

SHELF-LIFE EXTENSION OF PANEER USING SUPERCRITICAL FLUID
TECHNOLOGY

A Project Paper

Presented to the Faculty of the Graduate School
of Cornell University

in Partial Fulfillment of the Requirements for the Degree of
Master of Professional Studies in Agriculture and Life Sciences
Field of Food Science

by

Ragya Kapoor

August 2019

© 2019 Ragya Kapoor

ABSTRACT

The shelf-life of Paneer is limited to 1 to 3 days at room temperature and around 6-8 days at refrigeration temperature. This study was conducted to investigate the use of supercritical carbon dioxide (SC-CO₂) with added acetic acid (AA) for the shelf-life extension of Paneer at refrigerated as well as room temperatures. Freshly prepared Paneer was subjected to different pressure, temperature and treatment time to investigate their effects on the pH and moisture content of the final product. Efficacy of the process was established by textural, viscoelastic, and microbiological analyses.

The textural and viscoelastic properties of treated samples were within the acceptable range of literature reported values for traditional Paneer. The microbiological stability of treated Paneer stored at refrigerated and room temperature was evaluated over a period of 30 days. The results showed that the microbial count of all the samples under study were within the maximum limit prescribed by Food Safety and Standards Authority of India (FSSAI), indicating a shelf-life of up to 30 days during storage at both refrigerated and room temperatures. Although the microbial evaluation was terminated at 30 days of storage, the samples stored under refrigerated temperature showed consistently lower counts for the same storage time, indicating the possibility of attaining even a longer shelf-life at lower temperatures.

Overall, the findings show that treatment with SC-CO₂ containing acetic acid can be used as an effective processing technique to prolong the shelf-life of Paneer for up to 30 days at refrigerated as well as room temperatures, without compromising with the quality.

BIOGRAPHICAL SKETCH

Ragya Kapoor was born in Jammu and Kashmir, India. She received a Bachelor of Science (Honors) degree in Food Technology from University of Delhi, India in 2017. In August 2018, she entered the graduate program at Cornell University as an MPS student in the Department of Food Science.

ACKNOWLEDGMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Syed Rizvi, Professor Food Engineering, Department of Food Science, Cornell University, Ithaca, New York for the continuous support, motivation and immense knowledge. His guidance helped me through the process of researching and writing this project paper. I would also like to thank my fellow lab mates for their participation and insightful inputs.

Last but not the least, I would like to thank my family: my parents and brother, for always supporting and believing in me. This accomplishment would not have been possible without them.

TABLE OF CONTENTS

Biographical Sketch.....	iv
Acknowledgements.....	v
Chapter 1 Introduction.....	1
Chapter 2 Materials and Methods	
2.1 Preparation of Paneer.....	4
2.2 pH Measurement.....	4
2.3 Supercritical CO ₂ Treatment.....	4
2.4 Viscoelastic Analysis.....	6
2.5 Texture Profile Analysis.....	7
2.6 Microbiological Analysis.....	7
2.7 Statistical Analysis.....	7
Chapter 3 Results and Discussions	
3.1 Effect of SC-CO ₂ pressure and temperature on the final pH.....	8
3.2 Influence of acetic acid flow rate on the pH.....	9
3.3 Effect of SC-CO ₂ pressure, temperature and acetic acid on the final pH.....	11
3.4 Effect of pressure and temperature on the moisture content.....	12
3.5 Texture analysis.....	14
3.6 Viscoelastic Analysis.....	16
3.7 Microbiological Analysis.....	18
Chapter 4 Conclusion.....	21
References.....	22

CHAPTER 1

INTRODUCTION

According to a report published by FAO, India is the leading milk producing country in the world, accounting for almost 21% of the global market share. Apart from fluid milk, the revenue of the Indian dairy and milk processing sector is generated from several products like Paneer, butter, ghee, skim milk powder, etc. Paneer is a variety of soft cheese which is obtained by heat and acid coagulation of milk and is used widely for preparation of a variety of culinary dishes in India. It is estimated that out of 176.3 Million Metric Tons of milk produced annually in India, around 7% (12.31 MMT) is converted to Paneer (Edelweiss, 2017). As a rich source of animal protein for vegetarians with the biological value of protein in the range of 80 to 86 (Khan and Pal, 2011), consumption of dairy products such as Paneer is experiencing annual growth rates of around 15-20 percent (Zimmerman, 2017). Increase in the consumption of Paneer has also been accelerated by rising disposable income, urbanization, dual income households and other demographic shifts (Zimmerman, 2017). However, short shelf-life is a major problem faced by the Paneer processing industry in India. Paneer has a high moisture content (53-55%) and pH in the range of 5.4-5.9, which limits its shelf-life to only a day or two at ambient temperature and 6-8 days at refrigerated temperature (Khan and Pal, 2011). As a consequence of growing consumer demand, various preservation techniques like addition of chemical preservatives (Thakral et al., 1990), modified atmospheric packaging (Thippeswamy et al., 2011), and low-temperature storage have been proposed by different researchers for enhancing its shelf-life. However, the use of chemical/antimicrobial additives is not preferred as consumers nowadays are demanding clean label foods, free from chemical preservatives because of their possible adverse health effects. Modified

atmosphere packaging (MAP) may be used to enhance the shelf-life but it requires an extra temperature control system which may result in growth of anaerobic bacteria and production of microbial toxins if temperature is not properly controlled. Lack of cold-chain facilities and inefficient distribution system have also led to poor utilization of Paneer and resulted in wastage of the product (Sunil Kumar et al., 2014).

Sunil Kumar et al., 2014 , have reported that spoilage of Paneer is mainly associated with the growth of microorganisms. As is well known, the pH and the water activity of a food are two important factors that determine the survival and growth of microorganisms during processing, storage and distribution. Consequently, in this study the pH and water activity of Paneer was modified to a level that helps control the microbial growth and thus prevent product quality loss and spoilage. In order to achieve this, an innovative processing technology involving the use of SC-CO₂ in combination with acetic acid was used. It was hypothesized that this technique would result in enhanced storage stability of Paneer at refrigerated as well as room temperatures, without compromising the quality.

There have been numerous studies investigating the use of SC-CO₂ as an effective non-thermal decontamination technique for foods. Recent published data demonstrated that this technology can effectively inactivate microorganisms in culture media (Hong and Pyun, 1999, Erkmen, 2000), liquid (Ramírez-Rodrigues et al., 2013) , fresh cut fruits (Valverde et al., 2010, Ferrentino et al., 2012) , vegetables (Bi et al., 2011) and meats (Choi et al., 2009). The application of SC-CO₂ is considered to be favorable in the food industry because it is physiologically safe, inexpensive, and easily available in high purity and large quantities (Raventós et al., 2002). Moreover, the physiochemical properties of supercritical fluids such as adjustable densities, low viscosities, high diffusivities and low interfacial surface tension facilitates its penetration into various solid and

liquid matrices (Sikin et al., 2016, Amaral et al., 2017) However, unlike liquid foods, application of SC-CO₂ in solid foods suffers from a few limitations due to the complexity of the matrix which can limit the diffusion of CO₂ into solid matrices (Balaban and Duong, 2014). In this study, acetic acid, which is an organic acid found in natural food sources and classified as “generally regarded as safe (GRAS)” by FDA (21 CFR 184.1005) was used as a co-solvent along with SC-CO₂. The antimicrobial activity of acetic acid due to reduction in the pH of Paneer matrix posed as another hurdle to microbial growth and thus may result in better storage stability of the product. In this combined treatment, highly diffusive SC-CO₂ should serve as carrier for uniform and rapid penetration of acetic acid into the Paneer matrix. As acetic acid is a hydrophilic (polar) protic solvent, using it is believed to enhance the solubility of hydrophilic substrates through the formation hydrogen bonds (McGill, 2007). Therefore, along with acidification, another major effect of addition of acetic acid is increased solubility of water in SCF phase which will allow for enhanced moisture removal rate and lowering of water activity.

