COLD PLASMA-HURDLE TREATMENT FOR EXTENDED SHELF-LIFE OF PANEER

THESIS SUBMITTED TO THE

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE

(DEEMED UNIVERSITY)

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF DEGREE OF

MASTER OF TECHNOLOGY
IN
DAIRY ENGINEERING

BY

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This is to certify that the thesis entitled, **"COLD PLASMA-HURDLE TREATMENT FOR EXTENDED SHELF-LIFE OF PANEER"**, submitted by **Mr. VIPIN CHANDRA JEENGAR** towards the partial fulfillment for the award of the degree of **MASTER OF TECHNOLOGY** in **DAIRY ENGINEERING** of the **ICAR-NATIONAL DAIRY RESEARCH INSTITUTE (DEEMED UNIVERSITY), KARNAL (HARYANA), INDIA**, is a bonafide research work carried out by him under my guidance, and no part of the thesis has been submitted for any other degree or diploma.

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Abstract

Cold plasma (CP) is a novel non-thermal technology having potential to inactivate the surface microflora of food products. The main aim of the study was to harness the potential of CP and hurdle technologies to extend the shelf-life of paneer. Paneer was prepared from cow milk containing 3.5% fat and 8.5% solids-not-fat. The milk was heated to 95°C for 5 min, cooled to 70°C, coagulated using 1% citric acid solution and the whey was drained to get chhana. The chhana was pressed into paneer in a manual press to moisture levels of <55% and >55%, and the paneer thus obtained was cut into cubes of 2×2×2 cm size. The paneer cubes were treated with CP generated at 25 kV DC applied voltage with current flow in the range of 0.01-1.0A and 680 mm of Hg of vacuum. The CP chamber was made up of SS316 having a volume of 22 L, while the oil immersion type vacuum pump used had a capacity of 1 hp. Paneer was CP treated at exposure times of 3, 5 and 8 min, keeping moisture (water activity) as the hurdle. It was contemplated to destroy the microorganisms, while maintaining minimum moisture loss from paneer.

Paneer cubes showed moisture loss of 4.5-4.9% after 3 min of CP treatment, while 5.8 to 6.2% and 5.7 to 7.0% loss of moisture was observed after 5 and 8 min treatment, respectively. CP treatment resulted in reduction of total plate count (TPC) to 4.0±0.42, 3.15±0.21 and 3.0±0.15 log cfu/g after 3, 5 and 8 min treatment respectively from the initial population of 4.84±0.39 log cfu/g at <55% initial moisture content. In comparison, at initial moisture content >55%, the count reduced to 3.97±0.29, 3.18±0.16 and 3.0±0.18 log cfu/g after 3, 5 and 8 min treatment from the initial population of 5.13±0.19 log cfu/g. At both moisture levels, CP treatment resulted in inactivation of yeast and moulds. Based on various physicochemical and textural analyses, it was observed that there was no effect of CP treatment on whiteness index (WI) but hardness increased with exposure time. Paneer with >55% inital moisture content had relatively less hardness.

Paneer with >55% initial moisture content and 5 min of CP treatment was optimized using L_8 $(4^1 \times 2^1)$ Taguchi orthogonal array design and used for shelf-life evaluation. After 9 days of atmospheric packaging, CP treated paneer had TPC of 4.09 and 3.97 log cfu/g at refrigerated and frozen conditions as compared to 5.07 and 4.69 log cfu/g, respectively in untreated paneer. In CP treated paneer, yeast and mould counts were 2.7 log cfu/g when stored in refrigerated condition while no detectable growth was observed in frozen condition. Comparatively, in untreated paneer, 3.2 and 2.9 log cfu/g population was observed in refrigerated and frozen conditions. Free fatty acid (FFA) content of untreated paneer stored in refrigerated condition increased from 1.24 to 2.99 µ.eq/g after 9 days, while the increase in CP treated paneer was relatively higher at 3.21 µ.eq/g. In frozen condition, the rate of increase in FFA of untreated paneer after 9 days was relatively lower at 1.29 to 1.79 µ.eq/g. In comparison, for CP treated paneer stored under refrigeration for 9 days, the increase in FFA content was 1.22 to 2.01 µ.eq/g. TBARS value of CP treated paneer increased at relatively lower rate than that of untreated paneer under both refrigerated and frozen conditions. Similarly, vacuum-packaged paneer cubes also manifested increase in hardness, FFA and TBARS content during storage, while WI decreased. Also, it was observed that the rate of physicochemical and textural changes was slower in paneer cubes packaged in vacuum condition as compared to those stored under atmospheric packaging in refrigerated condition. Also, CP treated paneer samples had better microbiological quality than that of untreated paneer after 9 days of refrigerated and frozen storage when packaged under normal atmospheric and vacuum conditions.

सारांश

कोल्ड प्लाज्मा (सीपी) एक नई गैर-थर्मल तकनीक है जिसमें खाद्य उत्पादों के सतही माइक्रोफ्लोरा को निष्क्रिय करने की क्षमता है। अध्ययन का मुख्य उद्देश्य पनीर के शेल्फ जीवन को बढ़ाने के लिए सीपी और बाधा प्रौद्योगिकियों की क्षमता का दोहन करना था। पनीर गाय के दूध से तैयार किया गया था जिसमें 3.5% वसा और 8.5% वसा रहित ठोस पदार्थ थे। दूध को 5 मिनट के लिए 95 डिग्री सेल्सियस तक गर्म किया जाता है, 70 डिग्री सेल्सियस तक ठंडा किया जाता है, 1% साइट्रिक एसिड के घोल का उपयोग करके गाढ़ा किया जाता है और छाने को निकालने के लिए मट्ठा निकाला जाता है। छेना को मैनुअल प्रेस में <55% और >55% नमी के स्तर तक पनीर में दबाया गया था, और इस प्रकार प्राप्त पनीर को 2×2×2 सेमी. आकार के क्यूब्स में काट दिया गया था। पनीर क्यूब्स को 25 केवी डीसी एप्लाइड वोल्टेज पर उत्पन्न सीपी के साथ 0.01-1.0 ए की सीमा में विद्युत प्रवाह और वैक्यूम के 680 मिमी एचजी के साथ इलाज किया गया था। CP कक्ष SS316 से बना था जिसमें 22 L का आयतन था, जबकि तेल विसर्जन प्रकार के वैक्यूम पंप का उपयोग 1 hp की क्षमता के साथ किया गया था। नमी (पानी की गतिविधि) को बाधा के रूप में रखते हुए, पनीर को 3, 5 और 8 मिनट के एक्सपोजर समय पर इलाज किया गया था। पनीर से नमी की न्यूनतम हानि को बनाए रखते हुए सूक्ष्मजीवों को नष्ट करने पर विचार किया गया था।

पनीर क्यूब्स ने सीपी उपचार के 3 मिनट के बाद 4.5-4.9% की नमी की कमी दिखाई, जबकि 5 और 8 मिनट के उपचार के बाद क्रमशः 5.8 से 6.2% और 5.7 से 7.0% नमी की कमी देखी गई। सीपी उपचार के परिणामस्वरूप कुल प्लेट काउंट (टीपीसी) घटकर 4.0 ± 0.42, 3.15 ± 0.21 और 3.0 ± 0.15 लॉग सीएफयू/जी हो गया। <55% प्रारंभिक नमी सामग्री। इसकी तुलना में, प्रारंभिक नमी > 55% पर, 3.97 ± 0.29, 3.18 ± 0.16 और 3.0 ± 0.18 लॉग सीएफयू/जी की संख्या 3, 5 और 8 मिनट के उपचार के बाद 5.13 ± 0.19 लॉग सीएफयू/जी की प्रारंभिक आबादी से कम हो गई। . दोनों नमी स्तरों पर, सीपी उपचार के परिणामस्वरूप खमीर और मोल्ड निष्क्रिय हो गए। विभिन्न भौतिक-रासायनिक और टेक्सचरल विश्लेषणों के आधार पर, यह देखा गया कि श्वेतता सूचकांक (WI) पर CP उपचार का कोई प्रभाव नहीं पड़ा, लेकिन एक्सपोज़र समय के साथ कठोरता बढ़ गई। 55% से अधिक आंतरिक नमी वाले पनीर में अपेक्षाकृत कम कठोरता थी।

पनीर > 55% प्रारंभिक नमी सामग्री और 5 मिनट सीपी उपचार के साथ एल8 (41-21) तागुची ऑर्थोगोनल एरे डिजाइन का उपयोग करके अनुकूलित किया गया था और शेल्फ-लाइफ मूल्यांकन के लिए उपयोग किया गया था। वायुमंडलीय पैकेजिंग के 9 दिनों के बाद, सीपी उपचारित पनीर का टीपीसी 4.09 और 3.97 लॉग सीएफयू/जी था, जबकि अनुपचारित पनीर में क्रमशः 5.07 और 4.69 लॉग सीएफयू/जी था। सीपी उपचारित पनीर में, खमीर और मोल्ड की मात्रा 2.7 लॉग सीएफयू / जी थी जब रेफ्रिजरेटेड स्थिति में संग्रहीत किया गया था, जबकि जमी हुई स्थिति में कोई पता लगाने योग्य वृद्धि नहीं देखी गई थी। तुलनात्मक रूप से, अनुपचारित पनीर में, 3.2 और 2.9 लॉग सीएफयू/जी आबादी को रेफ्रिजरेटेड और फ्रोजन स्थितियों में देखा गया था। रेफ्रिजरेटेड स्थिति में संग्रहीत अनुपचारित पनीर की मुक्त फैटी एसिड (एफएफए) सामग्री 9 दिनों के बाद 1.24 से 2.99 μ.eq/g तक बढ़ गई, जबकि सीपी उपचारित पनीर में 3.21 μ.eq/g पर अपेक्षाकृत अधिक वृद्धि हुई। जमी हुई स्थिति में, 9 दिनों के बाद अनुपचारित पनीर के एफएफए में वृद्धि की दर अपेक्षाकृत कम 1.29 से 1.79 μ.eq/g थी। इसकी तुलना में, 9 दिनों के लिए प्रशीतन के तहत संग्रहीत सीपी उपचारित पनीर के लिए, एफएफए सामग्री में 1.22 से 2.01 μ.eq/g की वृद्धि हुई थी। सीपी उपचारित पनीर का टीबीएआरएस मूल्य प्रशीतित और जमे हुए दोनों स्थितियों में अनुपचारित पनीर की तुलना में अपेक्षाकृत कम दर से बढ़ा। इसी तरह, वैक्यूम-पैक पनीर क्यूब्स में भी भंडारण के दौरान कठोरता, एफएफए और टीबीएआरएस सामग्री में वृद्धि हुई, जबकि डब्ल्यूआई में कमी आई। साथ ही, यह भी देखा गया कि वैक्यूम स्थिति में पैक किए गए पनीर क्यूब्स में भौतिक-रासायनिक और बनावट परिवर्तन की दर रेफ्रिजरेटेड स्थिति में वायुमंडलीय पैकेजिंग के तहत संग्रहीत की तुलना में धीमी थी। इसके अलावा, सीपी उपचारित पनीर के नमूनों में 9 दिनों के रेफ्रिजरेटेड और जमे हुए भंडारण के बाद सामान्य वायुमंडलीय और वैक्यूम स्थितियों के तहत पैक किए जाने के बाद अनुपचारित पनीर की तुलना में बेहतर सूक्ष्मजीवविज्ञानी गुणवत्ता थी।

Abstract

List of Symbols and Abbreviations

Mathematical Operators

%	Percentage
×	Multiplication
°	Degree
<	Less than
>	Greater than
±	Plus or minus

Greek and Latin Symbols

µ.eq/g	Micro equivalent per gram
µm	Micrometre

Abbreviations

$^1O_2$	Singlet oxygen
A	Ampere
a*	Difference in red and green
AC	Alternating current
ACP	Atmospheric cold plasma
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APP	Atmospheric pressure plasma
APPJ	Atmospheric pressure plasma jet
b*	Difference in yellow and blue
°C	Degree Celsius
CAGR	Compounded annual growth rate
CFU	Colony forming unit
cm	Centimetre

CO_2	Carbon dioxide
CP	Cold plasma
d	Gap between electrodes
DBD	Dielectric barrier discharge
DC	Direct current
df	Degree of freedom
DNA	Deoxyribonucleic acid
e^-	Electron
Eq.	Equation
et al.	And others
etc.	Et cetera
eV	Electron Volt
FFA	Free fatty acid
Fig.	Figure
FSSAI	Food Safety and Standards Authority of India
g	Gram
GDP	Gross domestic product
GHz	Giga Hertz
GOI	Government of India
H	Hour
H_2O	Water
H_2O^+	Oxoniumyl
H_2O_2	Hydrogen per oxide
H_2SO_4	Sulfuric acid
He	Helium
Hg	Mercury
hp	Horsepower
HSD	Honestly significant difference
HVACP	High voltage atmospheric cold plasma
ICAR	Indian Council of Agricultural Research
IMARC	International Market Analysis Research and Consulting

K	Kelvin
kg	Kilogram
kHz	Kilo Hertz
KOH	Potassium hydroxide
kPa	Kilo Pascal
kV	Kilo Volt
L	Litre
L*	Difference in lightness and darkness
LDPE	Low density polyethylene
LLDPE	Linear low density polyethylene
Log	Logarithmic
LPM	Litre per minute
m	Metre
mA	Milli Ampere
MAP	Modified atmosphere packaging
mg	Milligram
MHCD	Micro hollow cathode discharge
MHz	Mega Hertz
min	Minutes
mL	Millilitres
mm	Millimetre
MSD	Mean square deviation
N	Normality
N	Newton
N	Atomic nitrogen
N_2	Dinitrogen
NaCl	Sodium chloride
NCDC	National Centre for Disease Control
NDDB	National Dairy Development Board
n_e	Density of electron
nm	Nanometre

NO	Nitric oxide
$NO^{\bullet}$	Nitric oxide radical
NO_2	Nitrogen dioxide
NO_3	Nitrate ion
NTP	Non-thermal plasma
O	Atomic oxygen
O^{-2}	Superoxide anion
O_3	Ozone
OAUGDP	One atmospheric uniform glow discharge plasma
OH^-	Hydroxyl ion
$OH^{\bullet}$	Hydroxyl radical
P	Pressure
PAW	Plasma activated water
PDA	Potato dextrose agar
pH	Potential of hydrogen
PPM	Parts per million
RF	Radio frequency
RH	Relative humidity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
s	Second
S/N	Signal to Noise ratio
SD	Standard deviation
SH	Sulfhydryl group
SNF	Solids-not-fat
Spp.	Species
SS	Stainless steel
T	Neutral gas temperature
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid

T_e	Temperature of electron
T_g	Overall temperature of gas
T_h	Temperature of heavy particles
T_i	Temperature of ions
TIFF	Tagged Image File Format
TOI	Times of India
T_p	Temperature of plasma
TPA	Texture profile analysis
TPC	Total plate count
UV	Ultraviolet
UV-Vis	Ultraviolet visible
V	Volt
v/v	Volume per volume
W	Watt
WHO	World Health Organization
WI	Whiteness index

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Introduction

1. Introduction

India is the leading producer and consumer of dairy products in the world. It produced 198.4 million metric tonnes of milk in the year 2019-20 (Economic Times, 2021), accounting for nearly 22.26% of the global market share. About 45% of the milk produced is used for the manufacture of traditional dairy products. Out of this share, 12% of the milk used for manufacturing of traditional dairy products is processed into paneer, comprising about 5% of the total milk produced (Edelweiss, 2017). A report by the IMARC group expects the market for paneer in India to grow at a CAGR of 15% in the period 2021-2026 (www.imarcgroup.com/paneer-market-india).

Paneer is an Indian variety of soft cheese obtained by heat and acid coagulation of milk. It is a non-fermentative, unripened, non-renneted and non-melting cheese. Paneer entraps almost all fats (23-26%), casein that is complexed with denatured whey protein (17-18%) and some amount of salt and lactose (1.5-2%) during heat-acid coagulation of milk. It is considered as one of the most extensively consumed dairy products in India. Paneer is used to prepare many sweets and various recipes along with other vegetables, thereby forming an important ingredient of Indian traditional dessert and cuisine. As a considerable population is vegetarian, paneer emerges as a staple food for them.

The high moisture content of paneer (about 50-60%), the rich source of nutrients, unhygienic environment and relatively high temperature prevailing in major parts of the country limit its shelf-life to just a day at ambient temperature and 6-8 days at refrigerated storage. However, even under refrigerated conditions, the freshness of paneer remains intact only for 3 days (Bhattacharya *et al.,* 1971). Surface spoilage is the major cause of deterioration of quality of paneer. It is mainly attributed to microorganisms that get established on the surface during post-heating exposure (Dongare *et al*., 2019).

The incidences of foodborne illnesses caused due to contaminated and sub-standard or poorly processed foods are common in India. They not only cause a threat to public health but also lead to economic losses. The consumption of unsafe food and its health consequences in 2019 alone cost us $15 Billion (FSSAI, Times of India, 2021). As per World Health Organization (WHO), only 1% of foodborne illnesses are recognized in developing countries

(NCDC, 2017). Milk products, poultry products and seafoods are the major causative agents of foodborne illnesses.

A wide variety of microorganisms can grow on food products causing spoilage and produce illnesses. *Staphylococcus aureus*, *Vibrio* spp., *Salmonella* spp., *Escherichia coli*, *Yersinia entercoclitica* and *Listeria monocytogenes* are identified as the pathogens of chief concern by the National Centre for Disease Control (NCDC, 2017). To overcome such diseases, it is recommended to provide hygienic conditions during food processing, packaging and storage. Achieving such a high degree of hygiene requires well-designed process equipment and processing lines with limited human interventions. In India, majority of the food industries are small or medium enterprises operating at low-profit margins. They cannot afford to develop adequate aseptic conditions in their process plants.

Though paneer is widely consumed in almost all the regions of the country, most of its market demand is fulfilled by local small enterprises or unorganized sectors with some exceptions. As a result, there are cases of foodborne disease associated with the consumption of paneer. Although paneer preparation involves heating milk at a near-boiling temperature under mildly acidic conditions, it is exposed directly to the environment during subsequent handling, pressing, cooling and packaging. Encountering unhygienic condition during pressing, packaging, storage and in the market chain of paneer shortens its shelf-life.

