तेजपुर विश्वविद्यालय
(केंद्रीय विश्वविद्यालय)
नपाम, तेजपुर - 784028, असम, भारत

TEZPUR UNIVERSITY
(A Central University)
Napaam, Tezpur - 784028, Assam, India

(सर्वोत्तम विश्वविद्यालय के लिए कुलाध्यक्ष पुरस्कार, 2016, भारत के 100 श्रेष्ठ उच्च शिक्षण संस्थानों में पंचम स्थान और 'नाक' द्वारा 'ए' ग्रेड प्राप्त विश्वविद्यालय)
(Awardee of Visitor's Best University Award, 2016, 5th among India's Top 100 Univesities, MHRD-NIRF-Ranking, 2016 and NAAC Accredited with "A" Grade)

A REPORT ON

In-vivo hypocholesterolemic effect of bioconjugates of starch nanoparticles with gamma oryzanol and tocotrienols extracted from rice bran

1. Name of the Collaborative Activity: **Externally funded Research Project**
2. Nature of Activity : Research activity
3. Name of the Collaborating Agency/ Individual with affiliation, and contact details:
   Dr. Charu Lata Mahanta, Professor, **(Principal Investigator)** Department of Food Engineering and Technology, Tezpur University, Tezpur, Assam, Contact no :

   Dr. Sanjay K Banerjee, Scientist E, Drug Discovery Research Center, Translational Health Science and Technology Institute (THSTI), Faridabad, Haryana.

4. **Summary of collaboration:**

A collaborative research project under the DBT Twinning scheme on the title, 'In-vivo hypocholesterolemic effect of bioconjugates of starch nanoparticles with gamma oryzanol and tocotrienols extracted from rice bran" was conducted between the Principal Investigators **Dr. Charu Lata Mahanta,** Professor, Department of Food Engineering and Technology, Tezpur University, Tezpur, Assam and **Dr. Sanjay K Banerjee,** Scientist E, Drug Discovery Research Center, Translational Health Science and Technology Institute (THSTI), Faridabad, Haryana. **DBT Sanction Order No. & Date are** No.BT/PR16804/NER/95/294/2015, Date: 10th Nov, 2016.

5. List of year-wise activities under the collaboration:

In this collaborative project, the animal experiment that formed the 4th objective was conducted under the supervision of Dr. Banerjee of THSTI. The objective was:

To determine anticholesterol effect of γ-oryzanol, tocotrienols and their bionanocaonjugates in vivo

Specific output

Very encouraging results were obtained from the collaborative work. The specific outcome are listed below:

1. The hydrophobic conjugate developed in the project showed good gastrointestinal stability, low serum levels of TGL, AST and ALT.
2. Conjugation of tocotrienol with starch nanoparticle-linoleic acid conjugate was effective in reducing liver fat accumulation and liver fibrosis besides lowering serum parameters.

Signature of Faculty	Signature and Seal of Head of Department/ Centre/ Cell
Name: Charu Lata Mahanta Designation: Professor	Name: Designation: Head Department of Food Engg. & Technology Tezpur University Napaam, Tezpur- 784028, Assam

2016 Research Project 2

Administrative
(Application No: MAP/2015/42)

File No. BT/PR16804/NER/95/294/2015
GOVERNMENT OF INDIA
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY
(NER-BPMC)
****

Block-2, 7th Floor,
CGO Complex, Lodhi Road
New Delhi-110003
Dated: 7 / 11 / 2016

ORDER

Sanction of the President is hereby accorded under Rule 18 of the Delegation of Financial Powers Rules, 1978 for the implementation of the project under **'DBT's Twinning programme for the NE'** titled **"In vivo hypocholesterolemic effect of bioconjugates of starch nanoparticles with gamma oryzanol and tocotrienols extracted from rice bran"** by Dr. Charu Lata Mahanta, Tezpur University, Napaam, Tezpur and Dr. Sanjay Kumar Banerjee, Translational Health Science and Technology Institute (THSTI), Faridabad, Gurgaon at a total cost of **₹ 79.60 lakhs (Rupees Seventy Nine lakhs Sixty Thousand only)** for a period of three years on the terms and conditions detailed as under:

2.0 The Project:

2.1 Project Title: In vivo hypocholesterolemic effect of bioconjugates of starch nanoparticles with gamma oryzanol and tocotrienols extracted from rice bran

2.2 Project Investigators

2.2.1 Principal Investigator: (Parent Institute)	**Dr. Charu Lata Mahanta** Professor Department of Food Engineering and Technology Tezpur University, Napaam, Sonitpur- 784028, Tezpur
2.2.2 Co-PI Name: (Parent Institute)	**Dr. Mattaparthi Venkata Satish Kumar** Assistant Professor Department of Molecular Biology and Biotechnology Tezpur University, Napaam, Sonitpur- 784028, Tezpur
2.2.3 Principal Investigator: (Collaboration Institute)	**Dr. Sanjay Kumar Banerjee** Scientist E Drug Discovery Research Centre (DDRC) Translational Health Science and Technology Institute (THSTI), NCR Biotech Science Cluster, 3rd Milestone, Faridabad-121001, Gurgaon Expressway PO box 04, Faridabad.

