COST EFFECTIVE PROCESS DEVELOPMENT FOR REDUCTION OF CHEESE ALLERGEN HISTAMINE BY OZONISATION FOR FOOD SAFETY

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Abstract
Food allergen consideration is a fundamental parameter for food safety. The scope of this work is to study the possibilities of reduction of biogenic amines by ozonization treatment. In the presence of histamine in Cheese, some people are prone to allergic responses. The Cheese was given a physical treatment of "Ozonisation." Cheese was exposed to ozone for different time segments of0,15,30,45,60 minutes. Histamine reduction was analyzed using a spectrophotometer. The sample was also studied for change in molecular weight, secondary structure, and thermal properties. The histamine content of Cheese was reduced from 106.25 mg/kg (untreated sample) to 20.87 mg/kg. It was seen through the electropherograms that the molecular weight of the treated, as well as the untreated sample, was ranging between 4000kDa to 20,100kDa.Thus, no significant changes were seen in the molecular weight of the samples. In FTIR analysis, the carboxylic group, fluoro group, was found to be deleted, and the carboxylic group was found added in treated samples. The heat required to break the bonds in the untreated sample is -2.09 J which is very high as compared to the heat required in breaking for the treated samples which are -45.37 J. It can be said that the treatment given to the cheese sample was effective to decrease its histamine content with enhancing the digestibility of protein.

Keywords: *Histamine, biogenic amine, allergen, ozonization, Cheese, digestibility*

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1. INTRODUCTION

The fermentation involves various metabolic processes like catabolism and anabolism, with a minor amount of undesired products. These compounds determine the food safety level of fermented products. The other metabolite compounds may produce toxicological or allergy effects. These metabolites may be a group of "Biogenic Amines (BA).Cheeseis considered as casein protein based product. The protein based fermentation leads in generation of histamine, Tyramine like biogenic aminesin cheese and which may raise a food safety concern. Histamine levels above 500 mg/kg considereda concern for food safety. The proteolytic activities in fermentation promote carboxylation reactions. These reactions produce histamine from free amino acids like histidine.Most of the fermented products havea concern with biogenic amines(ten Brink et al., 1990).Most of the consumer faced a product safety issue with fermented products. Cheese is considered as the most suitablefermentation conditions for decarboxylation reactions due to high proteolysis conditions and free amino acids. These optimum conditions can cross the level of histamine up to 2000 mg/kg, which can be a significant food safety concern(Linares et al., 2012). This food safety concern probability may linear proportion to the per capita cheese consumption. The consumption of Cheese in the world is around 19000000 tons per year, and hence the reduction of the BA approach for the dairy industry can reduce the risk of BA toxicity. The biogenic amines synthesis may depend onpasteurization, starter culture, saltconcertation, acidity, temperature, etc.(Fox et al., 2017).

The high-pressure processing is currently available technology, which found suitable to reduce the biogenic amines significantly. 45 to 76% BA reduction was observed at 400 and 600 MPa (Calzada et al., 2013). The high-pressure homogenization treatment also proved the reduction of BA at pressure 100 MPa (Lanciotti et al., 2007). The gamma radiation treatment can also reduce the BA at irradiation

dose 05, 10, 15 kGy (Kim et al., 2005). The use of high-pressure processing or gamma radiation or homogenization requires high capital investment and,which leads to high production cost than competitors. Hence economically feasible biogenic amine reduction method is the current need of dairy industry segment as well as fermented food producers.

Currently, ozone treatment is only limited for antimicrobial approach. The ozonisationhas proven results to reduce biogenic from poultry meat. The ozone treatment isa cost-effective process than gamma radiation, and high pressure(*Biogenic Amines in Food: Analysis, Occurrence, and Toxicity - Google Books*, no date, 2019)(Mercogliano, 2014) hence hypothesis was predicted for ozone treatment for Cheese to reduce the biogenic amines.

2. MATERIAL AND METHODS

Material:

Processed Cheese (Brand-Amul) purchased from the local market. Histamine dihydrochloride (98% pure), Sulphanilic acid (99.5% pure), Sodium carbonate anhydrous (99.9% pure), Sodium chloride (99.9% pure), Sodium phosphate tribasic dodecahydrate (98% pure), Sodium sulphate anhydrous (99.5% pure), Acetic acid Glacial (99.9% pure), n-Butyl Alcohol (99.5% pure), 2,2 – Diphenyl -1-picrylhydrazyl [DPPH] (95% pure) were procured from Sisco Research Laboratories Pvt. Ltd. (SRL) whereas Sodium nitrite purified (98% pure), Hydrochloric acid (35% pure), Methanol (99.7% pure) were procured from Merck Specialities Pvt. Ltd.

