

Apple Pomace as Potential Source of Natural Active Compounds*

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ABSTRACT

Apple pomace is a waste product of the apple manufacturing industry that has been in the focus of life sciences as it represents a low-cost source of fruit-derived compounds. High fruit consumption is associated with beneficial health effects, and therefore, apple pomace and its constituents raise therapeutic interest. The present work reviews (i) the chemical constituents of apple pomace, (ii) optimized extraction methods of apple pomace compounds, and (iii) biological activities of apple pomace. Current evidence of apple pomace influence on digestion and metabolism, cholesterol and triglyceride homeostasis, diabetes, and sex hormones is summarized. Furthermore, studies regarding its antioxidative, anti-inflammatory, antiproliferative, antibacterial and antiviral effects are presented. The review concludes that apple pomace is an underutilized waste product of the apple industry with the potential of being processed for its nutritional and pharmaceutical value.

Introduction

The average annual apple harvest of the European Union is about 10 million tons, with Poland, France, Italy, and Germany as the most productive countries [1]. The majority of the harvest is represented by table apples, which are used for direct consumption or juice production, while tannin-rich cider apples make up 15–40% of the harvest [2].

Apple pomace is an industrial waste product of apple manufacturing companies. Wet pomace generated by juicing and cider pressing represents up to 25% of the fresh fruit weight. In 2014, more than three million tons of apples were used for processing [3], of which about 800 000 tons of apple pomace might have accrued. Therefore, profitable ways of disposal or a value added use is of economic and environmental interest.

Scientists of various disciplines have been addressing the subject of apple pomace and a broad research field has developed over the years. This review focuses on the life science aspects of apple pomace research and on literature published in the past five years. In particular, the chemical composition of apple pomace

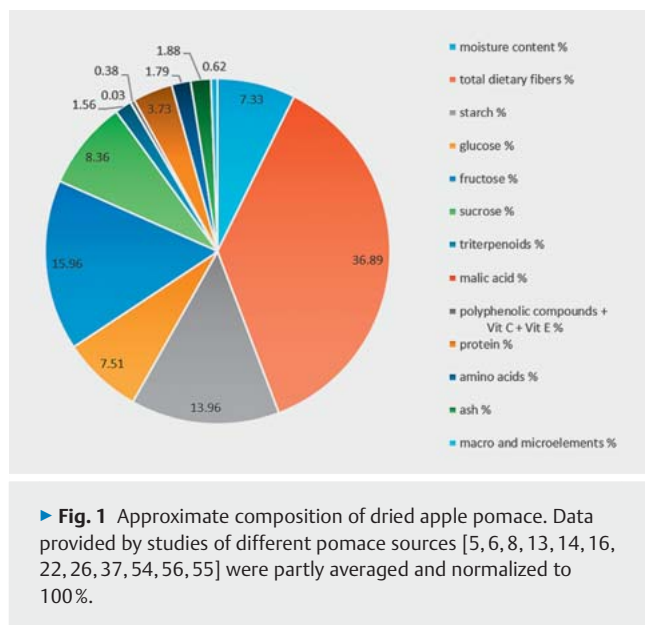
and biological activities mediated by apple pomace extracts and isolated compounds are reviewed.

Composition of Apple Pomace

Chemical constituents

Apple pomace consists of apple peels, leftover flesh, core with seeds, and stems. The moisture content after pressing is about 70–85% [4–7] and makes the pomace prone to microbial infestation. This can be used in the field of solid-state fermentation but should be avoided when intended for other applications. A reduction of water content to less than 10% should be performed immediately to sustain adequate pomace quality and storage stability [8]. Fresh apple pomace showed the highest moisture content of five investigated fruit pomaces but the shortest drying period to reach equilibrium moisture content. The drying kinetic follows the mathematical models of Midilli et al. or Page et al. [4,9]. Dry-

* This study is dedicated to Professor Dr. Max Wichtl in recognition of his outstanding contribution to pharmacognosy research.



ing rates linearly increase with the reduction of pomace layer thickness and temperature rise. The latter also leads to a reduction of equilibrium moisture content at the endpoint of the drying process [9]. These variations influence the composition and number of chemical constituents in apple pomace such as apple pectin [10] and selected polyphenols [8]. However, the originating apple cultivar and seasonal or environmental conditions during apple growth are the major factors that mediate the presence and content of compounds in apple pomace [11, 12]. After drying, the moisture content is around 10% [13, 14] but can be up to 20% [15], depending on the drying method.

The approximate composition of dry apple pomace is shown in **Fig. 1** and includes, dietary fibers, starch, glucose, fructose, sucrose, triterpenoids, malic acid, polyphenolic compounds, vitamin C, vitamin E, proteins, amino acids, macro- and microelements, and ash.

The chemical structures of compounds are presented in **Figs. 2–4**.

Dietary fibers

The major part per weight of dry apple pomace is made up by components that are indigestible for the human enzyme system and are therefore called dietary fibers (**Fig. 1**). The analysis of pomaces from 11 apple cultivars showed that the average content was 43.6% [16]. According to their dissolution behavior in water, dietary fibers are divided into soluble dietary fibers and insoluble dietary fibers [17]. Two-thirds of the apple's fibers refer to the insoluble group [16], which comprises cellulose (β -1,4-glycosidic linked glucoses), hemicelluloses (xyloglucan, galactomannan and glucuronarabinoxylan), and lignin (polymerized coniferyl, sinapyl and *p*-coumaryl alcohols) [17].

Apple pectin (**Fig. 2**), the main component of the soluble dietary fibers, is a three-dimensional macromolecule for which a smooth chain-hairy chain structure model was developed [18].

The smooth chains consist of homogalacturonan (**Fig. 2 [1a]**), built up from up to 100 α -1,4-glycosidic linked D-galacturonic acid moieties that could be methyl-esterified at C-6 or acetylated at C-2 and/or C-3. Xylogalacturonan (**Fig. 2 [1b]**), rhamnogalacturonan I (**Fig. 2 [1c]**), and rhamnogalacturonan II (**Fig. 2 [1d]**) form the hairy regions of the macromolecule. These are predominantly located at the start and the end of the smooth chains. Structural elements polymerize, as the free carboxyl groups of the galacturonic acid moieties from different chains could form salts with divalent cations or uronyl esters with a hydroxyl moiety from a different chain. Furthermore, free hydroxyl groups may form esters with 5,5'-diferulic acid or boric acid as a polymerizing step. Thus, the amount of methyl-esterification and the number of hairy chains, as well as the degree of polymerization, lead to differences in molecular weight and determine the three-dimensional expansion of the pectin macromolecule. This results in the varying physical properties known for apple pectin [19]. The increase of the grade of methylesterification requires more acid to be added for gelling. This in turn results in a gel that is more stable against thermal influences [20]. In the intact fruit, pectin is predominantly bound to other cell wall structures such as cellulose and hemicelluloses via calcium salts. For pectin extraction, these bindings must be cleaved, and thereby, the macromolecule partially degrades. Adapted extraction and purification conditions provide pectin with designated physical properties (e.g., swelling capacity or oil retention) [21].