Charu Lata Mahanta

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Inhibition mechanism of 3-hydroxy-3-methyl-glutaryl-CoA reductase by tocotrienol-rich rice bran fraction optimally extracted with ultrasonic energy

Gitanjali Gautam [a], Raj Kumar Duary [a], Kuldeep Gupta [b], Charu Lata Mahanta [a,*]

[a] Department of Food Engineering and Technology, School of Engineering, Tezpur University, 784 028, India
[b] Department of Molecular Biology and Biotechnology, School of Sciences, Tezpur University, 784 028, India

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ABSTRACT

Tocotrienols (T3) are vitamin E components that inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), a primary target for cholesterol management. T3 was extracted from rice bran (RBE) using ultrasonic energy keeping solute: solvent ratio, power and time on specific energy and T3 concentration as responses as per Box–Behnken Design. The lowest specific energy (52.38 ± 0.14 J mL^{-1}) uptake by the sample was most effective in enhancing the concentration of T3 in RBE (199.34 ± 0.63 μg mL^{-1}). In vitro HMGR kinetics and in silico binding interactions of the identified α-, δ- and γ-T3 fractions were studied. Enzyme kinetic studies revealed an uncompetitive mode of inhibition by α-T3, γ-T3, and RBE and a mixed mode of inhibition for δ-T3. γ-T3 showed lowest IC_{50} concentration (11.33 μg mL^{-1}) followed by α-T3 (16.73 μg mL^{-1}), RBE (20.45 μg mL^{-1}) and δ-T3 (23.16 μg mL^{-1}). Molecular docking studies highlighted the hydrogen bonding of δ-T3 with Gln766 and α- and γ-T3 with Met655 and Val805 amino acid residues at the NADPH binding site of HMGR. Results indicate the potential use of T3 enriched RBE optimally extracted using ultrasound as potent HMGR inhibitor.

1. Introduction

As per World Health Organization [1], the elevation in blood cholesterol leads to the rise of stroke and heart disease and is a serious concern for both developed and developing countries. 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR, EC 1.1.1.34) plays a crucial role in the biosynthesis of cholesterol in humans. It is a rate-limiting enzyme that catalyzes HMG CoA to mevalonate (an intermediate metabolite for sterols and isoprenoids synthesis) [2,3]. HMGR is a tetrameric enzyme having catalytic domain between residues from 426 to 888 with oligomeric active sites composed of Arg590, Lys690, Lys691 and Ser872 as major amino acids [4]. Statin, a commercial HMGR inhibitor acts as a competitive inhibitor with respect to the substrate (3-hydroxy-3-methyl-glutary-coenzyme A; HMG CoA) that limits the production of hepatic cholesterol [3]. However, there are side effects of synthetic drugs. Lowering the raised level of serum cholesterol using natural based biomolecules can be an effective strategy for reducing the incidence of cardiovascular disease (CVD) [1]. Active research is therefore, underway on natural compounds in order to find a safer alternative medicine.

Rice bran (RB), a by-product of rice processing industries, is currently under-utilized, in spite of having various functional components such as poly-unsaturated fatty acids, vitamin E, and γ-oryzanol. The functional components of rice bran have immense commercial value [5]. Vitamin E, a fat-soluble component, comprises of tocopherols and tocotrienols that exist in different stereo-isomeric forms cumulatively known as toco-chromanol. Structurally, tocopherols consist of saturated three isoprene units attached to the C2 position of chroman ring, whereas in tocotrienols, isoprene units contain three double bonds [6,7]. Tocotrienols (T3) exhibit anti-hypercholesterol property by inhibiting the activity of HMGR through post-transcriptional mechanism [7] and thus can be effectively used for the management of coronary artery disease [1]. Although RB is a rich source of T3, there is a demand for an efficient, effective, simple, and green process for extraction with maximum recovery retaining their functionality [5,8]. The ultrasound assisted extraction (UAE) method is an effective green technology that works on the principle of cavitations. It involves the micro-bubbles nucleation, growth, and collapse due to high acoustic power in the liquid system. This phenomenon leads to an increase in

Abbreviations: HMGR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; RB, rice bran; T3, tocotrienols; UAE, ultrasound assisted extraction; RBE, rice bran extract; DLLME, double dispersive liquid liquid microextraction; LDPE, low-density polyethylene; RSM, response surface methodology; P_a, ultrasonic actual power; MM, Michaelis-Menten; LB, Lineweaver-Burk; K_m, Michaelis-Menten constant; V_{max}, maximum velocity; PDB, Protein Data Bank; IC_{50}, half maximal inhibitor concentration; J mL^{-1}, joule per mL; ΔT, temperature difference; Δt, time difference.

