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Diacetyl problems in brewing and their control

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Abstract

Brewer's yeast, *Saccharomyces cerevisiae*, and some bacteria of *Lactobacillus* group (common contaminants of beer) are responsible for diacetyl (DA) production in beer, which results in 'cheesy' or 'buttery' off-flavour. While consumers dislike beer containing more than 0·07 ppm of DA, usually alcoholic beverages far exceed this limit (0·3 to 50 mg/l), making it necessary for brewers to exercise control over DA production in beer. The traditional methods of DA control in beer, lagering of beer and addition of fresh yeast cells to fermented beer, are tedious in operation and suffer from efficiency and economy. The newly proposed methods, immobilized yeast cells technique and diacetyl reductase technique, though expensive in their present form, are highly efficient and absolutely reliable.

Key words: Diacetyl, acetoin, brewing, beer, alcoholic beverages, diacetyl reductase, immobilized cell, yeast, valine, pH, rH, temperature, aeration, control, *S. cerevisiae*, *Aerobacter*.

Introduction

Diacetyl (DA) is an important primary metabolite of plants, animals and microorganisms[1–7]. Some of the important metabolic roles played by it include biosynthesis of riboflavin, valine and isoleucine[1–2] ; detoxification of excess pyruvate produced in cells[3–5], and provision of energy for sporulation in bacteria[6–7].

Diacetyl is also a secondary metabolite for a number of microorganisms which secrete it in their growth medium[8–24]. As such DA has acquired industrial importance: (1) as a raw material for synthetic rubber[9–11], (2) as a food preservative[12], (3) as

an important butter flavour (0·2 ppm of DA is a must)[5,13–15], and (4) as a serious off-flavour (off-flavour) problems of brewing industry and their control. flavour in alcoholic beverages and fruit juices[5,16–24]. This paper reviews the DA

2. Diacetyl problems in brewing

Flavour is the most important parameter to judge the quality of beer[18]. In breweries, flavour is usually judged by an expert taste panel which certifies the flavour as 'good' or 'off-flavour'. The 'cheesy' or 'buttery' off-flavour in beer is attributed to DA. It can be determined easily and precisely by chemical analysis[19–22, 25–28].

The mechanism of DA formation in beer has been studied extensively by many investigators[18–22]. Brewer's yeast, *Saccharomyces cerevisiae*, employed for beer fermentation, is the chief cause of DA in beer[18]. In addition, some bacteria such as *Lactobacillus pastorianus* and *Pedicoccus cerevisiae*, commonly contaminating beer, are also responsible for DA off-flavour in beer[29–33].

Consumers prefer mild-flavoured beer[25–34]. According to Drews *et al*[34], beer should not contain more than 0·07 ppm of DA. The reported[35–46] DA content of alcoholic beverages ranges from 0·3 to 50 mg/l (Table I). These data clearly show that the DA content of most of the common alcoholic beverages far exceeds the prescribed limit.

2.1. Control of diacetyl in beer

Lagering of beer, and addition of fresh whole yeast cells to fermented beer are the methods which have been used traditionally by brewers to remove DA from beer[32–33]. The lagering involves storing finished beer for long periods[18,32,33], requiring additional storage facilities. There is also the danger of beer getting contaminated with DA-producing lactic acid bacteria during extended storage[29–33]. With whole yeast cells, additional off-flavours are likely to be introduced as a result of yeast autolysis[29,30,32,33,47,48]. Valine may also inhibit DA synthesis by some yeast strains by feed-back repression when added to beer fermentation[18]. As discussed later, the performance of all these methods under modern brewery practice[49] is poor as the DA reduction in beer is incomplete. Immobilized yeast cells and stabilized diacetyl reductase (DRase) preparation, the methods successfully tried out recently by Elliker *et al*[32,33,47,48,50], are the methods of promise. They are quick in action and perfect in performance[32,33].

