Anti-Platelet Properties of Apple Cider

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Fermented alcoholic beverages, including apple cider, have been shown to provide several functional properties with health benefits when consumed in moderation. The beneficial functional properties of apple cider have been attributed to the plethora of its bio-functional compounds, including its phenolic content and anti-inflammatory and anti-platelet polar lipids (PL). More specifically, fermentation of apple juices from different apple varieties produced apple ciders containing PL and phenolic bioactives with anti-inflammatory and anti-platelet properties against human platelet aggregation induced by the inflammatory and thrombotic mediator, platelet activating factor (PAF), but also by a classic platelet agonist, adenosine diphosphate (ADP). Similar outcomes were also observed in the wastes, apple cider by-products (apple pomace), from all these apple cider production procedures.

apple cider fermentation yeast anti-inflammatory anti-platelet bioactives polar lipids platelet activating factor MUFA PUFA

1. Overview

Alcoholic beverages like apple cider are considered functional beverages with several health benefits, when consumed in moderation, which are mainly attributed to their microbiota and the plethora of their bioactive compounds. Among them, bio-functional polar lipids (PL) have recently been found in apple cider, which despite low quantities, have exhibited strong anti-inflammatory and anti-platelet properties, while fermentation seems to affect the functionality of apple cider's PL bioactives. The aim of the present study was to elaborate yeast strains isolated from the complex mixtures of apple surface and must yeasts for evaluating their effects on the anti-platelet functional properties of PL bioactives from their final fermented apple cider products. First, bio-functional PL were extracted and separated from the biomass of the different isolated apple surface/must yeast strains, and were further assessed for their anti-platelet potency against human platelet aggregation induced by the potent inflammatory and thrombotic mediator platelet-activating factor (PAF), or by a classic platelet agonist like adenosine diphopshate (ADP). Novel functional apple ciders were then produced from the fermentation of apple juice by elaborating the most bioactive and resilient yeast strains isolated from the apple must with optimum fermentation properties. PL bioactives extracted from these novel apple cider products were also further assessed for their anti-platelet properties against both the PAF and ADP pathways of human platelet aggregation. These novel cider products were found to contain PL bioactives with lower IC50 values (~40 µg) and thus increased anti-platelet potency against platelet aggregation induced by PAF and ADP. GC-MS analysis of the PL bioactives extracted from these novel apple ciders showed that apple cider PL bioactives are rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), such as the omega-6 linoleic acid (LA) and the omega-3

alpha linolenic acid (ALA), with favorably lower levels for their omega-6/omega-3 PUFA ratio, which further support the observed strong anti-platelet properties putative anti-inflammatory potency for the apple cider PL bioactives. However, further studies are needed in order to elucidate and fully characterize the apple yeast strains that can be utilized for increasing the anti-inflammatory, anti-platelet and cardioprotective functional properties of their fermented apple cider products.

2. Apple Cider

Fermented alcoholic beverages, including apple cider, have been shown to provide several functional properties with health benefits when consumed in moderation. The beneficial functional properties of apple cider have been attributed to the plethora of its bio-functional compounds, including its phenolic content and anti-inflammatory and anti-platelet polar lipids (PL) [1][2]. More specifically, fermentation of apple juices from different apple varieties produced apple ciders containing PL and phenolic bioactives with anti-inflammatory and anti-platelet properties against human platelet aggregation induced by the inflammatory and thrombotic mediator, platelet activating factor (PAF), but also by a classic platelet agonist, adenosine diphosphate (ADP) [1]. Similar outcomes were also observed in the wastes, apple cider by-products (apple pomace), from all these apple cider production procedures [2].

In addition, PL bioactives with similar anti-inflammatory potency have also been found in several yeasts/bacteria strains utilized for the production through fermentation of other alcoholic beverages like wine and beer, but also of bioethanol production, while both beer and wine products from such fermentations were also found to possess PL and phenolic compounds with strong antithrombotic and cardioprotective properties [3][4][5]. In all these alcoholic beverages including apple cider, it was reported that fermentation process affects the anti-inflammatory and anti-platelet functional properties of the final fermented product [1][3][4][5].