The conventional methods of thermal processing cannot be applied to paneer. The high protein content and solid consistency of paneer can cause localized surface heating during conventional thermal treatment and denatures the proteins, causing degradation of product quality. In the last few decades, many novel processing techniques have been explored to provide safe and shelf-stable foods with enhanced microbial quality. It is important to explore novel technologies such as hurdle technology, ohmic heating, high-pressure processing, infra-red heating, pulsed light, pulsed electric heating, ozone processing, ultrasound or cold plasma for paneer. Amongst them, cold plasma (CP) is highly suitable for surface decontamination of solid foods. It is an emerging non-thermal energy technique that can be used to preserve the nutritional value, enhance heat and mass transfer by altering the boundary layer structure, reduce the microbial load in paneer and save energy.

Plasma, considered as the fourth state of matter, refers to a quasi-neutral ionized gas. When materials acquire energy, they change their phase from solid to liquid and then to gas. At extremely high energies, matter undergoes a further transformation into plasma. The term ‘plasma’ was coined by Langmuir (1928). Matter in the plasma state consists of electrons, ions, neutrons, protons, reactive oxygen, atomic oxygen (O), ozone (O_3), hydroxyl radicals ($OH^•$) and nitrogen species (N_2, NO, NO_2 and nitric oxide radical $NO^•$). When plasma is produced without the application of thermal energy, it is called non-thermal or CP.

Cold plasma is produced by exciting a gas with high electric field strength. The active species in the ionized gas possess excellent bacterial inhibition properties, making CP a promising preservation technology. Generation of CP depends on several factors such as frequency, voltage, nature of working gas and treatment time. Cold plasma is generated under atmospheric or vacuum conditions at the temperature of about 30-60°C by applying a high-intensity electric field to a neutral gas between two electrodes. It requires less power for generation than thermal plasma but is equally effective for microbial decontamination of food products. Due to the ample amount of reactive species and charged particles in it, CP can destroy sporulating, spoilage-causing and pathogenic microorganisms (Butscher *et al*., 2016). It inactivates the microorganisms in food by any one of the following three mechanisms (Niemira, 2012).

1. Chemical interaction of radicals, reactive species or charged particles with the cell membrane.
2. Damage to the membrane and internal cellular components by UV radiation.
3. Breakage of DNA strands by UV radiation generated during the recombination of plasma species.

The advantage of employing CP in the food and dairy industry is its unique cocktail of reactive species. These reactive species are highly efficient in providing antimicrobial action. The species generated during CP have a broad spectrum of a lifetime (nanosecond to hours), depending on the conditions of plasma generation and available reactive matter. Initially, CP was produced using noble gases such as argon and helium because of their ease of breakdown and their common use with plasma jets. Commercial production of plasma using these gases as principal gas is however not economically feasible.

In contrast, reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by the breakdown of normal atmospheric air by high voltage are recognized for their antimicrobial effect, making air a potential option for commercialization. Researchers who have used air as a principal gas for CP generation observed good results with low processing costs. However, the reactive species are short-lived and rapidly return to their stable state. When this happens, microorganisms that survived CP treatment can easily recover and begin to grow. Therefore, the appropriate conditions should be selected for the generation of CP. Other hurdle technologies, especially modified atmosphere packaging (MAP), could be employed to enhance the preservation efficacy of CP treated foods during storage. As the microbicidal effect of CP is highly governed by ROS and RNS, the use of vacuum or MAP after treating the food with CP can be a second hurdle for the production of low cost and extended shelf-life products.

Hurdle technology refers to the simultaneous application of two or more food preservation techniques to secure food microbial safety and stability without losing the product’s organoleptic characteristics, nutritional quality and economic viability (Leistner, 2000). It is a method of ensuring that the product will be safe for consumption and will have an extended shelf-life. Hurdle-treated foods have additional safety as compared to conventionally processed foods. If the preservation effect is lost in the later processing stages such as handling, packaging and other treatments, safety of the product could be lost. Hence, the present study was contemplated to treat paneer with CP synergistically with moisture content (in turn water activity) as a hurdle factor and vacuum packaging the treated paneer (second hurdle) so that its shelf-life could be extended. In light of the above, the following objectives have been proposed in this study.

1. Evaluation and optimization of cold plasma-hurdle treatment of paneer.
2. Determination of storage stability of treated paneer at different temperatures.

Review of Literature

2. Review of Literature

The chapter discusses the state-of-the-art information on the potential of hurdle technology and CP as a promising technique for microbial destruction. The chapter also discusses the effect of CP on physicochemical and textural attributes of foods and other major changes in their constituents. Further, the effect of packaging on shelf-life of paneer is also discussed.

2.1 Paneer Definition and Characteristics

Milk is rich in essential nutrients and is a good source of energy, high-quality protein and fat. It forms a part of the staple diet of Billions of people. With an overall production of 198.4 Million metric tonnes (2019-20), India ranks first in the world’s milk production with 5-6% growth rate in the last decade. More than 25% of the country's agricultural gross domestic product (GDP) is contributed by the dairy sector. In terms of market value, it exceeds the value of wheat, rice and pulses put together. We are also the largest consumer of dairy products, utilizing more than 90% of our milk production internally. The per capita availability of milk is about 407 g/day (2019-20) (Economic Survey, 2020-21, GOI).

The composition of milk is quite complex, with more than 100 constituents in the form of emulsion, suspension or solution. Milk is also a rich source of fat-soluble vitamins (Vitamin A, D, E and K) and minerals like calcium, magnesium, potassium and sodium. The major constituents of milk are presented in Table 2.1. Milk can be converted into various fermented (curd, yoghurt, shrikhand, etc.), heat-desiccated (khoa, kunda, peda, etc.), concentrated (evaporated milk and sweetened condensed milk), heat and acid-coagulated (chhana, paneer, etc.), fat-rich (ghee and butter) products, milk powder, etc.

Table 2.1. Major constituents of cow milk (Fox *et al.*, 2017)

Constituent	State	Size (nm)	Content (%)
Fat	Emulsion	2000-6000	3.7-6.7
Casein	Colloidal	50-300	2.8-3.8
Whey protein	Solution	4-6	0.6-0.9
Lactose	Solution	0.5	4.8

Paneer is a heat and acid-coagulated unripened cheese consumed throughout India. According to the Food Safety and Standards Authority of India (FSSAI), paneer is the product obtained from cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid, malic acid and citric acid. It shall not contain more than 60 percent moisture and milk fat shall not be less than 50 percent of dry matter. Paneer contains whole milk casein, some part of whey protein, fat and colloidal solids in proportions of moisture retained. It is generally characterized by white marble colour and a little spongy body. It is a non-fermentative, non-renneted and non-melting cheese characterized by a mildly acidic flavour, slight sweet taste and a cohesive and compact texture (Kumar *et al.*, 2014). It contains less than 60% moisture, 22-26% fat, 16-18% protein, 1.9-2.2% lactose and 1.3-1.7% minerals. The microbiological standards of paneer are described in Table 2.2.

Table 2.2. Microbiological standards of paneer (FSSAI, 2019)

Parameter	Standard count
Total plate count	1.5×10^5 to 3.5×10^5 cfu/g
Coliforms	Not more than 100 cfu/g
Yeast and mould	Not more than 150 cfu/g
Escherichia coli	Less than 10 cfu/g
Staphylococcus aureus	Not more than 100 cfu/g
Salmonella	Absent in 1 g
Listeria monocytogenes	Absent in 1 g

2.2 Hurdle Technology

Hurdle technology refers to the simultaneous application of two or more preservation interventions that can maintain microbiological safety and stability and retain the organoleptic and nutritional qualities of the food economically (Leistner, 2000). It is a method of ensuring that the product will be safe for consumption and will have an extended shelf-life by reducing the multiplication and growth of pathogens in the product. Hurdle-treated foods are safer than conventional processed foods as conventional processing methods and post-processing operations provide limited microbial safety.

2.2.1 Food hurdles

Generally, hurdles are used in food applications to increase their shelf-life and quality. In the food industry, plenty of hurdles are adopted to provide consumers with safe food. Each hurdle aims to eliminate, inactivate or inhibit undesirable organisms from the food to improve food quality and consumers' safety. Physical, physicochemical and microbial hurdles are used for preservation of foods. Commonly used food hurdles are water activity, acidity, temperature, redox potential, preservatives and competitive microorganisms. For example, common salt and organic acids are used as humectants to control the microbes in food. Similarly, natural antimicrobial substances such as nisin, natamycin and other bacteriocins are also used to preserve foods (Pal *et al*., 2014). These hurdles preserve the food as well as influence the quality of food (Putnik, 2020). The intensity of hurdle employed or their concentration used affects the preservation effect.

2.2.2 Fundamentals of hurdle technology

There are four ways by which hurdles protect foods against both pathogenic and spoilage microorganisms.

2.2.2.1 Homeostasis

Microorganisms tend to maintain a stable and balanced internal temperature. The preservative factors functioning as hurdles can disturb the homeostasis mechanism to prevent the microbes from multiplication and making them remain inactive or even die (Raso *et al*., 1998). Low water activity, low pH and lower redox potential could act on the microbes synergistically. The interference with homeostasis of microbes forms an attractive and logical focus for improving food preservation techniques.

2.2.2.2 Metabolic exhaustion

The microbes in hurdle-treated stable products use their energy for homeostasis, thereby become metabolically exhausted. This leads to auto-sterilization of food products. Hence, microbiologically stable food becomes safe for storage at ambient temperature (Pundir and Murtaza, 2015). Synthesis of protective stress shock proteins is induced by several factors like water activity, pH, heat, ethanol, etc. Exposure to multiple stresses can cause the microorganisms to become metabolically weak. Therefore, multi-pronged preservation of foods could be the key to avoid synthesis of stress shock proteins (Leistner, 2000). The concept of multi-pronged preservation of food was introduced by Leistner (1995). Here, gentle hurdles that have synergistic effects are applied intelligently. Therefore, application of several hurdles simultaneously would lead to optimal microbial stability and effective food preservation. It is pertinent to mention that the multi-target attack of microbes may be a promising approach in food microbiology.

2.2.3 Techniques for extending shelf-life using hurdle technology

Rao and Patil (1999) investigated the effect of sodium chloride, sucrose and glycerol on the water activity of paneer. The authors observed that water activity decreased on applying the above hurdles at 1% rate, increasing the shelf-life of paneer to 1 month. Thippeswamy *et al.* (2011) showed that shelf-stable paneer could be produced by adopting hurdle treatment comprising 3% NaCl, 1% citric acid, 0.1% potassium sorbate and modified atmospheric packaging with a gas mixture of CO_2 and N_2 in the ratio of 1:1. Paneer could be stored for up to 12 days at ambient temperature and 20 days at refrigerated condition. Similarly, Eresam *et al.* (2015) evaluated the relative efficacy of black pepper, cardamom and clove in improving the shelf-life of paneer. The authors reported that at 0.6% concentration, cardamom provided the maximum shelf-life of 28 days for paneer, followed by that of addition of clove (21 days) and black pepper (14 days).

Gokhale *et al.* (2016) reported that pretreatment with acids, vacuum drying, vacuum packaging and storage under refrigerated conditions (hurdles) enhanced the shelf-life of paneer. Paneer cubes of 1.5 cm size were dipped in citric acid, lactic acid and vinegar of 4% concentration at 30±1°C for 10 min, and dried to an intermediate moisture content of 30-35% under vacuum (680-720 mm of Hg). Paneer treated with lactic acid and vinegar had shelf-life of up to 90 days under refrigerated conditions of 7±2°C, while paneer treated with citric acid was acceptable up to 60 days. Paneer when rehydrated with hot water had rheological properties comparable to that of fresh paneer.

Mishra *et al.* (2016) reported that dipping of paneer blocks in 0.5% citric acid solution for 12 h could increase the shelf-life up to 12 days at refrigerated temperature. Similarly, Mishra *et al.* (2017) investigated the effect of several hurdles such as pH, water activity and smoke. The authors reported that when these hurdles were applied together, the shelf-life of paneer improved to 29, 41 and 53 days at refrigerated temperature (7±1°C) when packed in polystyrene, LDPE and laminate, respectively. Sharma *et al.* (2019) demonstrated the effect of phytochemicals such as gallic acid, quercetin, piperine, eugenol and menthol against *E. coli* under *in vitro* and *in situ* conditions on the microbiological quality of paneer at concentration of 100 µg/mL. It was observed that maximum microbial reduction was 3 log by gallic acid followed by that of eugenol (1.9 log), quercetin (1.8 log), piperine (1.7 log) and menthol (0.9 log) at 5°C.

Kapoor *et al.* (2021) studied the combined effect of supercritical CO_2 and food-grade acetic acid on the shelf-life of paneer at ambient (25±1°C) and refrigerated (4±1°C) temperatures. The authors found that the final moisture content was 34.7g/100g, pH was 4.56 and water activity was 0.81 after 30 days. The microbial quality of paneer stored at ambient temperature was very poor, while the microbiological population in paneer stored at refrigerated temperature was within the permissible limit. Sarnaik *et al.* (2021) studied the effect of black pepper on the quality of paneer. The authors used black pepper at concentrations of 0.25, 0.50 and 0.75%. It was observed that paneer treated with 0.50% black pepper was highly acceptable by the panel of judges based on flavour, taste, colour and appearance.

2.3 Cold Plasma Technology

Cold plasma (CP) was originally employed for ameliorating the printing and adhesion properties of polymers, increasing the surface energy of materials, treating textiles, glass, paper and other products (Ekezie *et al.*, 2017a). Now, it is evolving as a novel non-thermal decontamination technology in food. The abundant reactive species in CP such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are considered as major contributors to the inactivation of a wide spectrum of microorganisms, including bacteria, fungi, viruses and spores. Due to its efficient microbicidal performance and low working temperature (<60°C), CP technology has gained increasing interest within the food industry for applications on fruits and vegetables, meat and poultry, dairy, beverages, cereals and spices (Misra *et al.*, 2015).

The amount and type of energy supplied to plasma cause changes in electron density and temperature of electrons, resulting in plasma being differentiated into two groups: high-temperature and low-temperature plasma or gas discharges. In high-temperature plasma, all the species (electrons, ions and neutral species) are in thermal equilibrium. In contrast, most of the applied electrical energy is channeled to electrons in low-temperature plasma, producing energetic electrons without heating the entire gas stream, thereby leaving the ions and neutrals cold (Niemira, 2012). This makes CP a potential technique for treatment of heat-sensitive foods. Low-temperature plasma is further subdivided into thermal plasma, also called quasi-equilibrium plasma, which is in local thermal equilibrium state and non-thermal plasma (NTP), also called non-equilibrium plasma or CP.

Table 2.3. Classification of plasma (Nehra *et al.*, 2008)

Plasma	**State**	**Example**
High-temperature plasma	$T_e \approx T_i \approx T_g$, $T_p \approx 10^6$–10^8 K; $n_e \geq 10^{20}$ m^{-3}	Laser fusion plasma
	Low-temperature plasma	
Thermal plasma (Quasi-equilibrium plasma)	$T_e \approx T_i \approx T_g \leq 2x10^4$ K; $n_e \geq 10^{20}$ m^{-3}	RF inductively coupled discharges, plasma torches and arc plasma.
Non-thermal plasma (Non-equilibrium plasma), NTP	$T_e >> T_i \approx T_g$ = 300 to 1000 K; $n_e \approx 10^{10}$ m^{-3}	OAUGDP, Glow, APPJ, DBD, MHCD, plasma needle and corona

T_e – temperature of electrons; T_h – temperature of heavy particles; T_g – overall temperature of the gas; n_e– density of electrons; T – neutral gas temperature; T_p– temperature of plasma; T_i – temperature of ions; OAUGDP – One Atmospheric Uniform Glow Discharge Plasma; APPJ – Atmospheric Pressure Plasma Jet; DBD – Dielectric Barrier Discharge; MHCD – Micro Hollow Cathode Discharge.

2.3.1 Plasma generation

Cold plasma is generated by energizing the neutral gas, causing the formation of charge carriers. Electrons and ions are produced in the gas phase when electrons or photons with sufficient energy collide with neutral atoms and molecules in the atmosphere or feed gas (electron-impact ionization or photoionization). The various ways to supply energy for plasma generation are listed below (Conrads and Schmidt, 2000).

a) Thermal energy, wherein exothermic reactions of the molecule are used as the primary energy source.
b) Energetic beam moderating in a gas volume is unperturbed by electrical and magnetic fields, which could generate sustained plasma.
c) Applying an electric field to a neutral gas. This is the most common method for generating and sustaining low-temperature plasma.

Any volume of neutral gas always contains a few electrons and ions formed due to the interaction of cosmic rays or radioactive radiation with the gas. The electric field accelerates these free charge carriers, and new charged particles may be created during the collision with atoms and molecules in the gas or with the surface of the electrodes. This leads to an avalanche of charged particles that are eventually balanced by charge carrier losses to develop a steady-state plasma (Conrads and Schmidt, 2000).

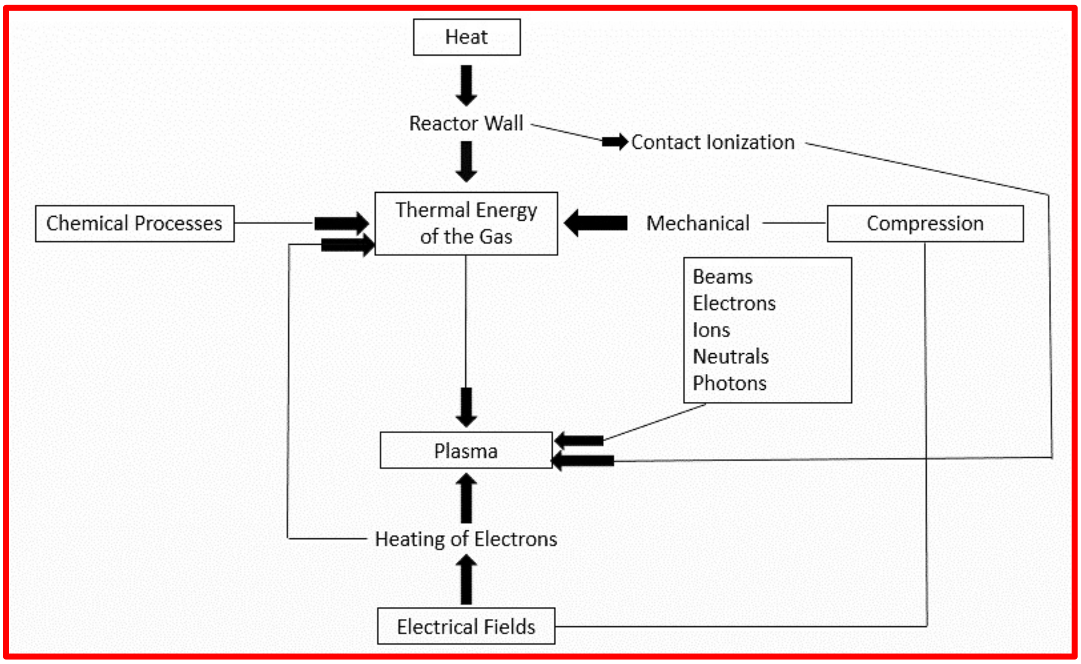

Fig. 2.1 Principle of plasma generation (Conrads and Schmidt, 2000)

Breakdown voltage, which is necessary to start a discharge or electric arc between two electrodes in a gas, is a function of pressure and gap length. It is described by **Paschen's Law** (Eq. 2.1).

$$V_B = \frac{Apd}{\ln(Bpd) + C} \qquad (2.1)$$

Where, 'V_B' is the breakdown voltage (kV) 'p' is the pressure in Pascal and 'd' is the gap between electrodes (m). A, B and C are coefficients that depend on both the gas and electrode material (Turner, 2016).

