

Analysis of Physicochemical Properties of corn starch based composite Biodegradable Cups Influenced by Ultrasonication Pretreatment of Casting Solutions

Abstract

Biodegradable cup is made using a casting solution which is made up of mixture of corn starch (7%), whey protein concentrate(3%), carboxy methyl cellulose (CMC), and glycerol. This research work focused on analysing the effect of ultrasonication treatment with varying amplitude levels (0%, 20%, 40%, and 60%) and time durations (0, 1, 2, 4, 8, and 16 minutes) on the casting solution for the preparation of biodegradable cups. The study examined the impact of ultrasonication on physical properties such as viscosity, thickness, density and chemical properties such as solubility in water, opacity of the cups. The viscosity of the casting solution decreased as ultrasonication treatment intensity increased, resulting in a decrease in the thickness of the cups. Higher ultrasonication intensity also led to an increase in solubility of cup samples in water. The density of cups, produced through the application of ultrasonication during the preparation process, exhibited an augmentation with elevated ultrasonication intensity and prolonged treatment duration. Ultrasonication treatment caused a decrease in opacity, resulting in a more transparent cup material. Overall, the findings indicate that ultrasonication may be used to streamline the preparation process while enhancing the physical characteristics of biodegradable cups.

Keywords: Ultrasonication treatment, Amplitude level, Time durations, Casting solution, Biodegradable cups, Physical properties

Introduction

Food packaging is a crucial subject in the field of food technology because it deals with the preservation and protection of various foods and their raw materials as well as the prevention of oxidation and microbial breakdown. Petrochemical-based plastics like polyolefin, polyester, and polyamide have grown in popularity as materials for packaging food due to their wide availability, low cost and advantageous functional characteristics like strong tensile and tear resistance, good barrier properties to gases like oxygen and aromatic compounds, and heat seal ability. In recognition of its negative effects on the environment, the use of single-use plastics and petroleum-based packaging materials for food packing in India has drawn attention (Wu et al., 2021). Growing interest in biodegradable packaging materials as a more environmentally friendly option has been seen recently.

Biodegradable materials come from plant-based origins and can naturally decompose over time, lessening their environmental effect. As substitutes for petroleum-based packaging, substances like corn-starch, sugarcane bagasse, and bamboo are becoming more and more well-liked. In terms of sustainability, these materials have several benefits. They may be composted and biodegraded, which helps prevent the build-up of plastic trash (Fuentes-Alonso & Martínez-Pañeda, 2020). They are also frequently made from renewable resources, which makes them more ecologically friendly.

Materials for packing that decompose naturally offer several benefits. They aid in the preservation of fossil fuel resources since they lessen reliance on polymers made from petroleum. In comparison to conventional plastic materials, biodegradable materials have a smaller carbon footprint and produce less greenhouse emissions during

manufacture(Muthuraj et al., 2018). They can also help reduce plastic pollution by organically degrading and providing less of a threat to wildlife and marine life.

In today's world, where the negative effects of non-biodegradable materials on the environment are a serious concern, the lack of research on biodegradable solid containers poses a significant challenge. The absence of sufficient studies and exploration in this area hinders the development of viable alternatives to conventional plastic containers. Biodegradable containers have the potential to reduce pollution, minimize waste accumulation in landfills, and mitigate the harmful effects of plastic on ecosystems. However, without dedicated research efforts, we are limited in our understanding of the most effective materials, manufacturing processes, and long-term degradation properties of such containers. The absence of comprehensive research impedes progress in creating sustainable packaging solutions and limits the availability of environmentally friendly options in various industries.

Ultrasonication is an effective method with a wide range of applications that includes exposing a liquid or solid sample to high-frequency sound waves (usually above 20 kHz). The mechanical agitation brought on by the sound waves causes small bubbles to develop and burst, creating strong shear forces and shockwaves inside the substance. Ultrasonication is a desirable method for altering the characteristics of materials because of the phenomena known as cavitation, which causes a variety of physical and chemical changes(Abral et al., 2020) . According to several research, ultrasonication successfully improved the mechanical barrier qualities of biodegradable films. For instance, Zhao et al. looked at how poly(lactic acid) (PLA) films' tensile strength and thermal characteristics were affected by ultrasonic treatment. In comparison to untreated films, the findings showed a considerable improvement in both mechanical and barrier qualities(Zhao et al., 2020). Similar results were found in Zhang et al.'s study into the use of ultrasonication to enhance the mechanical properties of starch-based films, which showed significant improvements in tensile strength and elongation at break. The use of ultrasonication to improve the mechanical barrier qualities of biodegradable films has attracted interest. Therefore, there is an increasing need to investigate cutting-edge techniques to enhance these films' mechanical barrier qualities(Zhang et al., 2019).

In the present research work, effect of ultrasonication of the casting solution on prepared biodegradable cup has been analysed. Specifically, the focus was on examining the impact of ultrasonication on the viscosity of the casting solution, which in turn affects the thickness of the final cup. The study also looked at how ultrasonication affected the properties of the resulting cup's solubility and opacity. By subjecting the casting solution to ultrasonication, the researchers aimed to determine how this treatment modality influences the various physical and visual characteristics of the biodegradable cup. Through careful analysis and experimentation, the study sheds light on the potential benefits of ultrasonication in optimizing the fabrication process and enhancing the overall quality of environmentally friendly cups.