With regard to consumer acceptability, it is worth mentioning that Paneer is generally fried in a light batter before consumption and as acetic acid is volatile, it can easily be evaporated from the matrix (Lan and HongZhang, 2011; Mariano et al., 2012).

Thus, the objective of the present study was to investigate the combined effect of SC-CO₂ and acetic acid on the shelf-life and quality characteristics of fresh Paneer stored at refrigerated as well as room temperatures over a period of 30 days. Microbiological, textural and rheological properties of treated Paneer samples were evaluated to establish product quality following treatment with SC-CO₂ and acetic acid.

CHAPTER 2

MATERIALS AND METHODS

Homogenized whole milk (4.0% fat, 8.5% SNF) produced by Ithaca Milk Company was obtained from Wegman's Supermarket. Food grade citric acid (99% purity) was used as a coagulant to make fresh Paneer samples. 4% solution of acetic acid was prepared in distilled water and was used as co-solvent.

2.1 Preparation of Paneer

Fresh samples of Paneer were prepared using method suggested by (Radhakrishnan, 2017). Whole milk was heated to 82°C for about 5 minutes and then cooled to 70°C. Citric acid (2%) was added with continuous slow agitation until whey separation occurred. The curd was allowed to settle for 5 minutes after coagulation. The whey was then drained out using a muslin cloth and the coagulated curd was collected and then pressed manually by applying a pressure of 1 kg per cm² for approximately 20 minutes followed by immersion in cold water (4-6°C) for 1 hour. The pressed coagulum was then cut into cubes of approximate size 1.5 cm x 1.5 cm x 1.5 cm. The cut samples were allowed to dry for about 30 minutes before any treatment.

2.2 pH Measurement

The initial pH of freshly prepared Paneer samples was measured at 20°C using a Fisher Scientific Accumet Excel XL20 pH meter (Fisher Scientific, Pittsburgh, PA), calibrated at the measurement temperature before use and found to be around 5.7.

2.3 Supercritical CO₂ Treatment

SC-CO₂ treatment of Paneer was carried out in an SFT-250 Processing System (Supercritical Fluid Technologies, Newark, DE, USA), shown in Figure 1. For each experiment, around 5-6 g

of Paneer cube was loaded into the 100 mL stainless steel extraction vessel. Effect of different pressure (150, 200, 250 Bar) and temperature (50°C, 60°C) on the moisture removal and change in pH were monitored and optimum conditions were determined based on the final pH and physical properties of the Paneer samples. For each experiment, the sample was subjected to 20 minutes of phase equilibration followed by 15 minutes of SC-CO₂ drying. After SC-CO₂ drying, an external pump attached to the system was used to inject the acetic acid into SC-CO₂ at 2 mL/minute for 20 minutes. During processing, the flowrate of SC-CO₂ was controlled by the metering valve. Different flow rates for the co-solvent were evaluated for their effectiveness on pH control. The acetic acid was delivered by the pump from a graduated 25 ml burette and was monitored to quantify the amount added. At the end of the experiment the acetic acid flow was stopped, and the product was treated with only supercritical SC-CO₂ for 10 minutes to remove any free acetic acid from the samples in the vessel. After treating the samples at the set conditions for a total of 65 minutes, they were vacuum packaged and stored at room (25 ± 1 °C) and refrigerated (4 ± 1 °C) temperatures for quality evaluation.

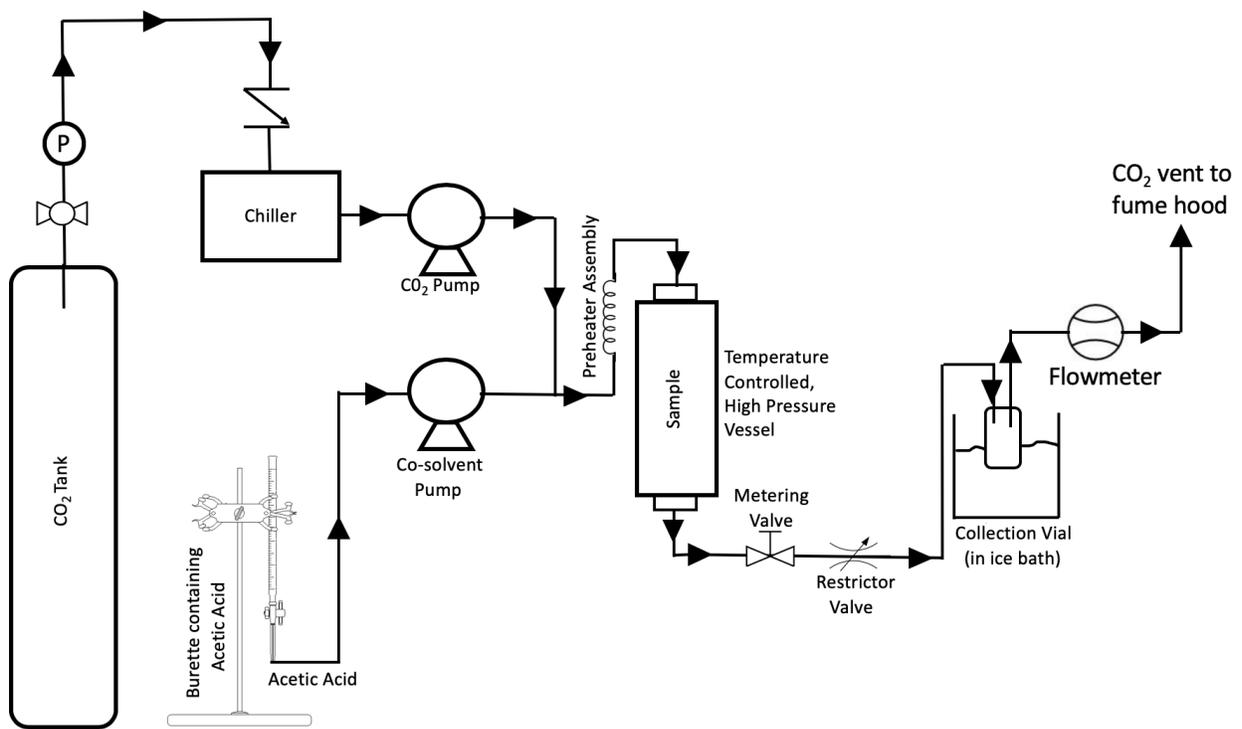


Figure 1: Process schematic for supercritical carbon dioxide treatment of Paneer with acetic acid as co-solvent.

2.4 Viscoelastic Analysis

The rheological characteristics of the control and experimental samples were evaluated using an AR N 1000 constant stress rheometer (TA Instruments, Leatherhead, Surrey, UK). Dynamic rheological measurements (frequency sweep) were used to determine the elastic or storage module (G') and viscous or loss module (G''). Paneer samples were equilibrated to room temperature (25 ± 1 °C) before analysis. A small piece of Paneer (around 1 mm thick), was placed on the lower plate and then the upper plate was slowly moved down until the pre-set gap size was reached. The frequency was oscillated from 0.1 to 100 Hz and the strain value was set at 100 Pa to be in the predetermined linear range.