Methods:

Ozone Treatment:

5-8gm of cheese samples were weighed and kept in a vessel filled with distilled water. The ozone gas was then passed through the water for different time intervals of 15,30,45 & 60 minutes by an ozone generator (Make-Kent) with a flow rate of 200 mg/hour at ambient temperature. An untreated sample (0 min) was kept as a control to check the difference between the treated and the untreated samples.

Histamine detection:

1 ml of saline (0.85% Sodium chloride) was added to 1 ml of cheese sample. A salt mixture of 1 g Sodium phosphate tribasic dodecahydrate and 6.25 g of anhydrous Sodium sulfate was prepared and kept ready for use. In the stoppered tube, 0.5 g of this salt mixture was added. The tubes were stoppered and were vortexed till the salt almost dissolved into the liquid. 2 ml of n-butanol was added into this aliquot, and then it was shaken for 1 minute and later allowed to stand for 2 minutes. Again, it was shaken briefly to break the protein gel. Lastly, the tubes were vortexed for 2-4 minutes and then centrifuged at 3100 rpm for 10 minutes at 4°C. After centrifugation, 1 ml of the upper butanol layer was removed and transferred to a new, clean, and dry stoppered tube. This liquid was evaporated to dryness in Petri plates. When there was no liquid left in the plates, the residue was dissolved in 1 ml of distilled water. This sample was further considered for histamine analysis by spectrophotometer. The histamine detection was carried out by spectrophotometric method based on the method developed and validated by (Patange, Mukundan, and Ashok Kumar, 2005),where Histamine dihydrochloride was used to make standard histamine graph; ranging from 0 µg/ml – 100 µg/ml concentration. The concentration of histamine in Cheese was determined using this standard curve with a spectrophotometer (Shimadzu UV-1700). At 496 nm.

Structural modification-FTIR Analysis:

1 gm each of untreated and treated cheese samples were given to perform FTIR. FTIR analysis was done by the Analytical "Chemie method," and the transmittance was recorded in the wavelength range of 3500-500 cm-1.

Molecular Weight Analysis-SDS PAGE:

Sodium Dodecyl Sulphate polyacrylamide gel electrophoresiswas done using the methods of (LAEMMLI, 1970)with 12% acrylamide resolving gel and 5% acrylamide stacking gel containing 0.1% SDS. The protein samples were dissolved in a solution of 1.6 ml D/W and 4ml Urea Buffer and 0.4ml 0f 2-Mercaptoethanol. It was run after 45 mins for

PAGE. After that, 10µl of the total protein was loaded in the well of the gel in a volume of 20µl. Electrophoresis was carried out until the bromophenol blue reached the bottom of the gel. After separation, the gels were stained with 0.1% Coomassie Brilliant Blue R-250 and de-stained several times with water: methanol (50:50,v/v) mixture until the bright background of the gel appeared, and protein bands became visible.

Differential Scanning Calorimetry:

The Differential Scanning Calorimetry (Shimadzu DSC 60) Was Executed With Heat Rate 20°C Per Minute, as mentioned in (Gliguem 2009).

Colour Analysis:

The color analysis l, a,b values of samples were determined by camera lens (Make-Zeiss), and color difference Δ E (Delta E) was carried out by "Colour Analysis- Research Lab Tool" Software in comparison with control samples.(Jung et al., 2015)(Kulkarni et al., 2020).

3. RESULTS AND DISCUSSION

Effect of ozone treatment on histamine concentration:

The untreated cheese sample showed 106.25mg/kg of histamine concertation. The 15 mins treated sample showed 20.85 mg/kg of concentration of histamine in Cheese (Fig.1). The significant reduction *($p<0.05$)* was observed in the 15, 30, 45, and 60 minutes treated samples than the control sample. Further 30, 45 and 60 minutes samples showed 35.76,39.48,42.09 mg/kg respectively (Fig.1). The same directional effect was observed (Mercogliano, 2014). The reduction was observed due to denaturation or alternation of histamine structure by nascent oxygen treatment. The reduction in the histamine level was probably because of the reactive oxygen in the ozone, which reacts with the H group and forms the -OH group and the results of the cyanide experiments suggest that the mechanism of this inactivation of histamine (Best & McHenry, 1930). The increased histamine level in 30,45 and 60 can be the counter effect of ozonization on protein metabolism. The high dose of ozonisations treatment like 8 hours per day can increase the amino acid levels. Further exposure after 15 minutes may lead to the generation of free amino acids and which leads to elevation in biogenic amines (Manderscheid et al., 1991). Hence 15-minute treatment can be considered as the optimum time for ozonization to reduce biogenic amines.