Material & methodology

In this paper, the impact of ultrasonication on the physical characteristics of the resulting composite biodegradable cup is examined. By layering corn starch(7%), whey protein concentrate(3%), carboxy methyl cellulose (CMC)(2%), and glycerol(4%), a composite biodegradable cup was created. The preparation method of composite biodegradable cup with ultrasonication was earlier discussed, referred to (Deshmukh et al., 2022).Figure 1

depicts the pictorial representation of preparation method. In this study, the impact of ultrasonication treatment amplitude and time on physical characteristics like casting solution viscosity, standability, were examined. Also, the changes due to ultrasonication on thickness, solubility, density, and opacity of composite biodegradable cup has been evaluated. Ultrasonication treatment was utilized for preparation of casting solution. Based on previous research and studies the range of amplitude and time was chosen. For the study three amplitude level 20, 40 & 60 % and the treatment time of 1, 2, 4, 8 & 16 min were applied at each amplitude level.

3.1 Viscosity analysis

Using the reference method outlined in Wang et al., (Wang et al., 2007) the viscosity of the casting solution was measured. The viscosity of test solutions was measured at 40 degrees Celsius using a Brookfield viscometer (DV2TLVTJ0 viscometer; Brookfield Engineering Labs Inc., Stoughton, MA, USA; Spindle: No. 4; Speed: 100 r.p.m.). With a thermostat water bath, the temperature of the casting solution is maintained.

3.2 Standability and Capacity of cup

To test the cup's standability, it was placed on a horizontal slab at 25°C for 5 days and checked to see whether it collapsed. The cup's capacity was measured in grams as well as the volume it could hold. To calculate the volume, water was poured from the cup into a graduated cylinder after it had been fully filled. To determine the gramme weight, an empty cup was placed on the weighing scale, followed by a cup filled with water till the brim and weighed in weighing scale. The difference between w_2 and w_1 represents the possible weight.

3.3 Thickness of biodegradable cup

Using a manual micrometre (Mitutoyo micrometre 2046F) with a 1m resolution, the thickness of the cups was measured. Calculations were based on the average of three thickness measurements taken at various places on each film sample.

Before testing, all cup specimens were conditioned for 48 hours in a humidity test room at 50% relative humidity (RH) and 25°C.