Starch

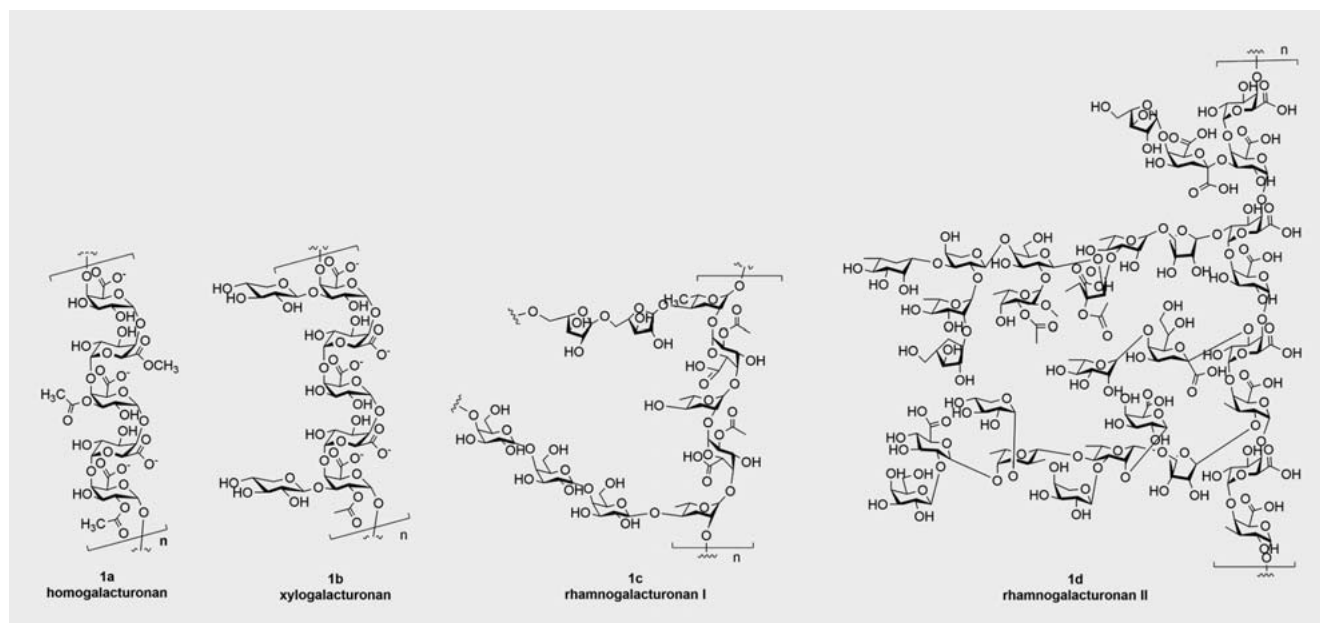
Beside indigestible glycosidic polymers, apple pomace contains about 14% starch [22]. Unripe apples have higher starch contents, which then degrade during apple maturation, increasing the sweetness and softness of apples [23]. This explains the low content of intact starch in apple pomace. The apple industry monitors the starch index for the determination of the optimal harvest time for each individual apple cultivar [24]. Apparently, apple starch is built up from approximately 40–48% amylose [25].

Mono- and disaccharides

Mono- and disaccharides account nearly the same share per weight in apple pomace as the total glycosidic polymer fraction (**Fig. 1**). An investigation of pomaces from 26 cultivars observed proportions of 18–31% fructose, 3.4–24% sucrose, and 2.5–12.4% glucose per absolute weight of dry apple pomace [26]. Another study of 11 cultivars measured average contents of 12.7% glucose, 17.9% fructose, and 7.0% sucrose [16]. Sucrose was the most variable component in amount as the variation coefficient calculated from the contents in the 11 cultivars was 73.4%. A study using an NMR-based method for quantitative determination reported constant relative proportions in four cultivars, which was fructose > glucose > sucrose [27].

Volatile compounds

The typical apple aroma is made up of more than 250 volatile substances that have been identified in apples [28]. They reach peak concentrations prior to full maturation of the fruit and are mainly biosynthesized via β -oxidation of fatty acids, leading to the formation of alcohols and acyl-coenzyme A, a precursor for ester forma-



► **Fig. 2** Structural elements of apple pectin. Homogalacturonan (1a), xylogalacturonan (1b), rhamnogalacturonan I (1c), and rhamnogalacturonan II (1d).

tion. Alcohol dehydrogenase and aspartate amino transferase are further involved in volatile compound formation. A rupture of the plant cell wall releases the enzyme lipoxygenase, leading to an increase in the formation of C_6 and C_8 aldehydes from linoleic and linolenic acid. Apple crushing, juice pressing and to a lower extent the normal ripening process alters the volatile pattern as different substrates are provided to the enzymatic system [29].

In dry apple pomace, Madrera and Valles [30] identified 48 esters, 30 aldehydes and ketones, 19 terpenes and norisoprenoids, nine acids, eight alcohols, four lactones, and six compounds of other chemical structure by stir-absorptive extraction followed by GC-MS analysis. The comparison of pomace volatile fractions from five cultivars revealed that the total number and the ratio of the constituents are variable. However, the main component of each chemical group was the same for all cultivars: benzaldehyde for the group of aldehydes and ketones; decanoic acid for the group of acids; a farnesol isomer for terpenes; and ethyldecanoate for the group of esters and lactones. The chemical group of alcohols represented an exception: in four of the five cultivars the main compound was 1-octen-3-ol, while in one cultivar it was 1-hexanol. In intact apples, the volatile compounds are embedded in the three-dimensional matrix of cutin (► **Fig. 3** [2]). It consists of hardly dissolvable glycerol polyesters of hydroxylated or epoxy hydroxylated C_{16} and C_{18} fatty acids [31].

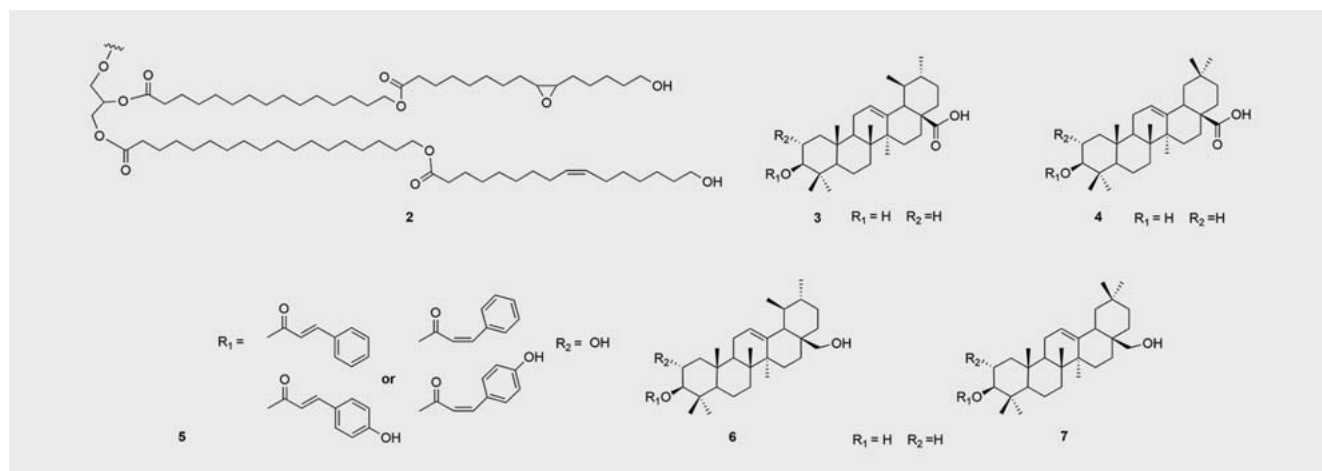
Triterpenoids

Triterpenoids are also located in the aforementioned layer of cutin. Ursolic (► **Fig. 3** [3]) and oleanolic acid (► **Fig. 3** [4]) are the main representatives but also numerous derivatives thereof (e.g., substituted with additional hydroxyl groups and *p*-coumaroyloxy- or cinnamoyloxy-moities (► **Fig. 3** [5])). In addition, their alcoholic derivatives have been isolated (► **Fig. 3** [6 and 7]) [32–36].

Although the number of structure-elucidated apple triterpenoids increases, their total quantity in apple pomace has hardly been investigated. Brieskorn and Klinger [33] reported that about 20% of the apple peel is extractable with petrol ether. The obtained extract contained 46% ursolic and oleanolic acid and at least another 24% of triterpenoid derivatives. Other authors postulated an extraction yield of pentacyclic triterpenoids from apple pomace of about 2% [37] or, more recently, of 7% by ultrasonic extraction with ethanol [38]. A quantification of 8 triterpenoid derivatives in frozen dried apple pomace resulted in a content of less than 0.01% [39]. These wide discrepancies are related to quantification and the different methods in use. Triterpenoid compounds only absorb around the wavelength of 200 nm, by which various compounds also have minor absorption rates. Thus, group determinations by UV spectrophotometry are less selective. Quantification by HPLC-UV/DAD only brings selectivity if the individual peaks can be accurately separated from other matrix peaks and identified (e.g., by LC-MS/MS). However, due to the existence of various structural isomers, a proper separation and identification is difficult. Derivatization could be a strategy for quantification. However, the accuracy depends on the completeness of the derivatization step. Therefore, the available data should be regarded as an estimation of absolute quantities.

