).

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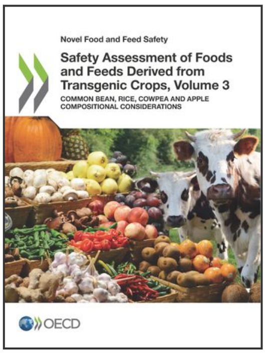

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