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EFFECT OF DIMETHYL DICARBONATE (DMDC) ON SURVIVAL OF MICROBIAL ACTIVITY IN BEVERAGES

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Abstract

This study investigated the effect of dimethyl dicarbonate (DMDC) on sensory, physico-chemical and microbial quality of the beverages. Study was carried out on lemon carbonated soft drink, 40% and 70% orange juice (Non-carbonated), it was found that the DMDC was more effective in glass bottle than thin plastic bottle and it does not affect on the colour, flavour, odour and taste of the beverages and it can be applied on conventional cold and hot fill lines with minor adjustments.

Keywords: Dimethyl Dicarbonate (DMDC), Beverage, Sensory analysis and Microbial analysis.

Introduction

During the hot fill, the beverage is heated close up to boiling point to create a stable beverage in regards to microbial load. Hot filled beverages can usually be managed without the addition of preservative and so do not need the addition of preservatives which apper on the label. While the hot fill process produce beverage in a stable manner, high amount of energy for the process and polyethylene therpthalet (PET) material for heat resistance bottles are required. Due to rising energy and raw material cost, this option becomes more costly. Another aspect of hot fill is that the beverages loses its natural and fresh taste.

In the production of soft drinks, microbial contamination is a crucial factor influencing sensory characteristics and is also a health risk for consumers. As alternative to the hot fill process is cold filling with Dimethyl Dicarbonate (DMDC) added to control microorganisms during the production process. It deactivates the enzymes, destroying the microorganism while in the beverage it rapidly break down into negligible amount of methanol and carbon dioxide.

It has also been observed that certain microorganisms showed adaptation to benzoic acid. In other words, there are certain microbes where persistent preservatives are not effective against and that actually eat up those preservatives.

DMDC, is a microbial control agent, it has been used in United States in wine, low alcoholic wine as well as juice, sparklers, sport drinks and ready to drink (RTD). It is very effective at low dosage against a broad range of yeast, molds and bacteria. Unlike other chemical preservatives, it can remain active for several hours (depends up on hydrolysis rate) there by helping to eliminate contamination for other sources such as bottles, closeres, and filling equipment.

It control the microorganisms by entering in the cell and inactivating some of the key enzymes required for cell functioning. Specifically, it is through to react with the histidly residues of proteins, including those involved in the active site of many enzymes. Susceptible enzymes are consequiently rendered functionless due to blockage of the active site and / or conformational chenges instructure. Excess DMDC then completely hydrolyzes in the presence of water.

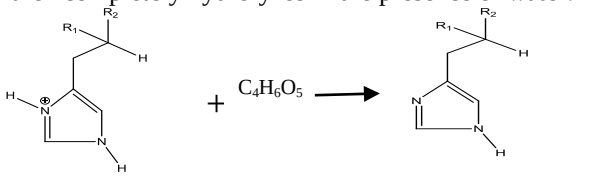

Figure 1: Inactivation of enzyme

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Application of DMDC

- To prevent refermentation in fresh wine, containing residual sugar is susceptible to fermentation in the bottle which can lead to off-odour, off-flavour and effervences. Addition it ito wine during bottling can help prevent fermentation. Also it can be used to replace or decrease the amount of sorbate which is sometimes used in wine containing residual sugar.
- To decrease the amount of sulphur dioxide used in wines, sulphur dioxide used in combination with DMDC has been shown to achieve microbial stability at lower overall sulphur dioxide levels.
- To reduce warehose holding times in early to market wine. It can be used decrease the amount of sulphur dioxide and / or decreases the degree of filtration. These wine speedier sulphur dioxide equilibrium and fewer bottles shock. They are therefore palatable sooner and can be released earlier.

Table 1: Standard dosage of DMDC

Product	DMDC (mg/lit)
Flavour water	125-250
Isotonic beverages	150-250
RTD	225-250
Still fruit based beverages	125-250
CSD (>10 % fruit juice)	175-250
CSD (<10 % fruit juice)	125-250
Alcohol free wine	125-250

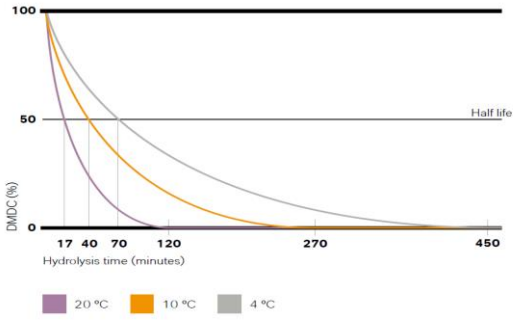

Figure 2: Hydrolysis & Time relation

Materials and methods

Materials

Hydrolysis strips & DMDC was obtained from LANXESS, Germany. (CAS No.: 004-525-33-1, INS: 242, Assay: 99.8%), Orange concentrate was obtained from Food system asia, Pune, India, this study was carried out at International Flavours and Fragrances India Pvt., Ltd., Gurgaon, India.

