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CONTENTS

Utilization of ultrasonicated edible coating to prolong shelf life of fresh cut-onion

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ABSTRACT: Plant tissues face considerable stress from mechanical damage, especially during cutting and handling, leading to changes in color, texture and flavor due to enzyme and substrate disruption. These challenges complicate the production of fresh-cut onions. Edible coatings offer a promising solution by forming a protective barrier against moisture loss, oxidation and microbial growth, while preserving product quality. In this research, chitosan was investigated as an edible coating for fresh-cut onion at varying concentrations (ranging from 0.5% to 1.5%). Other variables taken into consideration were dipping time (5 min to 15 min), ultrasonication power (50 to 250 W) and ultrasonication time (15 min to 45 min). Various coating properties such as thickness and viscosity were examined. Process conditions were optimized using response surface methodology and fresh cut onion was then coated with chitosan edible coating prepared with optimized processing conditions (Chitosan concentration- 1%, dipping time- 10 min, ultrasonication power- 150 W and ultrasonication time- 30 min). Weight loss, texture and microbiological analysis of the coated samples were done. It was observed that the fresh cut onion could retain their microbiological safety up to 15 days in comparison to untreated fresh cut onion which had shelf life of only 6 days under refrigerated conditions at a temperature of 5±2 !. The coating could slow the rate of weight loss and texture loss in comparison to the raw samples. Hence, chitosan edible coating can be promising technology to produce fresh cut onion for upto 15 days in refrigerated conditions.

Key words:Fresh cut onion, preservation, shelf life, ultrasonication

Onion is one of the most significant crops in the world and is widely used as a source of nutrients and other bioactive compounds that promote health in addition to its extensive use in cuisine (Zang *et al*., 2013). It is grown in almost 138 countries around the world, with a global production of around 106.6 million tonnes in the year 2021. Among the leading onion producing countries of the world, India ranks 1st with a production of 26.64 million tonnes followed by China, Egypt, USA and Turkey accounting for about 25, 23, 3, 3, 2 percent of the global production, respectively (FAO, 2021). Onion is marketed in three major forms i.e. fresh bulbs for the market, dehydrated onion as ingredient in food processing and onion for essential oil production (Wiczkowski, 2018). In the fresh-cut onion category, whole peeled, diced, sliced, slivered and pureed onions are typically made. Depending on the intended use, an onion can be either mellow or pungent. Typically, milder bulbs are chosen for seasoning or as a cooked vegetable. On the other hand, producers of goods like sauces, canned soups and extracts establish a market for extremely pungent varieties. Besides this, onion finds a great place in most of the Indian food. Chopping onions can be bothersome for many people because of the tear-inducing properties of propanethial S-oxide (lachrymatory factor) which is released while cutting along with the lingering smell they leave on hands (Bahram and Lim, 2018). Consequently, there is a growing demand for pre-cut, value-added and ready-to-eat onion products in households, retail, food service and various food industries, primarily for their convenience. However, despite the advantages, fresh-cut onion products face significant challenges such as tissue damage, which leads to chemical and physiological reactions that reduce shelf life. Common deterioration issues include rapid discoloration, microbial growth, softening and undesirable odors, all of which necessitate effective preservation methods to control. Edible coatings can act as a barrier to moisture loss, texture loss and reduce microbial growth without causing much change in any of the product characteristics due to processing. It is evident in various studies done on fresh cut fruits and vegetables. Sodium alginate coating when applied to fresh cut pineapple maintained the texture of the fruit 2.4 N in comparison to uncoated 1.3 N during 16 days of storage at 10±1 ! and 65± 10% RH. There was significant reduction in the lightness of uncoated pineapple L value- 48 in comparison to coated pineapple which had L value- 58 (Azarakhsh *et al*., 2014). Similarly, when sodium alginate coating was applied to fresh-cut lotus root ΔE value was found to be 12 for uncoated samples in comparison to coated ones which had ΔE values in the range of 2-5 indicating the lesser change in the color after storage (Gouda *et al*., 2021). When chitosan was used as edible coating in combination with clove essential oil it was observed that Psychrotrophic bacteria population was reduced to 4.16 log/CFU/g in coated strawberry from 5.93 log/CFU/g in uncoated, whereas Yeast and mould count was reduced from 6.19 log CFU g^{-1} in uncoated to 3.85 – 5.14 log CFU g^{-1} in coated strawberry samples (Pizato *et al.,* 2022). So, it can be inferred that edible coating has significant effect in maintaining the texture of the fresh fruits and vegetables. It also reduces the discoloration, microbial load and weight loss. As these are the characteristics that needed to be looked after in the fresh cut onion also, the edible coating can be used to produce fresh cut onion while maintaining its characteristic properties. The study was therefore done to apply edible coating as an effective method for enhancing the life of fresh cut onion causing minimum alteration to its biochemical and physical properties.