Even though apple cider can be simply defined as fermented apple juice, yet the process and specifications can be much more complex than its definition. Several parameters affect cider production, with yeast strains and yeast-available nutrients being crucial factors for the functional properties, nutritional value, and taste characteristics of apple cider [6][7][8][9][10][11]. There are specific yeast species that are associated with cider fermentation. In countries such as Ireland, Spain, and France, cider is mainly produced from naturally developing yeast species, derived from the fruit or sometimes from the surface of the processing equipment, stimulating alcoholic fermentation [9][10][11]. The most prevalent yeast specie in alcoholic fermentation has been shown to be the indigenous *S. cerevisiae*, which is also known as a budding yeast for its ability to bud off the parent cell. It is used in cider, beer, and wine making and is used as a model eukaryotic cell in the fields of molecular and cellular biology.

Nevertheless, apart from *S. cerevisiae*, several other yeast species, including *Debaryomyces, Candida, Hanseniaspora/Kloeckera, Metschnikowia, Pichia/Hansenula, Schizosaccharomyces, Torulaspora, Williopsis*, and *Zygosaccharomyces*, have also been found to supplement *S. cerevisiae* and thus enhance flavor complexity and aroma intensity [9][10][11]. Subsequently, these yeast species can be used in wine and cider fermentation to improve the complexity of the wine and cider flavor. Although most of these strains cannot perform complete alcoholic

fermentation individually, a mixture of these strains together with *S. cerevisiae* delivers a desirable method for alcoholic fermentation using the correct inoculation methods [9][10][11]. However, further research is needed on evaluating how each one of these different apple yeast species of cider fermentation affect the bio-functional properties of the final fermented apple cider products.

Thus, the purpose of this study was the isolation of different types of yeast species present in apple surface and apple musts from different apple varieties, depending of their tannin content, such as Jonagold, Dabinett, and Aston Bitter varieties with low, medium, and high in tannins content, respectively, in order to investigate their suitability for apple cider fermentations for producing fermented apple cider products with increased anti-inflammatory and anti-platelet functional properties.

For achieving such a goal, several yeast strains from the apple surface and apple musts of these three different apple varieties were isolated, while the yeast strains containing the strongest PL bioactives, were further utilized for producing different apple cider products by fermenting a commercially purchased controlled apple juice. The PL bioactives of the apple ciders produced by these fermentations were further studied for their potential anti-platelet properties against human platelet aggregation induced by either PAF or ADP, while structural elucidation and evaluation of their fatty acid profile was performed by GC-MS analysis, in order to evaluate structure activity relationships.

3.Conclusions

Specific bioassays based on a disease model of human platelet aggregation revealed that the PL, obtained from resilient and efficient for apple cider fermentation procedures apple must yeast strains, were more bioactive with strong anti-platelet properties. Moreover, by utilizing these most bioactive and resilient/efficient apple must yeast strains, several functional apple ciders were produced with PL bioactives that strongly inhibited platelet aggregation induced by PAF and ADP. The strong inhibition of the PL bioactives from these novel apple cider products against the inflammatory and thrombotic mediator PAF, in combination with their rich content in anti-inflammatory omega-3 PUFA like ALA and a subsequent favorable low omega-6/omega-3 PUFA ratio, further suggest putative anti-inflammatory and cardioprotective potency for these novel functional apple cider products.

Overall, these results further indicate that by choosing the more bioactive and resilient/efficient for fermentations yeast strains from apple must, then the anti-platelet functional properties of apple cider PL bioactives can be increased against thrombo-inflammatory mediators like PAF and ADP. Thus, these outcomes may facilitate the production of functional fermented products like apple cider, by valorizing the most optimum yeast strains, in terms of anti-platelet and anti-inflammatory potency and resilience/efficiency for fermentations. A wide range of applications for microbiological profiles of the fermentation industry through their yeast strains can thus improve the health characteristics and bioactive compound composition of alcoholic beverages like cider. As this is the first time that this has been researched a further in-depth study is required to accurately support the results and produce the parameters needed to efficiently characterize the apple must yeast strains (biochemical tests, sequencing, etc.)

that can be utilized for increasing the bio-functional properties of their fermented cider products and their subsequent health benefits.

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