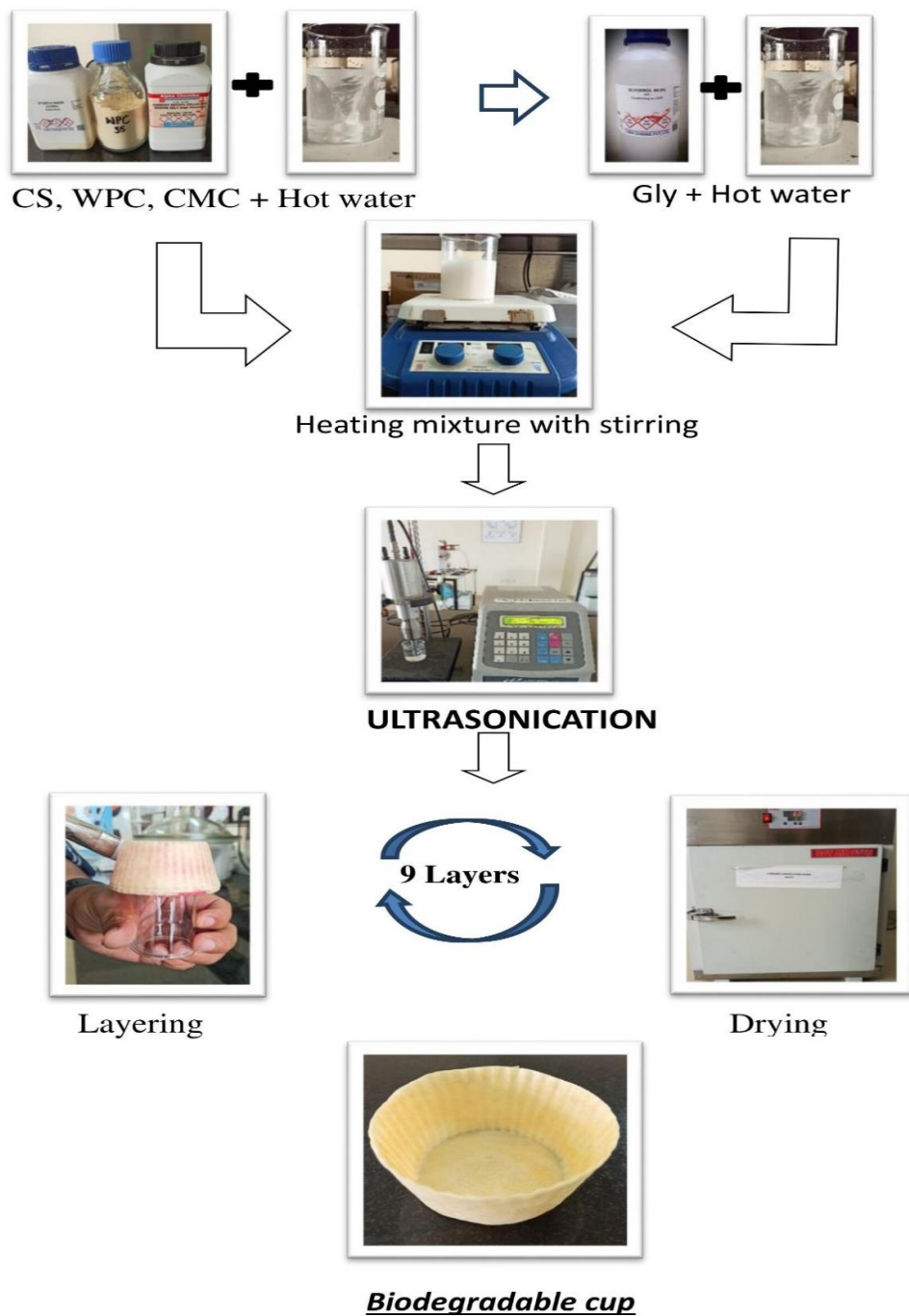


Figure 1: Visual representation of method for development of biodegradable cup

3.4 Opacity

Opacity of cup samples are calculated according to reference method of Ren et al.,[9], opacity is measured using spectrophotometer, cup sample were cut down into rectangular pieces, put in the sample into cuvette, such that it covers the width and length of cuvette, blank cuvette was taken as reference. The opacity was calculated as

$$\text{Opacity} = \frac{\text{Abs}_{600}}{X}$$

Where Abs₆₀₀ is absorbance at 600 nm and X is the thickness of the relevant sample, respectively.

3.5 Density

The composite cup sample was divided into pieces of varying sizes (s). A thickness gauge was used to measure the thickness (d), which was then calculated as the average of four points that were separated by 1 m and one point that was located in the middle of the specimen. With a resolution of 0.1 mg, an electronic scale was used to calculate the mass (m), the specimen area (s), and the film density using the equation shown below. Prior to testing, the samples were kept at a constant temperature of 23°C and 50% humidity. To obtain the mean values, we repeated the aforementioned procedures three times for each cup specimen (R. Li et al., 2019).

$$\rho = \frac{m}{s \times d}$$

Where,

ρ = density

m = the mass of specimen,

s = the surface area

d = the thickness of the specimen

3.6 Solubility

The method described by Romero-Bastida et al., (Romero-Bastida et al., 2005) was used to assess the solubility of the cup sample in water. Stripes of biodegradable cup measuring 20 mm by 20 mm were cut from the films (n = 3). The materials were dried for 24 hours at 105 °C to establish their initial dry weight (MAC India). Following the initial weighing, the samples were slowly stirred for an hour in a flask containing 80 ml of distilled water at 25°C. After being removed, the larger samples were dried at 60 °C until they attained a constant weight. A percentage loss of weight in an hour is used to measure weight loss. The solubility was calculated using the equation below;

$$\text{Solubility}(\%) = \frac{X_i - X_f}{X_i} \times 100$$

Where,

X_i = Initial weight of dried sample

X_f = Final weight

3.6 Experiment design

The experimental design plan (Table 1) is derived from the Response Surface Methodology (RSM) in Design Expert 13.0 Software. The experiments were conducted with the values of two numerical factors in table 2 (Design criteria): amplitude, which has levels 0, 20, 40, and 60, and time variation, which has levels 0, 1, 2, 4, 8, and 16 minutes. The experiments have been conducted with the values of these two factors. Full factorial design that had 24 runs, 9 of which were used as controls, these 9 runs had no amplitude and were of duration 0 minutes. The RSM was conducted using the Optimal (combined) Design method. The average of triplicates was recorded in responses.

Table 1 : Experimental layout of input parameter and resultant output

Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4	Response 5
A: Amplitude	B: Time	Viscosity	Density	Solubility in water	Thickness	Opacity
%	min	cP	g/mm ²	%	mm	AU/mm
0	0	2326	0.0738007	24.5614	0.813	3.017
20	1	2214	0.0752241	31.0833	0.776	2.893
40	1	2118	0.0752241	31.8694	0.76	2.84
60	1	1854	0.097493	33.6122	0.718	2.88
20	2	1841	0.0817	33.6667	0.719	2.85
40	2	1667	0.0914634	35.012	0.697	2.81
60	2	1514	0.100596	36.2093	0.671	2.77
20	4	1259	0.0901074	34.0024	0.652	2.784
40	4	116	0.0993253	37.7273	0.667	2.79
60	4	946	0.108075	37.913	0.609	2.68
20	8	868	0.0978435	39.7778	0.608	2.67
40	8	739	0.111426	42.6531	0.617	2.59
60	8	668	0.11289	42.907	0.609	2.56
20	16	686	0.104852	42.3333	0.608	2.267
40	16	592	0.118304	42.8889	0.56	2.75
60	16	536	0.126748	42.0755	0.572	2.7

Table 2: Experiment parameters and design Criteria

Factor	Name	Unit	Minimum	Maximum
A	Amplitude	%	0	60
B	Time	min	0	16

3.7 Statistical analysis

A minimum of three replicates of the data are used to calculate the average. The data was statistically analysed using the SPSS 17.0 programme. Two-way analysis of variance (ANOVA) was used to analyse the experimental data, and values are shown as mean standard deviation in various tables. The significance level for differences was 90% ($p < 0.1$).

Result & discussion

The level of amplitude and the duration of the ultrasonication are the two variables used in ultrasonic treatment. On the basis of earlier research and pilot experiments, the level of amplitude and duration were chosen. For time periods of 1, 2, 4, 8, and 16 minutes, amplitude levels of 20%, 40%, and 60% were selected to test the effects of ultrasonication on the characteristics of biodegradable cups.