2.5 Texture Profile Analysis

Texture profile analysis of control and treated samples was carried out using TA.XT Plus 2 texture analyzer fitted with 25 mm Perspex probe. Control and treated samples of size 1.5 cm x 1.5 cm x 1.5 cm were placed centrally beneath the probe. A crosshead speed of 10 mm/s with a trigger force of 10 gf was used to compress the sample to 70% of their original size. Each sample was compressed twice to give a two-bite texture profile curve. The force-time plots obtained were analyzed for hardness, springiness, cohesiveness, gumminess and chewiness, based on the method described by Bourne, M. C., 1978 and Dwarakanath et al., 2013.

2.6 Microbiological Analysis

Microbiological analysis of Paneer samples was carried out by plating before and after the treatments on day one and then on day 5, 10, 20 and 30 of storage at room (25 ± 1 °C) and refrigerated (4 ± 1 °C) temperatures. For total plate count, 5 grams of tryptone, 2.5 grams of yeast extract, 1 gram of dextrose and 15 grams of Agar were mixed in 1 liter of Milli- Q water and then autoclaved at 121°C cycle. The media was then aseptically dispensed into sterile petri plates and plates were stored in a refrigerator at 4-7°C until used.

For potato dextrose agar media, 39 grams of commercial potato dextrose agar was suspended in 1 liter of Milli-Q water and then autoclaved at 121°C cycle. The media was then aseptically dispensed into sterile petri plates and plates were stored in a refrigerator at 4-7°C until used. Phosphate buffer saline (PBS) solution was prepared and autoclaved to be used as a diluent. The stored samples were then placed in stomacher bags along with PBS. These samples were agitated and blended in order to get a homogenous mixture before plating.

2.7 Statistical Analysis

Experiments were carried out in triplicate and the data were presented as a mean \pm standard error for different samples. Microsoft Excel was used to plot graphs.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Effect of SC-CO₂ pressure and temperature on the final pH

The effects of SC-CO₂ treatment under various conditions on the reduction of pH in Paneer are shown in Figure 3.

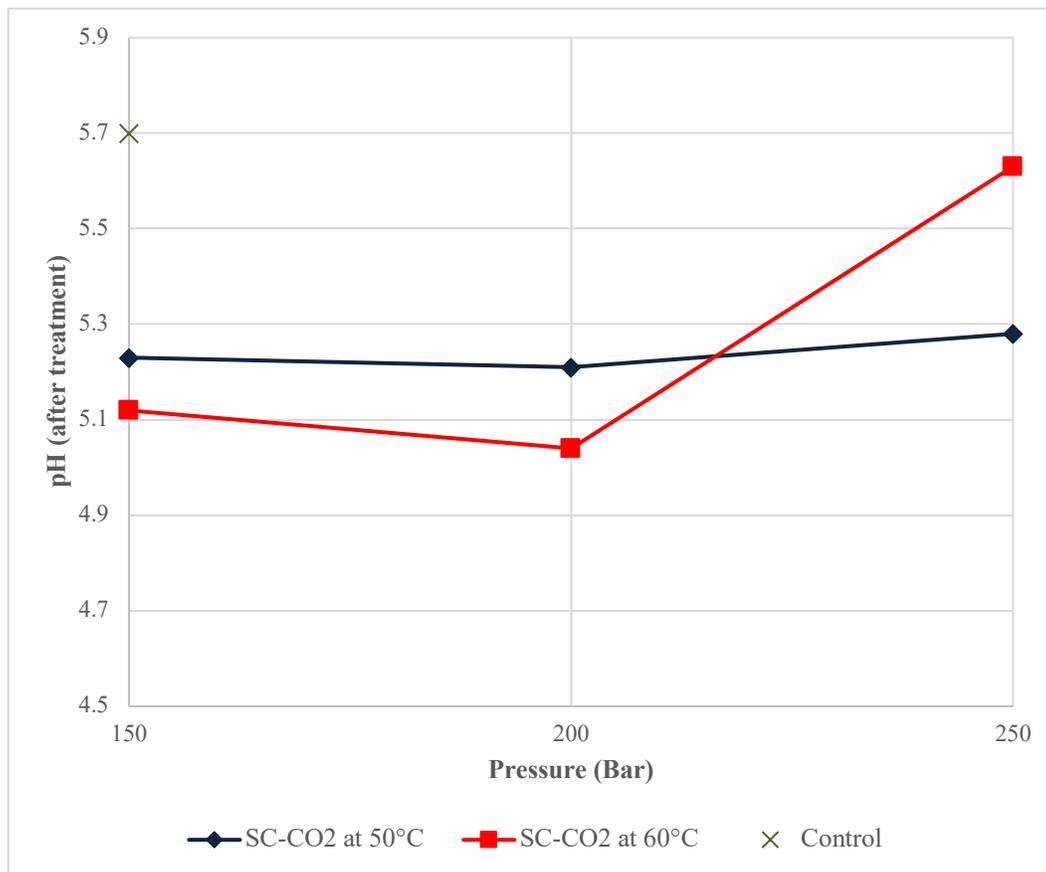


Figure 3: Effect of SC-CO₂ pressure and temperature treatment on the pH of Paneer after 65 minutes at flow rate of 3L/minute.

The initial pH of freshly made Paneer samples was 5.7. As observed from Figure 3, application of SC-CO₂ led to a decrease in the pH. It has been demonstrated that treatment with high pressure

SC-CO₂ is accompanied by a lowering of pH because of the formation of carbonic acid as carbon dioxide solubilizes in the aqueous phase of the food matrix (Wolfe, 1980). It can also be observed that at constant temperature, the pH of Paneer samples decreased with an increase in pressure up to 200 bar. This is because a higher pressure enhances the CO₂ solubility in water phase of the sample, which facilitates further acidification. However, Paneer structure was observed to disintegrate at 250 bar and the matrix was not uniformly exposed to SC-CO₂, resulting in lower pH drop. Analysis of data in Figure 3 indicates that the Paneer samples, when treated with SC-CO₂ alone, exhibited pH value as low as 5.04 at 200 Bar and 60°C.

However, SC-CO₂ alone is not enough to lower the pH below 5.04. Therefore, it is combined with acetic acid to facilitate further acidification so as to achieve better microbial inactivation under milder treatment conditions.

3.2 Influence of acetic acid flow rate on the pH

Figure 4 shows the effect of different flow rates of acetic acid on pH drop in Paneer, which increased with higher rate of flow of acetic acid.