Chitosan is a linear polysaccharide composed of β-(1→4) glycosidic bonds that connect D-glucosamine and N-acetyl-D-glucosamine units in a random fashion. It is primarily derived from the alkaline deacetylation of chitin (Şenel and McClure, 2004) which is predominantly present in arthropods, crustacean shells and fungal cell walls. It is a natural, non-toxic, biodegradable polymer and is widely used in the food industry for various applications due to its availability and cost-effectiveness, making it a viable alternative to non-biodegradable polymers. Previous studies have shown that chitosan coatings can effectively preserve fruit quality by delaying deterioration processes such as microbial growth, water loss, nutrient loss and firmness loss (Hernández *et al*., 2006; He *et al*., 2019). When used as an edible coating, chitosan offers several benefits, including retaining fruit color and firmness, maintenance of freshness, reduction in respiratory rate, mitigation of oxidative stressand inhibition of pathogenic microorganism growth. Thus, chitosan was used as edible coating in the fresh cut onion. However, chitosan is a long chain polymer, its microstructure can be modified by ultrasonication which results in more uniform granular microstructure and smaller surface area than the native form of chitosan. This is beneficial for enhancing the flow behavior of the chitosan coating, increased solubility and decreased viscosity leading to smoothness and homogeneity in the edible coating while application (Cui and Zhu, 2021). Thus, ultrasonication was used in the experimentto modify the chitosan coating.

MATERIALS AND METHODS

Raw material

Fresh red onion wasprocured from local market of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, District U.S. Nagar (Uttarakhand) and brought to the Department of Food Science and Technology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, District U.S. Nagar (Uttarakhand). Onionwas washed with 100 ppm sodium hypochlorite solution and then diced into pieces of 3 mm thickness using a hand-held commercial cutter.

Chemicals

All chemicals used during the extraction and product analysis were procured from HiMedia Laboratories Pvt. Ltd., Mumbai and SD Fine Chem. Ltd. Mumbai.

All the chemicals used were of analytical grade.

Preparation and application of coating material

Chitosan (from shrimp cells, with molecular weight 3800- 20000 Da and degree of deacylation e" 75 %) was purchased fromHiMedia Laboratories Private Limited (India). Coating solution was prepared by using the methods of previous researches with slight modification (Pizato *et al*., 2022).Chitosan coating was prepared by dissolving chitosan with concentrations between 0.5 to 1.5 % (w/v)and in 1% acetic acid followed by magnetic stirring at room temperature for 4 h. Glycerol in the ratio of 1:2(glycerol:chitosan) as plasticizer, was added and all the components were again mixed with magnetic stirring for 10 minutes. To the given solution, Tween 20 as surfactant was added at a concentration of 0.05 % (v/v) along with 2 % (w/v) calcium chloride as firming agent was added and again stirred from 5 minutes. The coating material with varying concentration of chitosan was subjected to different ultrasonication power for different interval of time pertaining to the experimental design made using Design expert software. The fresh cut onion was dipped in the coating material with varying dipping time (5 min to 15 min) as per the experimental design. After coating, the coated and diced onion was dried at room temperature (25±2!) for 4 h and then 100g of the sample was packed in polyethylene packages of 5 cm ×7 cm size,225-gauge thickness and 1 % ventilation. The whole experiment was optimized using Response Surface methodology and the optimal process conditions were used to coat fresh cut onion (stored for 18 days at 5±2 !) used later for microbial, textural and weight loss analysis within an interval of 3 days. Uncoated samples were considered as control and was used for comparison with the coated onion.

