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ENZYMATIC LIQUEFACTION OF APPLE POMACE

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Clarity is one of the most important quality criteria required in apple juices and wines. Haze formation during apple processing can be caused by homogalacturonan, rhamnogalacturonan, arabinogalactan, arabinan or xylogalacturonan. Manufacturers are looking for new technologies that guarantee high quality, decrease production costs and solve most technological problems. Nowadays production of apple juices is strongly connected with enzymes technology. Using pomace liquefaction with different pectinases and cellulases improves the yield of the apple juice, but on the other hand results in an increase in turbidity. Hence, inappropriate usage of enzymes can lead to filtration problems. Apple pomace liquefaction process need to be optimized. In this review, different methods of haze reduction was discussed.

Introduction

Fruit cells, particularly unripe, represents a complex structure of different elements linked together from a more or less rigid assembly [1]. This structure, consisted of several layers, protects the cell and gives it form. The first wall deposited after cell division is called “middle lamella” [2]. It is principally composed of pectic substances and forms the texture of fruit tissue. The next one, consisted of polysaccharides (cellulose, hemicelluloses and pectin) with small amount of proteins and phenolics, is primary cell wall.

At least five physiological roles can be attributed to the polysaccharide components: providing an alternate co-extensive network, cementing cells together at the middle lamella, limiting porosity of the cell wall, accommodating the symplast with an ionic and aquatic environment and transducing environmental signals to cells throughout bioactive fragments [2].

Pectins are mainly made up of homogalacturonan (1→4 linked α-D-galacturonic acid units) and rhamnogalacturonan regions which are interrupted by neutral sugar side chains such as arabinans, galactans or arabinogalactans [1].

In fruit and fruit juices pectins may be hydrolyzed by native tissue enzymes or enzymes introduced as pectolytic preparations [3].

The second ones, in fruit juice industry, are used in juice extraction to achieve better press yields and clarification of the extracts [4]. However, some of these enzymes can cause a haze formation. so careful selection is, therefore, critical [5].

Chemical structure of pectins

The precise chemical interconnections of pectins remain under debate, although their structural elements are mostly well described [6]. To understand its biological function and to use its properties in industry, we should widen our knowledge of this structure.

Pectins are heterogeneous and complex molecules, which together with hemicelluloses and cellulose build the cell walls and contribute to many cell wall functions [7]. The pectin contains so-called "smooth regions" and "hairy regions". The smooth regions are represented by homogalacturonan (HG), which consists of α-(1,4)-linked D-galacturonic acid residues [1, 8]. They are interrupted by ramified rhamnogalacturonan I regions (hairy regions, RGI) with a backbone build up by the →4)-α-D-GalAp-(1→2)-α-L-Rhap-(1→dimer. RGI can be associated with xylogalacturonan (XGA), arabinan, arabinogalactan or rhamnogalacturonan type II (RGII) [7].

Different amounts of the galacturonic acid regions can be methyl esterified, what greatly influence the physicochemical properties of the pectin [9]. Many researches of homogalacturonan structure show various distribution of methyl esters and acetyl groups over the galacturonan backbone, which contributes to the understanding of gelling behaviour and ripening processes of fruits [10]. The primary chain consists of α-D-galacturonate units linked α(1→4), with 2-4% of L-rhamnose units linked β(1→2) and β(1→4) to the galacturonic units [11]. Xylogalacturonan contains also a homogalacturonan backbone, but 25-75% of the galacturonic acid (GalA) units are substituted with xylose [12]. Xylose, arabinan and (arabino)galactan are attached to the main chain through their C1 and C2 atom [13-15]. Arabinan and (arabino)galactan are covalently linked to RGI [10], where RGII is thought to be covalently linked to HG [16]. Linkage between HG and RGI is presented in Fig. 1.