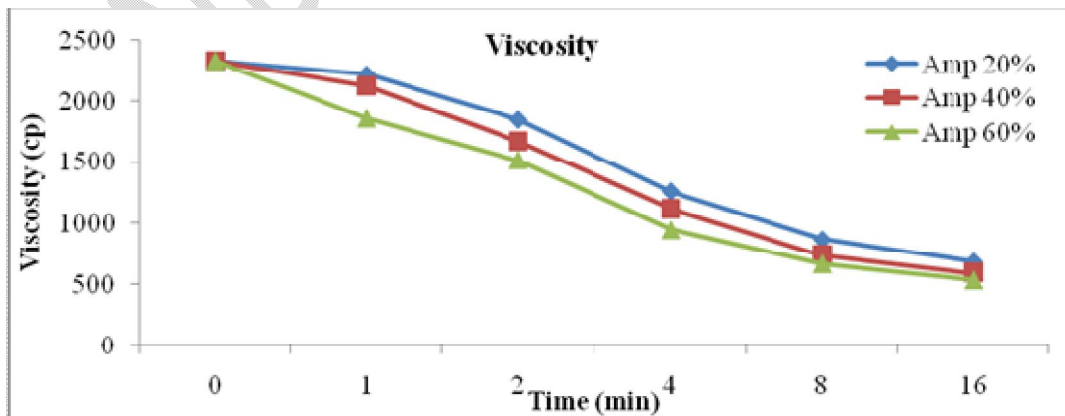
4.1 Viscosity

Table 1 presented viscosity values for ultrasonicated and non-treated casting solution samples. The non-treated or control sample found to be highest in viscosity value i.e. 2326 ± 14.8 cp. Viscosity of the casting solution decreased with rise in ultrasonication treatment. The viscosity of control sample was 2326 ± 14.8 cp. The Response surface plot figure 4(a) showing the impact of amplitude and time on viscosity of casting solution. Viscosity of decreased up to 686 ± 28.70 , 592 ± 26.98 cp for 20 and 40 % for 16 min. The least viscosity was observed with 60% and 16 min which was 536 ± 12.32 cp. The resulting 3D surface graph slopes downward as both time and amplitude increase, indicating this decrease (Fig 4a). The collapse of cavitation bubbles, causes severe heating in the remaining bubbles and significant mechanical consequences. This effect should cause fewer mobile polymers to break into tiny fragments (Kardos & Luche, 2001). It is well known that the starch dispersion needs to be heated before casting or spraying a starch-based film. When heated with a lot of water, the process of gelatinization causes starch granules to transform from being ordered to being chaotic (Lionetto et al., 2006). Gelatinization leads to elevate the viscosity significantly. Additionally, the gelatinized starch suspension contains a non-solubilized component known as "ghost" that makes it challenging to create a strong film using the suspension. Viscosity of casting solution is important in determining the further characteristic in developing cup. Viscosity assessment of all the sample including control sample has been done. According to the observations made about the viscosity of the casting solution, the values of viscosity gradually decrease when the amount of ultrasonication is increased. The

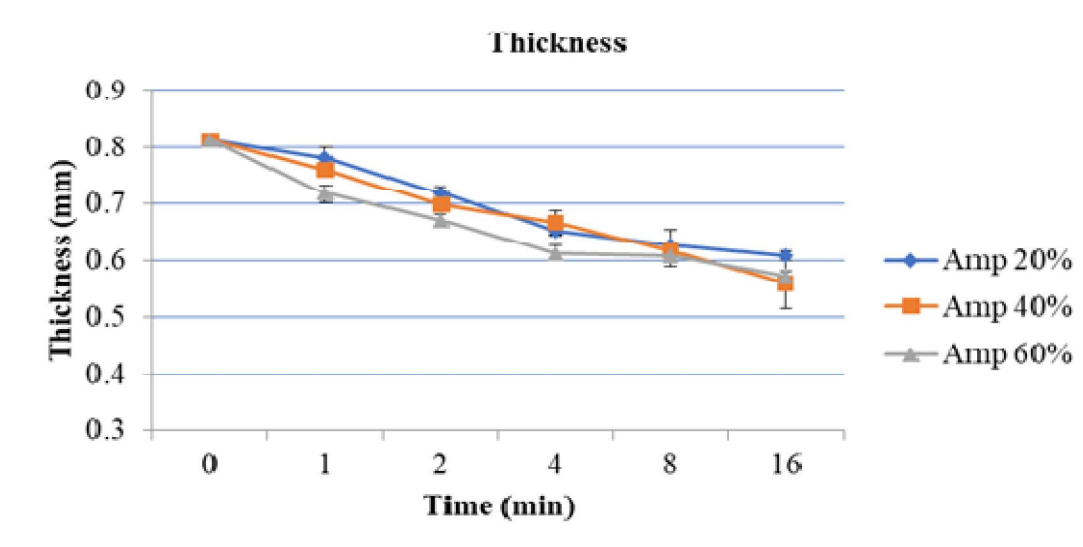
drop curve for viscosity value is depicted in Fig. 2(a) for various amplitude levels and time intervals. All amplitude levels, 20, 40, and 60%, experience a steady decrease in viscosity over time. The same behaviour was noted by (Cheng et al., 2010), who showed that ultrasound-induced decreases in the viscosity of maize starch solution. Similar results demonstrated that ultrasonication reduced non-solubilized portions on waxy gelatinized maize starch (Iida et al., 2008; Seguchi et al., 1994) and wheat starch (Seguchi et al., 1994). The production of ghost elements, which occurs when cross-linkages between polymers, primarily amylose/amylopectin, are disrupted by strong mechanical impact and heat from power ultrasonication, may be the cause of this phenomenon (Debet & Gidley, 2007). In addition, similar results have been reported for starch protein complex, it was observed there is decrease in viscosity value for whey protein concentrate-pectin complex suspension through ultrasonication treatment. Also, in another experiment (Zisu et al., 2010) reported protein suspension viscosity is reduced by particle size reduction through ultrasonication. Ultrasonication cause the reduction in size of complex by the virtue of cavitation, turbulence and shear force which cause decreased viscosity value. All the sample examination has been performed in triplicate and table shows the average value.