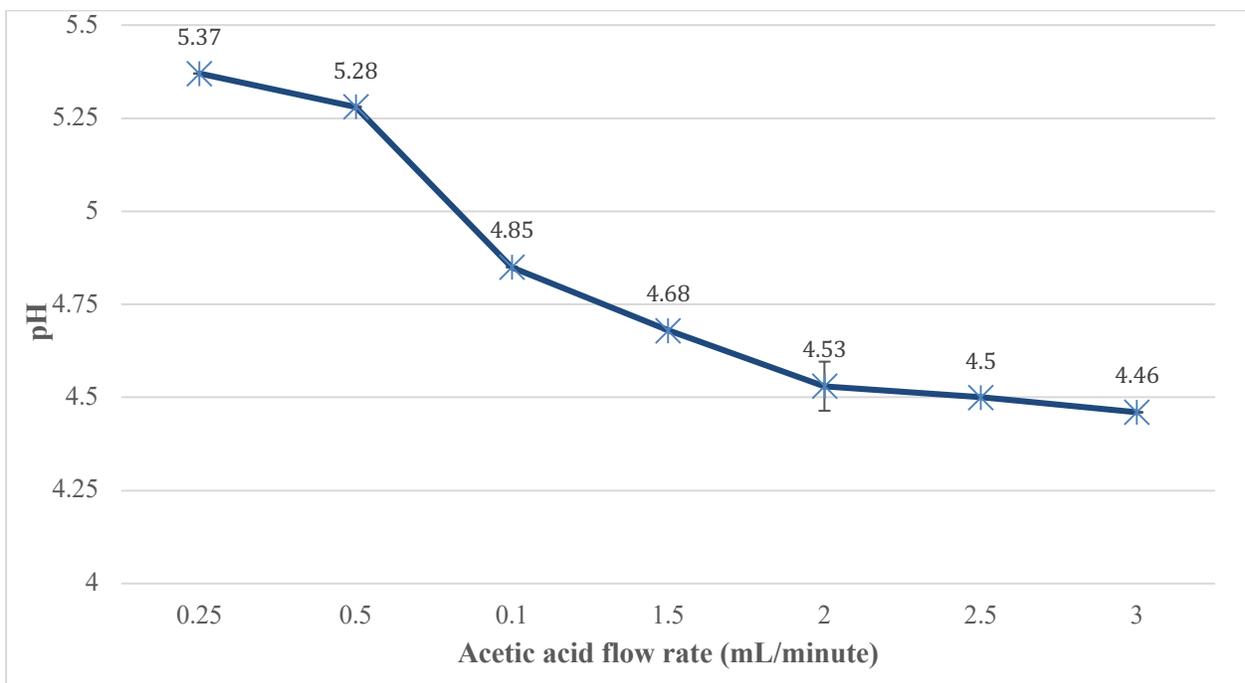


Figure 4: Effect of acetic acid (4% solution) flow rate on the pH of Paneer treated with SC-CO₂ at 200 bar; 50°C for 65 minutes and CO₂ flow rate of 3L/minute.

A high-acid food is defined as a food with a pH value of 4.6 or lower (McGlynn, 1999) and most micro-organisms are known not to grow at pH levels below 4.6. In order to lower the pH below 4.6 so as to limit microbial growth, acetic acid was injected at a flow rate of 2 ml/minute for 20 minutes which implies that about 40 mL of 4% acetic acid solution was required for a sample size of 5 gram. As mentioned earlier, this was done after 20 minutes of phase equilibration followed by 15 minutes of SC-CO₂ drying.

At the end of the experiment the acetic acid flow was stopped, and the product was treated with only supercritical CO₂ for another 10 minutes to remove any free acetic acid from the samples in the vessel.

3.3 Effect of SC-CO₂ pressure, temperature and acetic acid on the final pH

The effects of treatment of Paneer samples with SC-CO₂ with added acetic acid at three pressures and two temperatures on pH are shown in Figure 5.

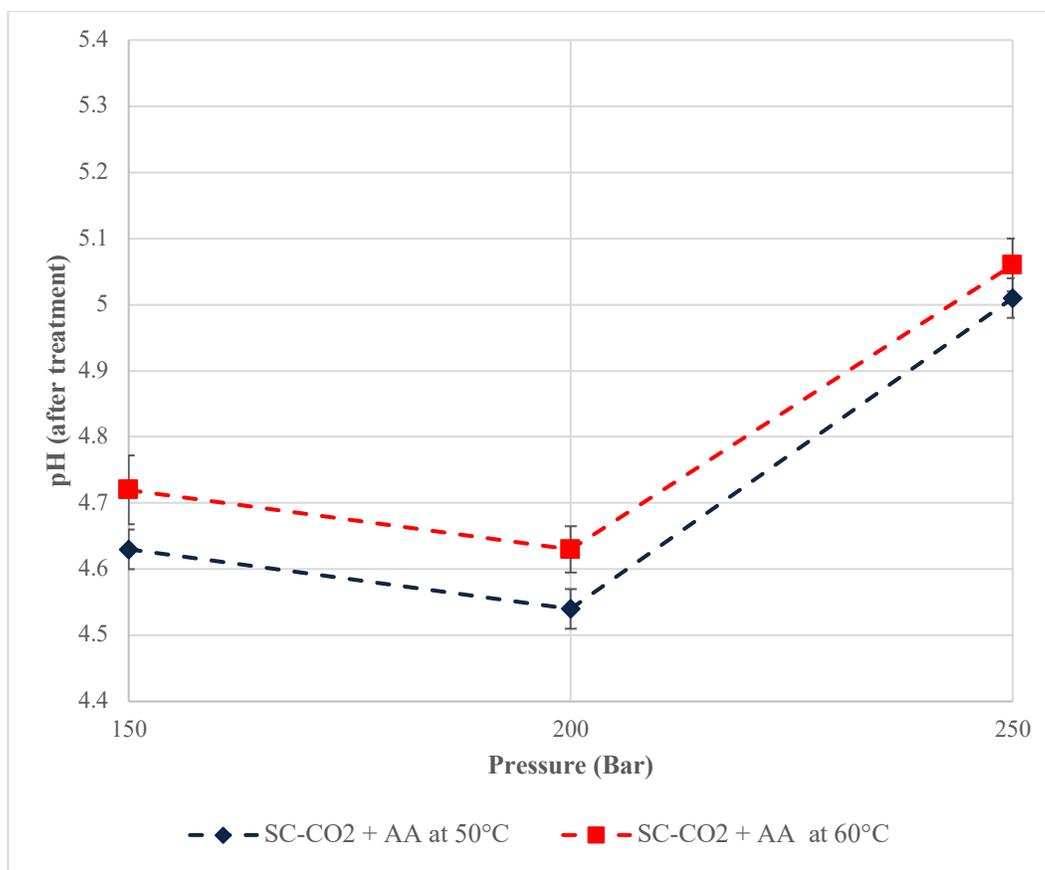


Figure 5 : Effect of SC-CO₂ pressure and temperature treatment with acetic acid on the pH of Paneer after 65 minutes at CO₂ flow rate of 3L/minute and acetic acid flow rate of 2mL/minute.

As shown in Figure 3, when SC-CO₂ alone was applied to the samples at 200 bar and 50°C for 65 minutes, a pH drop equivalent to 0.49 units was achieved. However, the pH drop was more in case of samples treated with 4% acetic acid solution. and SC-CO₂, indicating the effectiveness of this organic acid as a co-solvent. Analysis of data in Figure 5 indicates that the application of SC-CO₂ at 200 bar and 50°C in combination with acetic acid, resulted in maximum pH drop of 1.16 units with final product pH of 4.54. As discussed earlier, pH of Paneer samples decreased with an increase in the pressure up to 200 bar. However, limited exposure of Paneer to acetic acid loaded

SC-CO₂ due to disintegration of structure at 250 bar resulted in lower pH drop at this pressure. It can also be observed that at any constant pressure, the pH drop of the samples was observed to increase with a decrease in the temperature from 60°C to 50°C, in part due to higher solubility of CO₂ in the aqueous phase of Paneer.

Based on these results, the combined treatment of Paneer with SC-CO₂ and 4% solution of acetic acid in water was more effective in reducing the pH than treatment with SC-CO₂ alone. As the desired pH of below 4.6 was achieved at 200 bar, 50°C and SC-CO₂ flow rate of 3L/minute and acetic acid flow rate of 2mL/minute, these conditions were selected for further studies.