Ultrasonication

Probe sonicator of Labman Scientific Instruments Pvt. Limited, Kodungaiyur, Chennai (INDIA) Model No. Pro- 650 with probe no. 6 of diameter 6 mm was used for sonication of coating material. Coating material was subjected to ultrasonication with power ranging from 50 to 250 W for different interval of time (15 to 45 min).

Viscosity

Viscosity of the coating material was measured using Rotational Viscometer (Anton Paar, model no. ViscoQC 300, United States). Spindle type RH 4 was used with rotational speed of 100 rpm was used for estimating the viscosity and it was expressed in mPa.s.

Coating thickness

Coating thickness was measured using a Light microscope through 5x magnification lens manufactured by Olympus opto Systems India Pvt, Ltd, Noida, Model No: CX21iFS1 attached with the Magcam DC 10 vision camera with 10 MP range of vision. Methylene blue dye was added in the coating solution for differentiation, and diced onion was coated. Diced onionmeasuring 2 mm in thickness was cut using a stainless steel surgical blade and placed onto a glass slide.The sample was fixed using a cover slip. The microstructure of the coating was observed using Magcam DC 10 vision camera.The coating thickness was calculated using the software ImageJ (Wayne Rasband and contributers, National Institute of Health, USA). The micrometer (one graduation- 0.01 mm) was used for setting the scale and the sample image were analyzed taking scale measurement as standard.

Texture

The textural properties of sliced onions were assessed using a Stable Microsystem texture analyzer(Model No. TA- XT), following the methodology suggested by Aslam *et al*. (2022). Considering the fact that onion slices have structural deformity, onion cubes of same thickness were taken and many punctures at different positions on the samples were made. The average of force at each point was done and considered as the final firmness reading of the sample. Each onion cube (with a thickness of 3 mm) was placed on the platform, and a P/2N probe was used to puncture it to a depth of 1.5 mm. The penetration speed was set at 1.0 mm/s, with pre- and post-test speeds of 1 mm/s and 10 mm/s, respectively. The maximum force (Fmax) necessary to puncture through the onion slice was recorded as the firmness measurement. The firmness was expressed in gram force.

Microbial analysis

Microbiological population changes were assessed in both uncoated and coated fresh-cut onions stored over 18 days. Total bacterial counts were determined at intervals of 3, 6, 9, 12, 15 and 18 days. The count was conducted using plate count agar (PCA) medium(AOAC, 1990).

Weight loss

Physiological loss of weight was observed in treatedsamples by measuring their weight on a weighing balance having a least count of 0.01 g (AOAC, 2005).The following formula was used to calculate physio-logical loss in weight.

$$Weight\ loss\ (\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Statistical analysis

Analysis of the data was done using the statistical package DESIGN EXPERT7.0.0 (Stat-Ease, Inc. 2005, Minneapolis, United States) and graphs were plotted usingthe same. Box-Behnken Design was used in the experimental design. Multiple regression analysis was used to fit the model which wasrepresented by an equation of the experimental data.ANOVA was used to analyse the models. The adequacy of the model was examinedbased on two criteria i.e. lack of fit (LOF) and adequate precision error. Results wereinterpreted based on specific criteria laid down under the optimization session.

RESULTS AND DISCUSSION

Effect of ultrasonication on the viscosity and

Table 1 depicts the ANOVA for Response Surface Quadratic model for the effect of coating material concentration, dipping time, ultrasonication time and ultrasonication power on the viscosity of coating material. The viscosity of the samples ranged from 46.45 mPa.s to 499.61 mPa.s in the treated samples. The effect of A (Coating material concentration), C (Ultrasonication time), D (Ultrasonication power), AC, AD, BD, A^2, C^2 and B^2 was found significant as observed from the second order model (Table No. 1). The model equation (in terms of coded factors) showing the effect of variables on viscosity is depicted in equation no. (i).