NTP jets produce small ''plasma flames'' that are typically generated in the radio-frequency range. As the name suggests, a gas is blown through the discharge region and is exposed to excitation by plasma in this jet source. This configuration enables gas discharge in a non-sealed (open) electrode arrangement and plasma discharges are projected to an open environment (Nishime *et al.*, 2017). They consist of two electrodes (needle and ring electrode), with the outer electrode being grounded while the central electrode is excited by radio frequency power at 13.56 MHz. The gap is usually in some millimeters and noble gases such as helium or argon with high flow rates are usually used. Plasma jets have advantages like targeted applicability and penetration into narrow gaps due to their small dimensions (Weltmann *et al.*, 2008).

2.3.1.1 Dielectric barrier discharge (DBD)

It is also known as silent discharge or barrier discharge. For the generation of DBD plasma, alternating current is needed, wherein the two electrodes are kept apart using a dielectric material. Quartz, teflon and ceramic are commonly used as dielectric materials. The dielectric barrier material is used to stop the electric current from the formation of sparks. A neutral gas or any noble gaseous mixture is moved in a closed chamber and is ionized to generate plasma in between the two electrodes. One electrode is connected to a high voltage circuit and the other is grounded (Shimizu *et al.*, 2011). A sinusoidal voltage in the order of several thousand kV, with frequencies between 0.05 and 500 kHz, is applied on the high voltage side electrode. Consequently, electrons in the gap are accelerated, acquires enough energy, causing ionization. This triggers a cascade effect that exponentially increases the number of electrons in the gap. They flash towards the opposite dielectric plate in the same direction as the electric field. The usual gap between electrodes in DBD ranges from

0.1 mm to several centimeters and operates under reasonably high power levels (Rød *et al.*, 2012).

The main advantages of DBD are that a variety of gases can be used for plasma generation (noble gases, air and special mixtures), low or no gas flow is needed, homogenous discharge over a large area can be produced and good adaptability with different geometries of electrodes is possible (Phan *et al.*, 2017). The disadvantage is that a high ignition voltage of at least 2-10 kV, depending on the restricted electrode gap, is required and precautions or isolation are necessary.

2.3.1.2 Gliding arc discharge

Plasma is created in a reactor containing two or more diverging metallic electrodes operating at a high potential difference of 9 kV and 100 mA in atmospheric conditions. Inlet gas, generally humid air, is pumped into the discharge gap between the electrodes to form the arc between the narrower inter-electrode areas, which is subsequently blown away by the inlet gas into the diverging area**.** Gliding arc discharge produces both thermal and non-thermal plasma and could be used for both surface and liquid treatment (Ekezie *et al.*, 2017b). It can be used for the degradation of chemical contaminants like organic constituents, solvents and industrial waste present in water.

2.3.1.3 Corona discharge

It is also known as a diffused route for plasma ignition, which develops around a sharp-pointed electrode containing a substantial electric field for expediting the ionization energy of arbitrarily produced electrons to that of monoatomic gas atoms or molecules (Scholtz *et al.*, 2015). Plasma is generally generated at high voltage and occurs predominantly on one electrode. The technology is inexpensive and simple to implement, and has been exploited for microbial decontamination, surface treatment and electro-precipitation. The technology is restricted to the non-homogenous diminutive area. Corona discharge commonly occurs when highly asymmetric electrodes are employed such as a point and a plane. When the applied electric field is sufficiently large to accelerate randomly produced electrons up to the ionization energy level of surrounding gas atoms or molecules, corona appears near the sharp electrode geometry (Surowsky *et al.*, 2015). Corona is self-sustaining and stable for long periods. The sharp edge of the electrode leads to the formation of highly intense localized electric fields, which leads to decreased breakdown voltage (Bárdos and Baránková, 2010).

Typical geometries are point-to-plate (sharply curved electrode arranged counterpart to a flat one) and cylindrical configurations. Luminosity and ionization are mainly localized at a sharp electrode, which can have a positive or negative potential. This configuration works with both DC and pulsed voltages. The advantages are that the device is simple and less expensive but the application is limited to relatively small areas and is non-uniform (Scholtz *et al*., 2010).

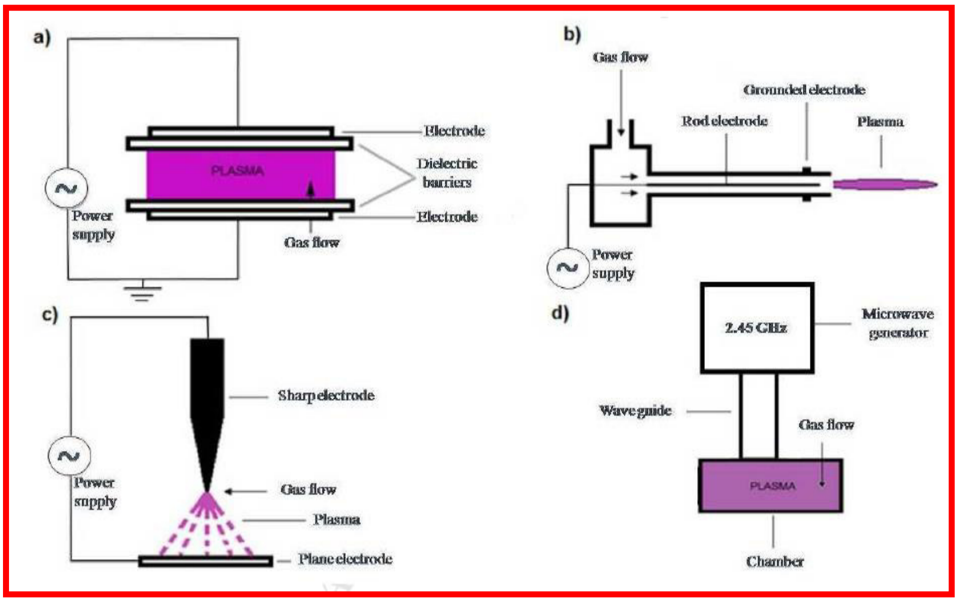

Fig. 2.2 Plasma generation at atmospheric pressure (a) dielectric barrier discharge (DBD), (b) plasma jets, (c) corona discharges and (d) microwave discharges (Niemira, 2012)

2.3.1.4 Radiofrequency plasma

Radiofrequency (RF) plasma is obtained when the gas is placed within an oscillating electromagnetic field produced by an induction coil or distinct electrodes that are kept outside the reactor. The plasma jet consists of two coaxial electrodes, between which gas flows at high rates. The outer electrode is grounded while the central electrode is excited by radiofrequency (RF) power at 13.56 MHz. The free electrons are accelerated by the RF field and collide with molecules of the background gas. These inelastic collisions can produce various reactive species (excited atoms, molecules and free radicals) that exit the nozzle at high velocity (Misra *et al*., 2016). Common gases used in the process include noble gases such as helium and argon at high flow rate. This is an expensive approach because of the high cost of gas, but this method is acceptable for some biomedical applications, rather than food processing, in which cost is an important consideration.

Table 2.4. Different plasma sources for food decontamination

Plasma source	Design features and electrode configuration	Reference
DBD cold atmospheric pressure plasma	27 kV voltage at a frequency of 27.8 kHz was applied between two aluminium electrodes of size 100×100 mm with an electrode gap of 10 mm. The plasma generated was used to treat ready-to-eat meat for the inactivation of pathogens.	Rød *et al.* (2012)
Microwave cold plasma	The chamber used for developing microwave plasma was of 120 mm diameter and 100 mm height. A microwave generator of 2.45 GHz was used for power generation. The plasma was generated for the determination of surface temperature of foods.	Knoerzer *et al.* (2012)
Atmospheric pressure needle plasma	Voltage in the range of 3.95-12.83 kV at 60 Hz was applied on 12 steel nickel-coated electrode needles with a radius of 50 µm. The plasma generated was applied to fresh produce to inactivate *E. coli.*	Bermúdez-Aguirre *et al.* (2013)
Cold jet plasma	The device consisted of the plasma jet itself (length: 170 mm; diameter: 20 mm; weight: 170 g), a gas flow controller and a DC power supply. The process gases were argon as well as mixtures of argon and oxygen, and the plasma exposure time was varied between 0 and 480 s. The plasma generated was applied to study the impact of plasma on *Citrobacter freundii* in apple juice.	Surowsky *et al.* (2014)

2.3.2 Mechanism of reactive species generation

Non-thermal plasma generated at atmospheric pressure contains a great variety of reactive species that are produced due to collisions between electrons, atoms and molecules.

Therefore, free charge carriers are accelerated by applying an electric or electromagnetic field, leading to elastic and inelastic collisions. Elastic collisions are accompanied by a redistribution of kinetic energy, whereas the fraction of transferred energy is very small. In contrast, inelastic collisions involve energy transfer up to 15 eV and more, leading to ionization excitation and dissociation reactions. Consequently, compounds such as ROS (atomic O, OH radicals and ozone), charged particles, electrons and ultraviolet photons are generated (Surowsky *et al.*, 2014).

Atomic oxygen is one of the most important ROS formed by electron impact dissociation of molecular oxygen.

$$O_2 + e^- \rightarrow O^+ + O^\bullet + 2e^- \quad (2.2)$$

$$O_2 + e^- \rightarrow O^\bullet + O^\bullet + e^- \quad (2.3)$$

$$O_2 + e^- \rightarrow O^- + O^\bullet \quad (2.4)$$

Hydroxyl radicals are formed from water molecules due to the mixing of process gas with the surrounding air. Their generation is based on either electronic dissociation or collisions with long-living species.

$$H_2O^+ + e^- \rightarrow H^\bullet + OH^\bullet + e^- \quad (2.5)$$

In contrast, reactive nitrogen species can be formed in plasma if the gas employed is nitrogen or ambient air.

$$\tfrac{1}{2} N_2 + \tfrac{1}{2} O^- \rightarrow NO \quad (2.6)$$

Nitric oxide (NO) cannot co-exist with ozone or atomic oxygen, thereby leading to the following oxidation reactions. The ultraviolet part of plasma is located in the wavelength range of 100-380 nm.

$$NO + O_3 \rightarrow O_2 + NO_2 \quad (2.7)$$

$$NO_2 + O_3 \rightarrow O_2 + NO_3 \quad (2.8)$$

$$NO + O^\bullet \rightarrow NO_2 \quad (2.9)$$

$$NO_2 + O^\bullet \rightarrow NO_3 \quad (2.10)$$

2.3.3 Mechanism of action of cold plasma

The chemical composition of CP is quite complex and multiple reactive agents present in the plasma are expected to play an individual role or act synergistically in the inactivation of target microorganisms. Atmospheric air CP is a good source of electrons, positive and negative ions, free radicals, stable conversion products, excited molecules and photons. The majority of reactive species produced by plasma include electronically and vibrationally excited oxygen, nitrogen and active forms of oxygen molecules and atoms. These include ROS consisting of atomic oxygen (O), singlet oxygen ($^1O_2$), superoxide anion (O^{-2}) and ozone (O_3). RNS are atomic nitrogen (N), excited nitrogen (N_2) and nitric oxide ($NO^•$). If humidity is present as H_2O, OH^- anion, $OH^•$ radical and H_2O_2 are also generated (Scholtz *et al.*, 2015). The generated CP products within the plasma gas phase play a vital role in producing bacterial inactivation. Most bacteria, particularly anaerobes, are considered to be very sensitive to ROS. The diffusion of oxygen species or oxygen-containing radicals (nitric oxide) through the bacteria cell wall causes local damage possibly by oxidation of cytoplasmic membrane, protein and DNA strands. Similarly, UV light leads to destruction or chemical modification of DNA, preventing replication of bacterial cells as the maximum absorption of DNA is at 254 nm. Guo *et al.* (2015) reported the role of UV photons in microbial destruction during plasma treatment. The UV irradiation induces photodesorption, which breaks the chemical bonds in the microorganisms.

ROS, which are extremely oxidizing agents, cause protein denaturation and cell leakage at equal amount in cells and spores. They react with membrane lipids, forming unsaturated fatty acid peroxides and disturb the transport of biomolecules across it. Species formed from the breakdown of air such as O_3, atomic oxygen, superoxide, peroxides and hydroxyl radicals play a leading role in microbial destruction, while nitrous oxide (NO) and nitrogen dioxide (NO_2) inactivate microorganisms by damaging the chemical constituents like proteins, lipids and nucleic acids. A chain of oxidation reactions leading to membrane lipid degradation is caused by $OH^•$ radicals. The C-O and C-N bonds in the cell wall component called 'peptidoglycan' get cleaved by oxygen species causing cell wall destruction (Misra *et al.*, 2016). RNS damages the nucleic acid and other chemical constituents of microorganisms.

The surface of bacteria also experiences intense bombardment by plasma radicals and form volatile compounds (CO_2 and H_2O), causing injury on the surface that the microorganisms cannot repair. When membrane integrity is compromised, it affects the DNA mainly due to the breakdown of interactions between membrane proteins and DNA as well as pore formation that causes the release of DNA and other critical contents from the cell.

2.3.3.1 Effect of cold plasma on bacterial cell

CP formed above the food acts on the surface of food. The ROS and RNS formed act on the bacterial cells and breaks down the chemical bonds holding the bacterial protective cell wall together. As the cell wall is damaged, the particles penetrate the bacterial cell wall and break apart the interior constituents of the cells including DNA, protein, lipids, cellular membrane and other cell components in microorganisms (Kaluwahandi *et al*., 2020).

Plasma mediated bacterial cell inactivation can be distinguished by three different mechanisms.

1. Direct permeabilization of the cell membrane or cell wall.
2. Critical damage of intracellular proteins from oxidative/nitrosative species.
3. Direct damage of DNA.

2.3.4 Factors affecting the efficiency of cold plasma

2.3.4.1 Processing variables

The effectiveness of CP is dependent on several factors that are primarily related to the techniques employed for their formation. For example, the type of gas determines the nature, quantity of reactive species produced and efficacy of treatment. Active species generated depend on the frequency and input voltage, wherein a higher value increases the energy density. The mode of exposure affects the efficiency of treatment as well. Direct exposure is more preferable in contrast to indirect and remote exposure.

2.3.4.2 Intrinsic attributes

Internal characteristics influence the efficiency of CP. For example, the sensitivity of microorganisms differs from species or strains. Generally, the microorganisms are more sensitive to CP treatment in the stationary phase than in the exponential phase. Gram-positive bacteria possess less sensitivity than gram-negative organisms. Sporulated bacteria are more resistant to plasma treatment than vegetative cells. Similarly, a high concentration of bacteria clusters reduces the penetration capacity of reactive species. Another observation was that the cell wall of fungi is chiefly composed of chitins that exhibit stronger resistance to plasma treatment than bacteria due to intricate rigidity imparted by the constituent of cell wall (Liang *et al.*, 2012).

2.3.4.3 Environmental factors

Ambient factors such as relative humidity (RH), pH and nature of the sample affect the effectiveness of CP treatment. For instance, solid and liquid matrices interact differently with reactive species, as most liquids can vaporize during treatment and take part in subsequent reactions. Also, foods with lower pH respond differently to heat, stress and other conditions as demonstrated by Muranyi *et al.* (2008), who observed 4.7 log reduction in *Bacillus cereus* after CP treatment at pH 5 as against 2.1 log at pH 7. Likewise, increasing RH enhanced the inactivation of microbes due to increase in the composition of hydroxyl radicals. Concurrently, the nature of the matrix also influences process efficiency. For example, an agar plate or filter membrane can be effectively decontaminated compared to a cheese slice or fruit surface due to migration of microorganisms from the outer to inner tissues at a definite velocity (Yong *et al.*, 2015)

2.3.5 Microbiological interaction with cold plasma

Bacterial pathogens are considered a critical food safety issue, followed by food-borne viruses, bacterial toxins, pesticide residues and mycotoxins (van Boxstael *et al.*, 2013). Many countries identify *E. coli* O157: H7, *Salmonella* spp. and *L. monocytogenes* as the target pathogens of concern. Also, the bacterial capacity for biofilm formation, internalization of contaminating cells within a host tissue or structure and/or formation of highly resistant spores often complicate or negate food disinfection processes. CP technology is a proven potential alternative to conventional methods as it can enhance microbiological safety and maintain quality characteristics in a broad range of foods with faster processing rates. The technique provides the advantage of reduced water usage, uses atmospheric gas for generation and leaves no chemical residue, which provides more scope for processing at the industrial level.

The interactions between CP, microorganisms and molecules are complex and depend on various systems, processes and target parameters. It includes plasma device, frequency, voltage level, working gas, humidity level, surface characteristics, distance between target and plasma emitter, gas flow rate, type of product, volume, concentration and physiological state of microorganisms. For example, CP combined with sodium dodecyl and lactic acid on *L. monocytogenes* and *E. coli* was found to reduce the microbial load below the level of detection when used synergistically (Trevisani *et al.*, 2017). Helgadóttir *et al.* (2017) reported that pretreatment with vitamin C inhibited the production of extracellular polymeric substances and subsequent CP treatment destroyed the bacterial cells. Biofilm pretreated with vitamin C for 15 min reduced the viability of *E. coli*, *P. aeruginosa*, *S. epidermidis* biofilms from 10 to 2%, 50 to 11% and 61 to 18%, respectively.