3.4 Effect of pressure and temperature on the moisture content

The initial moisture content of freshly made Paneer samples was 54.5%. The effect of SC-CO₂ pressure and temperature treatment of Paneer with and without acetic acid on moisture content is shown in Figure 6. It can be observed that at a constant temperature, the amount of moisture extracted increased as the pressure increases which is in accordance with the results reported by others (King, Mubarak, & Kim, 2005). This is stated to be due to the increase in SC-CO₂ density with pressure and thus its solvating power.

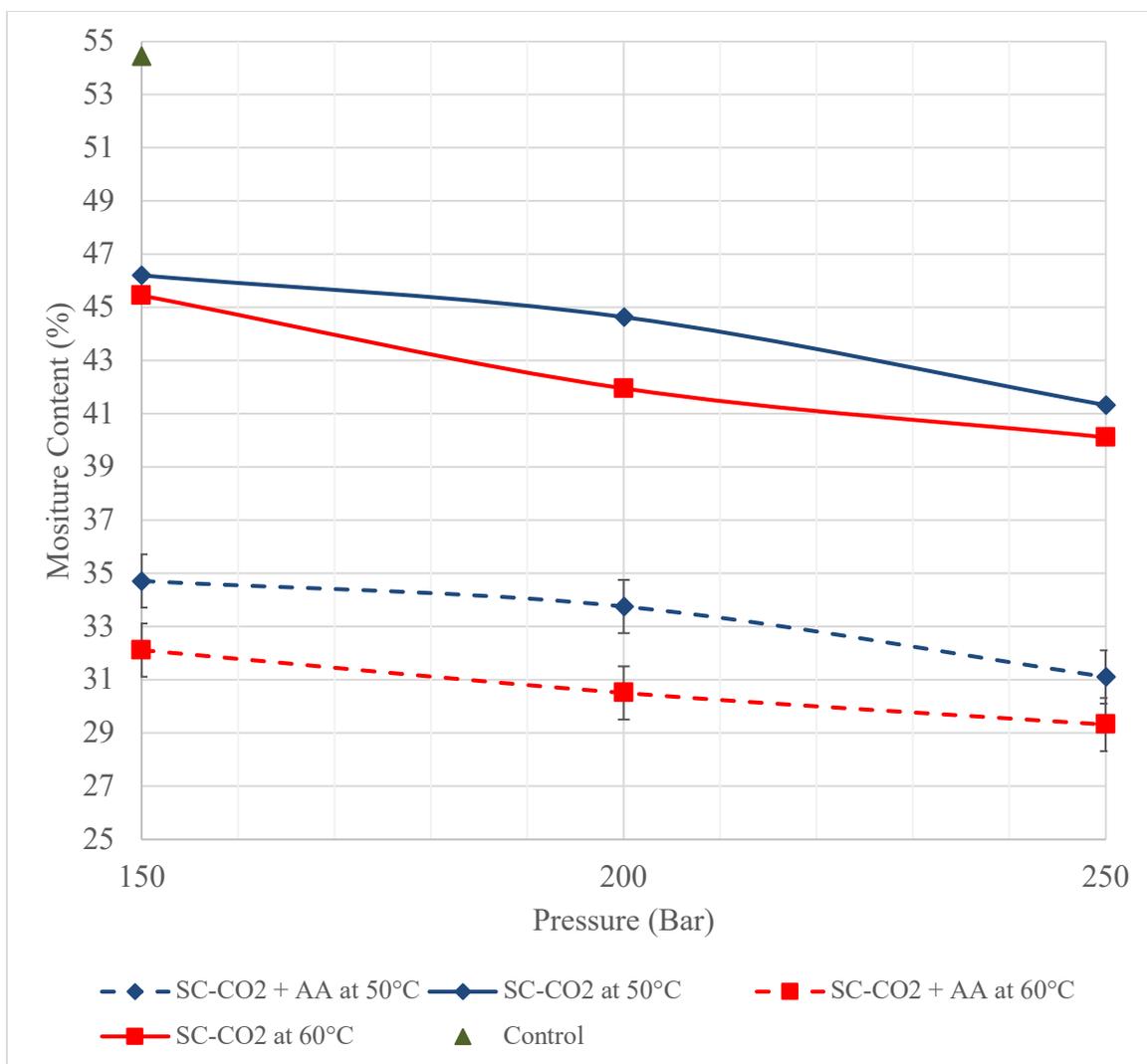


Figure 6: Effect of SC-CO₂ pressure and temperature on the moisture content of Paneer treated with and without acetic acid for 65 minutes at CO₂ flow rate of 3L/minute and acetic acid flow rate of 2mL/minute.

As discussed earlier, addition of polar co-solvent like acetic acid can favorably influence the solubility of polar substance like water in SC-CO₂. Ingrosso and Ruiz-López, 2017, suggested that this could be due to various chemical and physical interactions between co-solvent and solute,

including hydrogen bonding and dipole-dipole attractions. Therefore, as shown in Figure 6, the extent of moisture removal over 65-minute period increased with the addition of acetic acid. However, it can also be observed that the sample experienced considerable moisture loss when treated with SC-CO₂ alone, indicating the effectiveness of SC-CO₂ as a drying agent. As discussed earlier, the carbon dioxide in supercritical state has properties of the vapor as well as the liquid. Hence, SC-CO₂ penetrates the cellular structure of the sample and displaces the water outside and forcing the water molecules to be carried out in solubilized state (Brown et al., 2008). As expected, higher moisture removal was achieved at the higher temperature. However, the SC-CO₂ pressure has a greater influence on solubility when compared to the temperature (Vigano et al., 2015) due to the higher density effects.

The final moisture content and water activity of Paneer was 33.8% and 0.91, respectively when the samples were treated at 200 bar and 50°C and SC-CO₂ flow rate of 3L/minute and acetic acid flow rate of 2 ml/ minute. Intermediate moisture foods are characterized by a moisture content of around 15-50% and water activity between 0.62 and 0.92 (FAO, 1999). Therefore, from the above, it can be said that the treatment of Paneer with SC-CO₂ and acetic acid at 200 bar and 50°C at CO₂ flow rate of 3L/minute and acetic acid flow rate of 2 ml/minute resulted in Paneer becoming an intermediate moisture and a low pH food with improved microbiological safety.

3.5 Texture analysis

The textural properties of control and treated samples are presented in Table 1. Comparison among the control and treated samples suggests that SC-CO₂ treated samples showed a higher value for hardness, chewiness and gumminess. Control samples tended to be higher in springiness and cohesiveness. Desai et al. (1991) have previously reported that the hardness is inversely related to the moisture content of Paneer. Therefore, it is reasonable to believe that the increase in hardness

in case of treated samples is due to the loss of moisture during processing. Removal of fat also alters the protein matrix resulting in compact and dense appearance and as SC-CO₂ is a non-polar solvent, hardness could also be attributed to loss of some fat.

Springiness is defined as the and extent to which a deformed material goes back to its undeformed condition after the deforming force is removed. As seen from Table 1, the springiness value of the treated sample was less as compared to the control. It can also be observed that the treated sample were less cohesive than the control which means they seems to crumble and break more easily as compared to the control. This is in accordance with previously reported results which show that less moisture content is associated with lower springiness and cohesiveness of soft cheeses (Yuanrong et al., 2016; E. A. Foegeding et al., 2003 & M. D. Yates et al., 2007)

The values of textural parameters obtained following SC-CO₂ treatment of Paneer were comparable to those published by Kanawjia and Singh, 1996 who reported values of 13.2 N, 71 N-mm, 9.8 N, 7.8 mm and 0.68, respectively, for hardness, chewiness, gumminess, springiness and cohesiveness, However, these values are very subjective due to the empirical nature of the TPA tests and provide only a very rough and relative comparison.