Non-volatile acids

The non-volatile acids malic, citric, and quinic acid mediate the sour to astringent taste of apples [40]. Predominantly, gene expression of a malic acid transporter mediating malic acid uptake into the plant cell vacuole seems to influence the malic acid concentration in apples and consequently their acidity value. During juice production, the major content is transferred to the juice, from which malic acid was isolated for the first time by Carl Wil-



► **Fig. 3** Cutin constituents of apple present in apple pomace. Cutin (2), the triterpenoids ursolic acid (3), oleanolic acid (4), coumaroyl- and cinnamoyl derivatives of ursolic and oleanolic acid (5), uvaol (6), and erythrodiol (7).

helm Scheele in 1785 [41]. In apple pomace, an average amount of only 0.08% malic acid per absolute weight was detected in an investigation of 11 cultivars [26].

Polyphenols

Polyphenols, with the exception of lignin, are associated with the beneficial health effects mediated by fruits. Bitter-tasting cider apples, which are generally smaller and biennial, have higher total phenolic content than dessert apples, which are mainly used for direct consumption and juice production [42, 43].

By investigating 40 apple varieties, total phenolic contents between 66.2 and 211.9 mg/100 g were determined by the Folin-Ciocalteu method in fresh fruits. This demonstrates the wide range of polyphenolic content of different apple cultivars. The main compounds identified in the polyphenolic fraction were catechins and proanthocyanidins followed by hydroxycinnamates, flavonols, dihydrochalcones, and anthocyanins [43]. However, the number of single compounds varies up to 30% from one year to the other in the same cultivar [12].

Consequently, also in dry apple pomace, wide ranges of polyphenol contents were measured (e.g., 262 to 856 mg of total phenols/100 g or to a maximum concentration of only 350 mg/100 g, respectively) [16, 44]. The content of polyphenols per pomace weight increases compared to that per fresh fruit weight due to the water loss during pressing procedure. Polyphenols are partially transferred to the corresponding juices, but their predominant location is in the peel. Therefore, most polyphenols should remain in the pomace [45]. While all 13 compounds were detected in the peel, the recovery of some quercetin derivatives could neither be measured in the juice nor in the corresponding pomace indicating method deficiencies or degradation during manufacture. Other studies only measured the polyphenolic content of the apple juice and its pomace [44, 46]. The concentration of apple polyphenols in the pomace seems to be influenced by the method of juice production [44]. The polyphenol concentration was higher in the pomace obtained from clear juice production. However, allover polyphenol recovery was higher in the apple pomace from cloudy juice production as it accrues higher pomace mass. This suggests

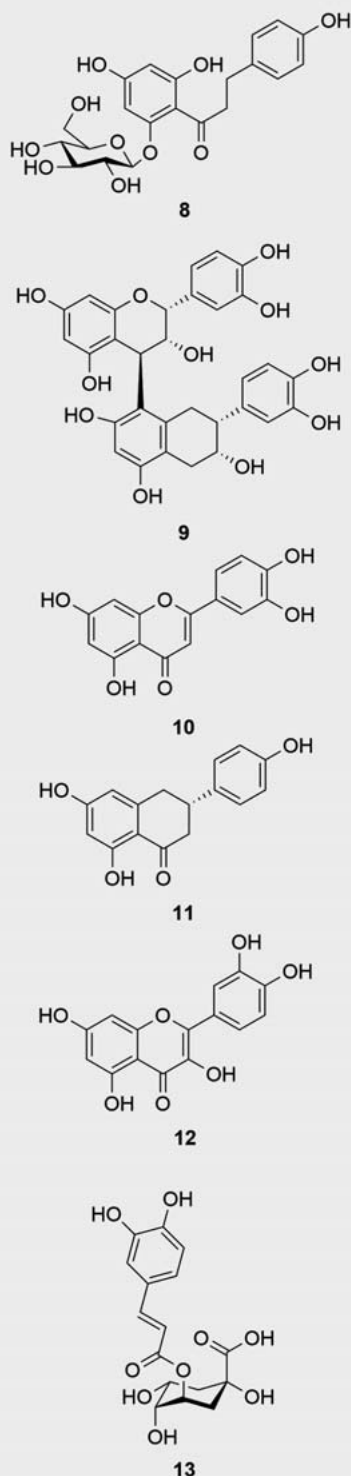
the binding of polyphenols to pectin or other cell wall structures [47]. Pectinases used for clear juice production cleave these bindings and increase the number of polyphenols available for extraction.

Extensive studies on the identification of apple pomace polyphenols were conducted [48, 49]. Detected compounds are listed in ► **Table 1**, and representatives from some structural classes are shown in ► **Fig. 4**.

In fresh apple pomace, the polyphenolic pattern resembles that of fresh apples, showing high contents of chlorogenic acid (► **Fig. 4** [13]), caffeic acid, (+)-catechin, (–)-epicatechin, rutin, and quercetin glycosides [50]. In contrast, phloridzin (► **Fig. 4** [8]) is the most prominent polyphenol in apple pomace after drying [51]. Phloridzin is absent in pomace obtained from other sources such as pears; thus, it is considered a good marker substance for dry apple pomace identification [52]. The method used for drying pomace has less impact on the content of polyphenols than the apple variety and the time to the onset of the drying process. Enzymes such as polyphenol oxidases and peroxidases are liberated from cell vacuoles or cell wall compartments during apple crushing and start the oxidation of o-diphenols to quinones, which then can condense with amino acid residues from proteins. These reactions are visible as a brown discoloration of the pomace after pressing [53]. Lavelli and Corti [8] revealed that phenolic compounds have highest stability in pomace samples with the lowest water activity. This supports the theory that enzymes mediate the degradation of polyphenols. Consequently, the most important step for polyphenol preservation in apple pomace is the immediate and effective drying process after pressing, which should inactivate the degrading enzymes. Gentle drying methods such as lyophilization additionally inhibit the formation of thermal degradation products such as 5-hydroxymethylfurfural from sugars [52].

Ubiquitous components

Macro- and microelements detected in apple pomace are sodium, potassium, calcium, phosphor and magnesium, iron, manganese, zinc, and copper with summed amounts of 0.79 g/100 g and



► **Fig. 4** Polyphenols of apple pomace. Phloretin-2-O-glucoside (phloridzin) (8), procyanidin B2 (9), apigenin (10), naringenin (11), quercetin (12), and 3-O-caffeoylquinic acid (chlorogenic acid) (13).

10.9 mg/100 g, respectively [54]. A protein content of 0.002–7.1% and an amino acid content of 2.3% were detected [16, 55]. The ash content varies from 1.5 to 4.0% [13, 16, 54]. Vitamin E

and vitamin C are present in amounts of 22.4 and 5.5 mg/100 g, respectively [54, 56].

Optimized extraction methods of selected apple pomace constituents

A growing number of apple pomace publications focus on the optimization of compound extraction methods. They intend to increase extraction efficacy or to reduce ecological or economical disadvantages entailed by conventional extraction methods. This chapter gives an insight into this field of research.

Pectin extraction

The major part of the extracted pectin goes into the nutritional and cosmetic industry as a gelling or thickening agent. Highly standardized or chemically modified pectin is used for drug delivery systems [57, 58]. Pectin can also be included in wound treatments due to its hemostatic effect [59]. Moreover, it showed potential to remove toxic heavy metals from human and animal biological systems [60].

The exploitation starts with a hot water-acid extraction, followed by the addition of ethanol to the up-concentrated extract, which leads to the precipitation of crude pectin. Acid treatments, filtrations, and washing steps lead to pectin with lower degrees of polymerization and esterification, which leads to high amounts of chemical waste. Therefore, alternative, environmentally friendly approaches of pectin extraction with high yields are of interest. Wang and Lu [61] used hot-compressed water extraction to replace the first extraction step performed with acids. The obtained pectin showed a lower viscosity rate, dry matter and protein content but higher ash and neutral sugar content.