4.2 Thickness

A digital Mitutoyo micrometre was used to measure the sample's thickness throughout. Control sample found thickest among all the sample. Thickness of the control sample was 0.813 ± 0.023 mm. As the ultrasonication intensity increased, the thickness of the ultrasonically induced sample significantly decreased. The thickness decreased up to 0.608 ± 0.016 mm, 0.56 ± 0.081 mm and 0.572 ± 0.016 mm for 20, 40 and 60% amplitude levels for the time duration of 16 minutes. Table 1 depicts the thickness values of the all the sample.



(a)



(b)

Fig. 2: Viscosity (a) and thickness (b) values of ultrasonicated and non-ultrasonicated samples

Figure 4(c) illustrates a response surface plot depicting the influence of amplitude and time on the thickness of the biodegradable cup sample. The corresponding 3D surface graph slopes downward with time, with lower thickness observed at higher amplitude regions. The film's thickness affects or determines a variety of film properties, including opacity and water vapour permeability. Given that the sample produced a more compact and dense structure after being ultrasonically processed, the decrease in thickness makes sense. Also, the viscosity of casting suspension decreases as amplitude and duration of treatment increased. Due to reduced viscosity of casting suspension, thickness of every single layer reduces which led to overall decrease in thickness of cup formed. The thickness of control or non-ultrasonicated sample was 0.813 mm, which is less than all the ultrasonicated sample. The figure for thickness value vs time for various amplitude levels is shown in Fig. 2(b). The thickness of ultrasonicated sample ranges in between 0.56 to 0.781 mm. the similar finding has been observed on rice starch and chitosan-based film, film thickness of ultrasonication treated film was lesser than non-sonicated sample (Brodnjak & Todorova, 2018). Also, in whey protein-based film similar behaviour was found on ultrasonication thickness of film get reduced (Cruz-Diaz et al., 2019)

Table 3: Viscosity and thickness of ultrasonicated and non-treated biodegradable cup samples

Parameters	Time (min)	Amplitude (%)			
		0%	20%	40%	60%
Viscosity (cp)	0	2326 ± 14.8 ^{qd}			
	1		2214 ± 30.59 ^{pc}	2118.67 ± 65 ^{pb}	1854 ± 27.27 ^{pa}
	2		1841.67 ± 25 ^{oc}	1667 ± 28.80 ^{ob}	1514.67 ± 12.5 ^{oa}
	4		1259 ± 32.89 ^{nc}	1116.67 ± 23.32 ^{nb}	946.67 ± 135 ^{na}
	8		868 ± 36.76 ^{mc}	739.67 ± 95 ^{mb}	668 ± 10.19 ^{ma}
	16		686 ± 28.70 ^{lc}	592 ± 26.98 ^{lb}	536 ± 12.32 ^{la}
Thickness (mm)	0	0.813 ± 0.023 ^{qd}			
	1		0.776 ± 0.035 ^{pc}	0.76 ± 0.021 ^{pb}	0.718 ± 0.028 ^{pa}
	2		0.719 ± 0.021 ^{oc}	0.697 ± 0.029 ^{ob}	0.671 ± 0.014 ^{oa}
	4		0.652 ± 0.014 ^{nc}	0.667 ± 0.037 ^{nb}	0.613 ± 0.024 ^{na}
	8		0.626 ± 0.049 ^{cm}	0.617 ± 0.024 ^{mb}	0.609 ± 0.035 ^{ma}
	16		0.608 ± 0.016 ^{lc}	0.56 ± 0.081 ^{lb}	0.572 ± 0.016 ^{la}

Values are the average and standard deviation with n=3, n=6 (thickness). The average is statistically different (p<0.1) when it is followed by different superscripts (a, b, c, and d in the row for amplitude variation and l,m,n,o,p,q in the column for time variations).

4.3 Standability of formed cup

Standability of cup defines the ability of the formed cup to stand without any external support. Which shows the capability of polymer matrix that it is enough strong to remain erected in as such form without any support, all the cup formed sonicated and non-sonicated all are found stand able. The volumetric capacity of the cup found was 55± 0.5 ml.