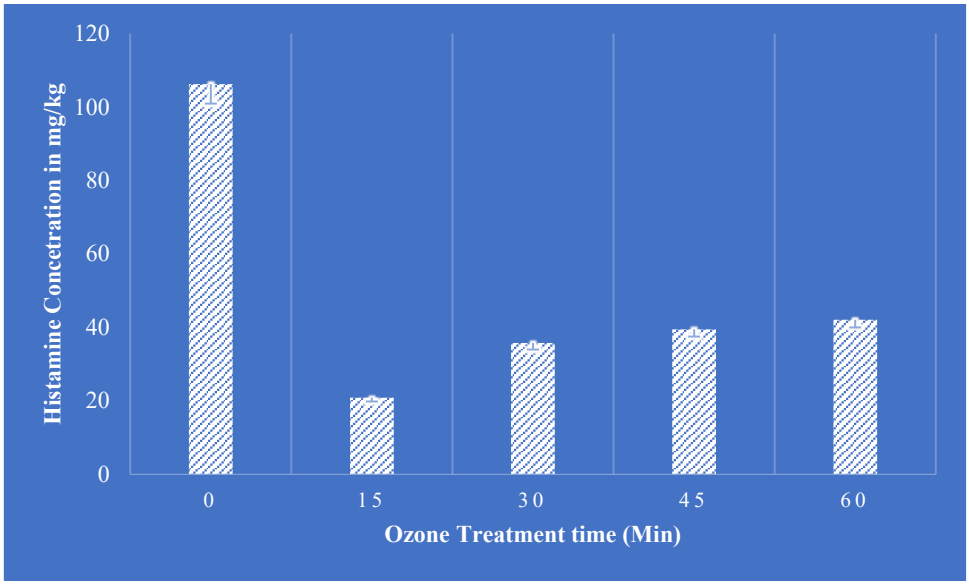


Fig.1. The effect of ozone treatment on histamine concertation of Cheese (n=3)

Structural modification -FTIR analysis-

FTIR was carried out to observe the structural changes in the untreated and treated samples of Cheese. The fingerprinting range was from 3500cm-1 to 500 cm-1 using Happ Genzel Apodization. The spectra show that many features are sensitive to the treatment applied.

Significant changes were observed in peaks of the treated sample, which completely disappeared were in range 3000-3400 cm-1 (Fig.2). The fingerprint region for the secondary structure of protein between 400-1800 cm-1observed significant changes, which indelicate the alternation of the secondary structure of the protein. Significant changes were observed in the -OH group(Alcoholic group). The alcoholic groups were deleted in the treated sample; also, the -NH(primary aliphatic amine) group was deleted in the treated sample. The amide one region (1700-1600 cm-1) showed the stretch of C=O whereas -CH(Alkane), C=O(Cyclopentanone), -OH(Carboxylic Acid), C-F (Fluoro compounds), and C-Cl (Halo compounds) were disappeared whereas -OH hydroxyl group addition was observed in treated sample. A similar result justified on whey protein exposure to ozone gas (Segat, Misra, et al., 2014).

Molecular Weight Analysis-SDS PAGE:

SDS-PAGE profile of both untreated and treated samples was run on a 12% resolving with a protein marker of broad range 0f 3000 Da – 205000 Da (Fig.3.). A similar pattern of bands was observed in both the samples. Thus it can be concluded that no fragmentation of protein took place due to the treatment given to the sample. The same results were observed by(Segat, Biasutti, et al., 2014), where no change in molecular weight was found after ozone treatment on whey proteins.