Enzymatic extraction seems to be a more promising alternative. Commercially available enzyme preparations contain mixtures of cellulases and proteases. Under optimal conditions, enzymatic extraction delivers higher yields than acidic extraction [62]. They break down cell wall lamellae liberating the water-soluble pectin. Furthermore, the efficacy of pectin extraction was tested for single enzymes. For example, a polygalacturonase of *Aspergillus kawachii*, which was recombinantly won from *Saccharomyces cerevisiae*, showed 1.3-fold pectin extraction yield compared to chemical extraction. Yields of other commercially-available polygalacturonases were below the amounts reached by chemical extraction, but all enzymatically-extracted pectin had a higher degree of esterification [63]. Further studied enzymes are hemicellulase [64], xylanase and cellulase [65] with the yield ranking of xylanase > hemicellulase > cellulase. Although one would expect that the products combining several enzymes deliver higher extraction yields, xylanase reached the maximum result of about 19%. The combination cellulase and xylanase decreased the pectin extraction yield [65].

To sum up, enzymatic pectin extraction depends on the individual enzyme activity. This may imply minor batch-to-batch variations of production yields, but it diminishes the use of strong acids and is able to produce pectin with favorable physical properties. Nevertheless, industrial application is limited by economic reasons, as extraction time increases up to six-fold.

Another way of optimizing pectin yield or quality is the modification of the precipitation step. While ethanol precipitates pectin

► **Table 1** Polyphenols identified in apple pomace [48, 49].

Compound class	Compound name	Number of not identified isomers
Dihydrochalcones	phloretin, phloretin-2-O-glucoside, phloretin-2-O-xylosyl-glucoside, phloretin-pentosyl-hexoside, phloretin-hexosyl-hexoside, 3-hydroxyphloretin-2'-O-xylosyl-glucoside, 3-hydroxyphloretin-2'-O-glucoside	2
Flavanols	(+)-catechin, (-)-epicatechin, procyanidin B1, procyanidin B2, procyanidin B3, procyanidin B5, procyanidin C1, ((epi)cat) <i>trimer</i>	6
	((epi)cat) <i>tetramer</i>	7
Flavons	apigenin, chrysoeriol, luteolin, luteolin-7-O-galactoside, luteolin-7-O-glucoside	
Flavanons	eriodictyol, eriodictyol-hexoside, hesperidin-O-pentoside, naringenin, naringenin-7-O-glucoside, naringenin-7-O-neohesperidoside, naringenin-7-O-rutinoside, naringenin-O-glucuronide, naringenin-O-hexoside	4
Flavonols	isorhamnetin-3-O-galactoside, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-arabinopyranoside, isorhamnetin-3-O-arabinofuranoside, isorhamnetin-3-O-rhamnoside, kaempferol-O-glucoside, quercetin, quercetin 3-O-diglucoside	
	quercetin-3-O-arabinofuranoside, quercetin-3-O-arabinopyranoside, quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, quercetin-3-O-rutinoside, quercetin-3-O-xylanoside, quercetin-O-hexoside, quercetin-O-pentosyl-hexoside, quercetin-O-pentoside, quercetin-O-xylosyl-pentoside, rhamnetin, rhamnetin-3-O-glucoside	
Hydroxycinnamic acids	caffeic acid-O-hexoside	2
	3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, x-O-caffeoylquinic acid, dicaffeoylquinic acid	8
	ferulic acid-O-hexoside	2
	p-coumaric acid-O-hexoside, 4-O-p-coumaroylquinic acid, 5-O-p-coumaroylquinic acid, p-coumaroylquinic acid, sinapic acid-O-glucoside	3
Anthocyanins	cyanidin-3-O-galactoside, cyanidin-3-O-hexoside	
Other	protocatechuic acid, salicylic acid	

quantitatively, but not selectively, sodium caseinate targets charged pectin of high molecular weight [66]. However, ethanol precipitation is still regarded as state of the art.

Microcrystalline cellulose

Microcrystalline cellulose is used as an excipient in the pharmaceutical and food industry. Guo and Luo [67] reported the optimum conditions for the isolation of microcrystalline cellulose from apple pomace: 6% hydrochloric acid, a solid-liquid ratio of 25, and a concentration of 2.5 mL sodium hypochlorite per 100 mL extraction mixture.

Dietary fiber

Instead of isolating a specific fiber type from apple pomace, the native apple pomace or the residue from pectin extraction, the so-called apple pomace fiber, can be used. It can be directly added to dietary products to modify the fiber content or the physical properties.

Ultra-fine pulverization of dry apple pomace fiber influences its physical properties, such as water solubility, swelling capacity, and product lightness, as well as its pharmacological effects such as cholesterol adsorption, which enhances, while fatty acid and bile acid absorption, water binding capacity, and cation exchange capacity lowers [68, 69]. Furthermore, the effect on dietary fibers by bleaching (H₂O₂-alkaline process) and desugaring (water ex-

traction at room temperature) was investigated [69]. Bleaching resulted in yellow cellulose-rich fiber concentrates with increased water-holding and swelling capacity. The same changes were observed for the desugared pomace.

Polyphenols

The extraction of polyphenols is a highly investigated field due to their low concentration in pomace. Sophisticated methods are needed to provide sufficient extraction yields.

The first factor to influence the extractable number of polyphenols is the drying process. Vacuum drying is generally regarded as a better alternative to oven drying as it preserves higher polyphenol contents [6]. However, this is not the case for anthocyanins and flavanols [8]. It seems that the drying efficacy has a higher impact on polyphenol stability than the drying method.

Polyphenol recovery further depends on the extraction solvent and the extraction technique. Investigations of the latter, in particular, lead to controversial results in the literature. ► **Table 2** lists extraction yields obtained with different extraction techniques on the basis of total phenolic contents in milligrams per 100 grams of pomace weight, determined by the Folin-Ciocalteu method.

Independently from the extraction technique, organic water mixtures provide extraction yields superior to pure water. However, when high amounts of raw material are intended for extraction, the latter should be preferred for environmental reasons. An

► **Table 2** Extraction techniques for polyphenols from apple pomace.

Technique	Optimized condition	Total phenolic content (dry weight) ¹	Reference
Stirring	acetone 65%, 60 min, 25 °C solid/liquid ratio (g/mL): 1 : 100	1415 mg GAE/100 g	[141]
	water, 37 min, 100 °C solid/liquid ratio (g/mL): 1 : 100	834.1 mg GAE/100 g	[142]
Shaking	water, 30 min, 85 °C solid/liquid ratio (g/mL): 1 : 20	118.6 mg GAE/100 g	[143]
Ultrasound	ethanol, 10 min, 65 °C, 503 W solid/liquid ratio (g/mL): 1 : 30	453 mg/100 g	[144]
	water, 40 °C, 0.764 W/cm ² solid/liquid ratio (g/mL): 1 : 6.7	551.7 mg CAE/100 g	[145]
	ethanol 50%, 45 min, 40.1 °C, 0.142 W/g solid/liquid ratio (g/mL): 1 : 6.7	971.3 mg CAE/100 g	[146]
Microwave	ethanol 62.1%, 53.7 s, 650.4 W solid/liquid ratio (g/mL): 1 : 22.9	62.7 mg GAE/100 g	[147]
	ethanol 60%, 149 s, 735 W solid/liquid ratio (g/mL): 1 : 10.3	1580 mg GAE/100 g	[148]
Subcritical fluid extraction	CO ₂ + ethanol 20%, 40 min, 54.6–57 MPa, 55.7–58.4 °C solid/liquid ratio (g/mL): 1 : 3	47 mg GAE/100 g	[149]

¹ expressed either as catechin equivalent (CAE) or gallic acid equivalent (GAE)

alternative approach to increase the polyphenol yield from apple pomace is fermentation with *Phanerochaete chrysosporium*. This fungus emits ligninolytic enzymes and β -glucosidase, liberating polyphenols bound to cell wall structures and increasing the extractable number of polyphenols [70]. Comparable to the use of pectinases during the production of clear apple juice, the addition of isolated enzymes such as cellulase leads to an increase in extractable polyphenols from apple pomace [71]. Polyphenols can also be gained by the use of β -cyclodextrin solutions [72] or resins [73] added to apple pomace slurries. Via a combination of empirical and statistical analysis, optimized conditions can be determined for every extraction technique. Based on the available literature, none of the presented techniques could be identified as superior. On an industrial scale, the selection of the extraction method depends on the amount of material intended for extraction.