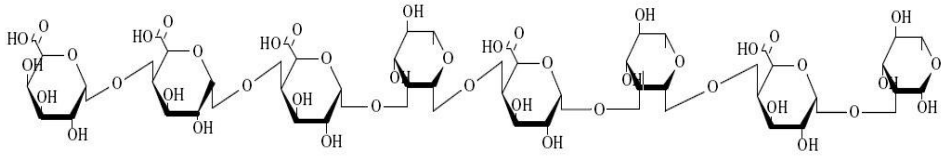

Fig. 1. Connection between homogalacturonan and rhamnogalacturonan I [7]

RGII contains twelve different glycosyl residues including several rate "diagnostic" monosaccharides such as apiose, 2-O-methyl-L-Fuc, 2-O-methyl-D-Xyl [17], aceric acid (3-C-carboxy-5-deoxy-L-Xyl) [18], Kdo (3-deoxy-D-manno-octulosonic acid) [19] and Dha (3-deoxy-D-lyxo-heptulosaric acid) [20]. Its backbone is built of 1,4-linked α-D-galacturonosyl residues carrying four oligosaccharide side-chains [21-22]. RGII has been shown to be rather as a dimer in plant cell walls [23]. This dimer is cross-linked by borate-diol diesters, located on apiosyl residue [24] and can be defined as an enzyme-resistant pectic polysaccharide [25]. The structure of pectins is presented in Fig. 2.

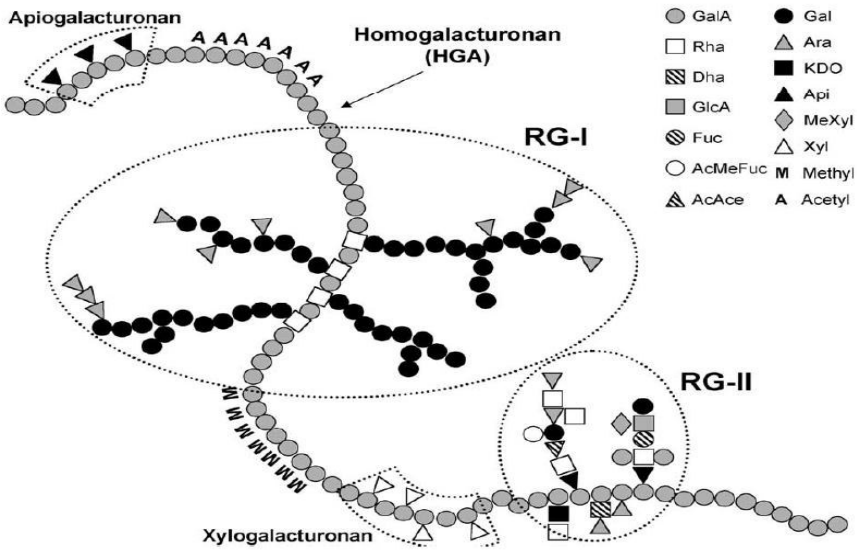

Fig. 2. Primary structure of pectins. It's assumed that HG, RGI and RGII are covalently linked [2]

Enzymes degrading pectin substances

Pectin is hydrolyzed by pectinases or pectolytic enzymes. Pectinases are widely distributed enzymes found in bacteria, fungi and plants [26]. It has been reported that microbial pectinases account for 25% of the global food enzymes sales.

Commercial preparations of pectinases are mostly produced from fungal sources, especially Aspergillus niger species [27-29].

Three following pectolytic enzymes can be distinguish [30, 31]

- Protopectinases: degrading the insoluble protopectin,
- Esterases: catalyzing the de-esterification of pectin and
- Depolymerases: catalyzing the hydrolytic cleavage of the pectic substances.

Protopectinase is also synonymous with protopectinase (PPase) and catalyses the following reaction:

$$\textbf{(insoluble) protopectin} + H_2O \xrightarrow{\textbf{PPase}} \textbf{pectin (soluble)}$$

PPases are classified into two types [32-34]: A-type PPases react with the inner site, i. e. of the polygalacturonic acid regions of protopectin, whereas B-type PPases react on the outer site, i. e., of the polysaccharide chains that may connect cell wall-constituents and galacturonic acid chain.