4.7 Density

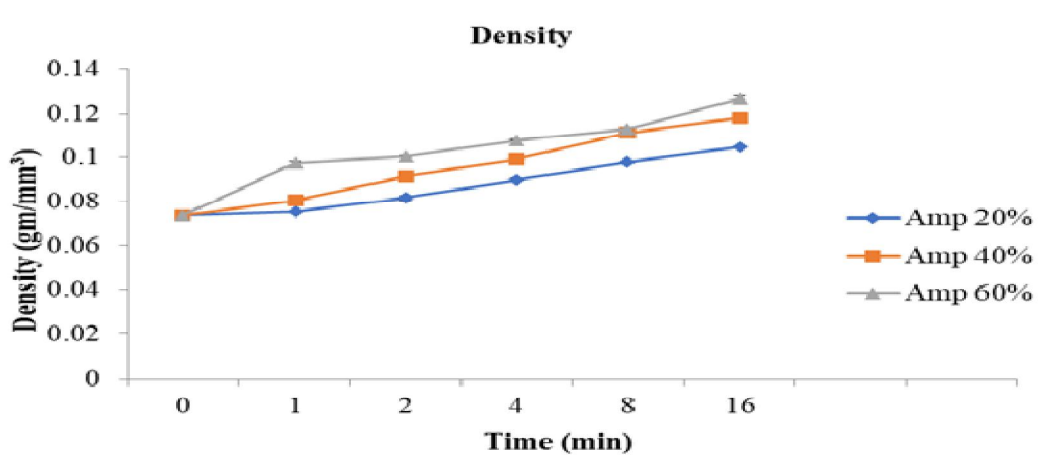
The density of the control sample was least for the 0.0738 ± 0.002 gm/mm³. As the amplitude intensity and time duration increased the density also increased significantly. Figure 4(b) displays a response surface plot that demonstrates the influence of amplitude and time on the density of the biodegradable cup samples. The maximum density observed

for 20 % and 40% at 16 min of time duration which was 0.1049 ± 0.0018 and 0.1183 ± 0.0019 gm/mm³. The peak density achieved was with amplitude level of 60 % and 16 min which is 0.1267 ± 0.0002 gm/mm³. The 3D surface graph for density displays peaks and troughs, but the overall trend is an upward slope, especially pronounced at higher amplitudes. Table 2 depicts the values for density of all the sample.

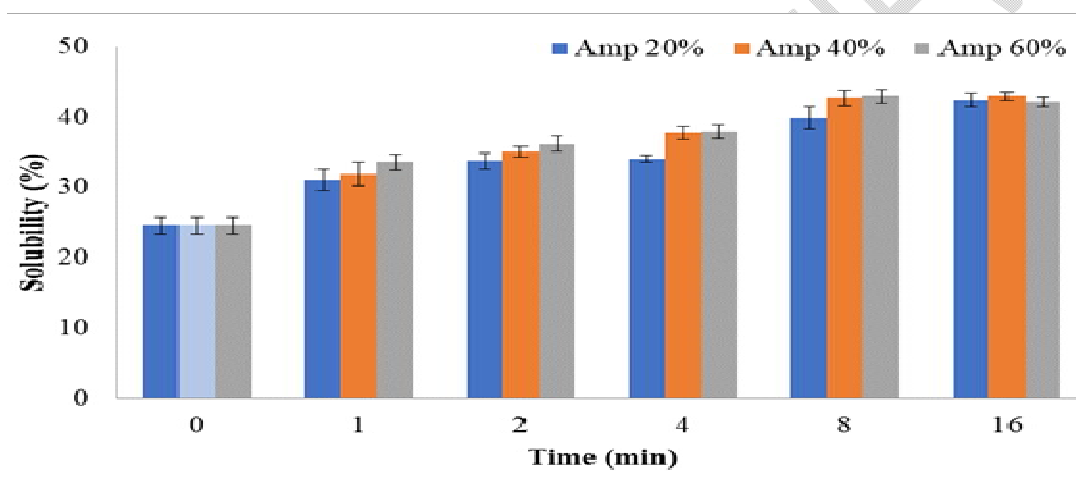
The density of composite material is an indicator of compactness in the film structure as it characterizes the intermolecular connections generated within the components. The weight and thickness of biodegradable film are proportionate to its density, which eventually corresponds to the thickness of the cup material, because the samples' cross-sectional areas were equal. The aforementioned growth pattern in density value could have occurred as a result of ultrasonic vibrations causing a denser network among the composite material's ingredients. The elimination of void air vacuoles may also result in an increase in density as has been confirmed with SEM images (Deshmukh et al., 2022). The increase in numerical value of density for composite cup material is aided by the lowered thickness of the cup material. Another possible explanation is that after ultrasonication, the increased interaction of WPC with glycerol causes a rise in the density of the resultant material, as described on (Abhishaben et al., 2019) plot for density values against time values for different amplitude level. All samples' triplicates were used to reach the result.