Table 1: Comparison of textural attributes of control and SC-CO₂ treated Paneer (200 bar, 50°C for 65 minutes at CO₂ flow rate- 3L/minute and acetic acid flow rate- 2mL/minute)

Parameters	Control	SC-CO₂ +AA Treated
Hardness (N)	10.11	21.34
Chewiness (N.mm)	94.69	135.95
Gumminess (N)	11.49	24.19
Springiness	8.24	5.62

Cohesiveness	1.53	1.15
--------------	------	------

3.6 Viscoelastic Analysis

To understand the flow behavior on a more fundamental level, frequency sweep test was performed to determine the viscoelastic behavior of Paneer. Changes in the flow properties of Paneer, before and after treatment based on its storage modulus and loss modulus are shown in Figures 7 and 8, respectively. As G' is greater than G'' at all frequencies, it can be said that elastic component plays a dominant role, reflecting the typical behavior for a solid viscoelastic material like Paneer (Madadlou et al., 2005).

The value of storage modulus (G') was higher for SC-CO₂ treated sample as compared to the control. This is likely due to the decrease in moisture, which is mainly responsible for the viscous properties of Paneer. It has been suggested that pH has a major role on the viscoelastic properties of soft cheeses (Tunick, 2000) and that soft cheeses with a lower pH show higher G' and G'' values (Karoui and Dufour, 2003), which is consistent with the data presented in Figures 7 and 8, respectively. (Vassal et al., 1986) also reported that pH plays a significant role in the rheological characteristics of soft cheeses due to the migration of calcium ions due to the solubilization of colloidal calcium phosphate (CCP), which may be responsible for altering the viscous and elastic components in soft cheeses.

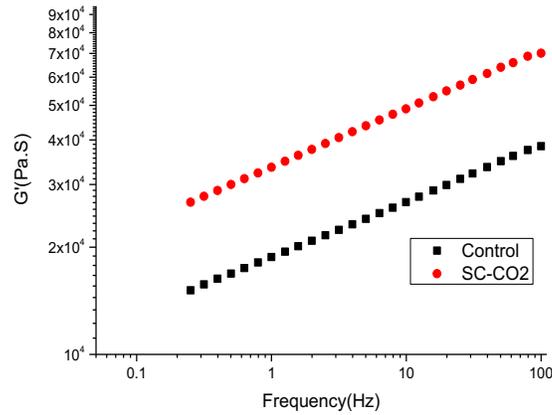


Figure 7: Variation of storage modulus (G') with oscillation frequency for control and SC-CO₂ treated samples of Paneer (following treatment with SC-CO₂ at 3L/min and acetic acid at 2 mL/min at 200 bar and 50°C for 65 minutes)

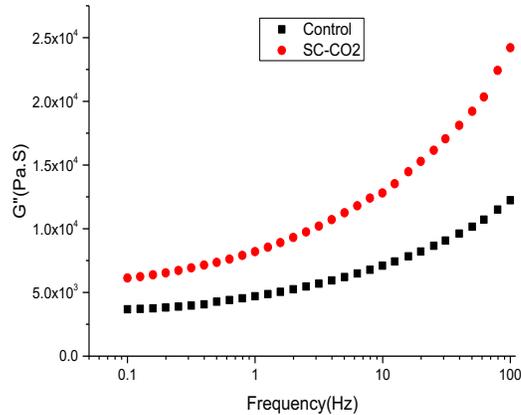


Figure 8: Variation of Loss loss modulus (G'') with oscillation frequency for control and SC-CO₂ treated samples of Paneer (following treatment with SC-CO₂ at 3L/min and acetic acid at 2 mL/min at 200 bar and 50°C for 65 minutes)

3.7 Microbiological Analysis

The treatment of Paneer with SC-CO₂ and acetic acid showed a decrease in total plate count (TPC) and total yeast and mold count (TYMC) as shown in Figures 9 and 10, respectively. There have been various studies done to determine the mechanism of microbial inactivation by SC-CO₂. (“Fraser, D. (1951). bursting bacteria by release of gas pressure. Nature, 33-34.,” n.d.)Fraser (1951) suggested the physical disruption of cells due to rapid pressure release and expansion of CO₂ could be the reason of microbial inactivation. Other researchers (Kamihira, Taniguchi & Kobayashi, 2014) have suggested that the extraction of the cytoplasmic material, such as the phospholipids, may be partly responsible for the lethality of SC-CO₂. Still others (Lin, Yang, & Chen, 1992) have hypothesized that once SC-CO₂ has penetrated into cells, it may extract out the cellular vital components of the cells and the membranes that could result in the inactivation of microorganisms.

The TPC and TYMC of freshly made Paneer was found to be 5.06 log₁₀ cfu/g 1.3 log₁₀ cfu/g respectively. Control samples stored at room as well as refrigerated temperature were observed to spoil within 5 days because of increased microbial activity. In comparison to that, the samples treated with SC-CO₂ and acetic acid showed less microbial activity. As shown in Figures 9 and 10, the application of supercritical CO₂ does lead to more inactivation due to the possible extraction of the cytoplasmic material from the inside of the microorganism. (Lin, Yang, & Chen, 1992). As shown earlier, the pH and water activity of the samples treated with SC-CO₂ and acetic acid have been lowered which decreases the chances for the growth of most microorganisms. From Figures 8 and 9, it may also be seen that the samples which are kept under the refrigerated showed consistently lower growth of the micro-organisms for the same storage time. By day 30 of storage,

TPC of treated Paneer samples stored at refrigerated as well as room temperature were still below $5.17 \log_{10}$ CFU/g, which is the maximal recommended limit for TPC in Paneer (FSSAI, 2015), thereby indicating a shelf-life of about 30 days for the treated samples stored at refrigerated as well as room temperature.

Similarly, the observed TYMC were lower than the standards prescribed for Paneer (FSSAI, 2015) at the end of the storage period of 30 days for samples stored under refrigerated as well as room temperature. Thus, microbiologically all the treated Paneer samples under study could be considered safe and acceptable.