The positive coefficients of factor 'A' i.e., Coating material concentration (%), shows that with increase in the coating concentration viscosity of the coating solution increases. On the other hand, the negative coefficients corresponding to factor 'C' i: e Ultrasonication time (min) and 'D' i.e., Ultrasonication power (W) indicates that with increase in the ultrasonication time and power the viscosity of the coating samples decreases (Fig 1 A).

This is attributed to the modification of chitosan structure by ultrasonication. Ultrasound degradation of polysaccharides occurs due to acoustic cavitation. The high stress and shear energy generated by cavitation (mechanical effects) lead to irreversible chain scissions. Depending on the composition and structural arrangement of the polysaccharides, ultrasound degradation may involve either random chain scission or midpoint chain scission (Vilkhu *et al.,* 2010).The extent of structural alterations induced by ultrasound is heavily reliant on the properties of polysaccharides, including monosaccharide composition, chain length, conformation, molecular weight and glycosidic linkage. Polysaccharides with a linear, rod-like conformation such as chitosan, are more susceptible to ultrasound-induced breakage. Thus, reduced chain entanglements and interactions led to viscosity reduction. Similar results were observed by researchers in different polysaccharides (Ding *et al*., 2019; Kaltsa *et al*., 2014; Feng *et al*., 2017; Gavahian *et al*., 2018). Thus, it can be inferred that the decrease in viscosity of polysaccharides after

$$Viscosity = +140.61 + 177.45 * A + 7.47 * B - 40.11 * C - 27.45 * D + 3.87 * AB - 38.30 * AC - 26.71 * AD + 1.33 * BC - 53.622.47 * BD + 14.43 * CD + 78.82 * A^2 - 11.94 * B^2 + 19.81 * C^2 + 36.52 * D^2$$

sonication is associated with the breakage of glycosidic linkages and weakening of the microstructural network (Sengar *et al*., 2020).

Coating thickness

Thickness of the coating in the samples ranged from 70.95 to 299.11 µm. Fig 1(B) shows the effect of coating concentration and dipping time on the coating thickness and it was observed that with increase in the concentration and dipping time coating thickness also increased significantly. It can be inferred that with increase in the concentration, higher amount of coating material is deposited on the surface of the onion per unit time, leading to increase in the coating thickness with the increase in dipping time.Fig 2 (A) depicts the coated onion surface. Similar results were found by Soares*et al.* (2016) where chitosan coating was used for coating salmon. They observed that longer dipping time lead to larger coating thickness. Also, research conducted *by El-Hefian et al.* (2010) demonstrated that as the temperature of the chitosan solution increases, its viscosity decreases. In conclusion, it can be said that higher the ultrasonication time and power lower will be the viscosity andwith higher concentration of the coating material and dipping time the coating thickness will be more.

Process optimization

The experimental parameters were optimized using the Response surface methodology. The criteria for optimization were selected within the range of experimentation and the concentration of 0.9 %, dipping time of 9 minutes, ultrasonication time of 23 minutes and ultrasonication power of 135 W was found to have the highest desirability (Fig. 3). These process conditions were then used to coat fresh cut onion which were then stored at 5±2 ^{0}C for 18 days.

Effect of edible coating on the shelf life of fresh cut onion

Textural loss, weight loss and microbiological spoilage are the major attributes of the shelf of fresh cut commodities. The weight of packaged fresh-cut onions typically diminishes over time due to various factors including respiration rate, temperature and microbial growth. The rate of weight loss is influenced by several factors such as the barrier properties of the packaging structure, relative humidity, surface/volume ratio, and microbial activity. Employing packaging films with optimal barrier properties can shield the product from water

Table 1: ANOVA showing the effect of coating material concentration (%), dipping time (min), ultrasonication time (min) and ultrasonication power (min) on the viscosity (mPa.s) of coating material