Methods

Orange juice 40% (Non-carbonated)

To obtain 40% orange juice, amount of orange concentrate taken was calculated.
Single strength (SS) of orange is 10.

$$\text{Quantity} = \frac{\text{Juice content (Desired)} \times \text{SS of Orange}}{\text{TSS of Orange Concentration}}$$

Amount of sugar required is also calculated according to final brix requirement. For the preparation of sample hot water of known quantity was taken, sugar was added and allowed to mix under continuous strring. Stabilizer was added slowly and mixed. After cooling it remaining sugar, acidity regulator, Ascorbic acid, Orange concentrate, stabilizer, remaining water and flavour was added under continuous mixing. The pasteurization of juice was carried out at 93^0C for 2-3 seconds. Control sample was prepared by fillied and suddenly cooled in glass bottle. After cooling the juice bellow 20^0C DMDC was added and sealed tightly. Shaking of each bottle for 10 inches 20 times in 10 seconds was done after addition of DMDC.

Lemon carbonated soft drink (CSD)

For the preparation of lemon CSD 56^0 Brix Sugar syrup was taken and filtered through muslin cloth. Acidity regulator, Lemon flavor, and preservative were added and allowed for mixing. For control sample desired quantity of beverage was taken in the glass bottles, pasturised suddenly cooled and carbonated. Remaining beverage was taken in glass bottles, DMDC was added, mixed well and carbonated.

Physicochemical analysis

Total Soluble Solid (TSS), Hydrolysis test, % Acidity, pH, specific gravity and sensory evaluation standard procedure by *Ranganna* was followed.

Microbial analysis

Total Plate count, yeast and mold, coliform of beverage was detrmined by using BIS methods.

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Results and discussion

This experiment began by testing the DMDC in beverages with different concentrations and different packaging materials.

Sensory data of 40 % orange juice beverage

Sensory data of 40 %, 70% orange juice and lemon CSD beverages were obtained by comparing with control sample which was pasteurized at 93^0 C.

Table 2 Effect of DMDC on sensory quality of 40 % orange juice, 70 % orange juice and lemon CSD

Parameters	Orange 40%				Orange 70%				Lemon CSD			
	15 days	30 days	60 days	90 days	15 days	30 days	60 days	90 days	15 days	30 days	60 days	90 days
Overall score	1	2	2	3	1	4	5	4	3	5	6	9
Appearance	1	1	1	1	1	1	1	2	1	1	1	2
Aroma	1	2	2	3	1	2	2	2	3	3	4	4
Flavor	1	3	2	3	1	3	3	3	3	5	5	6
Aftertaste												

Hedonic scale – 1 to 2 None to Very little difference, 3 to 5 Slight difference, 6 to 7 Medium difference, 8 to 9 Strong difference and 10 to 11 Extreme difference.

All sensory parameters of 40% and 70 % orange juice were compared with the control sample and found no change in sensory up to 15 days; there was very little difference up to 60 days and slightly difference found up to the 90 days. As DMDC had major role in inactivation of microbes, it had no absolute effect on sensory quality of the juices. DMDC was more effective against high concentration of juice up to 90 days. Above results indicate that DMDC was not more effective against Lemon CSD. Extreme changes found in overall sensory parameter. All sensory parameters of Lemon CSD were compared with the control sample and found slight change up to 15 days; there was extreme difference up to 90 days. It became oxidised after 30 days. That means DMDC presence not much affect on sensory character of the 40 % and 70 % orange juice. In lemon CSD, appearance was same but other parameters were changed, not because of microbial activity but due to oxidation of this beverage. That means DMDC is not much effective on control oxidation.

Physicochemical properties

pH, TSS and % Acidity was checked by Ranganna methods.