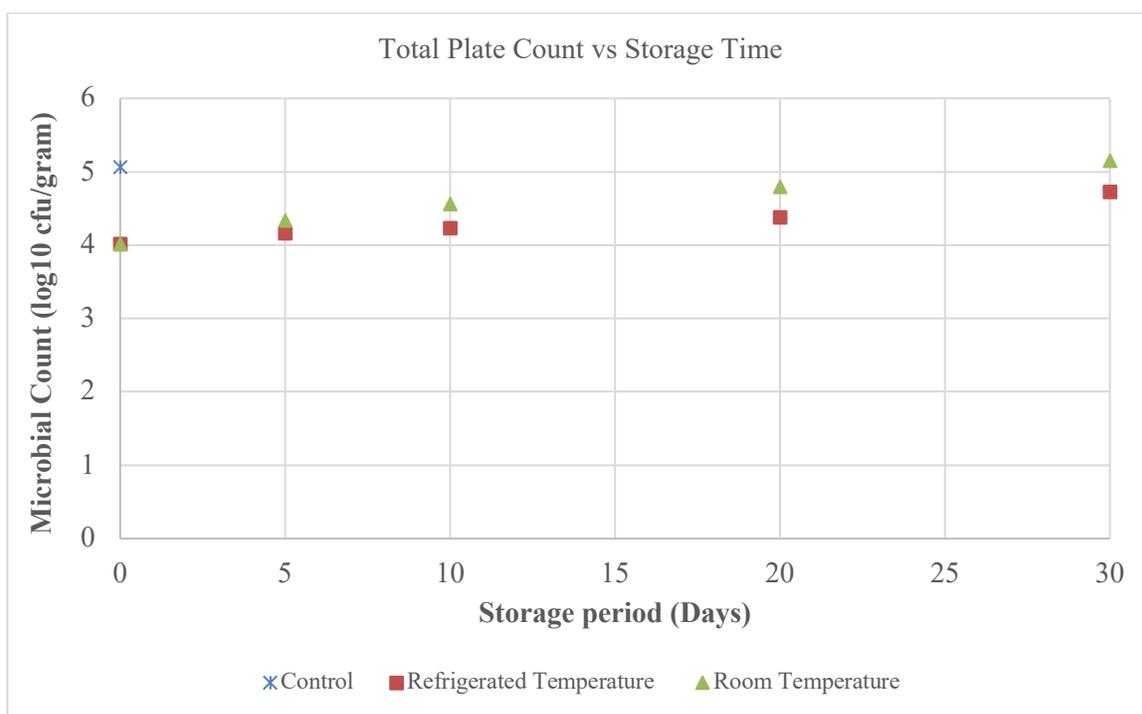


Figure 9: Total plate count during storage at refrigerated (4 ± 1 °C) and room (25 ± 1 °C) temperatures (following treatment with SC-CO₂ at 3L/min and acetic acid at 2 mL/min at 200 bar and 50°C for 65 minutes)

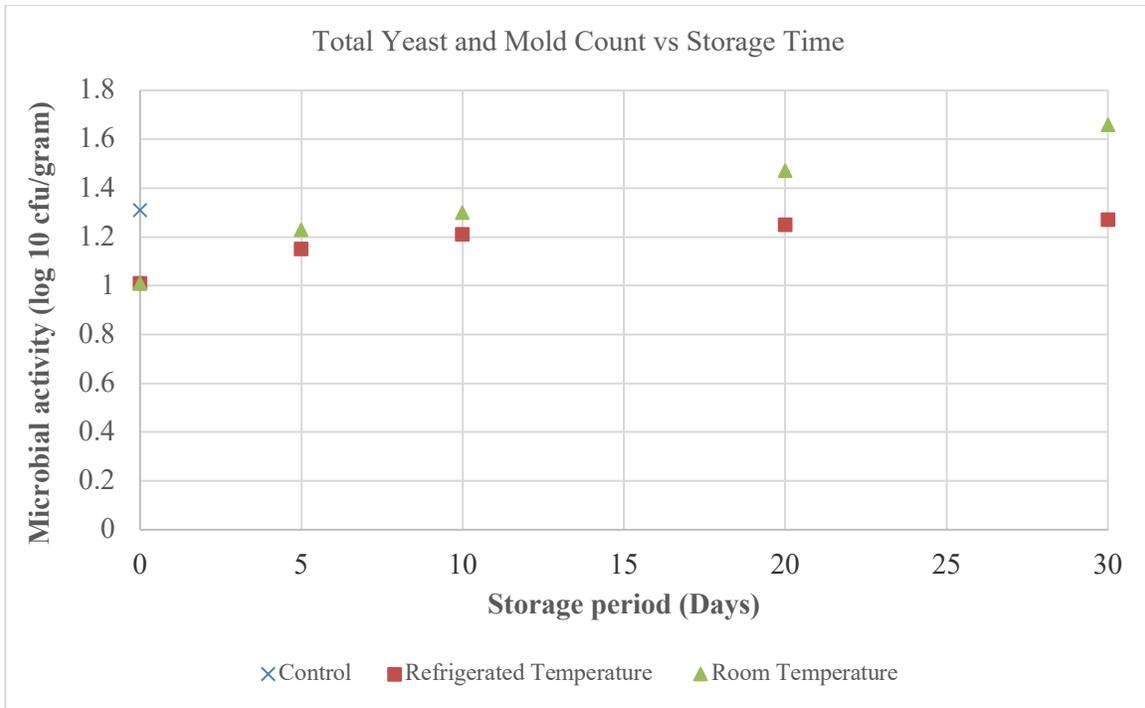


Figure 10: Total yeast and mold count during storage at refrigerated (4 ± 1 °C) and room temperature (25 ± 1 °C) (following treatment with SC-CO₂ at 3L/min and acetic acid at 2 mL/min at 200 bar and 50°C for 65 minutes)

CHAPTER 4

CONCLUSION

A combination of supercritical carbon dioxide and acetic acid has been established as an effective non-thermal approach for shelf-life extension of Paneer for up to 30 days stored at refrigerated as well as room temperatures. The findings of this study may be useful to reduce the dairy waste and economic losses incurred by dairy manufacturers due to spoilage of the product during transposition and storage. This approach of practical utility to dairy processors may also open up new opportunities to market product that meets consumer requirements of a convenient, shelf stable Paneer not yet available in the commerce.

References

1. Acetic acid [WWW Document], URL https://www.cs.mcgill.ca/~rwest/wikispeedia/wpcd/wp/a/Acetic_acid.htm (accessed 8.12.19).
2. Amaral, G.V., Silva, E.K., Cavalcanti, R.N., Cappato, L.P., Guimaraes, J.T., Alvarenga, V.O., Esmerino, E.A., Portela, J.B., Sant' Ana, A.S., Freitas, M.Q., Silva, M.C., Raices, R.S.L., Meireles, M.A.A., Cruz, A.G., 2017. Dairy processing using supercritical carbon dioxide technology: Theoretical fundamentals, quality and safety aspects. *Trends Food Sci. Technol.* 64, 94–101. <https://doi.org/10.1016/j.tifs.2017.04.004>
3. Balaban, M.O., Duong, T., 2014. Dense Phase Carbon Dioxide Research: Current Focus and Directions. *Agric. Agric. Sci. Procedia* 2, 2–9. <https://doi.org/10.1016/j.aaspro.2014.11.002>
4. Bi, X., Wu, J., Zhang, Y., Xu, Z., Liao, X., 2011. High pressure carbon dioxide treatment for fresh-cut carrot slices. <https://doi.org/10.1016/j.ifset.2011.04.001>
5. Bourne, M. C. (1978). Texture profile analysis. *Food Technology*,32(72),62–66, n.d.
6. Brown, Z.K., Fryer, P.J., Norton, I.T., Bakalis, S., Bridson, R.H., 2008. Drying of foods using supercritical carbon dioxide — Investigations with carrot. *Innov. Food Sci. Emerge. Technol.* 9, 280–289. <https://doi.org/10.1016/j.ifset.2007.07.003>
7. Chapter 4. Extension of the intermediate moisture concept to high moisture products [WWW Document], n.d. URL <http://www.fao.org/3/y4358e/y4358e07.htm> (accessed 8.12.19).
8. Choi, Y.M., Kim, O.Y., Kim, K.H., Kim, B.C., Rhee, M.S., 2009. Combined effect of organic acids and supercritical carbon dioxide treatments against nonpathogenic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and *E. coli* O157:H7 in fresh pork. *Lett. Appl. Microbiol.* 49, 510–515. <https://doi.org/10.1111/j.1472-765X.2009.02702.x>