Govaert *et al.* (2019) applied a combination of DBD (helium) plasma and H_2O_2 (0.05%, 0.2% v/v) for the treatment of *L. monocytogenes and S. typhimurium* biofilm on Petri dishes. The authors attributed the 5.42 log/cm^2 reduction in population to increase in porosity of biofilm provided by pretreatment of CP, which led to increased biofilm sensitivity and increased penetration of H_2O_2. Similarly, Xiang *et al.* (2019) conducted a study on the treatment of *E. coli* 0157:H7 with plasma-activated water (PAW) and combined it with mild heat treatment (60°C for 4 min). There was 8.28 log reduction in combined treatment, while there was only 0.77 log and 1.78 log reduction in the counts when *E. coli* was treated individually with PAW and mild heat, respectively. The authors explained the reason behind it by conducting scanning electron microscopy and a fluorescent assay of propidium iodide and N-phenyl-1-naphthylamine. Hurdle technology was found to produce a synergetic effect by damaging the outer structure and intracellular components of *E. coli* 0157:H7 cells.

Liao *et al.* (2020) studied the effect of plasma-activated water and mild heat (60°C) on *Bacillus cereus* species in *Oryza sativa L.* ssp. *japonica* (rice) and found a significant decrease in spore count. The authors also hypothesized that mild heat treatment might accelerate the reactive plasma species (ROS and RNS) to move and penetrate cells, thereby seriously disturbing the intracellular structure. Similarly, Timmons *et al.* (2018) studied the inactivation of *Salmonella enterica*, shiga toxin-producing *E. coli* and *L. monocytogenes* by surface discharge CP design. The transmission electron micrographs revealed that there was significant damage to cells after 2 and 4 min of CP treatment. The authors also observed that there was 3 log cfu/mL reduction in *Salmonella*, 3.6 log cfu/mL in *E. coli* and 2.6 log cfu/mL reduction in *L. monocytogenes* after 4 min of CP treatment. Also, treatment for 4 and 10 min to pecan and cherry tomato reduced the microbial load by 2 log cfu/mL.

Devi *et al.* (2017) studied the influence of CP on the growth of *Aspergillus parasiticus, Aspergillus flavus* and aflatoxin production in groundnut. There was a 97.9% and 99.3% reduction in the growth of *A. parasiticus* and *A. flavus,* respectively when treated with CP at 60 W. Also, 70% and 90% reduction in aflatoxin B_1 content was observed after treatment at 40 W for 15 min and at 60 W for 12 min, respectively. Similarly, Ott *et al.* (2021) demonstrated that high voltage atmospheric cold plasma (HVACP) could inactivate *A. flavus* and deoxynivalenol toxin. The authors observed that 1 min treatment could deplete the fungal culture in the pigments and inactivated 50% of spores. HVACP treatment for 20 min resulted in more than 99% reduction of deoxynivalenol toxin.

Niedźwiedź *et al.* (2020) investigated the effect of atmospheric CP on *Lentilactobacillus hilgardii* with helium and oxygen as working gas for 5, 10 and 15 min. The flow cytometry results showed the presence of 14.4% active cells, 77.5% mid active state and 8.1% dead cells. Some of the mid active state cells were able to grow again in culture medium, confirming the presence of *L. hilgardii* after CP treatment.

2.3.6 Interaction of cold plasma with food

Certain enzymatic, physical and chemical techniques are used to modify the functionality of food ingredients. Some studies suggested that CP processing could induce desirable changes in native ingredients (Thirumdas *et al.*, 2017). Precisely, CP alters the configuration of starch molecules leading to transformation in swelling power, pasting characteristics, water absorption, enzyme susceptibility, solubility and changes in compositional, structural and thermal properties (Thirumdas *et al.*, 2017). Typically, these modifications are initiated *via* increase in surface energy, incorporation of functional groups, cross-linking, depolymerization and changes in hydrophobic nature. The species present in plasma affect the microorganisms on the surface, but nutrients inside the food remain unaffected (Misra *et al.*, 2016). However, the effect of CP depends on product type, exposure time and process parameters.

Matan *et al.* (2014) used a combination of essential oils from lime, sweet basil and clove at low concentrations and CP to inactivate *E. coli, S. typhimurium* and *S. aureus* on chicken eggs. The authors observed that the above hurdle treatment could inactivate 6 log cfu/mL population against 1-3 log cfu/mL when only clove oil was used. Similarly, Cui *et al.* (2016) reported that the combination of CP and clove oil used synergistically could reduce the growth of *E. coli* 0157:H7 biofilm on fresh lettuce and achieved 5.48 log reduction. In contrast, the individual treatment of CP and clove oil only inactivated 0.81 and 2.2 log population of *E. coli*.

Chaplot *et al*. (2019) observed the effect of CP and peracetic acid on *Salmonella* in raw poultry, and reported that when peracetic acid (100 and 200 ppm) was used synergistically with CP, it produced inactivation of 3.8 and 5.3 log population, while individual treatment with peracetic acid at concentrations of 100 and 200 ppm produced only 0.6 and 1.3 log reduction in population, respectively. In the study on microbial decontamination of tiger nuts conducted by Muhammad *et al*. (2019), it was found that the sequential treatment of PAW and blanching (60°C/4 min) produced 3.65 to 3.7 log reduction in bacterial count, while individual treatments produced only 1.7 to 3.2 log reduction in the bacterial population.

Rana *et al*. (2020) investigated the effect of atmospheric CP on strawberries stored at ambient (25°C) and refrigerated (4°C) temperatures. The treatment was given for 10, 15 and 30 min at 60 kV, which increased the shelf-life of strawberries to 5 days at ambient temperature and up to 9 days at refrigerated temperature. Treatment for 15 min was found to be advantageous because it did not show any significant change in moisture, pH and total soluble solids but it was able to reduce the microbial load by 2 log scale. The treatment also enhanced the concentration of chlorogenic acid, hypri, phloretin, vanillin, gallic acid, 4-hydroxybenzaldehyde, rutin, total phenolic content and antioxidant activity during storage at ambient temperature.

Ahmadnia *et al*. (2021) studied the effect of CP on strawberries. The authors treated strawberry with CP for 5, 10 and 20 min, and found that 20 min treatment reduced the total aerobic bacterial count to 1.46 log cfu/g and yeast and mould to 2.75 log cfu/g. The study further showed that there was no significant effect on texture and colour, and treated strawberry was stable for 12 days at 6°C. Pérez-Andrés *et al*. (2020) studied the effect of CP on storage of mackerel fillets. The authors observed that treatment for 5 min at 80 kV did not have any significant effect on lipid oxidation as compared to the untreated fillets. Further, there were no observable changes in fatty acid composition or product quality after CP treatment. Also, there was formation of carbonyls when the fillets were stored at 4 and 8°C. Ukuku *et al*. (2019) investigated the combined effect of nisin-based antimicrobials and CP on

L. monocytogenes inoculated on apple surfaces. The authors treated the apples for 30, 40, 180 and 360 s. It was observed that 180 and 360 s exposure could inactivate microorganisms by 2.5 and 4.6 log cfu/g, but 3.5-4 log inactivation of the microbial load was achieved at 40 s of exposure when the apples were pre-treated with nisin. Kulawik *et al*. (2018) studied the effect of NTP on sushi (Nigri and Hosomaki) for total viable count, lipid oxidation and thiobarbituric acid (TBA) index. The authors observed that there was no significant change in total aerobic count as it was reduced by 1-1.5 mg/kg, while there was 0.4-1.5 mg/g increase in TBA index.

Rød *et al*. (2012) employed DBD plasma at 27 kV between two aluminum electrodes (100×100 mm) with a gap of 10 mm for inactivation of *L. monocytogenes* in meat slices. The treatment resulted in reduction of *L. monocytogenes* by 0.8±0.4 to 1.6±0.5 log cfu/g. Similarly, Kim *et al*. (2014) investigated the effect of corona discharge of 9 kV AC between two tungsten electrodes (radius of 0.8 mm) that were used for decontamination of red pepper powder. Results showed a reduction in the population of *A. flavus* by 2.5±0.3 log cfu/g and *B. cereus* by 3.5±0.7 log cfu/g. Atmospheric plasma at 18 kV killed *Salmonella* spp. on strawberries by 1.7-2.3 log cfu/g. Likewise, application of DBD plasma at 80 kV for 5 min successfully decontaminated cherry tomatoes containing *E. coli*, *S. typhimurium* and *L. monocytogenes* by 3.5, 3.8 and 4.2 log cfu/mL, respectively (Ziuzina *et al*., 2014). Moutiq *et al*. (2020) applied 100 kV of atmospheric cold plasma (ACP) treatment for 1, 3 and 5 min on chicken breast, and observed 2 log cfu/g reduction in microorganisms after 5 min treatment. The population of mesophiles, psychrotrophs and *Enterobacteriaceae* in chicken breast treated for 5 min at 100 kV after 24 days of storage was 1.5, 1.4 and 0.5 log, which were lower than that of control. Thus, it is evident that microbial decontamination of foods could be achieved using CP at low cost, with minimal changes in quality, thereby emerging as an alternative to conventional thermal processing techniques.

2.3.7 Application of cold plasma in the dairy industry

Song *et al*. (2009) applied atmospheric plasma on sliced cheese inoculated with three-strain cocktail of *L. monocytogenes,* and concluded that more than 8 log reduction could be achieved in 120 s at 150 W. DBD plasma discharge generated using helium and helium/oxygen mixture reduced *E. coli* population by 1.47 and 1.98 log cfu/cheese slice, respectively when treated for 15 min. Atmospheric low-temperature plasma treatment for inactivation of *E. coli* in milk at different fat contents was studied by Gurol *et al*. (2012).

Plasma generated with 9 kV of AC power was applied at time durations of 0, 3, 6, 9, 12, 15 and 20 min. About 54% reduction in the population of *E. coli* was observed after 3 min of treatment irrespective of the fat content of milk, while it did not affect pH and colour.

Lee *et al*. (2012) investigated the effect of CP on cheese. Significant reductions were observed in the population of *E. coli* ranging from 0.09 to 1.47 log and 0.05 to 1.98 log and in *S. aureus* from 0.05 to 0.45 and 0.08 to 0.91 log with CP generated using helium and He/O_2 mixture, respectively. The authors observed a significant change in L* value and increase in b* value of cheese. Also, it was reported that 10 and 15 min plasma treatment damaged the cheese slices. Kim *et al*. (2015) treated milk with DBD plasma, and analyzed the microbial and physicochemical changes. Results indicated that there was slightly noticeable colour difference and increase in the acidity of milk, but no significant change in the fatty acid profile was observed. Similarly, application of CP at intervals of 0, 3, 6, 9, 12, 15 and 20 min caused biochemical changes to protein, free fatty acids and volatiles of whole raw milk. The results indicated that there was no significant change in the lipid composition, ketone or alcohol levels. However, 20 min treatment increased the total aldehyde content (Korachi *et al*., 2015). Plasma treatment for 10 min resulted in a reduction of bacterial count by 2.4 log cfu/mL. It was observed that milk also underwent slight changes in physicochemical quality. Aslan (2016) concluded that the application of CP generated at a voltage of 3 kV for 3 min inactivated bacteria in raw milk.

Segat *et al*. (2015) studied the interaction between whey protein isolate and atmospheric CP for 1-60 min, and observed changes in the protein. The authors found that there was an increase in carbonyl groups and surface hydrophobicity, reduction in free SH groups in the interhelical structure and no change in the helical configuration of protein. Manoharan *et al.* (2020) applied low pressure plasma for decontamination of milk at 2 kV with 1.5 cm electrode gap. Milk was pumped at the rate of 6 and 3 mL/min. It was observed that flow rate of 3 mL/min resulted in a 95% reduction in microbial load when exposed for 5 min and no significant change in the physicochemical properties of milk was observed after treatment.

Wu *et al.* (2021) studied the influence of CP on sterilization of milk at 70 and 80 V for 120 s, and observed the changes in the microorganisms. The authors found that the sterilization effect was brought by breakage of cell membranes, reduction in the activity of metabolic enzymes and destruction of bacterial DNA. The treatment however decreased the micelle size and changed the structural characteristics of milk along with significant changes in colour, viscosity, pH, peroxidation of milk and titratable acidity. CP thus has the potential for use in the decontamination of dairy products, although the effect was limited (Lee *et al*., 2012)

2.4 Packaging

The product to be delivered to the consumer should be safe and should comply with the standards. Thus, adequate packaging is required to protect the physicochemical, nutritional and sensory properties of the product. Good packaging is one that lowers the product cost, reduces wastage, protects the product against spoilage and provides safety and comfort.

Vacuum packaging, where all the gas in the package is removed, is an alternative to atmospheric or modified atmosphere storage. In this process, the product is placed in high oxygen barrier film, the air is evacuated and the package is sealed. As the air gets evacuated, the package collapses around the product, lowering the pressure inside. Vacuum packaging helps in retarding chemical changes such as oxidative rancidity and bacterial growth. However, some deterioration could occur due to anaerobic organisms and non-oxidative reactions, which are usually minimized by storage at low temperatures. Although vacuum packaging involves complete removal of air, it is impossible to take out all the oxygen from the package.

The use of vacuum packaging could significantly increases the shelf-life of paneer. Kanawjia and Khurana (2006) reported that the limited keeping quality of paneer at refrigeration temperature could be enhanced by vacuum packaging. Paneer, when vacuum packed and stored at 3±1°C, showed an increase in shelf-life to 20 days (Shrivastava, 2007). Ahuja and Goyal (2012) observed that paneer *tikka* packed in LLDPE/BA/Nylon-6/BA/LDPE film under vacuum (0.70 kPa) had 40 days of shelf-life at 3±1°C. Bauer *et al.* (2017) reported an extended period for oxidation of plasma-treated beef by 10 days when vacuum packaged.

Rai (2004) investigated the effect of MAP paneer stored at 7±1°C. The paneer samples were packed in four different atmospheres namely atmospheric air (atm 1), vacuum (atm 2), 100% CO_2 (atm 3) and 100% N_2 (atm 4) in high barrier bags. The author concluded from the results of sensory attributes that samples packaged under the atmosphere of 100% CO_2 was rated the best, followed by those stored in atm 4, atm 2 and atm 1, respectively.

Srivastava (2004) studied the effect of MAP on the microbial quality of paneer stored for 45 days at 7±1°C. The author packaged paneer in four different atmospheric conditions namely, atmospheric air (atm 1), vacuum (atm 2), 100% CO_2 (atm 3) and 100% NO_2 (atm 4). The results showed that paneer packaged under 100% CO_2 showed the least increase in microbial count followed by those stored in atm 4 and atm 2. The initial total plate count of 3.462 log cfu/mL increased to 4.962 in atm 2, 3.817 log cfu/mL in atm 3 and 4.012 log cfu/mL in atm 4. The initial coliform count increased from 1.748 log cfu/mL to 3.953 log cfu/mL in atm 1, 3.772 log cfu/mL in atm 2, 2.141 log cfu/mL in atm 3 and 2.944 log cfu/mL in atm 4.

2.5 Summary

Paneer is a well-known and important traditional dairy product because of its extensive reach among the Indian population. It is used for making both sweets and culinary recipes. The market of paneer has been increasing continuously since 2016, but the organized sector has failed to increase the market share of paneer as compared to the unorganized sector due to its limited shelf-life. Various technologies have been applied to improve the shelf-life of paneer. With increasing concern of safety and standard food quality, researchers are pressed to introduce newer non-thermal technologies to improve the shelf-life of paneer. Among various non-thermal technologies, hurdle treatment and CP are known to be promising technologies for microbial decontamination and food preservation.

Also, many studies have been proven effective to improve the shelf-life of paneer such as incorporation of humectant or by applying hurdles such as pH, water activity, etc. NTP could be formed at ambient conditions upon application of high-intensity electric field to a neutral gas. Researchers have proved that microorganisms in fruits, vegetable, egg, meat and dairy could be inactivated with minimum or no effect on their quality attributes. Many authors also claimed that plasma effect was limited to surface only, and this unique feature provides promising impetus to apply plasma for microbial decontamination of various foods without compromising their physicochemical and sensory quality. The synergetic effect of hurdle and CP treatment along with vacuum packaging could be explored to extend the shelf-life of paneer.

Materials and Methods

3. Materials and Methods

The materials used and experimental procedures adopted to determine, evaluate and characterize various aspects of CP treatment of paneer are described in this chapter. The first part deals with the equipment and process parameters for CP treatment of paneer, while the later part covers various methods employed for analyzing the physicochemical, microbiological, textural and colour changes in paneer during CP treatment and subsequent storage.

3.1 Preparation of Paneer

Paneer was prepared by the methodology described by Gokhale *et al*. (2016) with minor modifications. Cow milk containing 3.5% fat and 8.5% solids-not-fat (SNF) was used for preparation of paneer. It was collected from the Livestock Research Centre, ICAR-National Dairy Research Institute, Southern Regional Station, Bengaluru, Karnataka. The milk was heated to 95°C for 5 min in a stainless steel (SS) container, cooled to 70°C and coagulated by 1% citric acid solution (70°C) along with slow stirring until a clear greenish tinge whey separated as shown in Fig. 3.1. The coagulum was then allowed to settle down for 5 min, followed by drainage of whey using a sterile cheese cloth. The coagulum was then hopped into a SS manual paneer press (Fig. 3.2). Adequate pressure was applied on the hoop by tightening the spring manually, and the force was kept for 20-30 min to facilitate drainage of whey from the coagulum. The pressure was adjusted in order to get the desired moisture level of 50-55% and 55-60% in paneer. The paneer block thus obtained was removed from the hoop and it was allowed to cool at 4°C before being used for CP treatment (Fig. 3.3). The SS vessels used for heating (95°C) and coagulation of milk were sterilized prior to use.

Fig. 3.1 Coagulum of milk solids for preparation of paneer

Fig. 3.2 Manual paneer press

Fig. 3.3 Paneer block obtained after pressing of coagulum in manual press

3.1.1 Process flow diagram

The process flow diagram for preparation of paneer is presented in Fig. 3.4.