Even when a quick and perfect method is available for DA removal from beer, it is in the interest of brewers to try to control DA formation in beer during fermentation, rather than to attempt its removal from the finished product. Lewish[18] has

Table I

Occurrence of diacetyl (DA) and acetylmethylcarbinol (AMC) in alcoholic beverages

Alcoholic beverage	DA (mg/l)	AMC (mg/l)	Ref. number	Alcoholic beverage	DA (mg/l)	AMC (mg/l)	Ref. number
Wines :				Red wines :			
Reisling	1·0	19·6	35	Burgandy	...	48·0	40
Whisky	15·0	...	36	Spanish	...	53·0	40
"	5·0	...	37	Beers :			
Cognac	15·0	...	36	Pale	...	9·1	41
Australian	7·5	...	38	Dark	...	9·1	41
Champagne	0·8	...	39	Lager	0·8	...	42
White wines :				Gris staut	...	14·6	41
Australian	0·5	...	40	Finnish staut	16·0	...	40
Moselle	0·29	16·0	40	Russian	0·96	...	43
Rhine	0·57	10·0	40	Alcohols/Spirits :			
Bordeaux	0·35	12·0	40	Sulphite	50·0	...	44
Burgandy	1·2	...	40	Potato	0·3	...	45
Hungarian	0·72	44·0	40	Barley	0·7	...	45
Red wines :				Vinegars :			
Bordeaux	...	53·0	40	Wine	42·0	800·0	46
Hungarian	...	40·0	40				

suggested the following four minimum measures for discouraging DA production during beer fermentation : (1) careful selection of a yeast strain for beer fermentation, (2) minimizing culture growth by low temperature, (3) maintenance of strong reducing conditions during the fermentation, and (4) provision of adequate wort nitroger.

2.2. Methods of diacetyl control in beer

Depending upon their approach, the methods of DA control in beer can be classified into two groups : (A) indirect and (B) direct.

(A) *Indirect methods of diacetyl control in beer*

The principle underlying these methods is that like all physiological reactions, DA formation by yeast is maximum at optimum conditions of pH, rH, temperature, aeration, etc., so that any change in the optimum conditions may decrease or inhibit DA formation. Thus the indirect methods involve the manipulation of the optimum

conditions during beer fermentation so as to inhibit DA formation by yeast or by spontaneous reaction.

(1) *Selection of raw materials* : Suomalainen and associates[22,26–28] observed that the nature of nitrogenous substances present in the raw materials used for beer production exerted profound effect on the formation of aroma compounds in beer. They found that the raw materials rich in valine and/or isoleucine were inhibitory to DA formation in beer by some strains of brewer's yeast under anaerobic conditions[26–28].

(2) *Manipulation of pH, rH and temperature* : The trend in the modern breweries is to enhance beer production by such practices as increase in seed yeast, sugar concentration, and running the fermentation at high temperature with intense aeration-agitation[49]. All these practices stimulate DA production by yeast.

α-Acetolactate (AL) is an immediate precursor of DA synthesis in yeast[19–21,29]. According to Suomalainen and coworkers[22,28], AL is excreted by yeast into growth medium (beer), where its oxidation to DA is nonenzymatic and depends on the fermentation conditions. Subsequent studies of Inoue *et al*[19–21] and Ingram[49] showed that AL accumulated in beer at rH less than 10 and at higher values, was converted to DA. Thus DA formation in beer can be controlled by the maintenance of strong reducing conditions during fermentation.

The reports on the effect of temperature on DA formation by yeast are quite contradictory but appear to be valid. Denshckikov *et al* (cited by Farrel and Rose[31]) observed decrease in DA level of beer with increase in temperature from 5° to 20° C, whereas Portno[52] and Chuang and Collins[3] observed increase in DA level with increase in fermentation temperature (from 55° to 75° F) (Table II). There are two possibilities for increase in DA level with decrease in temperature. DA is a volatile compound and at low temperature (5–10° C) its volatilization may be reduced[31]. The organisms that produce DA usually produce DRase[13]. DA destruction by the enzyme is maximum at 30° C ; decrease in reaction temperature from 30° to 5° C inactivates the enzyme linearly[4,13,32,33].