9. Dairy production and products: Production [WWW Document], n.d. URL <http://www.fao.org/dairy-production-products/production/en/> (accessed 6.27.19).
10. Draft_Regulation_on_Microbiological_standards_milk_and_milk_products_31_08_2015.pdf, n.d.
11. Dwarakanath, H., Gurumoorthi, P., Sutariya, H., Rao, K.J., Pagote, C.N., 2013. Effect of freezing on the textural attributes of paneer during storage. *INDIAN J. DAIRY Sci.* 66, 487–495.
12. Erkmen, O., 2000. Antimicrobial effect of pressurized carbon dioxide on *Enterococcus faecalis* in physiological saline and foods. *J. Sci. Food Agric.* 80, 465–470. [https://doi.org/10.1002/\(SICI\)1097-0010\(200003\)80:4<465: AID-JSFA550>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0010(200003)80:4<465: AID-JSFA550>3.0.CO;2-E)
13. Ferrentino, G., Balzan, S., Spilimbergo, S., 2012. On-line color monitoring of solid foods during supercritical CO₂ pasteurization. *J. Food Eng.* 110, 80–85.
<https://doi.org/10.1016/j.jfoodeng.2011.12.006>
14. Ferrentino, G., Spilimbergo, S., 2011. High pressure carbon dioxide pasteurization of solid foods: Current knowledge and future outlooks. <https://doi.org/10.1016/j.tifs.2011.04.009>
15. Fraser, D. (1951). bursting bacteria by release of gas pressure. *Nature*, 33-34., n.d.
16. Hong, S.-I., Pyun, Y.-R., 1999. Inactivation Kinetics of *Lactobacillus plantarum* by High Pressure Carbon Dioxide. *J. Food Sci.* 64, 728–733. <https://doi.org/10.1111/j.1365-2621.1999.tb15120.x>
17. Ingrosso, F., Ruiz-López, M.F., 2017. Modeling Solvation in Supercritical CO₂. *ChemPhysChem* 18, 2560–2572. <https://doi.org/10.1002/cphc.201700434>
18. J. Vigano, A.P.F. Machado, J. Martínez Sub- and supercritical fluid technology applied to food waste processing *The Journal of Supercritical Fluids*, 96 (2015), pp. 272-286, n.d.
19. Kamihira, m., taniguchi, m., & kobayashi., t. (2014). sterilization of microorganisms with supercritical co₂. *agricultural and biology chemistry*, 407-412., n.d.

20. Kanawjia, S.K., Singh, S., 1996. Sensory and textural changes in paneer during storage [WWW Document]. Buffalo J. URL <https://eurekamag.com/research/002/954/002954794.php> (accessed 8.12.19).
21. Karoui, R., Dufour, É., 2003. Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses. *Int. Dairy J.* 13, 973–985.
[https://doi.org/10.1016/S0958-6946\(03\)00121-3](https://doi.org/10.1016/S0958-6946(03)00121-3)
22. Khan, S.U., Pal, M.A., 2011. Paneer production: A review. *J. Food Sci. Technol.* 48, 645–660.
<https://doi.org/10.1007/s13197-011-0247-x>
23. King, M., Mubarak, A., & Kim, J. &. (2005). the mutual solubility of water with supercritical and liquid carbon dioxide. *Journal of supercritical fluids*, 296-302., n.d.
24. Lan, W., HongZhang, C., 2011. Increased fermentability of enzymatically hydrolyzed steam-exploded corn Stover for butanol production by removal of fermentation inhibitors. *Process Biochem.* 46, 604–607.
25. Lin, H., Yang, Z., & Chen, L. (1992). Inactivation of *saccharomyces cerevisiae* by supercritical and subcritical Co₂. *biotechnology. prog*, 149-154., n.d.
26. Madadlou, A., Khosroshahi, A., Mousavi, M.E., 2005. Rheology, Microstructure, and Functionality of Low-Fat Iranian White Cheese Made with Different Concentrations of Rennet. *J. Dairy Sci.* 88, 3052–3062. [https://doi.org/10.3168/jds.S0022-0302\(05\)72986-6](https://doi.org/10.3168/jds.S0022-0302(05)72986-6)
27. Mariano, A.P., Qureshi, N., Filho, R.M., Ezeji, T.C., 2012. Assessment of in situ butanol recovery by vacuum during acetone butanol ethanol (ABE) fermentation. *J. Chem. Technol. Biotechnology.* 87, 334–340. <https://doi.org/10.1002/jctb.2717>
28. Market_Dairy_Edelweiss_12.12.17.pdf, n.d.
29. McGlynn, W., n.d. The Importance of Food pH in Commercial Canning Operations 8.

30. Paneer (contributed by Sunil Radhakrishnan) | Food Science [WWW Document], n.d. URL <https://www.uoguelph.ca/foodscience/book-page/paneer-contributed-sunil-radhakrishnan> (accessed 6.28.19).
31. Ramírez-Rodrigues, M.M., Plaza, M.L., Ferrentino, G., Balaban, M.O., Corcuera, J.I.R.-D., Marshall, M.R., 2013. Effect of Dense Phase Carbon Dioxide Processing on Microbial Stability and Physicochemical Attributes of Hibiscus Sabdariffa Beverage. *J. Food Process Eng.* 36, 125–133. <https://doi.org/10.1111/j.1745-4530.2011.00663.x>
32. Raventós, M., Duarte, S., Alarcón, R., 2002. Application and Possibilities of Supercritical CO₂ Extraction in Food Processing Industry: An Overview. *Food Sci. Technol. Int.* 8, 269–284. <https://doi.org/10.1106/108201302029451>
33. Sikin, A.M., Walkling-Ribeiro, M., Rizvi, S.S.H., 2016. Synergistic effect of supercritical carbon dioxide and peracetic acid on microbial inactivation in shredded Mozzarella-type cheese and its storage stability at ambient temperature. *Food Control* 70, 174–182. <https://doi.org/10.1016/j.foodcont.2016.05.050>
34. Yuanrong Zheng, Zhenmin Liu, and Beihong Mo, “Texture Profile Analysis of Sliced Cheese in relation to Chemical Composition and Storage Temperature,” *Journal of Chemistry*, vol. 2016, Article ID 8690380, 10 pages, 2016. <https://doi.org/10.1155/2016/8690380>.
35. Sunil Kumar, Dinesh Chandra Rai, Keshavan Niranjana, Zuhaib Bhat, 2014. Paneer - An Indian soft cheese variant: A review. *J. Food Sci. Technol. -Mysore-* 515. <https://doi.org/10.1007/s13197-011-0567-x>
36. Thakral S., Prasad MM., Ghodekar, DR., 1990. Effect of incorporation of potassium sorbate and nisin for improvement in shelf-life of paneer. XXIII International Dairy Congress, Montreal, 8–12 October, Vol 1, p 150

37. Thippeswamy, L., Venkateshaiah, B.V., Patil, S.B., 2011. Effect of modified atmospheric packaging on the shelf stability of paneer prepared by adopting hurdle technology. *J. Food Sci. Technol.* 48, 230–235. <https://doi.org/10.1007/s13197-010-0155-5>
38. Tunick, M.H., 2000. Rheology of Dairy Foods that Gel, Stretch, and Fracture1. *J. Dairy Sci.* 83, 1892–1898. [https://doi.org/10.3168/jds.S0022-0302\(00\)75062-4](https://doi.org/10.3168/jds.S0022-0302(00)75062-4)
39. Valverde, M.T., Marín-Iniesta, F., Calvo, L., 2010. Inactivation of *Saccharomyces cerevisiae* in conference pear with high pressure carbon dioxide and effects on pear quality. *J. Food Eng.* 98, 421–428.
40. Vassal, L., Monnet, V., Bars, D.L., Roux, C., Gripon, J.C., 1986. Relation entre le pH, la composition chimique et la texture des fromages de type Camembert. *Le Lait* 66, 341–351. <https://doi.org/10.1051/lait:1986422>
41. Zimmerman, J., n.d. *India Dairy and Products Annual* 2017 10.