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	4.780E+05	14	34140.91	133.31	< 0.0001	significant
A-Coating material concentration (%)	3.778E+05	1	3.778E+05	1475.34	< 0.0001	
B-Dipping time (min)	669.46	1	669.46	2.61	0.1282	
C-Ultrasonication time (min)	19303.1	1	19303.1	75.37	< 0.0001	
D-Ultrasonication power (W)	9042.14	1	9042.14	35.31	< 0.0001	
AB	60.03	1	60.03	0.23	0.6358	
AC	5867.56	1	5867.56	22.91	0.0003	
AD	2853.96	1	2853.96	11.14	0.0049	
BC	7.02	1	7.02	0.027	0.8708	
BD	11501.06	1	11501.06	44.91	< 0.0001	
CD	832.52	1	832.52	3.25	0.093	
A^2	40294.95	1	40294.95	157.34	< 0.0001	
B^2	924.39	1	924.39	3.61	0.0783	
C^2	2546.46	1	2546.46	9.94	0.007	
D^2	8653.46	1	8653.46	33.79	< 0.0001	
Residual	3585.5	14	256.11			
Lack of Fit	3325.53	10	332.55	5.12	0.0648	not significant
Pure Error	259.96	4	64.99			
Cor Total	4.80E+05	28				

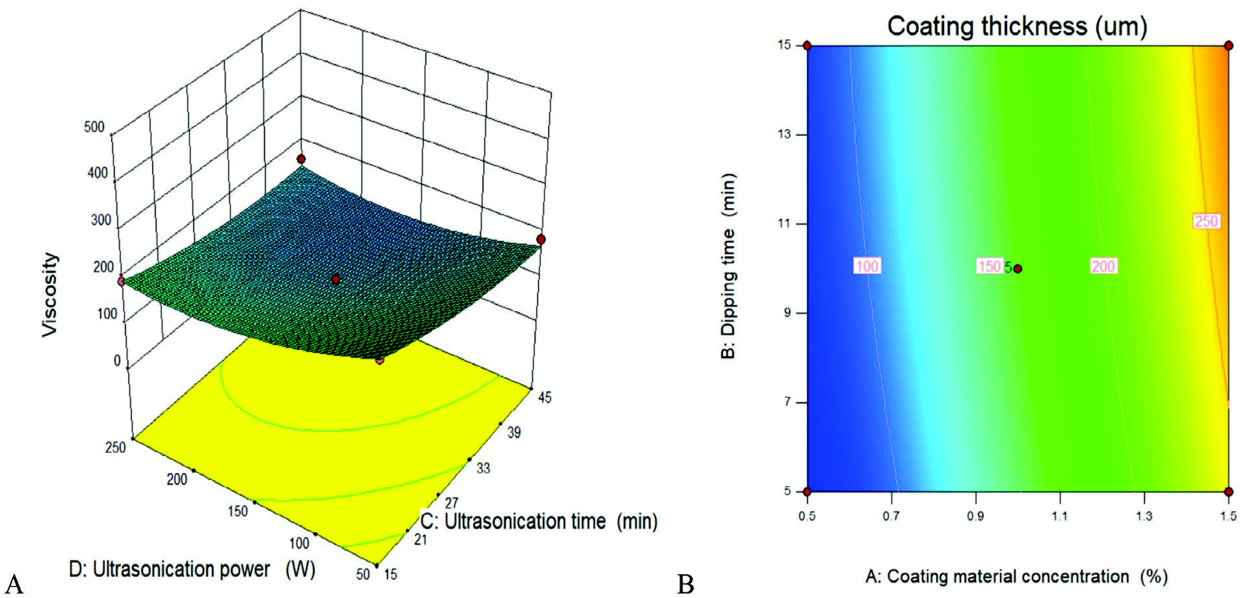
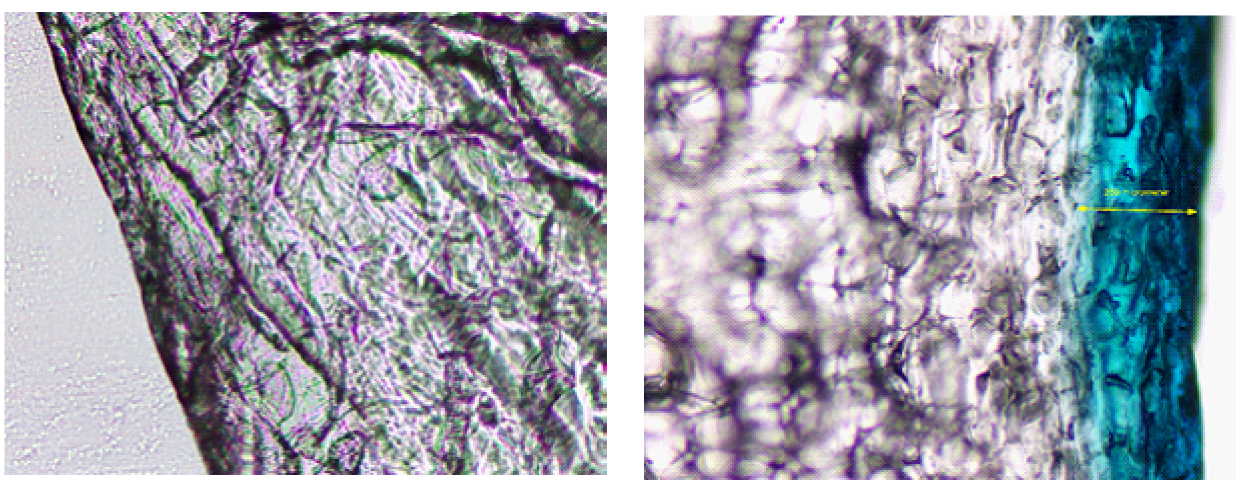