In yeast, DA production and growth are parallel, the temperature optima being 21° to 25° C[3,8,16,18,53]. Outside the temperature optima, both growth and DA production are affected adversely. Good yeast growth is necessary for both pyruvate production and lowering of medium pH below 4·9 as prerequisite to DA synthesis[3,18,32,53]. At 10° C and less, yeast growth is poor, proper acidity does not develop and consequently DA production is poor.

(3) *Exclusion of air* : Lewish[18] has recommended maintenance of anaerobic conditions during beer fermentation for the control of DA production by yeast. Burger *et al*[29–30] suggested that excess of air during beer fermentation may enhance DA formation by

yeast. Later, Portno[52] found that under aerobic conditions, yeast utilized valine (present in wort) much faster than the wort sugar and *vice versa*. Valine, as pointed out earlier, is an allosteric inhibitor to DA pathway[18], and its early exhaustion from the wort derepresses DA pathway. Portno[52] showed that DA production by yeast decreased from 0·62 to 0·02 ppm with increase in wort valine level from 0·7 to 2·8 μM/ml (Table II).

However, not all yeast strains are sensitive to allosteric inhibition by valine [5,18], and yet DA production by them is stimulated by aeration–agitation[15]. This fact suggests the existence of a mechanism other than valine repression for the action of aeration on DA formation by yeast. According to Suomalainen *et al*[54–57], aeration can change the thiamin status of yeast cell dramatically. Thiamin pyrophosphate (TPP) is the cofactor for pyruvate decarboxylase (PDase), which catalyzes the first reaction of DA pathway[5,15,18]. Under anaerobic conditions, the thiamin level in yeast cell is reduced drastically[54–57]. Thus the exclusion of air could arrest DA synthesis in yeast by depriving PDase of its cofactor. Similarly, Yadav and Gupta[53] confirmed that aeration, pH and temperature exerted profund effect on acetoin and diacetyl production of yeasts and bacteria. They noted a dramatic 700-fold increase in acetoin plus diacetyl production of *Torulopsis colliculosa* NRRL 172 due to aeration–agitation (Table II).

Table II

Effect of fermentation conditions on diacetyl production

Organism	Factor	DA (ppm)	Factor	DA (ppm)	Factor	DA (ppm)
	Adjunct		Pitching		Temperature	
S. cerevisiae[25,33]	Control wort	0·28	2 lb/bbl	0·20	55° F	0·26
	5% glucose	0·40	4 „	0·48	75° F	0·66
	Valine		Aeration		Growth (SPC/ml)	
	0·0 μM/ml	0·93	Air	0·93	Poor	0·10
	2·8 „	0·02	CO_2	0·52	$1·7 \times 10^7$	0·54
T. colliculosa[53]	Aeration–agitation		pH		Temperature	
	Control	5	5	5100	20 ± 1° C	4800
	240 rpm	3800	7	4300	24 ± 1° C	2800
E. cloacae[53]	Control	3600	5	1000	20 ± 1° C	940
	240 rpm	7900	7	9600	24 ± 1° C	9200

(B) Direct methods of diacetyl control in beer

(1) *Selection of yeast strain* : The application of the modern methods of chemical analysis has made possible the detailed analysis of aroma fraction of alcoholic beverages[26]. Suomalainen *et al* [25–28] have identified more than 100 aroma compounds occurring in alcoholic beverages. It is interesting to note that beverages of quite different origins, such as beers from malt, wines from grapes and berries, brandies and cognac from grapes, and whiskies from grain and malt, contain qualitatively the same spectrum of aroma compounds. This fact clearly indicates that the alcoholic beverages owe their aroma chiefly to brewer's or wine yeast, the role of the raw materials being of secondary importance[22,25,28]. Sherry yeast, for example, produces the same aroma ccmpounds whether grapes or berries are used as the substrates[22,25,28].

There are several reports to suggest that the nature of aroma compounds produced in alcoholic beverages is the inherent property of the yeast strain employed for their production. Sihto and Arkima (cited by Suomalainen and Ronkainen[27]) reported that some yeast strains produced large amounts of fusel alcohol and isoamyl acetate, and imparted strong ester flavour to beer, whereas Burgandy yeast strains produced more of propanol and little or no isoamyl acetate. Similarly, the capability of DA production of yeast varies markedly from strain to strain[18,22]. For example, DA production of six yeast strains studied by Portno varied from 0·4 to 1·5 ppm. From his studies, he concluded that other factors affecting DA production are of secondary importance to the yeast strain selected for beer fermentation. This was recently corroborated by Tolls *et al*[33]. The eight strains of *S. cerevisiae* studied by them produced DA varying from 32 to 79 ppm.