Heating of milk to 95°C for 5 min followed by cooling to 70°C

↓

Addition of 1% citric acid solution at 70°C with gentle stirring for coagulation

↓

Settling of coagulum for 5 min

↓

Draining of whey by cheese cloth

↓

Pressing of coagulated solids in manual paneer press for 20-30 min

↓

Cooling of paneer block

Fig. 3.4 Process flow chart for preparation of paneer

3.2 Selection of Parameter Levels for Cold Plasma Treatment

Paneer cubes of 2×2×2 cm size were cut and treated by CP at 25 kV following the process parameters at different levels (Table 3.1). With these parameter levels, an experimental design consisting of combinations of each level of independent parameter was formulated with three replications.

Table 3.1. Levels of treatment parameters

Parameter	No. of levels	Parameter levels
Moisture content (%)	2	50-55 & 55-59
Time of exposure (min)	3	3, 5 & 8

3.3 Cold Plasma Treatment

Freshly prepared paneer of the above two moisture content ranges was cut into cubes of 2×2×2 cm size (Fig. 3.5) with the help of a knife in a clean environment.

Fig. 3.5 Paneer cubes of 2x2x2 cm size for cold plasma treatment

3.3.1 Specifications of cold plasma unit

3.3.1.1 Plasma chamber

Make : Hydro Pneovac Technologies, Bengaluru, Karnataka, India

Material : SS316

Chamber diameter : 30 cm

Chamber height : 30 cm

Electrode diameter : 15 cm

Electrode thickness : 1 mm

Chamber thickness : 3 mm

Top plate thickness : 16 mm

Bottom plate thickness : 16 mm

Chamber volume : 22 L

3.3.1.2 Vacuum pump

Type : Oil immersed

Capacity : 1 hp

Maximum vacuum : 700 mm of Hg

Make : Nidec Leroy Somer, Newtown, Powys, UK

3.3.1.3 Power supply unit

Voltage range	: 0-30 kV
Output type	: DC
Current	: 0.01-0.02 A

Fig. 3.6 Paneer samples placed on sample holder for treatment

For CP treatment, the paneer cubes were arranged on a dry sterile Petri dish, and it was placed on the sample holder inside the plasma chamber between the two electrodes (Fig. 3.6). The chamber was then closed by placing the top cover in position, and a relatively air-tight joint was ensured (Fig. 3.7). The vacuum release valve was closed, and the vacuum pump was started to create vacuum inside the treatment chamber. The oil-based vacuum pump had suction capacity of 450 LPM.

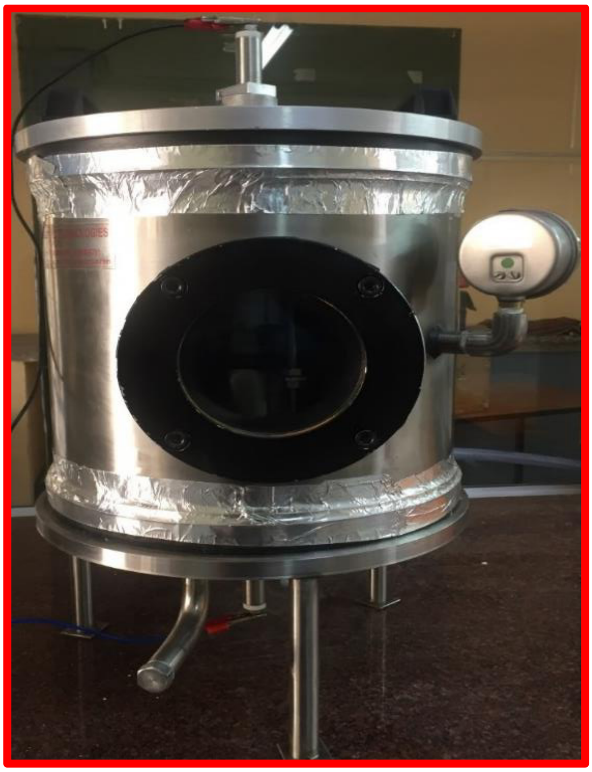

Fig. 3.7 Plasma chamber loaded with paneer cubes for cold plasma treatment

As the vacuum of 680 mm Hg was reached (Fig. 3.8), high voltage power supply to the electrodes was switched on. The DC voltage applied was increased steadily to 25 kV in a stepwise manner. At this voltage and vacuum level, atmospheric cold plasma was formed inside the treatment chamber (Fig. 3.9). No other gases were used for generation of CP. The time of exposure of paneer cubes to CP treatment was considered after attaining the set voltage of 25 kV. The time of exposure of CP was 3, 5 and 8 min. The applied voltage was stopped at about half exposure time of 1.5, 2.5 and 4 min, respectively and the vacuum inside the chamber was released. The top plate was gently removed, and the paneer cubes on the Petri dish were inverted. The paneer cubes were inverted so as to expose their bottom side to CP. The vacuum was regenerated and the voltage was applied to generate the plasma again for the remaining treatment duration. This step was done to ensure exposure of all the surfaces of paneer cubes to CP. After treatment, the paneer cubes were immediately transferred to phyta jars (Fig. 3.10) for microbiological and other analyses.

Fig. 3.8 Pressure gauge showing vacuum of 680 mm Hg

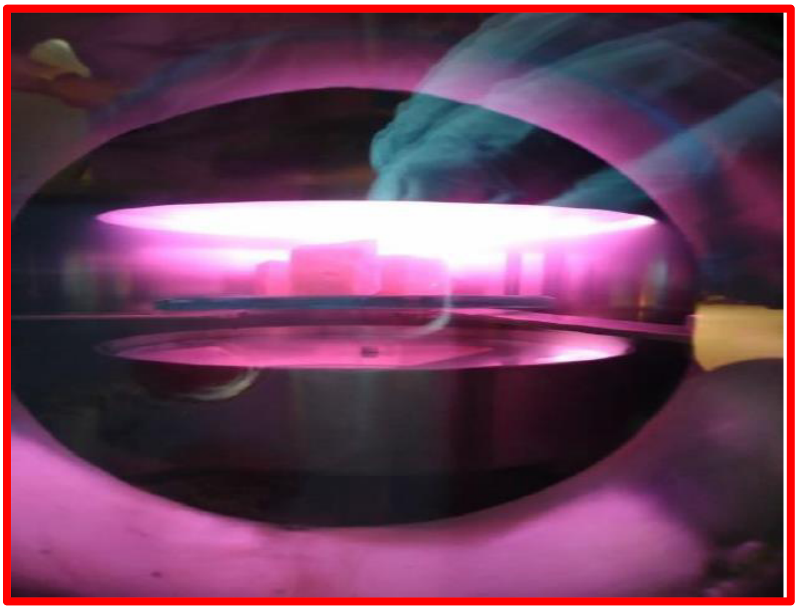

Fig. 3.9 Cold plasma treatment of paneer

Fig. 3.10 Packaging of cold plasma treated paneer for analysis

Fig. 3.11 Cold plasma unit

3.3.2 Process flow diagram

The flow diagram for CP treatment is presented in Fig 3.12.

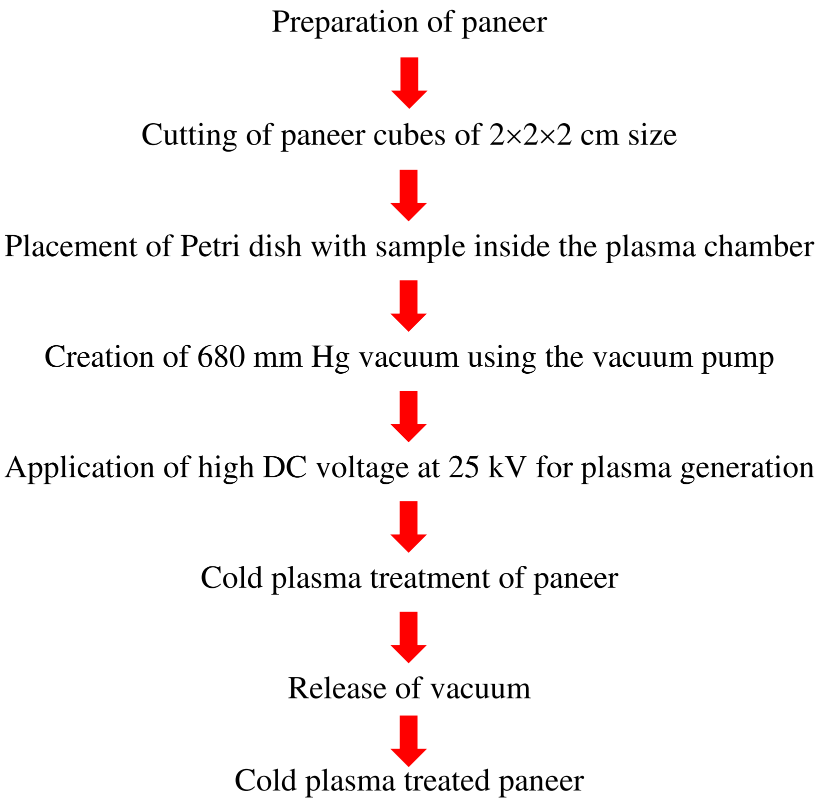

Fig. 3.12 Process flow chart for cold plasma treatment of paneer

3.4 Physicochemical Analyses of Paneer

3.4.1 Moisture content

The moisture content of CP treated paneer was determined by AOAC method 926.08 (AOAC, 2016). Dried and cooled flat bottom Petri dishes were taken, and about 3 g paneer was weighed on to them. The sample was crushed with the help of glass rod, and 4 mL of warm double-distilled water was used to crush and make a uniform paste. The dishes were then placed in a hot air oven kept at 102±2°C for 4 h, and subsequently, they were transferred to a desiccator, cooled and weighed accurately. The moisture content of paneer was calculated by Eq. 3.1, and was expressed in dry basis.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1 - W} \times 100 \qquad (3.1)$$

Where,

W = Weight of dish in g

W_1 = Weight of dish and sample together in g

W_2 = Weight of dish and dried sample together in g

The weight loss of paneer during CP treatment was calculated using Eq. 3.2.

$$\text{Weight loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \qquad (3.2)$$

Where,

W_1 = Initial weight of paneer in g

W_2 = Weight of paneer after CP treatment in g

3.4.2 Titratable acidity

The titratable acidity of CP treated paneer was determined by AOAC method 920.124 (AOAC, 2016). Exactly 10 g sample was weighed in a porcelain dish, and it was crushed into a fine paste using warm distilled water. The paste was transferred into a volumetric flask of 100 mL capacity, and the contents were filtered using Whatman No. 42 filter paper. Exactly 25 mL of filtrate was titrated against 0.1 N NaOH using phenolphthalein indicator. The filtrate taken for analyses represented 2.5 g sample, and the titratable acidity of sample was expressed as percentage lactic acid (Eq. 3.3).

$$\text{Lactic acid (\%)} = \frac{9 \times N \times V}{W} \times 100 \qquad (3.3)$$

Where,

N = Normality of NaOH

V = Volume of NaOH consumed for titration in mL

W = Weight of sample in g

3.4.3 Free fatty acid

Exactly 3 g of CP treated paneer was crushed and taken in a 50 mL stoppered test tube. Ten mL of extraction mixture (iso-propanol: petroleum ether: 4 N H_2SO_4 ratio in 40:10:1) was poured into the test tube, and 6 mL of petroleum ether and 4 mL of distilled water were added and mixed. The test tube was agitated vigorously for 20 s and left undisturbed for 10 min. Two layers got separated with a clear thin line, and the volume of upper layer was measured. Subsequently, 5 mL of upper layer was taken and transferred into a 50 mL flask. Six drops of methanolic phenolphthalein indicator (1 g phenolphthalein in 100 mL methanol) were added into the flask and the contents were titrated against 0.02 N-methanolic KOH solution. Similarly, a blank with distilled water was used to obtain the background titre value. Concentration of methanolic KOH solution used was noted by titrating it against 0.02 N oxalic acid. Free fatty acid content (FFA) was determined based on Eq. 3.4.

$$\text{FFA}\left(\frac{\mu\text{eq}}{g}\right) = \frac{\text{T}\times\text{N}}{\text{P}\times\text{W}}\times 100 \tag{3.4}$$

Where,

T = Net titration value (titre volume for sample – titre volume of blank)

N = Normality of methanolic KOH

P = Proportion of aliquot taken (mL/mL)

W = Weight of sample in g

3.4.4 Oxidative rancidity (TBA content)

Thiobarbituric acid (TBA) reaction was used to determine the extent of oxidative rancidity in treated paneer. The procedure suggested by Zhang (1991) for milk lipids was followed. Exactly 3 g of sample was taken and mixed with 15 mL of warm distilled water taken in a stoppered tube. To this, 1 mL of 50% trichloroacetic acid (TCA) was added, followed by addition of 2 mL of 95% ethanol. The contents were stoppered and vigorously shaken for 10 s. The contents were left undisturbed for 5 min before filtering through Whatman No. 42 filter paper. To 4 mL of clear filtrate obtained, 1 mL of TBA reagent (1.4 g of 2-thiobarbituric acid in 95% ethanol to 100 mL) was added and the contents were mixed well before placing in a water bath being maintained at 60ºC for 60 min. The contents were cooled, and the optical density was measured at 532 nm in the UV-Vis spectrophotometer (Model-UH5300, Hitachi High-Technologies, Tokyo, Japan).

3.4.5 Colour measurement

The colour of treated paneer was determined in terms of L*, a* and b* values. Adobe Photoshop (v. 22.1.1) was used to analyze the ‘L’, ‘a’ and ‘b’ values of paneer. The images were acquired using an Epson scanner (Model-Epson Perfection V39, Cilandak, South Jakarta, Indonesia) in Tagged Image File Format (TIFF) at 1200 dpi resolution. The ‘L’, ‘a’ and ‘b’ values derived from analyzing the images in the histogram window were used to calculate machine independent L*, a*, b* values, and whiteness index (WI) was calculated based on Eqs. 3.5 to 3.8.

$$L^* = \left(\frac{L}{255}\right)\times 100 \tag{3.5}$$

$$a^* = \left(\frac{240a}{255}\right)-120 \tag{3.6}$$

$$b^* = \left(\frac{240b}{255}\right)-120 \tag{3.7}$$

$$\text{Whiteness index} = 100-\left[\left(100-L^*\right)^2+a^{*2}+b^{*2}\right]^{0.5} \tag{3.8}$$

3.4.6 Texture profile analysis

Texture profile analysis (TPA) of CP treated paneer was done to determine the effect of CP treatment and moisture content on textural quality of paneer. TPA was done using the texture analyzer (Model-TA-XT plus, Stable Micro Systems, Godalming, Surrey, UK) (Table 3.2). The texture analyzer was fitted with a 50 kg load cell, and the samples were tested by two-bite linear compression. A circular platen of 75 mm dia. (P/75) was used for compression of the samples.

Paneer samples of 2×2×2 cm were positioned centrally over the platform and subjected to uniaxial compression. The platen was allowed to compress 50% of sample height before returning to the original position. After a gap of 5 s, the second compression was done and the time-force curve as shown in Fig. 3.13 was generated. The texture profile parameters of paneer were determined from the force-time curve.

Table 3.2. Programme settings for texture profile analysis

Parameter	Value
Test type	TPA
Test mode	Compression
Pre-test speed	1 mm/s
Test speed	5 mm/s
Post-test speed	5 mm/s
Distance	50% of sample height
Time	5 s
Triger type	Auto (Force)
Trigger force	1 g
Break mode	Off
Advanced option	On

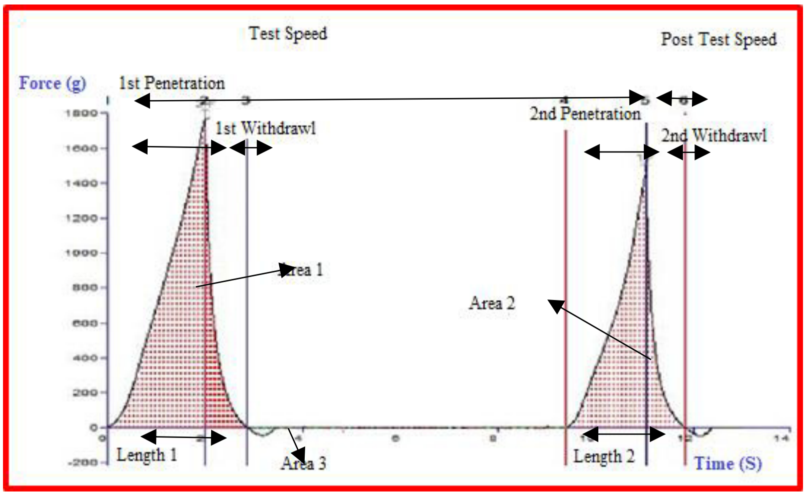

Fig. 3.13 Force-time curve of texture profile analysis

3.4.6.1 Hardness

Hardness (N) was measured as the peak force that occurred during the first compression of the sample.

3.4.6.2 Cohesiveness

Cohesiveness tells how well the product withstood the second deformation relative to its resistance under the first deformation. It is the area of work during second compression divided by the area of work during first compression as expressed in Eq. 3.9.

$$\text{Cohesiveness (\%)} = \left(\frac{A_2}{A_1}\right)\times 100 \tag{3.9}$$

Where,

A_1 = Area of work done during first compression

A_2 = Area of work done during second compression

3.4.6.3 Adhesiveness

Adhesiveness is a measure of negative work done between two cycles. It is the area under the curve of the first negative peak.

3.4.6.4 Springiness

Springiness is how well a product physically recovers and springs back in the time duration of holding between the first and second compression. It is measured at the down stroke of the second compression (Eq. 3.10).

$$\text{Springiness (\%)} = \left(\frac{L_2}{L_1}\right)\times 100 \tag{3.10}$$

Where,

L_1 = Height attained during first compression (mm)

L_2 = Height attained during second compression (mm)

3.4.6.5 Gumminess

Gumminess was calculated as the product of hardness and cohesiveness (Eq. 3.11).

$$\text{Gumminess (N)} = \text{Hardness} \times \text{Cohesiveness} \tag{3.11}$$

3.4.6.6 Chewiness

Chewiness is the energy required to masticate a solid food product to make it ready for swallowing. It is calculated as the product of gumminess and springiness, as expressed in Eq. 3.12.

$$\text{Chewiness (N)} = \text{Gumminess} \times \text{Springiness} \qquad (3.12)$$

3.5 Microbiological Analysis of Paneer

Paneer treated by CP was analyzed for total plate and yeast and mould counts to evaluate the effect of treatment. Pour plate method was used for microbiological examination. The saline solution for dilution used was tri-sodium citrate buffer at 2% concentration.