Biological activities

Ethnopharmacological studies indicate the usage of apple against several ailments. Teas or decocts containing dried apple fruits or apple peels have been used as antitussives [74, 75]. Apple vinegar intake was intended to “clean the kidneys” and to treat indigestion and arthritis. The vinegar has also been used externally against burn, wasp stings, and headache. The fresh fruit has been consumed for the treatment of constipation [76].

More recently, epidemiological and intervention studies emphasize that apple consumption leads to beneficial health effects [77, 78], while those of apple juice consumption are controversially discussed [79, 80]. This leads to the assumption that the pomace could contain especially those apple constituents that mediate the beneficial health effects. This section summarizes

the health-promoting properties of apple pomace and its constituents.

Digestion and metabolism

Back when farmers used to produce small amounts of apple juice from their own orchards, the pomace was commonly fed to the animals on the farm in addition to their normal diets. With the increasing livestock of fattening animals, apple pomace soon became an inferior feed due to its low protein content, although the up-scale to industrial production of apple juice provides tons of low-price apple pomace every year. Consequently, it has been mainly fed to deer held in hunting reserves. Following the aforementioned hypothesis that the consumption of apple residues may mediate beneficial health effects, research efforts have been expanding to the topic of human consumption of apple pomace.

Several animal and human intervention studies measured a decreased pH of cecal and colonic digesta and the feces after the ingestion of native apple pomace and preparations thereof. This is the result of an enhanced production of short-chain fatty acids from the gut microbiome during apple pomace consumption [55, 79, 81–83]. Ethanol-extracted and native apple pomace decreased the intestinal pH [55]. The fiber content seems to be mainly responsible for the effect. Fibers could act as prebiotics favoring the growth of saccharolytic bacteria and resulting in an adaptation of the microflora to the new substrate availability during the apple pomace diet. Indeed, this was measured in feces samples of weaning pigs. A supplementation of the control diet with 3.5% of apple pomace led to enhanced rates of *Lactobacilli*, while *Streptococci* and *Enterococci* numbers were tentatively reduced [84].

Concurrently, the relative proportions of fatty acid composition changes during apple pomace diet. In Wistar rats the number of intestinal branched-chain fatty acids decreases, while the number of total short-chain fatty acids increases (except propionic and valeric acid) [55]. Other studies also postulated the increase of acetic, propionic, and butyric acid content during apple pomace diet [85,86]. The consumption of apple pomace with reduced polyphenol content led to the most effective reduction of intestinal isobutyric acid and isovaleric acid concentration [85].

A human comprehensive cross-over study with a treatment duration of four weeks tested the intake of either whole apples, clear or cloudy apple juice, or 22 g of apple pomace per day. The study revealed lower intestinal amounts of 3-hydroxybutyric acid, 2-hydroxy-3-methylbutyric acid, and α -ketoisovaleric acid [87]. Recently, isobutyric and isovaleric acid were associated with a reduction of cyclic adenosine monophosphate (cAMP)-mediated lipolysis and insulin-stimulated *de novo* lipogenesis, as well as the potential to increase insulin-dependent glucose uptake [88]. The reduction of branched short-chain fatty acids could be interpreted as a negative effect of apple pomace consumption. However, the same study emphasized the higher potential of non-branched short-chain fatty acids to mediate these aforementioned effects.

Propionic and butyric acid are associated with the prevention of metabolic syndrome and diabetes, and high intestinal concentrations are inversely correlated to inflammatory bowel disease [89,90]. The influenced physiological mechanisms could be multivariant. First, when absorbed by colonocytes, they serve as an energy source stimulating mucosa production, which results in an improved gut barrier function against pathogens. They play a role in gut bacterial cross-feeding mechanisms, PPAR γ down-regulation, differentiation of T-cells, and gut hormone concentration regulating the appetite [89].

Dietary fibers, pectin in particular, seem to increase the non-branched short-chain fatty acid concentration in the gut. However, an impact of polyphenols that are embedded or bound to dietary fibers can also be hypothesized [55,85,86].

A further indicator of the nutritional influence from apple pomace on the bacterial milieu of the intestine is the detection of bacterial enzymes in the cecal digesta. Two studies investigated the effect of apple pomace diet on the level of intestinal α - and β -glucosidases, α - and β -galactosidases, and β -glucuronidase in Wistar rats. While one study reported moderate increases of α - and β -glucosidases, as well as α - and β -galactosidases [85], a decrease of bacterial β -glucosidase activity and no significant effects on the other enzymes were observed by the other study [55]. β -glucuronidase was significantly decreased in all apple pomace intervention groups of both studies. This is associated with a reduced risk of colon carcinogenesis [91]. However, the extrapolation of these results from rat studies to the human body must be considered carefully, and further studies investigating the effects on the human microbiome activity should be performed.

Another observation during apple pomace intervention in rats was the increase of cecal N-excretion [55,81]. This occurred together with a decrease in urinary N-excretion and a tentative reduction of ammonia content in feces, which could be associated with a repression of proteolytic bacteria growth during apple pomace diet. The hypothesis is reinforced by the aforementioned

measured decrease of intestinal branched short-chain amino acid concentrations, which are metabolites of aromatic and branched-chain amino acids.

Furthermore, lowered rates of medium- and short-chain acylcarnitines, the storage forms of L-carnitine, which is *de novo* synthesized from the amino acids lysine and methionine, were measured [87]. The effect was significant during consumption of whole apples and apple pomace, but not for apple juices. Therefore, apple dietary fibers or pectin could be associated with the effect.

Ingestion of apple pomace influences not only bacterial colonization and bacterial enzymes but also the mucosal activity itself. For example, maltase and sucrase activity were lowered under apple pomace consumption [81]. *In vitro*, it could be demonstrated that pectin, isolated from apple pomace via citric acid or hydrochloric acid extraction, is a competitive inhibitor of the pancreatic lipase [92]. The resulting lipase-pectin complex degrades after approximately 30 min. While the administration of a pectin-lipase ratio of 2:1 did not prolong the effect, physicochemical properties make up a difference. The lipase-pectin complex formed with pectin isolated by citric acid extraction was more stable.

A positive side effect of apple pomace ingestion is the water-binding activity of apple fibers resulting in an enhanced cecal mass under unvarying or even smaller dry mass. This could be beneficial for peristaltic effects, and therefore, the apple pomace is tested not only for human nutrition and the feed of herbivorous animals, but also as an addition to, for example, dog feed [83]. Grinded stale apple slices have been traditionally used to treat diarrhea, especially in children. Preparations containing apple pectin and a chamomile extract showed clinical efficacy for the treatment of diarrhea in children [93]. The pharmacological mechanism may involve bacterial toxin adsorption by pectin and the aforementioned effects on gut microbiome and intestinal mucosal activity.

Cholesterol and triglycerides homeostasis

The most evident effect mediated by the consumption of apple pomace or preparations thereof is on cholesterol and triglyceride homeostasis.

Despite the existence of studies reporting a negative effect on serum total and HDL cholesterol level after apple pomace intake [81], studies postulating a positive effect prevail (► **Table 3**).

These positive effects are mainly associated with the soluble dietary fiber content of the pomace [94,95]. The mechanism of action may be an increase of cholesterol excretion and an inhibition of gastrointestinal reabsorption of primary bile acids [87]. This promotes cholesterol uptake, synthesis, and turnover in the liver, leading to a decrease of cholesterol serum levels [96]. The molecular mechanism behind the reduction of serum triglyceride levels may be explained via the aforementioned inhibition of the lipoprotein lipase [92].