4.8 Solubility in Distilled water

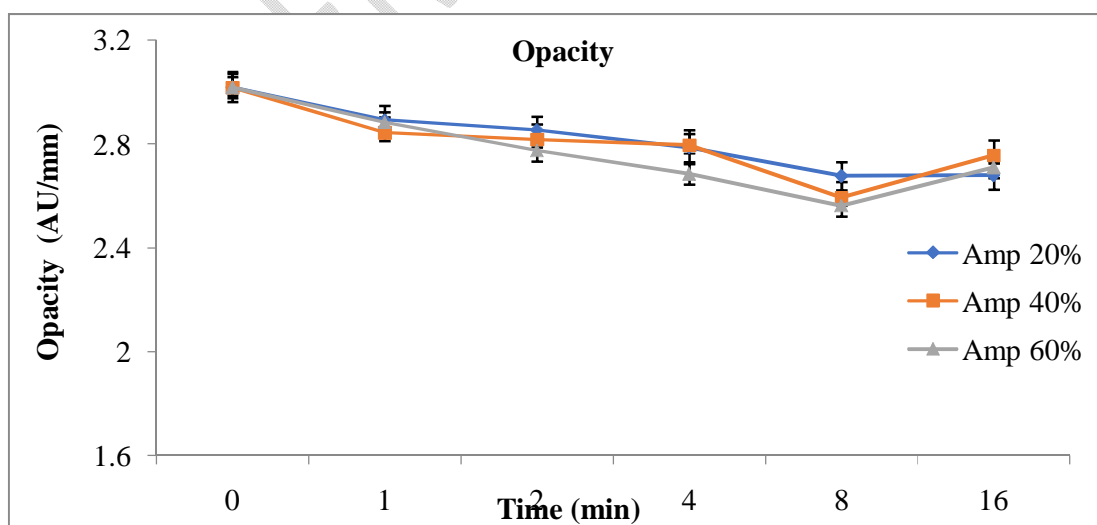
The solubility of control biodegradable cup sample was 24.5 % and the maximum value for solubility 42.89% which is attained by sample 10 (Amplitude level 40% and time duration 16 min). Solubility % of biodegradable cup sample increases for 20, 40 and 60 % amplitude level, the solubility percentage for 1 min samples 31.083 ± 1.516 , 31.869 ± 1.632 and 33.612 ± 1.121 % respectively which is increased up to 42.333 ± 0.99 , 42.889 ± 0.59 and 42.075 ± 0.691 % for respective amplitude level. Figure 4(d) illustrates the influence of amplitude and time on the solubility of the biodegradable cup sample through a response surface plot. The 3D surface graph for solubility in water shows an upward slope, illustrating the increase in solubility as both factors rise.



(a)



(b)



(c)

Fig. 3.: Density (a), solubility in water(b) and opacity(c) of ultrasonication treated and non-treated biodegradable cup samples

The ability of starch-based films to dissolve in water is an essential characteristic. For some applications, water insolubility may be necessary in order to increase product dependability, moisture barrier properties, and shelf life. However, in some circumstances, preceding product usage, the water solubility of films may be advantageous for food encapsulation, for instance. any additional chemicals or additives (Bertuzzi et al., 2007). The depolymerization of macromolecules brought on by the high pressure associated with cavitation bubble collapse leads to severe heating in the remaining bubbles and has significant mechanical effects. Less mobile polymers should fragment more as a result of this mechanism (Kardos & Luche, 2001). The solubility of all the sample has been examined to check the effect of ultrasonication on solubility of composite cup material. The result obtained shows that with the increase of ultrasonication intensity that is amplitude level and time the solubility of composite cup material increases. The solubility of non-ultrasonicated was found to be least among all the ultrasonicated sample. The depolymerization and structural breakdown of starch granules brought on by the oxidation process is the reason of the aforementioned rise in solubility percentage of sample with increase in ultrasonic amplitude and time duration (Adebowale et al., 2002). Oxidation of starch granule increases the carbonyl and carboxyl group which weaken the structure of starch granule by degrading the amylose molecule into short chains which is soluble in water(Uthumporn et al., 2010).

After starch has been gelatinized, the inner amylopectin and amylose-containing non-solubilized remain (Han & Hamaker, 2002). Rise in solubility can be further explained by the fact stated that the ghost material is destroyed after ultrasonication which led to increased number free mobile polymer which are ready for hydration reaction. The ghost-forming polymer may be completely hydrated and cause solubility in water(Cheng et al., 2010).

The solubility of solely maize starch-based film can reach up to 100% after ultrasonication of 15 min at amplitude level of 20%(Cheng et al., 2010). The added CMC in composite material form matrix with starch and resulting in relatively less solubility in water(Ghanbarzadeh et al., 2010). Furthermore, in a study of whey protein suspension, due to the protective effect of lactose present during pressure treatment by ultrasonication solubility of wpc suspension not change significantly(Jambrak et al., 2008). Fig. 3(b) shows the plot for solubility % of all the sample of different amplitude and time duration.

4.11 Opacity

Opacity of the packaging material is of prime importance as it determine the product visibility in the package. The opacity of control sample was 3.0171 ± 0.02 which was decreased to the minimum level of 2.56 ± 0.38 due to ultrasonication. Figure 4(e) illustrates

the influence of amplitude and time on the solubility of the biodegradable cup sample through a response surface plot. The 3D surface graph for opacity presents a slight downward trend, more evident over time than with amplitude changes. The least value was attained by ultrasonication treatment of amplitude 60% for the duration of 8 min. However, the opacity values for cup sample with 40 and 60% at 16 min were slightly increased with time i.e., 2.75 ± 0.316 & 2.70 ± 0.316 respectively. These might be because the dense structure obstructs the light transmission path. The table 2 displays the opacity values for all the cup sample.

The outcome from the test suggests that the opacity of cup sample decreases as the ultrasonication amplitude and duration increases. The ultrasonication cause the maximum decrease in opacity value by 15%. the decreasing trend in opacity values was explained by (Cheng et al., 2010) as the high amplitude causes the thorough destruction of the starch ghost and also removes the air bubbles in the film which increase the transparency of the film which ultimately decreases the opacity of film (Cheng et al., 2010). This suggests that the light transmission through the cup sample made from ultrasonic treated CS/WPC/CMC/GLY composite suspension is significantly better than via native corn starch film.