3.5.1 Preparation of media and saline

Purified tri-sodium citrate (SD Fine-Chem Ltd., Mumbai) was added to distilled water to achieve 2% concentration solution. The saline solution was plugged tightly with non-absorbent cotton and autoclaved at 121°C (pressure was 1.03421×10^5 Pa) for 15 min. Potato dextrose agar (PDA) and plate count agar (Hi-Media Laboratories Pvt. Ltd., Mumbai) were used for yeast and mould and total plate count analyses, respectively. PDA was added to distilled water at the rate of 39 g per 1000 mL, while plate count agar was added at 23.5 g per 1000 mL. The agars were also individually autoclaved as described above before plating.

3.5.2 Sample preparation

Exactly 11 g of paneer was aseptically crushed in a pre-sterilized pestle and mortar, and the paste was transferred to 99 mL sterile saline solution and mixed well to get 1:10 dilution. From the stock solution, aseptically 1 mL was added to 9 mL of saline to obtain 1:100 dilution. The dilutions were done such that no flakes or paneer particles were allowed to come into the solution. From the diluted solution, 1 mL was poured onto sterilized Petri dishes followed by pouring of sterilized agar media at near ambient temperature, and the agar was allowed to cool and solidify. For yeast and mould analyses, 1% tartaric acid solution was added to PDA before plating, and the contents were mixed well. The dilutions and plating of media were performed inside UV-sterilized laminar airflow chamber. Exactly, 1:10 dilution was used for determination of yeast and mould count and 1:100 dilution was used for total plate count. Incubation time and temperature for total plate count and yeast and mould were

37±2°C for 24 to 48 h and 25±2°C for 72 to 120 h, respectively. All the samples were plated in duplicates (as replication) to minimize error. After counting of colonies, the plates were decontaminated and sterilized in the autoclave.

3.6 Optimization of Cold Plasma Treatment of Paneer

3.6.1 Taguchi orthogonal array design

The CP treatment conditions were optimized based on factors such as exposure time and initial moisture content. L_8 (4^1x2^1) Taguchi mixed-orthogonal array design was applied for optimization of these selected independent variable factors (Table 3.3), while moisture loss and microbial destruction were selected as response factors. A combination of statistical, mathematical and heuristic techniques is used in this design. Eight runs were carried out in triplicate, and Minitab 17 software (Minitab, LLC, Pennsylvania, USA) was used for analysis of data.

Table 3.3. Cold plasma treatment parameters and their levels

Parameter	Level 1	Level 2	Level 3	Level 4
Moisture content (%)	<55	>55	--	--
Exposure time (min)	0	3	5	8

The "Loss function" is used to compute the deviation between experimental and predicted values in Taguchi optimization design. The loss function was converted into Signal-to-Noise (S/N) ratio (Eq. 3.13) where 'S' denotes signal (desirable value) and 'N' denotes noise (undesirable value). S/N ratios are classified into three categories namely 'smaller is better', 'nominal is best' and 'larger is better'. The attempt was made in this study to minimize the moisture loss and maximize the microbial destruction in paneer. Therefore, 'smaller is better' option was selected for both moisture loss and microbial population as optimization criteria.

$$\frac{S}{N} = -10 \times \log(\mathrm{MSD}) \tag{3.13}$$

$$\mathrm{MSD} = \frac{1}{N}\sum_{i=1}^{n} y_i^2 \tag{3.14}$$

Where,

MSD = Mean square deviation

y_i = Value observed for i^{th} test

N = Total number of observations

The S/N ratios for each type of characteristics were computed by Eq. 3.15:

$$\text{"Smaller is better"(minimize)}\left[\frac{S}{N}\right]_{SB} = -10\times\log\left(\frac{1}{n}\sum_{i=1}^{n} y_i^{\,2}\right) \tag{3.15}$$

Where,

$\bar{y}$ = Mean of the observed data

S_y^2 = Variance of 'y'

n = Number of observations

y = Observed data

The optimal combination of cold plasma treatment conditions was predicted based on S/N ratios. Finally, a confirmation experiment was conducted to validate the optimal process parameters obtained from the experimental design (Table 3.4). The significance of the parameters for minimum moisture loss and maximum microbial destruction was determined using 95% confidence intervals.

Table 3.4. Taguchi optimization design of cold plasma treatment of paneer

Run	Moisture content (%)	Exposure time (min)
1	<55	0
2	<55	3
3	<55	5
4	<55	8
5	>55	0
6	>55	3
7	>55	5
8	>55	8

3.7 Proximate Composition of Paneer

Paneer after cold plasma treatment was also analyzed for protein, fat, ash content, etc.

3.7.1 Moisture content

The moisture content of paneer was determined by the method described in Section 3.4.1.

3.7.2 Fat content

The fat content of paneer was determined by AOAC method 933.05 (AOAC, 2016) recommended for cheese. Exactly 10 g of paneer was weighed and transferred into an extraction tube, and to that 1 mL of concentrated ammonia solution was added and mixed well. Further, 10 mL ethyl alcohol was added and mixed thoroughly, followed by the addition of 25 mL diethyl ether and mixing for at least 1 min and 25 mL petroleum ether. The contents were vigorously shaken for 30 s. The tube was left undisturbed for about 30 min so that the liquid portion appeared as a separated layer. The liquid was transferred into a pre-weighed dry flask and 15 mL of di-ethyl ether and petroleum ether were added for second extraction. The process was repeated up to 3 times and the liquid formed was transferred to the original extraction tube. The blank was made simultaneously using distilled water. The extraction flask was kept for distillation at 98-100°C for 1 h, and the fat content was estimated using Eq. 3.16.

$$\text{Fat (\%)} = \left(\frac{\text{Weight of flask and sample} - \text{Weight of empty flask}}{\text{Sample of weight}}\right)\times 100 \tag{3.16}$$

3.7.3 Ash content

The ash content of paneer was determined by the method of Suleiman *et al.* (2018). Approximately 3-4 g of paneer was taken into a pre-weighed crucible dish, weighed precisely and placed on a water bath for complete drying of moisture. The dried paneer was then transferred to a muffle furnace, and heated to 550°C for 5 h, cooled in a desiccator and weighed. The percentage ash content was estimated using Eq. 3.17.

$$\text{Ash (\%)} = \left(\frac{W_2}{W_1}\right)\times 100 \tag{3.17}$$

Where,

W_1 = Weight of ash (weight of dried sample and dish – weight of empty dish) in g

W_2 = Weight of sample in g

3.7.4 Protein content

The protein content of paneer cubes was determined by AOAC method 2001.14 (AOAC, 2016) using 6.38 as conversion factor (Eq. 3.18). In this method, 0.3 g of paneer was taken in the digestion flask followed by addition of 2-3 g digestion mixture and 15 mL of concentrated sulphuric acid. The contents in the flask were digested for 3 h in the Kjeldahl digestion unit until a clear solution was obtained. After cooling, the Kjeldahl flask was washed with 10-15 mL of distilled water and the contents were transferred to the distillation unit. Twenty mL of 40% NaOH was added to make the solution alkaline. The contents were steam-distilled and the liberated ammonia was collected in 30 mL of 4% boric acid solution added with 2-3 drops of mixed methyl red and methylene blue indicator. The distillate was titrated against 0.1 N H_2SO_4, and the colour change from green to purple was taken as the endpoint. A blank digestion was carried out parallelly using all the reagents without the addition of paneer.

$$\text{Protein (\%)} = \frac{(A - B) \times N \times 1.4007 \times 6.38}{W} \qquad (3.18)$$

Where,

A = Volume of 0.1 N H_2SO_4 required for titration of sample in mL

B = Volume of 0.1 N H_2SO_4 required for titration of blank in mL

N = Normality of H_2SO_4

W = Weight of paneer in g

3.8 Statistical Analyses

The experiments were performed in triplicate. All statistical analyses were performed using SPSS software with level of significance at 0.05. Two-way analysis of variance (ANOVA) was used to determine the effect of main and interaction factors. If the factor effects were significant, the differences among treatment means was compared using Tukey's HSD Post-hoc test.

3.9 Shelf-life Evaluation of Cold Plasma Treated Paneer

On the basis of preliminary trials and evaluation of microbiological, physicochemical and textural changes occurring in CP treated paneer cubes at different moisture levels and treatment times, an optimized combination was selected on the basis on minimum moisture loss and maximum microbial inactivation.

Paneer cubes treated at the optimum time duration and moisture content was stored in low-density polyethylene (LDPE) pouches under normal and vacuum atmosphere. Two different storage temperatures were applied to evaluate the shelf-life quality of paneer after 9 days as given below.

Control : Atmospheric packaged untreated paneer
Sample 1 : Atmospheric packaged CP treated paneer stored in refrigerated condition
Sample 2 : Atmospheric packaged CP treated paneer stored in frozen condition
Sample 3 : Vacuum packaged CP treated paneer stored in refrigerated condition

Paneer treated under optimized CP conditions was packaged in UV-sterilized (LDPE) pouches and stored. Physicochemical properties such as moisture content, titratable acidity, oxidative rancidity, hydrolytic rancidity, textural properties and microbiological quality of paneer cubes were determined at the regular intervals for refrigerated and frozen paneer as per methodology described in Sections 3.4 and 3.5.

Results and Discussion

4. Results and Discussion

The present study was undertaken to extend the shelf-life of paneer using hurdle and cold plasma technologies. This chapter discusses the results that were obtained from various experiments conducted to fulfill the objectives of the study.

4.1 Preparation and Yield of Paneer

The method for preparation of paneer from cow milk was discussed in detail in Section 3.1. The yield of fresh paneer was 14.13 to 14.90% when prepared from cow milk with 3.5% fat and 8.5% SNF. The yield of paneer was in accordance with the values of 10.17-14.15% reported by Jadhavar *et al.* (2009), and was satisfactory. The moisture content of freshly prepared paneer was in the range of 50-60%. Moreover, the obtained moisture content was within the acceptable limit specified in the Food Safety and Standards Act (FSSAI, 2011), which stipulates maximum moisture content of 60% (wet basis) in paneer obtained from milk.

4.2 Effect of Cold Plasma Treatment on Moisture Loss in Paneer

In the present study, paneer cubes of 2×2×2 cm were treated with CP at 680 mm of Hg vacuum and applied voltage of 25 kV for 3, 5 and 8 min. Paneer cubes of two different initial moisture contents, namely <55% and >55%, were taken for study. It was observed that CP treatment resulted in moisture loss in paneer (Table 4.1). Paneer cubes with initial moisture content of <55% showed 4.5, 5.8 and 6.7% moisture loss, while those with >55% initial moisture content had 4.9, 6.2 and 7.0% reduction in moisture content after 3, 5 and 8 min of CP treatment, respectively. It could be stated that moisture loss primarily occurred due to high vacuum (and consequently low water vapour pressure) prevailing in the treatment chamber, which facilitated the release of water molecules from the product depending on the initial moisture content and exposure time (Sarangapani *et al.*, 2015). Also, CP processing, being a surface treatment, removed moisture from the surface of paneer. Sarangapani *et al.* (2015) also reported post treatment moisture loss in rice when exposed to low pressure plasma (0.05 mbar) for 5, 10 and 15 min at 30, 40 and 50 W. Similarly, Ahangari *et al.* (2021) observed 0.72% reduction in moisture content in walnut kernels treated by plasma at pressure less than 500 mTorr for 20 min at 50 W.

Table 4.1. Effect of cold plasma treatment on moisture content of paneer

Treatment time (min)	Initial moisture content (<55%)		Initial moisture content (>55%)	
	Moisture content after treatment (%)	Moisture loss (%)	Moisture content after treatment (%)	Moisture loss (%)
0	53.06±1.96	-	57.05±1.65	-
3	54.44±1.70	4.57	50.46±2.20	4.90
5	53.71±1.11	5.85	49.73±2.25	6.27
8	49.51±2.29	6.69	53.03±1.80	7.04

Results are expressed as Mean±SD

4.3 Effect of Cold Plasma Treatment on Microbial Destruction of Paneer

It was observed that the initial microbial population was relatively higher in paneer cubes with initial moisture content >55%. After CP treatment, the paneer cubes at both moisture contents showed a significant reduction in total plate count (TPC) and yeast and mould count as shown in Figs. 4.1 and 4.2. However, the microbial destruction was comparatively severe in paneer cubes with >55% initial moisture content. For paneer cubes with >55% initial moisture content, the initial TPC of 5.13±0.19 log cfu/g reduced to 3.97±0.29, 3.18±0.16 and 3.00±0.18 log cfu/g at 3, 5 and 8 min of CP treatment, respectively. In comparison, in paneer cubes with <55% initial moisture content, the initial TPC of 4.84±0.39 log cfu/g reduced to 4.00±0.42, 3.15±0.21 and 3.00±0.15 log cfu/g at corresponding treatment times.

The initial moisture content of food products affected the destruction of microorganisms by CP. According to Liao *et al.* (2017), higher moisture content of food products could enhance the effect of CP on microbial destruction owing to the increased production of peroxyl acid and hydroxyl radicals formed from the decomposition of water molecules. The additional hydroxyl radicals produced by decomposition of water molecules was probably utilized as reactive species for enhanced destruction of microorganisms. However, an excessive amount of water could produce dilution effect in the treatment, and hence, moisture content of paneer needs to be optimized to realize better effect of CP treatment.

It was also observed that mere 3 min of CP treatment reduced the microbial population by nearly one log count. Further increase in exposure of paneer to CP did not bring drastic reduction in the TPC. The microbial load in paneer after 8 min of treatment was statistically same as that of 5 min treatment. This could be due to the solid body and texture of paneer cubes, which the reactive species of CP were not able to penetrate and also entrap the microorganisms present in pores. CP treatment is a surface treatment, and the penetration depth is limited regardless of exposure time (Surowsky *et al.*, 2016). Yeast and mould count showed complete destruction after CP treatment of 3 min of exposure. The initial moisture content had no effect on yeast and mould count as complete destruction was observed in both cases. Similar results were reported by Gurol *et al.* (2012) during low temperature plasma treatment at 9 kV AC for 20 min on milk containing *E. coli* ATCC 25922. The authors observed 54% reduction in microbial population. Similarly, Wan *et al.* (2019) applied high voltage atmospheric CP using DBD for 5 min at 100 kV for inactivation of *L. innocua* in queso fresco cheese and cheese model, which resulted in 1.6 and 3.5 log cfu/g reduction in TPC, respectively.

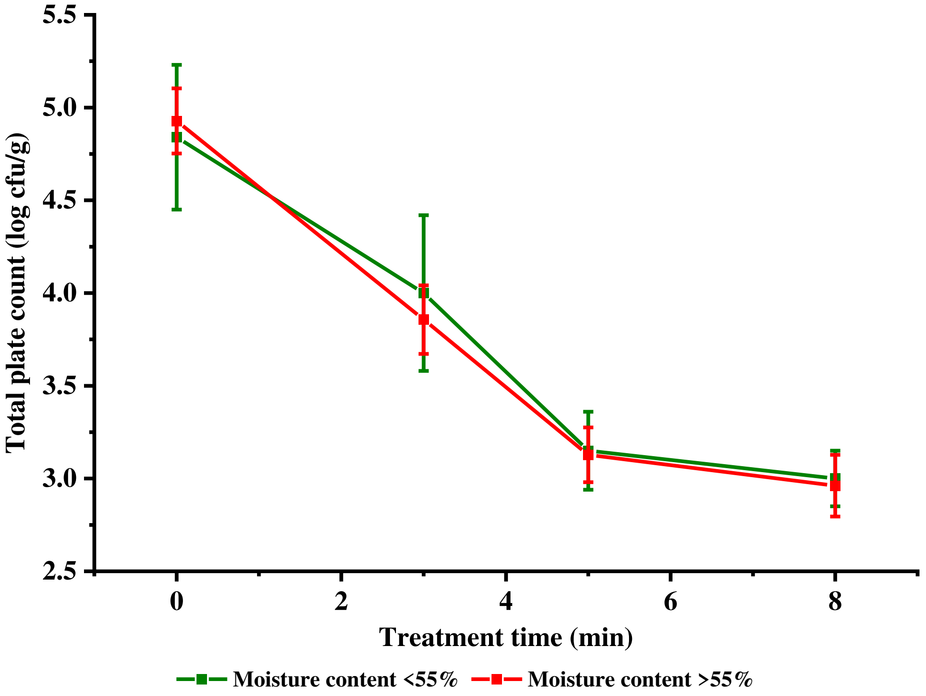

Fig. 4.1 Effect of cold plasma on total plate count of paneer

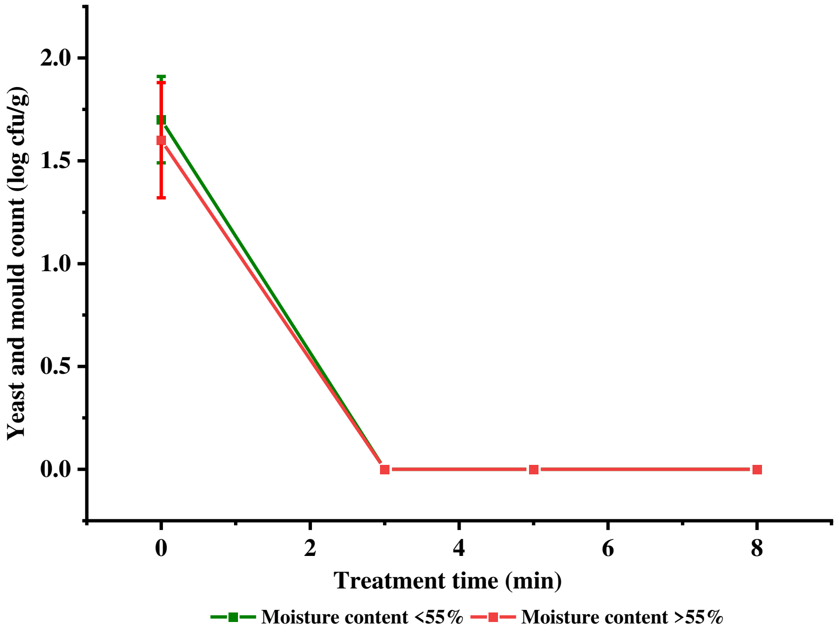

Fig. 4.2 Effect of cold plasma on yeast and mould count of paneer

4.4 Effect of Cold Plasma Treatment on Physicochemical Properties of Paneer

4.4.1 Effect of cold plasma on colour

Whiteness index of paneer cubes decreased after CP treatment (Table 4.2). In paneer cubes with <55% initial moisture content, the WI reduced from 84.93±6.32 to 84.09±5.05, 84.03±5.79 and 81.8±7.24 after 3, 5 and 8 min of CP treatment, respectively. The corresponding decrease in WI of paneer cubes with >55% initial moisture content was 85.97±6.35 to 85.47±6.01, 84.96±6.82 and 84.96±7.06 after 3, 5 and 8 min of CP treatment, respectively. Thus, it could be stated that the effect of CP on WI was not significant ($p>0.05$). Lee *et al.* (2012) attributed change in a* value to induced oxidative reactions and decrease in L* to moisture loss from the product. Grzegorzewski *et al.* (2011) observed that reactive species from plasma helped in the disintegration of cell membrane that resulted in oxidation of cellular components, which reduced the whiteness. Similar results were reported by Dasan *et al.* (2018), on application of atmospheric pressure plasma (APP) to apple, orange and tomato juices at 3×400 V for 30, 60, 90 and 120 s. CP treatment resulted in decreased L* and increased a* values. Bermúdez-Aguirre *et al.* (2013) also investigated the effect of atmospheric pressure CP on lettuce and tomatoes, but observed no significant change in colour values of tomato, while L* value of lettuce increased after 7 min of exposure.