Growing evidence indicates that also the apple pomace polyphenols contribute to the positive effects [97]. The combination of apple pectin and a polyphenol-rich apple concentrate significantly decreased plasma cholesterol and triglyceride levels, as well as intestinal cholesterol absorption. Furthermore, liver cholesterol storage level lowered more significantly under the combined diet

► **Table 3** Rat studies investigating the effect of apple pomace intake on cholesterol- and triglyceride level.

Test model	Test regime		Effects on serum concentration or indicated biological activities (significance)	Reference
Sprague Dawley rat (8)	10 days Control (CP): 1% cholesterol and 0.25% sodium cholate, 5% cellulose; apple pomace powder (AP): 1% cholesterol and 0.25% sodium cholate, 5% apple pomace powder; insoluble dietary fiber (IDF): 1% cholesterol and 0.25% sodium cholate, 3.73% insoluble dietary fiber from apple pomace; soluble dietary fiber (SDF): 1% cholesterol and 0.25% sodium cholate, 1.27% soluble dietary fiber from apple pomace	SDF	↓ total cholesterol concentration (p < 0.05 vs. CP) ↓ liver cholesterol concentration (p < 0.05 vs. IDF)	[94]
Wistar rat (60)	40 days Control: basal diet; Cholesterol (Chol): basal diet + 3 g/kg cholesterol; Apple pomace fiber (AP): basal diet + 100 g/kg apple pomace fiber; Apple pomace fiber + cholesterol (AP+Chol): basal diet + 100 g/kg apple pomace fiber+ 3 g/kg cholesterol	AP + Chol	↓ total cholesterol concentration (p < 0.05 vs. Chol) ↓ LDL-cholesterol concentration (p < 0.05 vs. Chol) ↓ triglyceride concentration (p < 0.05 vs. Chol) ↓ total phospholipid concentration (p < 0.01 vs. Chol) ↑ HDL-phospholipid concentration (p < 0.05 vs. Chol) ↓ liver total cholesterol concentration (p < 0.0005 vs. Chol)	[95]
Wistar rats (male, 10)	21 days Control: basal diet; Apple pectin (PEC): basal diet + 5% apple pectin; Apple (cider) (PL): basal diet + 10% apple (cider, rich in catechin, epicatechin, procyanidins, chlorogenic acid, coumaroylquinic acid and phloridzin); Mixed (PEC+PL): basal diet + 5% apple pectin + 10% apple (cider)	PEC PL PEC + PL	↑ portal vein bile acids (p < 0.05 vs. control) ↓ fecal bile acid excretion (p < 0.05 vs. control) ↑ fecal cholesterol, coprostanol, total steroid excretion (p < 0.05 vs. control or trend) ↓ cholesterol absorption (trend) ↑ body weight gain (p < 0.05 vs. control) ↓ plasma triglyceride concentration (p < 0.05 vs. control) ↓ body weight gain (p < 0.05 vs. control) ↑ portal vein bile acids (trend) ↓ fecal bile acid excretion (p < 0.05 vs. control) ↑ fecal cholesterol, coprostanol, steroid excretion (p < 0.05 vs. control or trend) ↓ cholesterol absorption (trend) ↓ plasma cholesterol and triglyceride concentration (p < 0.05 vs. control)	[97]
Wistar rats (male, 48)	4 weeks Control: basal diet + water; Intervention group (IV): basal diet + apple pomace extraction juice	IV	↓ food intake after 2 weeks (p < 0.05 vs. control) ↑ liquid intake after 2 and 4 weeks (p < 0.001 vs. control) ↑ urine excretion after 2 weeks (p < 0.05 vs. control) ↑ urine excretion after 4 weeks (p < 0.001 vs. control) ↑ cecal, colonic, and fecal concentration of primary and total bile acids (p < 0.001 vs. control) ↓ cecal, colonic, and fecal concentration of secondary bile acids (p < 0.001 vs. control) ↑ (cecal) and fecal cholesterol, coprostanol, coprostanon, and (cholestanon) concentration (p < 0.001 vs. control or trend)	[98]
Wistar rats (male, 32)	4 weeks basal diet + cellulose (control) basal diet + natural apple pomace (AP); basal diet + ethanol-extracted apple pomace (containing 0.10% polyphenols) (APE); basal diet + ethanol and acetone extracted apple pomace (containing 0.01% polyphenols) (APA)	AP, APE, APA APE APA	↓ triglyceride concentration (p < 0.05 vs. control or trend) ↓ total cholesterol concentration (p < 0.05 vs. control or trend) ↑ HDL% of total cholesterol (p < 0.05 vs. control or trend) ↓ LDL-cholesterol concentration (ns) ↓ log (triglycerides/HDL cholesterol) (p < 0.05 vs. control or trend) ↑ triglyceride concentration (p < 0.05 vs. AP, APA) ↑ total cholesterol concentration (p < 0.05 vs. AP, APA) ↑ log (triglycerides/HDL cholesterol) (p < 0.05 vs. AP) ↑ HDL% of total cholesterol (p < 0.05 vs. AP, APE) ↑ log (triglycerides/HDL cholesterol) (p < 0.05 vs. AP)	[85]

continued

► Table 3 Continued

Test model	Test regime		Effects on serum concentration or indicated biological activities (significance)	Reference
Sprague-Dawley rats (32)	5 weeks Negative control: normal diet (ND); Positive control: high fat diet (HFD); Apple pomace intervention: HFD + 10% apple pomace (AP) Apple juice concentrate intervention: HFD + 10% apple juice concentrate (AC)	AP	↓ weight gain ($p < 0.05$ vs. ND and HFD) ↓ food intake ($p < 0.05$ vs. ND) ↓ total cholesterol concentration ($p < 0.05$ vs. HFD) ↑ HDL cholesterol concentration ($p < 0.05$ vs. ND and HFD) ↓ LDL cholesterol concentration ($p < 0.05$ vs. HFD) ↓ triglyceride concentration ($p < 0.05$ vs. ND and HFD) ↓ liver total cholesterol concentration ($p < 0.05$ vs. HFD) ↓ liver triglyceride concentration ($p < 0.05$ vs. HFD)	[15]
		AC	↓ weight gain ($p < 0.05$ vs. HFD) ↓ food intake ($p < 0.05$ vs. ND) ↓ total cholesterol concentration ($p < 0.05$ vs. HFD) ↑ HDL cholesterol concentration ($p < 0.05$ vs. HFD) ↓ LDL cholesterol concentration ($p < 0.05$ vs. HFD) ↓ triglyceride concentration ($p < 0.05$ vs. ND and HFD) ↓ liver total cholesterol concentration ($p < 0.05$ vs. HFD) ↓ liver triglyceride concentration ($p < 0.05$ vs. HFD)	
Wistar rats (male, 20)	2 weeks Semi-purified casein diet + cellulose fiber + maize starch (control); Apple fiber preparation: semi-purified casein diet + 6.7% dried apple pomace without seeds (AFP)	AFP	↑ total cholesterol (trend) ↓ HDL cholesterol (trend) ↓ HDL/total cholesterol ratio ($p < 0.05$ vs. control)	[81]
Wistar rats (male, 32)	34 days Control: basal diet + 50 g/kg cellulose apple pomace (AP): basal diet + 69 g/kg apple pomace	AP	↓ triglyceride concentration ($p < 0.05$ vs. control) ↓ total cholesterol concentration (<i>ns</i>) ↓ liver cholesterol concentration ($p < 0.05$ vs. control) ↓ liver fat (<i>ns</i>)	[150]

than under single apple dietary fiber diet. The reduction of serum triglycerides was significant only in the group receiving the polyphenol-rich apple preparation in this study. This indicates that soluble dietary fibers seem not to mediate the positive effects on cholesterol homeostasis alone and that polyphenols may have a major impact on the reduction of plasma triglyceride concentration.