* Corresponding author.
E-mail address: charu@tezu.ernet.in (C.L. Mahanta).

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Atmospheric cold plasma inactivation of *Escherichia coli* and *Listeria monocytogenes* in tender coconut water: Inoculation and accelerated shelf-life studies

Nikhil Kumar Mahnot[a,b], Charu Lata Mahanta[a], Brian E. Farkas[b], Kevin M. Keener[b,c,d,*], N.N. Misra[d]

[a] Department of Food Engineering and Technology, School of Engineering, Tezpur University, Assam, India
[b] Department of Food Sciences, Purdue University, West Lafayette, IN, USA
[c] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, USA
[d] Center for Crops Utilization Research, Iowa State University, Ames, IA, USA

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Keywords:
Barrier discharge
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ABSTRACT

In the current work we evaluated the effect of atmospheric cold plasma (ACP) generated in air and M65 (65% O_2, 30% CO_2, 5%N_2) as working gases for inactivation of *Escherichia coli* and *Listeria monocyotogenes* in tender coconut water. A 5 $\log_{10}$ reduction in the population of both microbes was achieved in tender coconut water with addition of 400 ppm citric acid and plasma treatment with M65 gas for 120 s at 90 kV, followed by a 24 h post-treatment storage under refrigerated conditions. Optical emission spectroscopy was utilized in identifying reactive gas species of nitrogen and oxygen which were accounted for cellular leakage and morphological changes in microbes on plasma treatment. The plasma treatments on tender coconut water caused a lowering of pH and small changes in Hunter color parameters (a^* and b^*), while total soluble solids and total titratable acidity did not change significantly. Accelerated shelf-life studies (ASLS) carried out at 10 °C, 20 °C and 30 °C compared three batches: Batch 1(tender coconut water), Batch 2 (Tender coconut water + citric acid + ascorbic acid) and Batch 3 (Tender coconut water + citric acid + Plasma treatment with M65 + ascorbic acid). ASLS revealed that the rate constants for parameters namely percent transmission, ascorbic acid content, total titratable acidity and total color change decreased in the order Batch 1 > Batch 2 > Batch 3. Estimation of rate constants following Arrhenius model for Batch 3 at 5 °C was comparable to experimental results. A shelf-life of 48 days was predicted for Batch 3 at 5 °C considering 75% ascorbic acid content degradation. Thus, ACP was concluded to be a novel technology for tender coconut water processing.

1. Introduction

In recent times atmospheric cold plasma technology is being explored as a nonthermal intervention for decontamination and shelf-life extension of foods. From the research standpoint, ample work has been carried out revealing the promising applications of cold plasma technology in the field of microbial decontamination in liquid food products, mostly fruit juices of apple, orange, pomegranate, tomato (Almeida, Cavalcante, Cullen, Frias, Bourke, Fernandes, 2015; Shi et al., 2011; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014; Kovacevi, Putnik, Dragovic-Uzelac, Pedisic, Rezek-Jambrak, & Herceg, 2016; Ma & Lan, 2015), milk (Korachi et al., 2015) and coconut liquid endosperm (Gabriel et al., 2016). Of these, only a few studies employing plasma jets, dielectric barrier discharge, and radio–frequency technology of plasma generation were effective in inactivating 5-log reduction in *Escherichia coli, Staphylococcus aureus, Candida albicians, Citrobacter freundii* in different fruit juices were reported (Montenegro, Ruan, Ma, & Chen, 2002; Surowsky et al., 2014). The U.S. Food and Drug Administration (FDA, 2001) suggests a need of 5-$\log_{10}$ reduction performance standard against hardy pathogenic microbes for an effective juice processing. Within this context, *E. coli* and *L. monocytogenes* have been reported to be responsible for disease outbreaks from the consumption of fruit and vegetable juices (Han & Linton, 2004; Vojdani, Beuchat, & Tauxe, 2008).