Polygalacturonase (PGase): catalyzes the hydrolytic breakdown of the polygalacturonic acid chain with the introduction of water across the oxygen bridge and has biological, functional and technical applications in food processing and plant-fungal interactions. PGases are divided in two groups: endo-PGases and exo-PGases.

Lyases (transeliminases) perform non-hydrolytic cleavage of pectic substances, characterized by a trans-eliminative split of the pectic polymer [35]. They break the glycosidic linkages at C-4 and simultaneously eliminate H from C-5, producing a Δ4:5 saturated product [36-37]. Lyases can be classified into following types:

I endopolygalacturonate lyase (EndoPGL),
II exopolygalacturonate lyase (ExoPGL),
III endopolymethylgalacturonate lyase (EndoPMGL),
IV exopolymethylgalacturonate lyase (ExoPMGL).

Pectinesterases (PEs) catalyze the hydrolytic deesteryfication of methyl ester linkages of galacturonan backbone of pectins to release acidic pectic substances and methanol [38]. Fungal PEs act by a multi-chain mechanism, removing the methyl groups at random, whereas plant PEs tent to act either at the non-reducing end or next to a free carboxyl group and proceed along the molecule by a single chain mechanism. The PE reaction is presented as follows [39]:

$$\textbf{Pectin} + n\textbf{H}_2\textbf{O} \xrightarrow{\textbf{PE}} \textbf{pectate} + n\textbf{C}_2\textbf{H}_5\textbf{OH}$$

Fruit juice industry

The most popular application of pectinases is in fruit juice extraction and clarification [26]. Pectins play important role in fruit juice viscosity and turbidity. Combinations of pectinases, cellulases, arabinases and xylases are used to clarify fruit juices and increase extraction yields [40]. It decreases also filtration time up to 50%.

Apple juices can be produced by traditional enzyme treatment of the mash with pectinases for the premium juices and pomace liquefaction for the extraction juices [41]. The production of clear apple juices and apple juice concentrates with pectolytic enzymes was rather uncommon until the 1970s. When the raw material basis of juice production changed (table apples, warehoused fruit), the enzyme treatment of mash took off [42-45]. Another method of enzyme technology is mash liquefaction, where due to strong pectolytic and cellulolytic enzyme activities, oligo- and polysaccharides are released from the apple pomace, resulting in high colloid concentrations in the extraction juices, what can lead to technological problems [46].

Another disadvantages of this process are a lower sensory quality of the juices, an increased release of polyphenols with a tendency to browning and high enzyme doses (costs). In the European Union, this process is banned because no nutritional assessment of the apple juices and liquefaction extract is available. On the other hand pomace liquefaction can also be useful for obtaining value-added foods. Hence, all these aspects should be developed to solve technological and sensory problems of apple juice production [41].

Haze formation in apple pomace

Hazes are mostly caused by larger particles suspended in a solution. Particles of greater than colloidal size settle out, if there is no agitation to suspend them. The colloids arise when both the size of particles is sufficiently small and their density sufficiently similar to the suspending liquid that the particles are kept suspended by Brownian motion [47]. Colling may result in settling of larger colloidal particles and also cause the formation another particles due to a reduction in solubility.

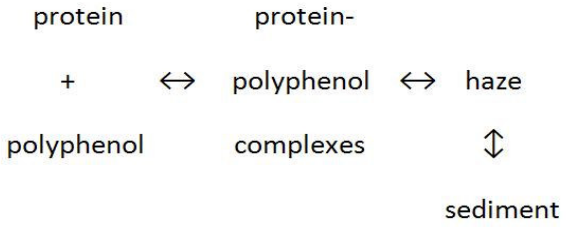

Fig. 3. Protein-polyphenol interactions leading to haze and sediment formation

The most frequent cause of haze in beverages is protein-polyphenol interaction (Fig. 3) [48]. Even if initial combination of protein and polyphenol is soluble, the complex may grow to sufficient size that results in turbidity.