There are several reports suggesting that DA production by yeast is linear with the amount of its growth[3,18,50]. Herein lies the possibility of DA control by the selection of a yeast strain which produces little or no DA. DA-negative mutants of *S. cerevisiae* have been described by Chuang and Collins[3].

(2) *Use of live yeast cells* : This is the most traditional method used by brewers for DA control in beer[18,32,33,58]. There are a number of reports regarding its successful use in breweries and distilleries. Kobuyama *et al*[59] were able to achieve DA reduction in shaké from 3 to 1 ppm in 24 h by mixing it with fresh yeast cells at the rate of 3 lb/bbl. The efficiency of this approach depends upon the species, quality and physical conditions of yeast strain employed. The reduction of DA was observed in a beer inoculated with live yeast (120 g/l) but not with heat-killed yeast[18,30,33,4]. One of the limitations of this approach is that yeast cells may autolyze giving rise to additional off-flavours[32–33].

3) *Immobilized yeast cells* : The traditional method of incubating beer with live yeast cells, as pointed out earlier, is unreliable because of the danger of yeast auto-

lysis. To overcome this problem, Tolls *et al*[33] have recently proposed use of immobilized yeast cells. It consists of percolating beer through a bed of diatomaceous earth impregnated with live yeast cells. The laboratory model was constructed by packing 50 g wet brewer's yeast mixed with 200 g diatomaceous earth (commercially used for beer filtration) in a glass column (5 × 60 cm) to a height of 18 cm. A DA solution (0·5 ppm) was then passed (12 drops/min) through the column, which eliminated all the DA, and yeast autolysis did not pose any problem. For faster flow rates, the authors recommend larger columns with shallow bed. As a further safeguard against yeast autolysis, the authors recommend the use of two filters alternately, so that old cells can be washed off and filters charged with fresh cells periodically. The life span of any such filter will depend upon the quality of yeast strain.

Beer is customarily filtered through diatomaceous earth when it leaves the aging tank and during pumping from finished tank to holding tank[32,33]. The yeast filter can be used at either stage. The yeast cells required for impregnating diatomaceous earth can be obtained from the fermenting wort[33]. Thus the method is neither intrinsically mechanistic in design nor elaborately complicated in operation. It is simple, economical, efficient and reliable.

(4) *Feed-back inhibition by valine* : Owades *et al*[60] were the first to report the feed-back inhibition of DA pathway by valine in yeast. This observation has since been confirmed by others[3,18,19,22,52]. As pointed out earlier, not all yeast strains are repressible by valine, and those which are repressed, do so only under anaerobic condition. Further the mechanism of DA formation by bacteria is totally different than that operating in yeast[5,15,18]. Addition of valine, therefore, is not recommended as a means to control DA production as it is not reliable and economical because of high cost.

(5) *Enzymatic removal of diacetyl from beer* : Seitz *et al*[61,62] were the first to suggest use of DRase for the removal of DA from alcoholic beverages. *Aerobacter aerogenes* 8724 strain is a rich source of the enzyme with a specific activity of 345[62]. A number of other bacteria including lactic streptococci, *Leuconostoc*, psychrophiles and coliforms were also evaluated as a possible source of DRase. The specific activity varied from 3 to 100 for *Streptococcus diacetilactis*, 0 to 8 for *S. lactis* and *S. cremoris*, and *Leuconostoc*, and 3 to 64 for psychrophiles.

Bavisotto *et al*[48] extracted DRase from *A. aerogenes* 8724 strain, and observed reduction of DA level from 1·25 to 0·1 ppm in just an hour. This initial success prompted extensive studies on the use of DRase for commercial beer production[32,33]. In comparison to DRase of brewer's yeast, *Aerobacter* is superior in action ; however, it is not suitable since it is sensitive to acidity (pH < 7·0). This is a very serious limitation to its use for DA reduction in beer, the pH of which is normally 4·1 which appears to precipitate the enzyme[50].