Table 3: Effect of DMDC on - pH, TSS and % Acidity of beverage

Product	pH				TSS				% Acidity			
	15 days	30 days	60 days	90 days	15 days	30 days	60 days	90 days	15 days	30 days	60 days	90 days
40 % orange juice	3.64	3.65	3.65	3.82	14.2	13.80	13.8	13.9	0.33	0.34	0.33	0.33
70 % orange juice	3.81	3.82	3.82	4.10	15.2	15.50	15.9	15.2	0.42	0.44	0.49	0.46
Lemon CSD	2.90	3.30	3.25	3.30	11.5	11.40	11.2	11.5	0.24	0.38	0.35	0.35

From the results, it was observed that in 40 % and 70% orange juice pH was remained same until 60 days but at 90 days it change slightly, that means microbial activity increases. Total soluble solids were increased due to inversion and decreased due to microbial activity. Above results clearly shows that, negligible changes of total soluble solid in all three beverages. Results were varying in the range of 0.2-0.3^0 brix due to room temperature and calibration. As stated by the results, the acidity of 40% orange juice remained almost unchanged. But acidity of 70% orange juice and Lemon CSD slightly changed. Changes were due to the microbial activity in 70 % orange juice and due to oxidation of Lemon CSD.

Microbiological Analysis

Analysis data are representing in following table and figures, below represented results after 90 days from processing and result of products at zero day was "0 cfu /ml".

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Table 4 Effect of DMDC on - Microbial analysis

Product	Y&M	TPC	Coliform
40% Orange Juice Control	10 Cfu/ml	20 Cfu/ml	0 Cfu/ml
40 % Orange Juice Sample	25 Cfu/ml	30 Cfu/ml	0 Cfu/ml
70 % Orange Juice Control	40 Cfu/ml	20 Cfu/ml	0 Cfu/ml
70 % Orange Juice Sample	350 Cfu/ml	2000 Cfu/ml	0 Cfu/ml
Lemon CSD Control	0 Cfu/ml	0 Cfu/ml	0 Cfu/ml
Lemon CSD Sample	0 Cfu/ml	3 Cfu/ml	0 Cfu/ml

The effect of DMDC was tested under the same physical conditions of beverages practices. Effect of the maximum legal amount of DMDC (250 mg/lit) on the indigenous microbial population of beverages prepared. In all samples yeast count was up to acceptable level, except 70 % orange juice sample. Coliform count was nil in all samples. In 70 % orange juice sample TPC and Y&M population was not controlled by 250 mg/lit amount of DMDC. These results indicate that DMDC may not more effective against high concentration of juice or due to packaging material (thin plastic).

Conclusion

This experiment led to the conclusion that microbial cells were died in glass packaging material but not in thin plastic material. Moreover, three months after addition of DMDC, percentage of microbial cells increases in 40 % and 70 % orange juice beverage, but not in lemon CSD. This was only due to packaging material, lemon CSD was packed in glass bottle. 40 % and 70 % orange juice was packed in thin plastic bottle. The effectiveness of DMDC was found more in glass bottle comparing to thin plastic bottle, it also depends on the added concentration and type of packaging material. Finally, DMDC might be effective to cure microbial count even at low concentration (250 mg/l), but it is not effective to control oxidation in beverage.

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References

Aline Lonvaud-Funel *et al.* (2005), Effectiveness of dimethyl dicarbonate to stop alcoholic fermentation in wine, Food Microbiology 22, 169-178.

Filipa *et al.* (2004), Target Selection in Designinh Pasteurisation Processes for Shelf- Stable High- Acid Fruit Product, Taylor & Francid, Food Science & Nutrition, 44:353-360.
Food and Beverage Asia, February/March 2012, ISSN: 2010-2364, page 56.

FSSAI, ILBCO's, Food Safety & Standards Acts 2006, Rules 2011, Regulation 2011, 11th Edition.

M. Malfeito- Ferreira *et al.* (2007), Evaluation of the inhibitory effect of dimethyl dicarbonate (DMDC) against wine microorganism, Food Microbiology 25, 422-427.

Rangana, S. (1986). Handbook of Analysis and Quality Control For Fruit And Vegetable Products, 2nd Ed., 88-92, Tata McGraw Hill publishing Co. Ltd, New Delhi , India.

Rosnah Shamsudin *et al.* (2014), Effect of repetitive ultraviolet irradiation on the Physico-chemical properties and microbial stability of pineapple juice, Innovative Food Science and Emerging Technologies, Elsevier.

R.W. Worobo *et al.* (2009), Inactivation of different strains of Escherichia coli O157:H7 in various apple ciders treated with dimethyl dicarbonate (DMDC) and sulfur dioxide (SO_2) as an alternative method, Food Microbiology, Elsevier.

Vincent Renouf *et al.* (2008), Effectiveness of dimethyl dicarbonate to prevent Brettanomyces bruxellensis growth in wine, Food Control 19, 208-216.

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