A fiber- and polyphenol rich preparation from apple pomace, namely a juice obtained by the use of pectinases and cellulases, increased fecal and fecal neutral sterol and primary bile acid excretion [98]. Intervention groups in a rat study, receiving either native apple pomace, ethanol-, or ethanol-and acetone-extracted apple pomace showed significantly reduced blood levels of triglycerides and total cholesterol. The HDL proportion increased compared to a control diet group with cellulose as single fiber source. Most effective on triglycerides and total cholesterol level was the natural pomace diet, offering both soluble dietary fibers and polyphenols [85].

Sprague-Dawley rats under high fat diet either supplemented by 10% apple pomace or 10% apple juice concentrate showed decreased levels of blood triglyceride levels and an enhanced HDL cholesterol concentration. Additionally, body weight gain, white adipose tissue proportion, subcutaneous and epidermal adipocyte size, as well as subcutaneous fat pads size was smaller. All effects were more evident in the groups supplemented with apple pomace than in the apple juice concentrate group, which again in-

dicates that a combination of dietary fibers and other secondary metabolites may have additive or synergistic effects [15].

Forty male NIH mice were divided into a control group and four hyperlipidemia groups induced by intravenous injection of Triton WR-1339. Three of the intervention groups were additionally treated with fenofibrate, 200 mg or 400 mg of an apple pomace polyphenol extract per 10 g body weight for 7 days. In all treatment groups, serum triglyceride concentrations were lower. As in this study hyperlipidemia was induced by intravenous application, the protective effect mediated by apple pomace polyphenols cannot be based on an inhibition of triglyceride resorption. Indeed, the authors measured enhanced serum lipoprotein lipase activity, hepatic triglyceride lipase activity, and hepatic PPAR α mRNA level [99].

Current evidence is predominantly based on animal studies. In healthy human volunteers, effects were shown on plasma total and LDL cholesterol concentrations for a daily dose of 22 g of apple pomace, 550 g of whole apples, and 500 mL of cloudy apple juice, but not for 500 mL of clear apple juice [79]. Here as well, apple pomace showed the highest significance. Thus, it can be hypothesized that the limiting parameter for additive or synergistic effects of soluble dietary fibers and polyphenols is the polyphenol content. In pomace, polyphenol concentration per gram is higher than that in the same weight of whole apple due to the water loss.

However, one must consider the bioavailability as the limitation of their physiological effects. Decreased serum triglyceride level, total, LDL, and especially oxidized LDL cholesterol concen-

trations of a Portuguese apple cultivar has been associated with the apple polyphenols (–)-epicatechin, (+)-catechin, procyanidin B1, and β -carotene [100]. Good gastrointestinal bioavailability of procyanidins and their ability to bind HDL cholesterol was reported for *in vitro* digestion [101].

Indirectly, livestock animals under apple pomace diet could have beneficial effects on human blood cholesterol levels. Broiler chickens under apple pomace diet had significantly lower meat cholesterol as well as thiobarbituric acid reactive substances content [102].

Cardiovascular effects

Effects of apple pomace consumption on cardiovascular health have hardly been investigated. Long-term apple consumption has been associated with a reduction of coronary mortality [103], but instant effects on blood pressure by apple consumption are controversially discussed [104, 105]. An apple peel extract and the majority of its constituents, predominantly flavonoids and especially some quercetin derivatives, inhibited the angiotensin converting enzyme activity *in vitro* [106]. In a previous study, we demonstrated that a mixture isolated from apple pomace containing various triterpenic acids and quercetin is able to enhance the activity of the endothelial nitric oxide synthase in EA.hy926 cells [36]. Both of these molecular mechanisms are associated with vasodilation effects and, therefore, could be physiologically relevant.

Diabetes

Apple (pomace) consumption may counteract disorders in glucose metabolism at an early stage. It has been shown that after four weeks of daily apple or apple preparation intake the plasma concentration of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) declines [87]. Risen CMPF plasma levels have been measured in patients with gestational or type-2 diabetes and impaired glucose-tolerant patients, indicating that CMPF is a sensitive marker for disorders in glucose metabolism [107].

In an open-label, randomized cross-over study, six female test persons underwent an oral glucose tolerance test with and without the additional intake of 25 g apple pomace preparation [108]. The preparation from blanched unripe apples was served in powdered form. The maximum blood glucose concentration was on average two-fold lower in the apple pomace preparation intaking group. Concurrently, the urinary excretion of glucose increased 20-fold during the first 2 h after glucose intake and was still elevated five-fold after 2–4 h. Phloridzin and its metabolites phloridzin-2'-O-glucuronide, phloretin-O-glucuronide, and phloretin were detected in urine. The whole study was designed to achieve high intake concentrations of phloridzin which is a well-known specific inhibitor of intestinal and renal glucose uptake [109]. The elucidated molecular mechanism of this effect is the competitive inhibition of sodium-linked glucose transporter (SLGT) 1 and 2, of which SLGT-1 is predominantly located in the small intestine and SLGT-2 on the apical domain of the proximal convoluted renal tubule [110]. Due to this non-selective effect, isolated phloridzin could not be used as an antidiabetic drug, as it causes severe gastrointestinal side effects. Additionally, rapid deglycosidation by intestinal glycosidases lowers its oral bioavailability. In 2012, the

first selective SLGT-2 inhibitor, dapagliflozin, was registered in the European Union, which is structurally based on phloridzin. This led to a new class of antidiabetic drugs: the gliflozins [111].

Another potential target of phloridzin might be the mitogen-activated protein kinase 1 (MAPK1), according to computational studies [112].

Quercetin could also exhibit antidiabetic properties, especially in early-stage type-2 diabetes. Intraperitoneal injection of quercetin prevented pancreatic β -cells from damage caused by streptozotocin-induced diabetes in albino rats [113]. Additionally, the consumption of soluble dietary fibers such as pectin was associated with a reduction of blood glucose levels [114].

All of the mentioned compounds seem to be stable throughout the manufacturing processes of juice production and, therefore, could mediate physiological effects through pomace consumption. The apple pomace matrix seems to increase the tolerance against the negative effects of, for example, phloridzin. Consequently, efforts are taken to find new ways for the inclusion of apple pomace in the recipes of glucose-rich snacks in order to limit their postprandial blood glucose increase [115–117].

Anti-oxidant activity

Reactive oxygen species (ROS) play an important role in cell signaling cascades. They are highly reactive, and therefore, the concentration, duration, and cellular location of ROS are essential to prevent proteins, lipids, and nucleic acids from major damage. Prolonged imbalances in cellular ROS status are associated with the development of chronic and neurodegenerative diseases [118]. Lambs fed a fattening diet including the addition of 10.91% of fermented apple pomace showed higher plasma antioxidant activity and higher leukocyte counts than the control group. An observed positive side effect of the apple pomace diet was the enhanced growth of multilayered epithelium and enhanced dimensions of papillae [119].

Wistar rats on native apple pomace diet showed increased serum antioxidant capacity of lipid-soluble substance and an enhanced activity of superoxide dismutase in the hemolysate of erythrocytes [55]. These studies indicate that apple pomace contains compounds able to trigger physiological antioxidant effects.

The effect of apple juice, apple pomace, and apple peel extracts on the redox status and DNA damage of CaCo-2 cells was investigated *in vitro* [120]. The antioxidant activity of the extracts showed a linear correlation to the content of total low molecular weight polyphenols in the ORAC assay. In the TEAC assay, flavan-3-ols and procyanidins were most effective. The apple pomace extract achieved a 53% reduction of DNA strand breaks measured by the Comet assay and a potent reduction of intracellular ROS level under test concentrations of 1–10 μ g/mL and 1–30 μ g/mL, respectively. Concurrently, the concentration of GPx, a ROS-degrading enzyme that is controlled via the gene regulatory sequence antioxidant responsive element, decreased. The protective effects diminished and even changed to pro-oxidant conditions at higher tested concentrations. Based on these results, the authors hypothesize that nutritional polyphenol concentrations mediate protective effects on intestinal cells via an interaction with cellular ROS-regulating mechanisms. Despite the fact that *in vitro* ROS scavenging effects of phloretin, epicatechin, and an apple pom-

apple polyphenol fraction have been postulated, it is more likely that polyphenols induce an intracellular defense mechanism against ROS than have a direct scavenging effect on cellular ROS [14, 121, 122].