Fig 1: (A)Effect of ultrasonication time and power on coating viscosity and (B) Effect of coating concentration and dipping time on coating thickness



A. B.

Fig 2: Chitosan coating on the fresh cut onion observed by Light Microscope at 5x magnification A) Uncoated onion B) Coated onion

loss. However, it is crucial to prevent the accumulation of moisture from the product inside the package (Ayala-Zavala *et al*., 2008).Undesirable biochemical reactions occurring post-cutting can lead to texture alterations in fresh-cut onions, with soft texture being the primary defect observed during processing and storage, resulting in consumer dissatisfaction. Texture softening is primarily attributed to enzymatic activity, particularly by enzymes like pectin methyl esterase and polygalacturonase, which degrade pectin. Pectin methyl esterase partially demethylates pectin, while polygalacturonase depolymerizes it into polygalacturonic acid, consequently causing a loss of firmness (Rico *et al*., 2007). Apart from the factors mentioned above, surface microbial growth can also impact the visual appearance of fresh-cut onions (Blanchard *et al*., 1996; Liu and Li, 2006). In the given experiment, it was observed that chitosan coating could increase the microbiological shelf life, retain the texture up to optimum level and reduce the weight loss in fresh cut onion during storage days. The detailed effect is explained in the sub-sections below.

Microbiological analysis

In this experiment, the coated samples were compared with uncoated samples. Food Safety and Standard Authority of India has laid microbial limit of 6 log cfu/g for the cut or minimally processed fruits and vegetables (FSSAI, 2011). The uncoated samples lost microbiological shelf life after 6 days of storage with micro biological load of 1 log cfu/g on the first day and 6.7 log cfu/g on the 6th day of storage. However, the treated samples retained its microbiological shelf life upto 15 days with 5.8 log cfu/ g microbial load. After 15 days of storage the microbial load was higher than 6 log cfu/g which was beyond the acceptable limit.Thus, the results of the investigation justify the antibacterial effect of chitosan as edible coating. The antibacterial activity of chitosan is linked to the creation of a polyelectrolyte complex. This occurs when the protonated amine groups of chitosan selectively adhere to the negatively charged cell surfaces of microorganisms. As a result, intracellular components are lost, leading to the inhibition of