Table 4.2. Effect of cold plasma treatment on whiteness index of paneer

Treatment time (min)	Whiteness index (initial moisture content <55%)	Whiteness index (initial moisture content >55%)
0	84.93±6.32	85.97±6.35
3	84.09±5.05	85.47±6.01
5	84.03±5.79	84.96±6.82
8	81.80±7.24	84.58±7.06

Results are expressed as Mean±SD

4.4.2 Effect of cold plasma treatment on texture profile of paneer

The initial hardness of paneer cubes was less at >55% moisture content (15.16±1.60 N) than at <55% moisture content (17.08±3.01 N) (Table 4.3). It was on expected lines as higher solids content in paneer cubes at <55% moisture content would contribute to increased hardness. It was observed that hardness of paneer cubes increased with increasing exposure time to CP (Fig. 4.3). The hardness of <55% moisture content paneer cubes increased to 17.64±3.98, 17.93±2.45 and 18.47±2.98 N after CP treatment of 3, 5 and 8 min, respectively. In comparison, the hardness of paneer cubes with >55% initial moisture content increased to 16.91±2.42, 17.30±2.88 and 17.77±3.23 N at corresponding exposure times.

Though the main effect of CP treatment on hardness was significant (Table 4.4), the simple effect comparisons (comparison between treatment times) as evident from Fig. 4.3 are not significantly different. Similar results were reported by Jayasena *et al.* (2015) for plasma treated pork butt and beef loin at 2.5, 5, 7.5 and 10 min, wherein increase in hardness with treatment time was reported to be insignificant. In comparison, the effect of initial moisture content on the hardness of CP treated paneer cubes was significant (Table 4.4). However, the effect of CP treatment on other texture profile parameters was not significant.

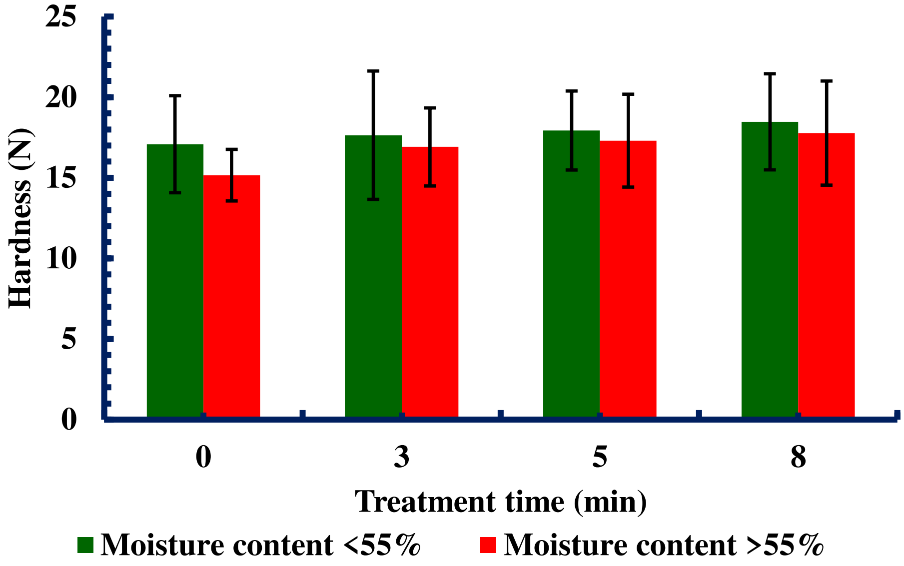

Fig. 4.3 Effect of initial moisture content and cold plasma treatment on hardness of paneer

Table 4.3. Effect of cold plasma treatment on texture profile analysis of paneer

Treatment time (min)	Moisture content	Hardness (N)	Adhesiveness	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
0	<55%	17.08±3.01	-0.12±0.05	0.82±0.02	0.62±0.05	11.22±2.88	9.29±2.37
3	<55%	17.64±3.98	-0.18±0.18	0.82±0.02	0.63±0.04	11.57±2.93	9.52±2.41
5	<55%	17.93±2.45	-0.12±0.07	0.82±0.02	0.65±0.05	11.03±2.42	9.17±2.09
8	<55%	18.47±2.98	-0.16±0.14	0.81±0.02	0.63±0.05	11.30±2.22	9.25±1.97
0	>55%	15.16±1.60	-0.22±0.22	0.83±0.03	0.65±0.06	9.74±2.21	8.08±1.84
3	>55%	16.91±2.42	-0.30±0.27	0.81±0.02	0.68±0.05	11.29±2.33	9.19±1.88
5	>55%	17.30±2.88	-0.18±0.17	0.82±0.01	0.69±0.05	11.72±2.30	9.64±1.88
8	>55%	17.77±3.23	-0.21±.021	0.81±0.02	0.64±0.05	11.26±3.25	9.20±2.72

Results are expressed as Mean±SD

Table 4.4. ANOVA for cold plasma treatment on hardness of paneer

Source	Type III sum of squares	df	Mean square	F-value	Significance
Corrected model	1522119.456[a]	7	217445.637	2.412	0.022
Intercept	5.695×10^8	1	5.695×10^8	6316.328	0.000
Moisture	472997.540	1	472997.540	5.246	0.023
Time	982821.025	3	327607.008	3.633	0.014
Moisture × Time	128018.625	3	42672.875	0.473	0.701
Error	16050035.609	178	90168.739		
Total	5.959×10^8	186			
Corrected total	17572155.066	185			

4.5 Optimization of Cold Plasma Treatment Time for Extended Shelf-life of Paneer

Process optimization of CP treatment of paneer was carried out, and product properties such as moisture loss, microbial count, texture profile and colour were analysed. Taguchi L_8 orthogonal array design was used to optimize the conditions for CP treatment of paneer. The optimization criteria were minimization of moisture loss and maximization of microbial destruction. The experiments were carried out based on the Taguchi Orthogonal Array design to determine the optimum initial moisture content and CP exposure time.

In S/N ratio, ‘S’ denotes ‘Signal’ which is desirable output, while ‘N’ represents ‘Noise’, the extent of deviation from desirable output. In general, the S/N ratio is used to determine the extent of variation of control factors, and delta values are used for ranking the influence of independent variables on the control factor (Tables 4.5-4.7). Higher S/N ratio with ‘Smaller is Better’ criteria was selected for both moisture loss and microbial population. The mean S/N value for moisture loss, total plate count and yeast and mould count is tabulated (Tables 4.5-4.7). From the delta values, it was observed that CP exposure time had higher influence on both moisture loss and TPC with delta value of 3.23 and 5.982 than that of initial moisture content (0.55 and 0.150).

The influence of independent variables on moisture loss, TPC and yeast and mould count is illustrated in Figs. 4.4 to 4.6. For achieving minimal moisture loss and maximum destruction of microorganisms together, exposure time at level 2 (5 min) and initial moisture content at level 1 (>55%) are recommended. These conditions would ensure complete destruction of yeast and mould in paneer, while destroying other bacteria to the maximum extent. The optimized conditions of CP treatment were initial moisture content of >55% and CP exposure time of 5 min were selected for shelf-life study.

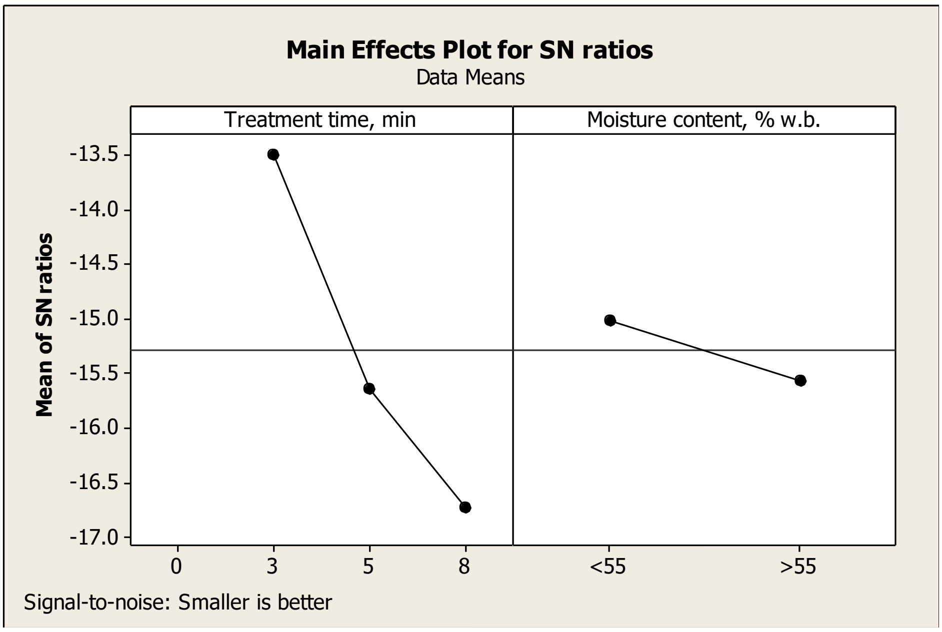

Fig. 4.4 Taguchi S/N ratio plot for moisture loss

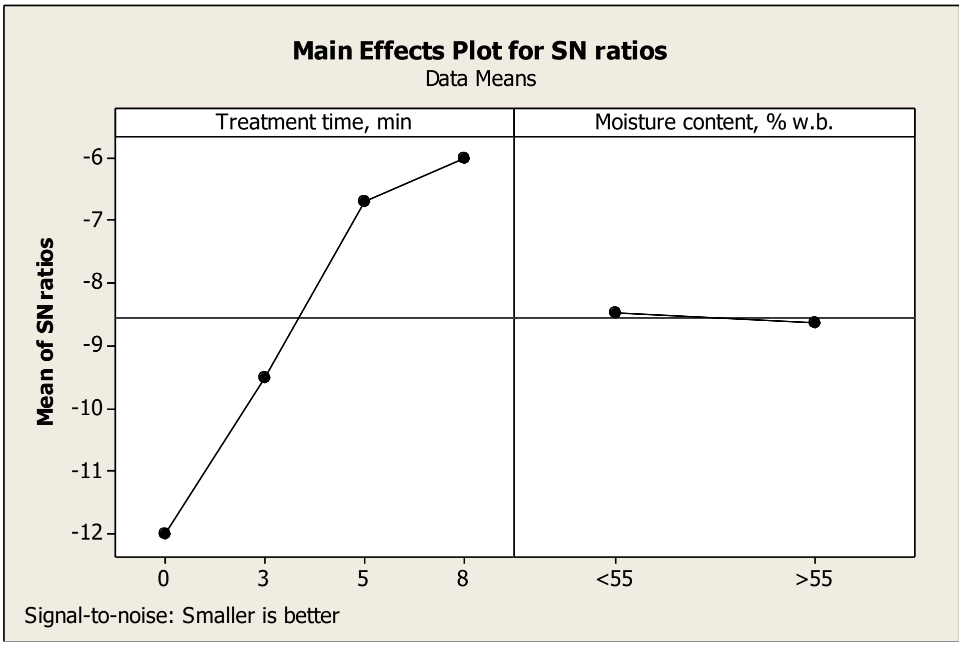

Fig. 4.5 Taguchi S/N ratio plot for total plate count

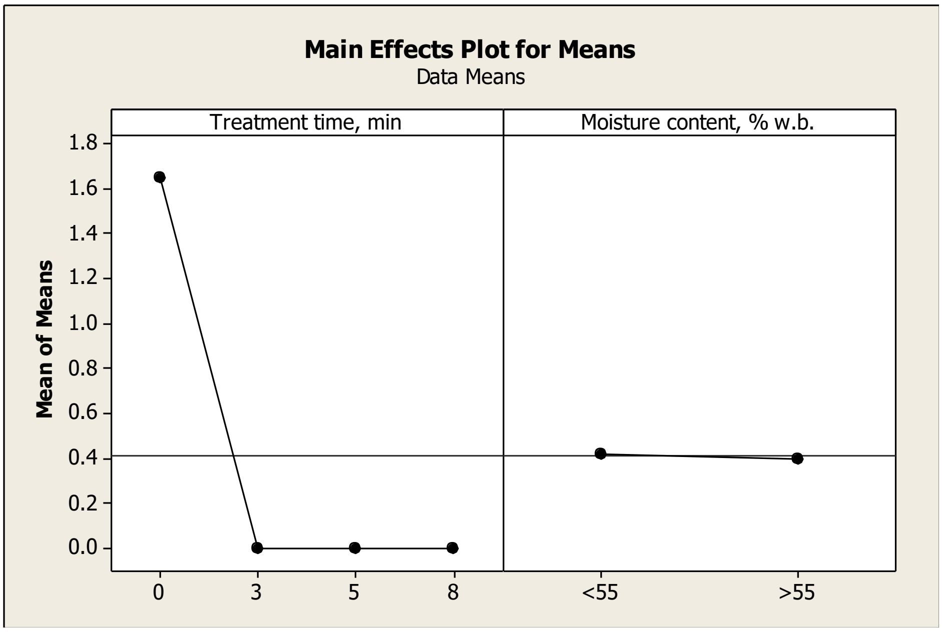

Fig. 4.6 Taguchi S/N ratio plot for yeast and mould count

Table 4.5. Taguchi response for moisture loss

Level	Treatment time (min)	Moisture content (%)
1	--	-15.02
2	-13.50	-15.57
3	-15.64	--
4	-16.73	--
Delta	3.23	0.55
Rank	1	2

Table 4.6. Taguchi response for total plate count

Level	Treatment time (min)	Moisture content (%)
1	-12.003	-8.483
2	-9.499	-8.633
3	-6.709	--
4	-6.021	--
Delta	5.982	0.150
Rank	1	2

Table 4.7. Taguchi response for yeast and mould count

Level	Treatment time (min)	Moisture content (%)
1	-4.346	-4.609
2	--	-4.082
3	--	--
4	--	--
Delta	0.000	0.527
Rank	2	1

4.6 Evaluation of Shelf-Life of Cold Plasma Treated Paneer

The paneer cubes treated at optimized conditions were stored for 9 days, and the changes in various physicochemical, textural, colour and microbial load were analysed. The paneer cubes that underwent CP treatment were packaged in UV-sterilized LDPE pouches under vacuum and atmospheric condition. Also, they were stored in refrigerated and frozen conditions. The changes in physicochemical and textural properties that occurred during storage are presented in Tables 4.8 and 4.9. Fresh paneer had 55.24% initial moisture content, 24.32% fat, 17% protein, 1.99% lactose, 1.45% ash content, and 0.25% lactic acid. Thus, the total solids content of paneer was found to be 44.76%. The hardness of fresh paneer was 16.69 N, while WI was 88.94.

After CP treatment at the optimized condition of 5 min, hardness increased to 23.57 N, while moisture content and WI decreased to 53.43% and 88.83, respectively. The titratable acidity of paneer remained the same after CP treatment. Fresh paneer prepared for vacuum packaging had initial moisture content of 58.19%, which reduced to 56.22% after CP treatment. Meanwhile, hardness increased from 16.36 to 18.01 N and WI reduced from 82.73 to 81.91.

4.6.1 Effect of storage on moisture content of cold plasma treated paneer

In the present study, paneer cubes packaged and stored in LDPE pouches at refrigerated and frozen conditions experienced drip (moisture) loss. It was observed that untreated paneer cubes had 3.1 and 3.9% drip loss, while CP treated paneer cubes had 3.7 and 3.9% drip loss after 9 days of storage under refrigerated and frozen conditions, respectively

(Table 4.8). It is evident that the drip loss was higher in samples stored under frozen conditions. During frozen storage, large ice crystals could have been formed in paneer due to slow freezing as ample time was available for the growth of large ice crystals. When the frozen paneer was taken out for sampling, drip loss occurred due to melting of the large frozen ice crystals. However, there was no significant difference ($p>0.05$) in the drip loss between untreated and cold plasma treated paneer even though their initial moisture content at the time of storage was statistically different. In contrast, the drip loss was considerably less at 1.20 and 1.36%, respectively in untreated and CP treated paneer cubes that were vacuum packaged and stored under refrigerated condition (Table 4.9). Thus, vacuum packaging reduced the drip loss in paneer cubes considerably during storage.

4.6.2 Effect of storage on FFA content of cold plasma treated and untreated paneer

The FFA content of CP treated paneer cubes increased under atmospheric condition at a rate higher than that of untreated paneer under both refrigerated and frozen conditions. The FFA content of CP treated paneer cubes after 9 days was 3.61 and 2.01 µ.eq/g, while for untreated paneer cubes, it was 2.99 and 1.79 µ.eq/g at refrigerated and frozen conditions, respectively (Table 4.8). Similarly, the FFA content of paneer cubes packaged under ambient atmosphere and stored at refrigerated condition was found to be higher than those stored in frozen condition. In comparison, the rate of increase in FFA content in vacuum packaged paneer cubes was relatively slower than that packaged under normal atmosphere. It increased to 1.07 and 1.34 µ.eq/g from the initial value of 0.75 and 1.05 µ.eq/g for untreated and CP treated paneer cubes, respectively (Table 4.9). Similar results were presented by Korachi *et al*. (2015) when milk was treated with 9 kV AC plasma for 3 and 6 min. The authors reported that increase in FFA might be the result of dehydrogenation of stearic acid caused by oxygen radicals produced during plasma treatment, which led to increase in oleic acid. Rai (2004) also reported that FFA content of paneer increased by 33.33% during 15 days of storage.