Apple pomace consumption leads to higher concentrations of uric acid/urate in the plasma of healthy volunteers than in volunteers under control diet [87]. In physiological concentrations, uric acid works as a selective plasma antioxidant for, for example, hydroxyl anions and hypochlorous acids or as an oxidizable co-substrate of endothelial enzymes [123]. Permanently elevated levels of uric acid, however, are associated with a growing risk to develop hypertension, gout, and chronic renal disease. The intake of fructose has been associated with an elevation of uric acid plasma levels. During fructose metabolism in the liver, the ketohexokinase catalyzes phosphates and the formed adenosine monophosphate (AMP) is degraded to inosine monophosphate and further to uric acid [124, 125].

Native apple pomace contains high concentrations of fructose. This side effect should be kept in mind if a diet supplementation with apple pomace is intended. However, theoretically, apple pomace products with reduced monosaccharide content and up-concentrated apple pomace polyphenols and fibers could be promising nutritional candidates for the prevention of chronic diseases induced by cellular ROS imbalances.

Anti-inflammatory activity

Polyphenol-rich fractions from an apple pomace ethanol extract significantly reduced the lipopolysaccharide (LPS)-induced expression of cyclooxygenase (COX)-2 in murine RAW 264.7 macrophage cells. The authors could associate the effect with the compounds procyanidin B2, quercetin, and its 3-O-galactoside. Chlorogenic acid, caffeic acid, and syringin stimulated COX-2 expression, while no effect was observed for (–)-epicatechin, phloridzin, and cinnamic acid [126]. Another study, conducted with a purified subfraction of apple pomace containing up to 72% procyanidin B2, confirmed the COX-2-inhibiting results [127]. Rana and Bhushan [128] summarized the anti-inflammatory effects of apple polyphenols. Procyanidins, phloretin, kaempferol, and quercetin were identified as potent anti-inflammatory agents. The effect was due to an inhibition of inflammatory enzymes such as COX-2, CYP3A4, transcription factors such as NF- κ B, and STAT1, and pro-inflammatory cytokines. Furthermore, ursolic acid exhibited anti-inflammatory potential *in vitro* and *in vivo* as summarized by Cargnin and Gnoatto [129].

These studies underline the importance of apple pomace as a source of individual compounds with anti-inflammatory potential. Isolation of these compounds would be needed for therapeutic applications. Their recovery depends not only on the extraction technique but also on the initial compound content at harvest time.

Anti-proliferative activity

Apple pomaces from six varieties were tested for their antiproliferative activities. All pomace extracts exhibited strong antiproliferative activities in the cervical cancer cell line HeLa. In HT-29 human colon cancer cells, the pomace extracts of *Pinova*, *Iduna* and *Braeburn* apple varieties were especially potent proliferation inhib-

itors. The antiproliferative effect could not be correlated to the polyphenol content of the extracts [130].

Two studies from the same author demonstrated the *in vitro* antiproliferative effects of pectin obtained from apple pomace via supercritical fluid extraction. However, compared to pectin isolated from citrus peels, the growth inhibition rate of apple pomace pectin was 31.3% lower [131]. Surprisingly, the subcritical water extracted apple pectin showed higher growth inhibition rates of HT-29 cells than commercially available apple pectin [61]. This in turn leads to the presumption that not pectin itself mediates the growth-inhibiting effects, but other compounds that are bound to pectin or associated to it by other mechanisms. Other reviews summarize the antiproliferative effects of apple compounds. Procyanidins and phloretin, as well as ursolic acid, are regarded as potent candidates [128, 129].

Current evidence of antiproliferative activity from apple pomace compounds is based mainly on *in vitro* studies. *In vivo* studies are awaited.

Anti-bacterial and anti-viral activity

Plants evolutionary evolved a sophisticated chemical defense arsenal to combat pathogens, predators, and overgrowth by other plants.

Apple pomace was tested on growth-inhibiting effects against *Helicobacter pylori* strains, a pathogen that enhances the possibility to develop gastritis, and gastric or duodenal ulcer in infected patients. The 10% hot-water pomace extract did not exhibit any growth-inhibiting effect in agar diffusion test when administered alone. However, in combination with quince juice and wild or cultivated cranberry juice, synergistic growth-inhibiting effects were observed [132].

Furthermore, the effects of several food-based powders against food-borne pathogens were investigated [133]. A 4% stock solution of apple skin extract was prepared and diluted to evaluate the bactericidal activity against *E. coli* O157:H7, *Lactobacillus monocytogenes* RM2199, *Salmonella enterica* RM1309, and non-MRSA-resistant *Staphylococcus aureus* 1200. Dilutions of the apple skin extract stock solution, killing 50% of the aforementioned pathogens, were > 2.7%, 1.39%, 0.007%, and 0.002%, respectively. The apple skin extract revealed extraordinary growth inhibition of *S. aureus* strains. The authors elucidated procyanidins and phloridzin as the main compounds in the extract.

Ursolic acid is associated with antimicrobial effects against methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococci*, *Streptococcus* sp., *Actinomyces* sp., and *Listeria monocytogenes* [134–137]. Synergistic effects of ursolic and oleanolic acid against *Mycobacterium tuberculosis* were postulated [138].

Methanol and acetone extracts from apple pomace were used to treat *Herpes simplex* 1 and 2 (HSV-1, HSV-2) infections in Vero cells. EC₅₀ of HSV-1 growth inhibition was 710.9 μ g/mL and 576.7 μ g/mL, respectively. For HSV-2, the EC₅₀ was 629.6 μ g/mL and 450.7 μ g/mL. The IC₅₀ of cell viability was significantly higher with 7281.8 μ g/mL for the methanol extract and 5494.9 μ g/mL for the acetone extract [139]. A follow-up study investigated the impact of the apple cultivar on the antiviral effect. A significant difference between the effective concentrations of the different cultivars was identified. The cultivar *Meana* was highly active

against HSV-1, while the *Carrió* cultivar showed the best efficacy against HSV-2 infections. The best inhibition rates were achieved when administered at the time point of virus infection. The elucidated mechanism of action was the inhibition of virus adsorption and entry in the host cell. No effects were observed on virus DNA replication for the extracts. Two compounds showed potent antiviral activities, procyanidin B2 against HSV-1 and quercitrin against HSV-2 infections [140].

Hormones

Human plasma concentration of sulphated dehydroepiandrosteron decreases during apple pomace diet (22 g/day). Sulphated dehydroepiandrosteron is a shared progenitor of male and female sexual hormones and structurally related to bile acids. This leads to the hypothesis that its levels could be decreased via the same mechanism as for the decrease of plasma cholesterol levels during apple pomace diet [87].

Conclusions and Future Perspectives

Apple pomace is an underutilized waste product of the apple manufacturing industry with potential nutritional and pharmaceutical applications. It is a heterogeneous product impacted by the manufacturing process, the cultivar, and the harvest year, and it represents a source of compounds belonging to numerous structural classes. Despite extensive research of apple pomace, discovery of new compounds is still expected. However, their recovery from the complex matrix remains a challenging task.

Apple pomace extracts, fractions, or isolated compounds thereof have been shown to impact pharmacological targets. Preliminary studies point to promising antioxidative, anti-inflammatory, antibacterial, and antiviral activities. However, the available information to date indicates that the lead structures and mechanisms behind their activity are still to be elucidated. The intake of apple pomace was shown to beneficially influence digesting enzymes, intestinal microbiome, and plasma cholesterol and triglyceride levels. Hence, it could play an important role in the prevention of lifestyle diseases such as type-2 diabetes, hypercholesterolemia, and hyperglyceridemia. Also in this case, the characterization of the beneficial effects and the potential risks must be further investigated in order to achieve a safe and reproducible effect of apple pomace consumption. Future studies of the physiological effects of apple pomace should have an emphasis on the chemical characterization of the material. This would facilitate the correlation of biological activities with pomace constituents and the standardization of the material when intended for therapeutic use.

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Conflict of Interest

The authors declare no conflict of interest.

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