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INFLUENCE OF FERMENTATION CONDITIONS ON CONGENER COMPOSITION AND METHANOL CONTENT IN DISTILLED SPIRITS

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ABSTRACT

This study elucidates the multifaceted influence of raw materials, yeast strains, and fermentation conditions on the formation of congeners and methanol in distilled spirits. The research underscores the role of the raw material's quality and its pretreatment processes, such as deacidification, pasteurization, and depectinization, in determining the spirit's congener profile and methanol content. It is demonstrated that specific pretreatments can significantly alter methanol levels, with deacidification and depectinization increasing methanol content, whereas pasteurization reduces it by inactivating pectolytic enzymes. Furthermore, the selection of yeast strains and the optimization of fermentation conditions, including temperature and duration, are pivotal for controlling the development of congeners and enhancing desirable flavor profiles. The importance of nutrient supplementation in the fermentation medium to prevent efficiency losses is also highlighted. Finally, innovative approaches such as the use of genome editing for yeast strain development and co-fermentation techniques are discussed for their potential to optimize spirit quality. This analysis provides insights for the industry on manipulating fermentation variables to achieve desired product characteristics while ensuring consumer safety.

Keywords: Fermentation techniques. Pretreatment outcomes. Yeast strain selection.

1 INTRODUCTION

In a fermentation process, numerous compounds are produced besides the final product. These compounds are called congeners and they are the responsible for giving the unique taste of each spirit or beverage. The reason they are produced is due to the yeast defense mechanisms from stress, secondary metabolic products (Figure 1), incomplete fermentation, reactions and other factors. It can be higher alcohols, esters, carbonyl compounds, organic acids, polyols, vicinal diketones, Sulphur compounds and phenolic compounds. Esters are particularly important because their fruity and floral characteristics¹. Understanding how each raw material is going to influence the congener formation can be used to enhance the final product quality by designing systems, like distillation or other treatments, to maintain the desirable aromas or remove the undesirable compounds. The main objective on this review is to gather the information on why the congeners are formed and the influence of the raw material and fermentation conditions on it.



Figure 1 Outline of metabolic inter-relationships in the synthesis of secondary yeast metabolites. 1.

2 MATERIAL & METHODS

The survey was conducted using the Web of Science database with the keywords being "SPIRIT" AND ("AROMA COMPOUNDS" OR "CONGENERS" OR "VOLATILE COMPOUNDS") AND "FERMENTATION", searched at the "Topics" section, filtering for articles since 2020, which found 57 articles. In further depth, 9 were chosen for this review.

3 RESULTS & DISCUSSION

The raw material can be indeed an important factor on congener formation. This was the conclusion of reached by researchers who tested four apple cultivars for apple spirits, where not only the quality of the spirit was due to the raw material, but to its pretreatment as well. In the study deacidification, pasteurization, and depectinization were tested. Deacidification and depectinization increased the methanol content in the spirit by ten times when comparing to the raw pulp, but half when using pasteurization, possibly due to deacidification and depectinization increasing the activity of pectolytic enzymes while pasteurization inactivating it due to the high temperature employed. ². Besides the quality of the raw material, it is important to know if it have sufficient nutrients to sustain the whole fermentation without lowering the efficiency and having a high content of sugar, which can be disadvantageous for the distillation step. ³. This shows the importance of understanding the raw material to conduct pre-treatments and fermentation.

Another factor on congener formation can be the yeast strain used. For neutral spirits beverages like gin and vodka the need is to minimise the flavour-active volatile compounds, especially because it leads to higher alcohol yields, while in other flavoursome brown spirits such as whisky and rum it's the opposite, and yeast-strain and fermentation conditions have an impact. The fermentation process control can be fermentation time or temperature. An increase in fermentation temperature decreases the number of congeners, while prolonged fermentation increases their development. E Esters, which give fruity and floral characteristics, can be produced through condensation reactions between alcohols and acyl CoAs or by lactic acid bacterial metabolism, which highlight the importance of having uncompetitive yeast strains if the flavour is desirable¹. There are different variants that can be developed by genome editing, to optimize the production and have better robustness when dealing with thermal stress, osmotic stress and ethanol stress. ⁴. Another approach involves utilizing co-fermentation by isolating Kveik—a consortium of yeast traditionally used in Norway for spirit production—and combining it with non-conventional yeasts. This method has been shown to enhance the production of specific volatile aroma compounds. ⁵. This is in agreement with other study that concluded that mixed culture fermentations lead to higher concentration of volatile compounds and better sensory attributes when comparing to the pure culture of *Saccharomyces cerevisiae*. ⁶. Overall, the yeast strain chosen and fermentation conditions should be in agreement with the characteristic of the final product.