Haze-active (HA) proteins – contain proline [49] and the relative haze-forming activity of a protein has been shown to be largely a function of the proline content [50]. Proline prevents the formation of an α-helix what facilitates access by polyphenols, but proline is also involved in the binding sites. An HA protein isolated from apple juice was shown to contain 5% of proline.

Haze-active polyphenols – their activity depends on the number and location of the hydroxy groups on an aromatic ring [51]. Monophenols hardly bind to proteins, meta-diphenols weakly attach to protein, but ortho-diphenols bind with moderate strength and vicinal triphenols act quite strongly.

Flavanols (catechin and procyanidins) have the strongest association with haze-forming activity, because they are large enough to encompass two attachment sites (each an aromatic ring with at least two phenol groups). Haze-forming activity has been shown to increase with molecular complexity (trimers>dimers>monomers) and with increasing numbers of phenol groups on a ring [52].

Protein-polyphenol interaction, at least initially, is reversible and so not covalent [49]. Four factors have been noticed to influence this phenomenon: protein concentration, polyphenol concentration, pH and alcohol content [53]. Numerous stabilization methods are used against haze formation. One of them is storage of a beverage at low temperature. This leads to the settling out of material that would otherwise lead to haze in the package. The product is decanted from the storage tank and filtered. Fining can be conducted with the addition of a protein (gelatin or isinglass) or a polyphenol (gallokatechin). Some adsorbents may reduce the amount of either HA-protein (bentonite or silica) or HA polyphenol (polyvinylpolypyrrolidone – PVPP) [47].

Fining has been shown to be a critical processing step. Varying the temperature used for pasteurization of raw juice, on the other hand, does not appear to have much of an effect on haze development. Present studies do not show much difference in haze formation with or without using amyloglucosidase. On the contrary, the temperature used for ultrafiltration had a marked effect on the rate and amount of haze [5].

Apart from protein-polyphenol interactions, increased colloid concentration is caused by oligo- and polysaccharides obtained through pomace liquefaction [41]. To receive clear apple juice concentrate, these substances cannot be removed by fining agents or filtration. These steps of apple processing may results in, for example, increased fouling during ultrafiltration. Hence, filtration and clarification problems have to be expected.

Conclusion

The production of clear apple juices or apple juice concentrates is closely linked with industry fields based on enzymes. Apple pomace liquefaction results in higher juice yields, but on the other hand causes clarification problems. Oligo- and polysaccharides, released from apple cell material under the influence of pectolytic and cellulolytic enzyme activity, are responsible for haze formation.

Nowadays, scientists are investigating the question of whether or not these substances are suitable for nutritionally useful dietary fibre in human nutrition. This method also leads to apple polyphenols release. In apple juice industry, various combinations of pectolytic and cellulolytic enzymes should be developed to prevent or retard haze formation. Studies referring to apple pomace liquefaction are under way.

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ENZYMATYCZNE UPŁYNNIANIE MIAZGI JABŁKOWEJ

Streszczenie

Konsumenci wymagają produktów, które gwarantują dobrą jakość. Klarowność soków i win jabłkowych jest jednym z pożądanych kryteriów jakości. Tworzenie się zmętnień podczas przerobu jabłek może być spowodowane obecnością homogalakturonianów, ramnogalakturonianów, arabinogalaktanów, arabinanów czy ksylogalakturonianów, które zostają uwolnione z tkanki jabłek. Możemy zapobiec występowaniu zmętnień w gotowych produktach poprzez zastosowanie enzymatycznego upłynniania z użyciem różnych pektynaz i celulaz. Co więcej, możemy dzięki temu zwiększyć zysk otrzymywanego soku. Aby otrzymać najlepsze wyniki, musimy odpowiednio zoptymalizować warunki upłynniania (rodzaj stosowanego enzymu, dawka, temperatura i czas działania). W tym przeglądzie zostały poruszone różne metody przerobu jabłek oraz redukcji zmętnień.