Sensitivity of DRase to ethanol is another limitation to its use in beer. The normal alcohol content of beer is about 3·6%, which is enough to inhibit DRase activity by 42 to 50%[32,50].

Thompson *et al*[32] studied the stability of DRase in crude and in semipure states. Lyophilization inactivated semipure enzyme, but crude enzyme was stable for at least 4 months at –20° C. The specific activity of crude enzyme was reduced 50% either at pH 5·5 or at 5% ethanol. The authors concluded that for optimal functioning, the enzyme will require protection against both ethanol and acidity normally found in beer. Tolls *et al*[33] reported that the enzyme required NADH as a cofactor for optimal functioning in beer. The prohibitive cost of NADH could be a serious limitation to the commercial use of DRase unless it becomes possible to regenerate the cofactor.

Thompson *et al*[32] suggested several measures to overcome the limitations to the commercial use of DRase in breweries. Coating the enzyme with gelatin (1·5%) makes it resistant to both acidity (pH 4·1) and alcohol (5%). Yeast cells can be substituted for NADH. The gleatin–yeast–DRase complex is not only stable to store at 25° C and –20° C but also recoverable after use. However, the complex is not as active in fruit juices and distiller's products as it is in beer[32,33].

3. Economic implications

The crux of DA problem is the yeast strain employed for beer fermentation. Therefore, the employment of a DA-negative strain may prove to be the cheapest, most reliable and least cumbersome method for DA control in beer. Isolation of such a strain is the most logical approach to tackling this problem in the industry.

The immobilized yeast cell technique[33] is also reliable and economical. Yeast cells required in this method are available as a by-product of beer fermentation. Diatomaceous earth needed to immobilize yeast cells is a material routinely used in breweries for beer filtration. The removal of DA from beer using this method is complete, but some precautions against yeast autolysis must necessarily be taken. This will involve the removal of old cells periodically by interruption of the filtration process, and hence the decrease in production efficiency.

The enzymatic removal of DA from beer is quick and complete. While *A. aerogenes* has been shown to be a rich source of DRase[32,33,50], bulk of the enzyme required for commercial use is not available as yet. The enzyme necessary for complete reduction of DA normally present in a barrel of beer is about 0·15 lb (about $2·35 \times 10^7$ units based on 345 specific activity). In addition, the enzyme must be protected against acidity and alcohol normally encountered in beer[32,33] until the immobilized whole cell technology similar to yeast is developed for *A. aerogenes*[33].

4. Summary and conclusions

While DA is an important metabolite for plants, animals, and microorganisms, many yeasts and bacteria produce it as a secondary metabolite and excrete in their growth medium. In this way DA is produced in beer chiefly by brewer's yeast and occasionally by the lactic acid bacteria which occur commonly as contaminants in beer.

DA imparts buttery or cheesy off-flavour to beer, wines, vinegars and fruit juices. The good quality beer should not contain more than 0·07 ppm of DA, while the reported DA content of alcoholic beverages ranges from 0·3 to 50 mg/l, which is much more than what the consumers could tolerate.

Since brewer's yeast is the chief cause of DA off-flavour in beer, the use of DA-negative strain for beer fermentation is the simplest solution to DA problem in beer provided beer is simultaneously protected from contamination by the lactic acid bacteria. Where these two measures are difficult to achieve, DA production in beer could be minimized by resorting to adequate operational methods. *i.e.*, by using minimum amounts of each of seed yeast, sugar and air, and by maintaining low temperature (10° C), low rH (< 10) and high pH (6·0).

Lagering of beer and addition of fresh yeast cells to beer are the traditional methods which have been used by brewers since long for the control of DA in beer. They are time-consuming, unreliable and uneconomical. Filtration of beer through an immobilized yeast filter bed or treatment of beer with DRase are the new methods for DA control in beer which are quick and reliable. However, in their present form, they may appear costly and tedious as they require specialized material, apparatus, and also operational skill when applied on a commercial scale.

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