Tender coconut water (TCW), not to be confused with coconut milk, is the inner clear liquid portion also called the liquid endosperm of the

* Corresponding author. Department of Food Sciences, Purdue University, West Lafayette, IN, USA.
E-mail addresses: nikhil.mahnot@gmail.com (N.K. Mahnot), charu@tezu.ernet.in (C.L. Mahanta), bfarkas@purdue.edu (B.E. Farkas), kkeener@iastate.edu (K.M. Keener), misra.cftri@gmail.com (N.N. Misra).

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Strategy to achieve a 5-log *Salmonella* inactivation in tender coconut water using high voltage atmospheric cold plasma (HVACP)

Nikhil Kumar Mahnot[a,b], Charu Lata Mahanta[a], Kevin M Keener[b,c,d,*], N.N. Misra[c]

[a] Department of Food Engineering and Technology, School of Engineering, Tezpur University, Assam, India
[b] Department of Food Sciences, Purdue University, West Lafayette, IN, USA
[c] Center for Crops Utilization Research, Iowa State University, Ames, IA, USA
[d] BioCentury Research Farm, Iowa State University, Ames, IA, USA

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ABSTRACT

This study examined high voltage atmospheric cold plasma (HVACP) technology as a non-thermal intervention for inactivating *Salmonella enterica* serovar Typhimurium LT2 (ST2) in tender coconut water (TCW). Treatment with HVACP in air at 90 kV for 120 s inactivated 1.30 $\log_{10}$ of ST2. Development of a TCW stimulant suggested an interfering role of magnesium and phosphate salts with HVACP inactivation. Generation of reactive gas species, viz. ozone and hydrogen peroxides were found to be responsible for microbial inactivation. The addition of 400 ppm citric acid to the TCW effectively reduced ST2 by 5 $\log_{10}$ during HVACP treatment. Under these conditions, higher cellular leakage and morphological damage were observed in ST2. Minimal physico-chemical changes in TCW were observed with HVACP treatment, except for an 84.35% ascorbic acid loss (added externally). These results demonstrate a potential pathway for developing highly effective cold plasma treatments to preserve fruit and vegetable juices.

1. Introduction

The increasing market demand for minimally processed, safe and high-quality food products has paved the way for development of novel non-thermal technologies for food processing. Atmospheric pressure cold plasma is an emerging process intervention for preservation of food products with advantages of being a low-cost technology that is non-toxic, leaves no known residues, and causes minimal damage to foods. Thus, it shows promise in replacing or at least, complementing conventional pasteurization technologies (Pankaj, Wan, & Keener, 2018). There are various methods of cold plasma production, viz. dielectric barrier discharge (DBD), plasma jets, coronas, and microwave discharges. Individual researchers have preferences toward specific plasma devices based on the application(s) of interest. It is worthwhile mentioning that the reactive gas species generated in plasma devices are device dependent. Thus, success of one device in one application does not guarantee success of a different plasma device in that same application. High voltage atmospheric cold plasma devices have been demonstrated to be very effective in a variety of food applications. Examples include plasma decontamination of raw and dried produce, solid foods like cheese, ham, eggshells, bacon and liquid foods like milk and juices (e.g. apple, pomegranate, orange and grape) (Pankaj et al., 2018; Xu, Garner, Tao, & Keener, 2017).

The antimicrobial nature of cold plasma treatment has been demonstrated by several researchers in the past (Liao et al., 2017). The microbial inactivation mechanisms which have been reported and suggested includes generation of reactive gas species, viz. reactive oxygen species (ROS), reactive nitrogen species (RNS), and minor contributions from ultraviolet light. This cocktail of reactive species enables the inactivation of microbes on food surfaces, and could further diffuse into liquid media, thereby effecting the acidification and microbial inactivation in liquids (Gaunt, Beggs, & Georghiou, 2006; Oehmigen et al., 2010). The use of gases like He, He/O_2, Ar, Ar/O_2, N_2/O_2, $N_2/O_2/CO_2$ and air has been shown to be effective in microbial inactivation with plasma (Misra, Keener, Bourke, Mosnier, & Cullen, 2014; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014).

The demand for processing and preservation of tender coconut is on the rise due to its natural rehydrating properties, nutritive composition, unique flavour and rising awareness among consumers regarding the deleterious health effects of artificial carbonated drinks. The nutritive composition of tender coconut water renders its high sensitivity towards microbial growth and thus, a very short shelf-life. Thermal processing is effective in killing microbes, but changes the nutritive and flavour characteristics of tender coconut water. Thus, the industry is in

* Corresponding author at: Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, USA.
E-mail addresses: charu@tezu.ernet.in (C.L. Mahanta), kkeener@iastate.edu (K.M. Keener), misrann@iastate.edu (N.N. Misra).

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