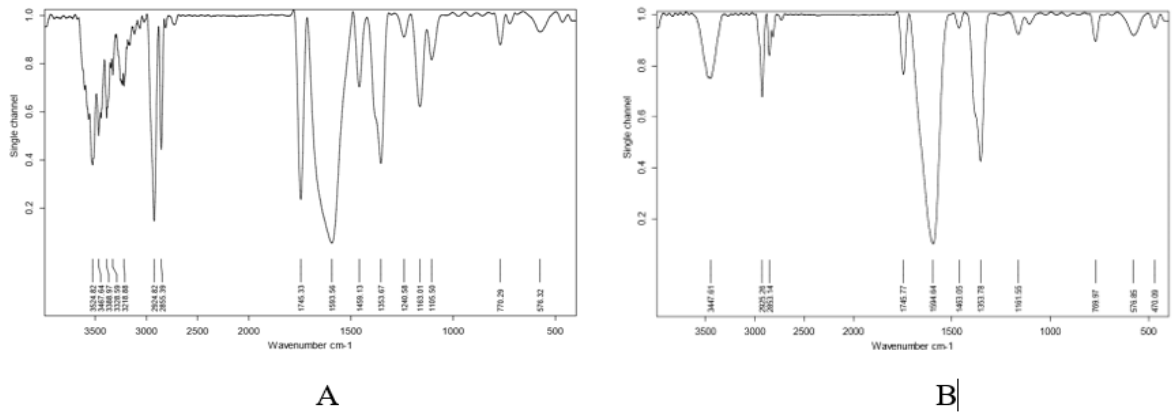


Fig.2. FTIR analysis of A: Untreated Cheese and B: 15-minute treated Cheese

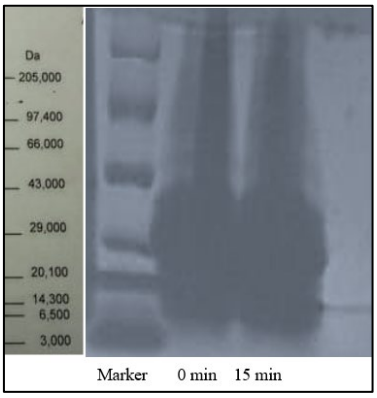
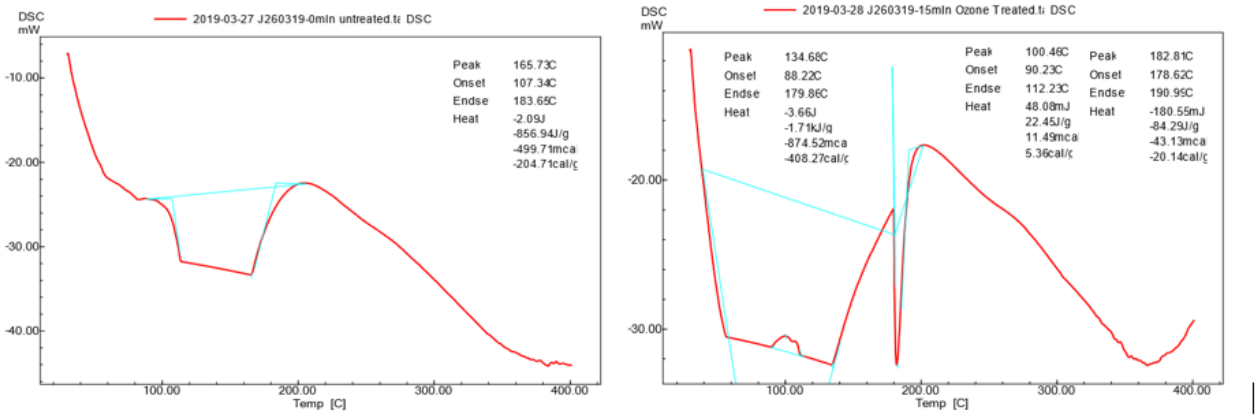


Fig 3. Effect of ozone treatment on the molecular weight of protein (SDS-PAGE)



A B

Fig.4. DSC thermogram for A- Untreated Cheese Sample and B-15 minutes Ozone Treated Cheese Sample

Table 1- DSC characteristics values for Treated and Untreated Samples

Sample	To (°C)	Tp (°C)	Tc (°C)	Tc-To	(J/g)
Untreated	107.34	165.73	183.65	76.31	-2.09
15 minutes Ozone Treated	119.02	139.31	161.02	42	-45.37

Table 2-The color changes (Δ E) in ozone-treated cheese samples. (n=3)

Time (Min)	Δ E
0	0±0
15	13.925±0.44
30	28.703±1.66
45	17.946±4.94
60	14.73±5.52

Differential Scanning Calorimetry:

The DSC thermograms for both Untreated and Treated samples of Cheese are shown in Fig 4 representing their corresponding thermal parameters To, Tp, Tc, Tc-To. Table 1 shows that the thermal parameter values for both samples have been altered. The variation in To, Tp, Tc confirms that alternation in secondary structure and composition of the cheese protein. The energy used by the Untreated sample was -2.09 J/g, whereas the energy used by the Treated sample was -45.37 J/g. It can be seen that the untreated sample needs higher energy to break the bonds than the treated sample. It may be the representation of bond energy reduction and which may lead to better digestibility.These results justify the results (Manderscheid et al., 1991), where free amino acid concentration was found increased.

COLOUR ANALYSIS:

The ozone treated sample was shown a significant amount of color changes with a comparison with the control sample (Table.2). The color change is proportional to the ozone time exposure.

4. CONCLUSION

The reduction of histamine by ozonization can be a potential, economical method for the dairy industry as well as the other biogenic amine concerned food industries. While the increase in protein digestibility adds a significant advantage in the process and which needs to be proved. The 15-minute ozonization time can be considered as the optimum time to reduce a significant amount of biogenic amine like histamine. The only color compromise may be

required from the sensory point, but the cost of processing may consider this method of reduction for industrial processing choice. These preliminary results show that the ozone in food processing can be used for tailor maid operations.

Declarations:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgement:

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