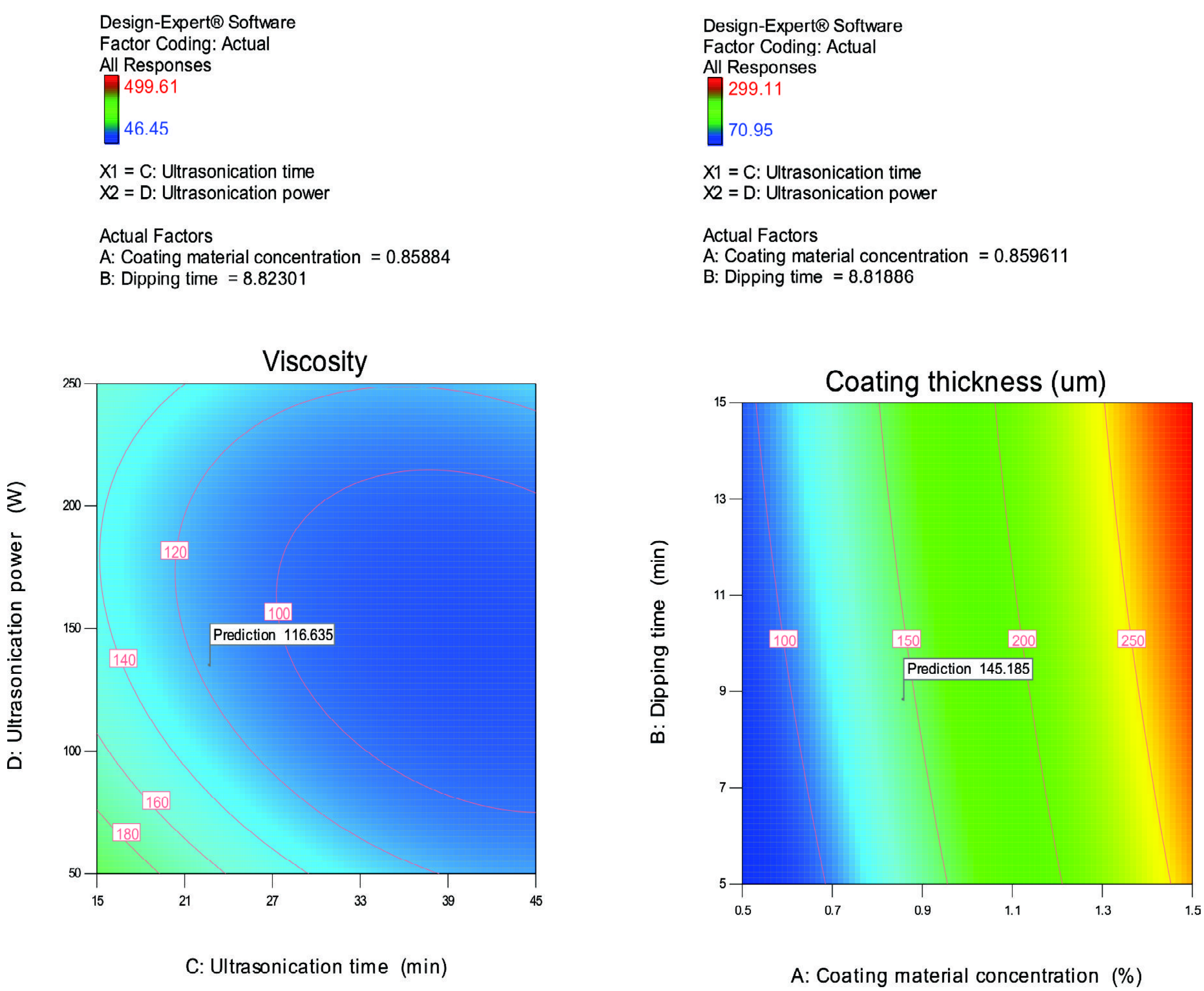


Fig. 3: Optimzation and point prediction post analysis of the experiments

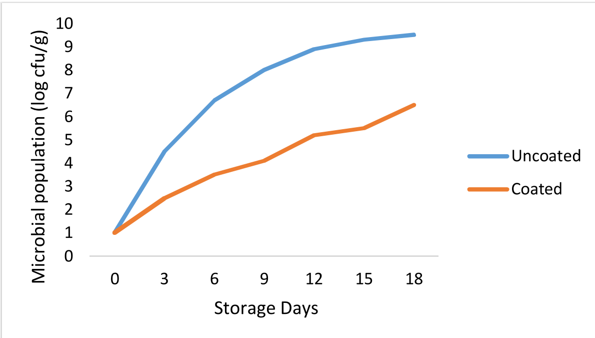


Fig 4: Effect of Coating on microbial population in fresh cut onion

microbial growth (Pizato *et al*., 2022). Similar results were observed by other researchers in minimally processed strawberry, watermelon, bell pepper etc. (Pizato *et al*., 2022; Salsabiela *et al*., 2022; Hu *et al*., 2020).