4.6.3 Effect of storage on oxidative rancidity of cold plasma treated paneer

The TBARS content of paneer cubes was not affected by CP treatment but it increased during storage. Both CP treated and untreated paneer cubes stored at refrigerated condition did not show any change in TBARS content after 3 days of storage. The formation of TBARS as a by-product of lipid peroxidation is not an instantaneous process. Therefore, there was no change in TBARS content within 3 days of storage. However, after 9 days, the TBARS content of untreated and atmospheric packaged paneer cubes increased during storage to 0.0065 and 0.016, respectively (Table 4.8), and for CP treated paneer cubes, it increased to 0.003 and 0.009 under refrigerated and frozen conditions. In comparison, in vacuum packaged paneer cubes, the TBARS content increased to 0.005 and 0.003 for untreated and CP treated samples at refrigerated conditions after 9 days (Table 4.9). Based on TBARS content, it could be stated that the rate of increase in oxidative rancidity was much slower in vacuum packaged paneer cubes. The reduced availability of oxygen under vacuum limited the development of oxidative rancidity in paneer during storage. Similar results were reported by Kim *et al.* (2011) when pork was treated with 100 W atmospheric plasma for 1.5 min and stored. The authors observed no effect of cold plasma on TBARS content, but it was found to increase during storage. The authors also reported that the change in TBARS content was due to the reaction of ROS with food lipids. Das *et al.* (2018) observed increase in TBARS content of paneer from 0.286 to 0.775 during refrigerated storage for 9 days. Similarly, Ahmed *et al.* (2014) observed increase in TBARS content of paneer when stored for 28 days.

4.6.4 Effect of storage on hardness of cold plasma treated paneer

The hardness of paneer stored under normal atmosphere increased from 16.69 to 23.57 N after CP treatment. In comparison, the hardness of untreated paneer cubes increased to 22.64 N and 31.12 N, while CP treated paneer increased to 28.0 N and 32.33 N after refrigerated and frozen storage, respectively (Table 4.8). However, the increase in hardness of CP treated paneer stored under vacuum increased from 16.36 N to just 18.01 N. During storage of 9 days, the hardness increased to 22.73 N and 25.96 N for untreated and treated paneer cubes at refrigerated condition, respectively (Table 4.9). Similar results were reported by Sukumar (2020) for CP treated paneer cubes vacuum packaged and stored under refrigerated and frozen conditions for 15 days. The increase in hardness was mainly ascribed to moisture loss that occurred in paneer cubes during storage.

4.6.5 Effect of storage on whiteness index of cold plasma treated paneer

The WI of paneer cubes decreased after CP treatment. For atmospheric packaged paneer cubes, WI remained almost the same (Table 4.8). During storage for 9 days at refrigerated and frozen conditions, the WI of untreated paneer cubes decreased to 87.65 and 83.68, while for CP treated paneer cubes, it decreased to 86.35 and 85.36, respectively. The decrease in WI under frozen condition was higher than that of samples stored in refrigerated condition. As the drip loss was more in frozen paneer cubes, the WI index decreased as compared to refrigerated and atmospheric air packaged paneer cubes. For CP treated paneer cubes stored under vacuum, the WI reduced from 82.73 to 81.97, and during storage at refrigerated condition for 9 days the WI of untreated and treated paneer cubes reduced to 77.79 and 75.79, respectively (Table 4.9). It was observed that WI decreased at a relatively slower rate in paneer packaged under vacuum in comparison to those stored in atmospheric packaging. Similar results were reported by Shrivastava (2007) when paneer was stored for 10 days at 3±1°C under 100% CO_2, 50% N_2 and CO_2, 100% N_2, vacuum and air. The reduction in L* value was the least for 100% CO_2 followed by those in 50% N_2 and CO_2, 100% N_2, vacuum and air.

Table 4.8. Effect of atmospheric packaging on physicochemical, textural and colour attributes of cold plasma treated paneer

Days	Treatment	Storage condition	Moisture content (%)	Drip loss (%)	FFA (µ.eq/g)	TBARS	Hardness (N)	Whiteness index
0	Control	-	55.24	-	-	-	16.69	88.94
0	Plasma	-	53.43	3.28	-	-	23.57	88.83
3	Control	Refrigerated	54.56	1.20	1.44	0.002	18.63	86.65
3	Control	Frozen	54.12	2.02	1.29	0.007	22.21	86.73
3	Plasma	Refrigerated	51.53	3.40	1.79	0.002	25.50	86.62
3	Plasma	Frozen	52.09	2.50	1.46	0.004	25.76	86.09
6	Control	Refrigerated	54.48	1.37	1.98	0.004	20.15	86.19
6	Control	Frozen	53.58	3.00	1.43	0.009	26.47	81.43
6	Plasma	Refrigerated	51.39	3.80	2.26	0.005	26.82	86.57
6	Plasma	Frozen	51.82	3.01	1.83	0.027	29.27	85.61
9	Control	Refrigerated	54.09	2.10	2.99	0.007	22.64	87.65
9	Control	Frozen	53.04	3.90	1.79	0.016	31.12	83.68
9	Plasma	Refrigerated	51.42	3.90	3.61	0.003	28.00	86.35
9	Plasma	Frozen	51.41	3.7	2.01	0.009	32.33	85.36

Each observation is mean of three replications (n=3) ; FFA – Free fatty acids content

Table 4.9. Effect of vacuum packaging on physicochemical, textural and colour attributes of cold plasma treated paneer

Days	Treatment	Storage condition	Moisture content (%)	Drip loss (%)	FFA (µ.eq/g)	TBARS	Hardness (N)	Whiteness index
0	Control	-	58.19	-	0.75	0.002	16.36	82.73
0	Plasma	-	56.22	3.38	1.05	0.002	18.01	81.97
3	Control	Refrigerated	58.11	0.13	0.90	0.002	18.01	80.50
3	Plasma	Refrigerated	55.71	0.90	1.23	0.002	19.07	79.18
6	Control	Refrigerated	57.68	0.87	1.00	0.004	19.37	80.04
6	Plasma	Refrigerated	55.56	1.18	1.30	0.003	21.69	78.84
9	Control	Refrigerated	57.49	1.20	1.07	0.005	22.73	77.79
9	Plasma	Refrigerated	55.45	1.36	1.34	0.003	25.96	75.79

Each observation is mean of three replications (n=3); FFA – Free fatty acids content

4.6.6 Effect of storage on microbiological quality of cold plasma treated paneer

The TPC of untreated and treated paneer cubes increased during storage (Table 4.10). The TPC of untreated paneer cubes packaged in normal atmosphere increased to 5.07 and 4.69 log cfu/g after 9 days of storage under refrigerated and frozen conditions, whereas for CP treated paneer, the corresponding increase was 4.09 and 3.57 log cfu/g, respectively. The yeast and mould count of CP treated paneer did not show any visible growth in 0.1 g sample under frozen storage. However, in the case of untreated paneer, it increased to 2.9 log cfu/g. Under refrigerated storage for 9 days, the yeast and mould count increased to 3.2 log cfu/g and 2.7 log cfu/g for untreated and CP treated paneer cubes, respectively. For CP treated paneer cubes stored under vacuum packaging, the TPC decreased to 3.60 log cfu/g from the initial population of 4.38 log cfu/g (Table 4.11). After 9 days, TPC increased to 4.90 and 4.05 log cfu/g for untreated and CP treated paneer cubes under refrigerated condition, respectively. The yeast and mould count did not show any growth in 0.1 g sample after CP treatment, while in fresh paneer cubes, yeast and mould count of 2.30 log cfu/g was obtained. The count increased during refrigerated storage for both untreated and CP treated paneer cubes, and it was 2.77 and 2.35 log cfu/g after 9 days of storage, respectively. Thus, it could be concluded that CP treatment had destructive effect on the microorganisms of paneer. Similar results were obtained by Sukumar (2020) for CP treated paneer at 25 kV for 5 min, followed by storage under refrigerated and frozen conditions for 15 days.

Table 4.10. Effect of atmospheric packaging on TPC and yeast and mould count of cold plasma treated paneer

Storage period (days)	Treatment	Storage condition	TPC (log cfu/g)	Yeast and mould (log cfu/g)
3	Control	Refrigerated	4.30	2.40
3	Control	Frozen	4.24	1.40
3	Plasma	Refrigerated	2.54	1.00
3	Plasma	Frozen	2.17	Absent in 0.1 g
6	Control	Refrigerated	4.65	2.97
6	Control	Frozen	4.50	1.60
6	Plasma	Refrigerated	3.01	1.30
6	Plasma	Frozen	2.77	Absent in 0.1 g
9	Control	Refrigerated	5.07	3.20
9	Control	Frozen	4.69	2.90
9	Plasma	Refrigerated	4.09	1.70
9	Plasma	Frozen	3.57	Absent in 0.1 g

Each observation is mean of three replications (n=3)

Table 4.11. Effect of vacuum packaging on TPC and yeast and mould count of cold plasma treated paneer

Storage period (days)	Treatment	TPC (log cfu/g)	Yeast and mould (log cfu/g)
0	Control	4.38	2.30
0	Plasma	3.60	Absent in 0.1 g
3	Control	4.44	2.47
3	Plasma	3.77	0.69
6	Control	4.60	2.65
6	Plasma	3.95	1.07
9	Control	4.90	2.77
9	Plasma	4.05	1.35

Each observation is mean of three replications (n=3)

Summary and Conclusion

5. Summary and Conclusions

The study was focused on harnessing the combined effect of initial moisture content of paneer as hurdle and cold plasma (CP) treatment on minimizing the physicochemical, textural, colour and microbiological changes of paneer. The aim of the first part of the study was to find the optimum CP processing conditions and initial moisture content in paneer to achieve minimum moisture loss and maximum microbial destruction by CP.

The range of process conditions required for generation of plasma was selected based on preliminary trials. Accordingly, voltage of 25 kV, oil immersion vacuum pump of 450 LPM and capable of creating 700 mm Hg of vacuum were selected. The initial moisture content of paneer was kept at <55% and >55%, while the exposure time was 3, 5 and 8 min. Food-grade stainless steel (SS 316) was used as the material of fabrication of plasma chamber. Air was selected as the medium for plasma generation at vacuum of 680-700 mm of Hg. The vacuum pump could generate the required level of vacuum in the treatment chamber in less than 2 min. Taguchi L_8 Orthogonal Array Design was used for optimizing the exposure time and initial moisture content for CP treatment. Paneer cubes treated under optimized conditions were packaged under atmospheric and vacuum conditions in LDPE pouches. Evaluation of shelf-life of CP treated paneer that was stored in refrigerated and frozen conditions was conducted for the period of 9 days.

The salient findings of study are summarized below:

1. Moisture loss of paneer was observed after CP treatment, which increased with increase in exposure time. The minimum and maximum moisture loss observed were 4.57% at 3 min and 7.04% at 8 min of CP treatment in paneer cubes having >55% initial moisture content. For paneer cubes having initial moisture content of <55%, 4.90 and 6.69% moisture loss was observed after 3 and 8 min of CP treatment, respectively.

2. Paneer with initial moisture content of >55% had more microbial population than its counterpart with <55% initial moisture. However, microbial destruction was

pronounced in paneer cubes having initial moisture content above 55%. For paneer cubes with >55% initial moisture content, 1.10, 1.95 and 2.13 log reduction in TPC were achieved, while for <55% initial moisture content paneer, 0.84, 1.69 and 1.84 log reduction was observed after 3, 5 and 8 min of CP treatment, respectively. After CP treatment, no yeast and mould growth was observed in the paneer cubes, regardless of the initial moisture content.

3. Paneer cubes with initial moisture content of >55% was found to have higher value of WI as compared to paneer with moisture level of <55%. With increase in exposure time to CP treatment, the WI decreased. For paneer cubes with <55% initial moisture content, WI decreased from 84.93 to 84.09, 84.03 and 81.8 after 3, 5 and 8 min of CP treatment, respectively. The corresponding decrease in paneer with >55% moisture content was 85.48, 84.96 and 84.58. Thus, the reduction in WI was more pronounced in paneer with lower initial moisture content.

4. The initial hardness was relatively less for paneer cubes with >55% initial moisture content. However, the overall change in hardness after CP treatment was significant ($p<0.05$) at both moisture levels, but within the exposure times, the treatment was not significantly different. For paneer cubes with initial moisture content >55%, the hardness increased from 15.16 N to 16.91, 17.30 and 17.77 N, while for paneer cubes with <55% initial moisture content, the hardness increased from 17.08 N to 17.64, 17.93 and 18.47 N at 3, 5 and 8 min of CP treatment, respectively. However, the effect of CP treatment on other texture profile parameters was not significant ($p>0.05$).

5. Based on Taguchi L_8 orthogonal array design, paneer having initial moisture content >55% and CP treatment time of 5 min was selected as optimized treatment conditions. The optimization design also revealed that CP exposure time was the major factor influencing moisture loss and destruction of bacteria. However, for destruction of yeast and mould, initial moisture content was found to be the major factor rather than exposure time. After 3 min of exposure, all the yeast and mould in the product were destroyed.

6. Paneer cubes treated at the optimal CP conditions were used for shelf-life evaluation, which was done based on minimal moisture loss and maximal microbial inactivation. The paneer cubes treated at these conditions had minimal changes in physicochemical and textural properties during storage.

7. Paneer cubes used for shelf-life evaluation had 55.24% moisture content, 24.32% fat, 17% protein and 1.45% ash content. The moisture content of paneer cubes decreased to 53.43% after CP treatment, while the hardness increased from 16.69 to 23.57 N. In contrast, WI of fresh paneer cubes did not change considerably after CP treatment.

8. Cold plasma treated paneer cubes were packaged in LDPE pouches under normal atmosphere and stored at refrigerated and frozen conditions for 9 days. One set of CP treated cubes were vacuum packaged and stored under refrigerated conditions. During this period, various physicochemical, textural, colour and microbial changes of paneer were evaluated and compared. During storage, the moisture content of untreated and treated paneer cubes had moisture loss as dripping occurred. The minimum and maximum drip losses observed were 2.1% for untreated paneer cubes and 3.9% for CP treated paneer cubes in refrigerated conditions, respectively.

9. The FFA content of paneer cubes increased during storage. The rate of increase in FFA content of CP treated paneer cubes was relatively higher at 3.61 and 2.01 µ.eq/g as compared to 2.99 and 1.79 µ.eq/g in untreated paneer cubes under refrigerated and frozen conditions, respectively.

10. The oxidative rancidity (TBARS content) did not show significant change till 3 days of storage. Later, it increased at a slower rate but the rate of increase was higher for CP treated paneer cubes. It increased from 0.002 to 0.0065 and 0.016 under refrigerated and frozen conditions, respectively after 9 days of storage.

11. The hardness of CP treated as well as untreated paneer cubes increased during storage. After 9 days, the hardness of CP treated paneer cubes increased to 28 and 32.33 N at refrigerated and frozen conditions, respectively. Thus, the hardness increased at a higher rate in CP treated paneer cubes. Whereas for untreated paneer

cubes, hardness increased to 22.64 and 31.12 N under refrigerated and frozen conditions, respectively.

12. The WI decreased during storage. It decreased in frozen untreated paneer cubes, to 83.68 after 9 days. Comparatively, in CP treated paneer cubes, WI decreased to 85.36 and 86.35 after 9 days of storage under frozen and refrigerated conditions, respectively.

13. TPC increased during storage in both CP treated and untreated paneer cubes. In frozen condition, the yeast and mould count was absent in 0.1 g sample of CP treated paneer even after 9 days of storage. Contrastingly, in untreated paneer, it increased to 2.9 log cfu/g. Similarly, under refrigerated condition, the yeast and mould count increased at higher rate for untreated paneer cubes (3.2 log cfu/g) while for CP treated paneer cubes, it increased to only 2.7 log cfu/g. During storage for 9 days, the TPC of untreated paneer cubes increased from 3.38 log cfu/g to 5.07 and 4.69 log cfu/g under refrigerated and frozen conditions, respectively. The corresponding increase for CP treated paneer cubes was only 4.09 and 3.57 log cfu/g.

14. During storage, vacuum packaged paneer cubes stored under refrigerated condition had less drip loss as compared to those stored under atmospheric pressure. After 9 days of storage, 1.20 and 1.36% moisture loss occurred in untreated and CP treated paneer cubes, respectively.

15. The FFA content of vacuum packaged paneer cubes stored for 9 days increased to 1.07 and 1.34 μ.eq/g from the initial value of 0.75 and 1.05 μ.eq/g in untreated and CP treated paneer cubes, respectively. The rate of increase of FFA content for vacuum packaged paneer cubes was significantly lower than that of atmospheric pressure packaged paneer.

16. The oxidative rancidity (TBARS content) of vacuum packaged paneer cubes did not show significant change after 3 days of storage even though there were indications of slower rate of increase in both CP treated and untreated paneer cubes. However, after 9 days, it increased to 0.005 and 0.003 for untreated and CP treated paneer cubes, respectively.

17. The hardness of CP treated and untreated vacuum packaged paneer cubes also increased during refrigerated storage. After 9 days, hardness of untreated and CP treated paneer cubes increased to 22.73 and 25.96 N from the initial value of 16.36 and 18.01 N, respectively. Thus, the rate of increase in hardness of both samples was quite similar.

18. The WI of vacuum packaged paneer cubes decreased during refrigerated storage After 9 days, it reduced to 77.79 and 75.79 from the initial value of 82.73 and 81.97 for untreated and CP treated paneer cubes, respectively.

19. As expected, the TPC and yeast and mould count of both CP treated and untreated vacuum packaged paneer increased during refrigerated storage. However, the rate of increase in vacuum packaged paneer was lower than those stored under atmospheric pressure. The TPC of vacuum packaged untreated and CP treated paneer cubes increased to 4.90 and 4.05 log cfu/g from the initial value of 4.38 and 3.6 log cfu/g, respectively after 9 days. Similarly, the yeast and mould count of untreated paneer increased from 2.3 log cfu/g to 2.77 log cfu/g. The yeast and mould count of CP treated paneer cubes increased to 1.35 log cfu/g. In contrast, no growth of yeast and mould was observed after CP treatment in fresh paneer cubes.

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