The natural fermentation of some raw materials, such as in plum spirit production, can have a significant impact on final congener composition of the fermented mash, resulting in higher concentrations of total acids, ethyl acetate and benzaldehyde. Benzaldehyde, for instance, is formed from cyanogenic glycoside, an amygdalin present in the stone, making it more concentrated in plum spirits obtained from mashed that the stone is not removed. Additionally, the storage time of the mash before distillation can increase its concentration. The traditional method of plum fermentation takes place in open vessels, which create favourable conditions for growth of acetic acid bacteria (acetic acid is the most abundant volatile acid in traditionally produced plum spirits), lactic acid bacteria (contributing to a high concentration of ethyl lactate) and larger formation of volatile acids, which do not occur in closed vessels. Using commercial yeasts, pulping of plum fruits and distilling immediately after fermentation can lower the total acid and ethyl lactate content. ⁷. Overall, the choice of fermentation method and the handling of the mash significantly influence the chemical profile and quality of the final plum spirit.

Methanol formation is attributed to enzymatic reactions facilitated by enzymes present in the raw materials, which can be modulated by various pre-treatment and fermentation conditions. Specifically, the application of heat during pre-treatment, such as must heating, can result in decreased methanol content by inhibiting enzymatic activity. Moreover, fermentation conditions that involve contact with the solid parts of raw materials, as opposed to fermenting only with the juice, are associated with increased methanol production. Factors such as the pH level during fermentation, the quality of the raw material (with ripe fruits being more prone to producing methanol), the presence and degradation of pectin, the activity of pectin methylesterase, and the choice of yeast strain also play significant roles in determining methanol levels. The distillation process has been identified as a method for reducing methanol content in the final product. ⁸. The storage time of fermented mashes, like plum fermented mash, can significantly increase the methanol content in the final product, because the fruit pectin demethylation begins during plum fruit mashing and continues during alcoholic fermentation and storage. ⁷ For volatile compounds, it was demonstrated that whey supplemented with high levels of nutrients produces higher concentrations of most volatile compounds compared to whey with no or moderate supplementation. Notably, spirits derived from highly supplemented whey have the advantage of containing no methanol in the final product. ⁹.

The raw material, pre-treatments and fermentation conditions of some articles can be seen in Table 1.

Table 1 Raw material, pre-treatments and fermentation conditions.

Reference	Raw material	Pre-treatments	Fermentation conditions
2	Apples	Pasteurization, depectination and deacidification	18± 1°C for 14-20 days

3	Cassava flour	Liquefaction and saccharification	28 °C at 180 rpm stirring
5	Malt	-	20 °C or 28 °C at 300 rpm stirring
6	Apples	Pectin decomposition	16± 1°C for 20 days
9	Whey samples	Pasteurization	36 °C at 200 rpm for 24,30 and 36h
7	Plum	Stones removal	20± 2°C for 2-12 days, depending on the variety

4 CONCLUSION

The composition of congeners in distilled spirits is significantly influenced by the methodologies employed in fermentation and its preceding treatments. Examination of various studies reveals that several factors impact this profile, including the type of raw material utilized, which can lead to elevated methanol levels if it contains pectin and pectolytic enzymes that remain active or lacks sufficient nutrients, necessitating supplementation. The choice of yeast strain is also important and varies based on the desired outcome, whether it is the maximization of ethanol production or the enhancement of flavor-active compounds. The duration of fermentation also plays a role; extended fermentation periods tend to augment the formation of congeners. Similarly, the temperature at which fermentation occurs can significantly reduce the presence of certain congeners. The metabolic activity of bacteria in the context of non-competitive yeast strains has been shown to increase ester production. Moreover, co-fermentation employing mixed cultures and the addition of supplements can further influence the congener profile. Overall, a comprehensive understanding of the intended product's requirements is essential for manipulating these variables effectively to achieve the optimal congener composition.

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