Texture

The texture of fresh cut onion was observed in terms of the maximum force (F_{max}) necessary to puncture through the onion cubes completely. The firmness was expressed in gram force. It was observed the firmness was lost both in coated and uncoated samples however the rate of firmness loss was lower in coated samples with 43 g force on the 15th day and 35 g force for the uncoated samples on the same day. Edible coatings establish physical barriers around fruits or vegetables, separating them from their surroundings. This semipermeable barrier regulates water movement, gas exchange, and modifies internal atmospheres. Additionally, it influences physiological processes such as enzymatic breakdown of cell walls and the production of ethylene gas (Maftoonazad *et al*., 2008). Thus, justifying the higher firmness in coated samples as compared to uncoated samples.

Weight loss

Weightloss in coated samples was 4.2 percent on 15th day of storageand went upto 6 percent at the end of the storage period of 18 days. However, in the uncoated samples weight loss was rapid with 8percent weightloss at 15 days of storage and 10 percent at the 18th day of storage. The weight reduction observed in fresh-cut fruit commodities is linked to their thin skin and the rate of transpiration, which makes them susceptible to rapid water loss, leading to deterioration and shriveling (Hu *et al*., 2020). Chitosan coatings serve as semipermeable barriers, establishing an appropriate atmosphere for fresh-cut onions and delaying water loss.

CONCLUSION

The current study infers that ultrasonication leads to change in the rheological property of the coating material such as viscosity pertaining to the modification in the structure of different types of polysaccharides like chitosan in current case. Thus, it is recommended to use ultrasonication for edible coating preparation. This study also highlights that chitosan edible coating can be used as a technology to enhance the shelf life of fresh cut onion for about 15 days while maintaining the firmness,

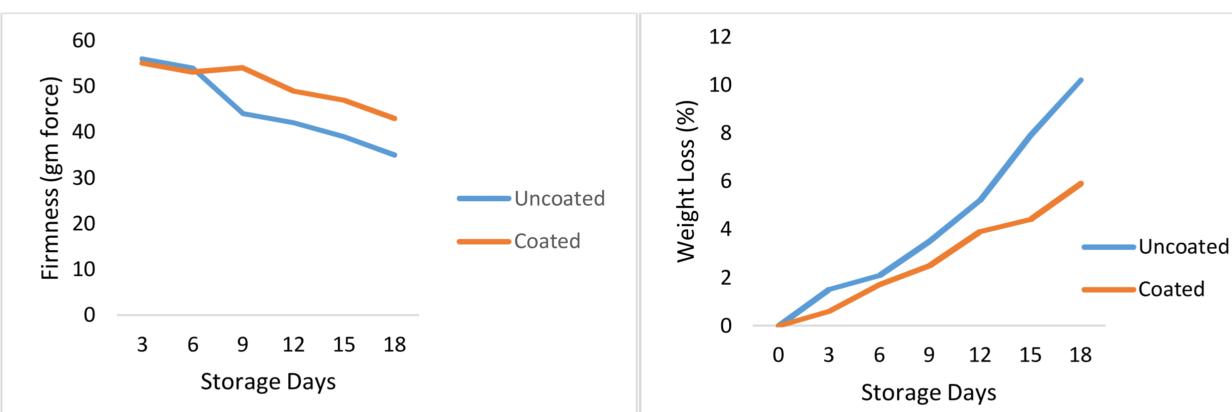


Fig. 5: Effect of Coating on Firmness and Weight loss in fresh cut onion

microbiological safety and reducing the weightloss.

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