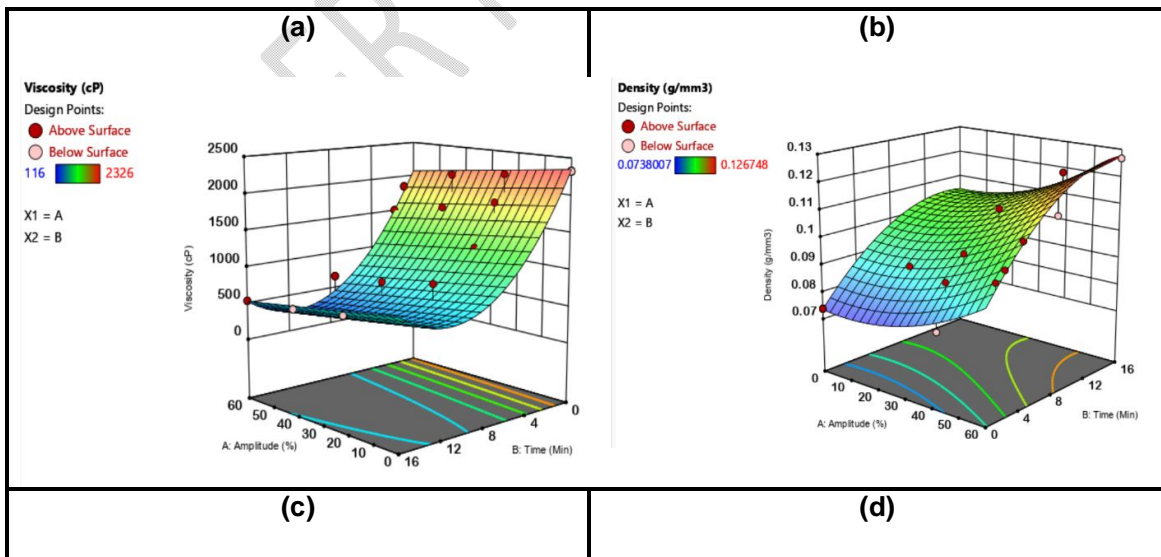
Table 4: Solubility, density and opacity of ultrasonicated and non-treated biodegradable cup samples

Parameters	Time (min)	Amplitude (%)			
		0%	20%	40%	60%
Solubility (%)	0	24.561 ± 1.166 ^{la}			
	1		31.083 ± 1.516 ^{mb}	31.869 ± 1.632 ^{mc}	33.612 ± 1.121 ^{mc}
	2		33.667 ± 1.09 ^{nb}	35.012 ± 0.792 ^{nc}	36.209 ± 1.152 ^{nc}
	4		34.002 ± 0.507 ^{ob}	37.727 ± 0.92 ^{oc}	37.913 ± 1.015 ^{oc}
	8		39.778 ± 1.612 ^{pb}	42.653 ± 1.135 ^{pc}	42.907 ± 1.01 ^{pc}
	16		42.333 ± 0.99 ^{pb}	42.889 ± 0.59 ^{pc}	42.075 ± 0.691 ^{pc}
Density (gm/mm3)	0	0.0738 ± 0.002 ^{la}			
	1		0.0752 ± 0.0015 ^{mb}	0.0806 ± 0.0006 ^{mc}	0.0975 ± 0.0007 ^{md}
	2		0.0817 ± 0.0006 ^{nb}	0.0915 ± 0.0015 ^{nc}	0.1006 ± 0.0020 nd

	4		0.0901 0.0003 ^{ob}	±	0.0993 0.0011 ^{oc}	±	0.1081 0.0013 ^{od}	±
	8		0.0978 0.0001 ^{pb}	±	0.1114 0.0009 ^{pc}	±	0.1129 0.0007 ^{pd}	±
	16		0.1049 0.0018 ^{qb}	±	0.1183 0.0019 ^{qc}	±	0.1267 0.0002 ^{qd}	±
Opacity (AU/mm)	0	3.017 0.02 ^{qd}		±				
	1		2.893 0.023 ^{pc}	±	2.84 ± 0.31 ^{pb}		2.88 ± 0.31 ^{pa}	
	2		2.85 ± 0.04 ^{oc}		2.81 ± 0.32 ^{ob}		2.77 ± 0.32 ^{oa}	
	4		2.784 ± 0.22 ^{nc}		2.79 ± 0.34 ^{nb}		2.68 ± 0.34 ^{na}	
	8		2.67 ± 0.26 ^{lc}		2.59 ± 0.38 ^{lb}		2.56 ± 0.38 ^{la}	
	16		2.267 ± 0.13 ^{mc}		2.75 ± 0.316 ^{mb}		2.70 ± 0.316 ^{ma}	

Values are the average of triplicates ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statistically different ($p < 0.1$)

Control sample light transmission was lower. The most likely explanation for this is that imperfections, such as clumps of partially gelatinized starch, may obstruct the transmission of light (X. Li et al., 2015) and that is removed by ultrasonication cause in reduction of opacity value. Furthermore, the presence of CMC in the starch-based composite enhances the transmission of light there by reduces the opacity (Abdorrezza et al., 2011; Battezzore et al., 2015).



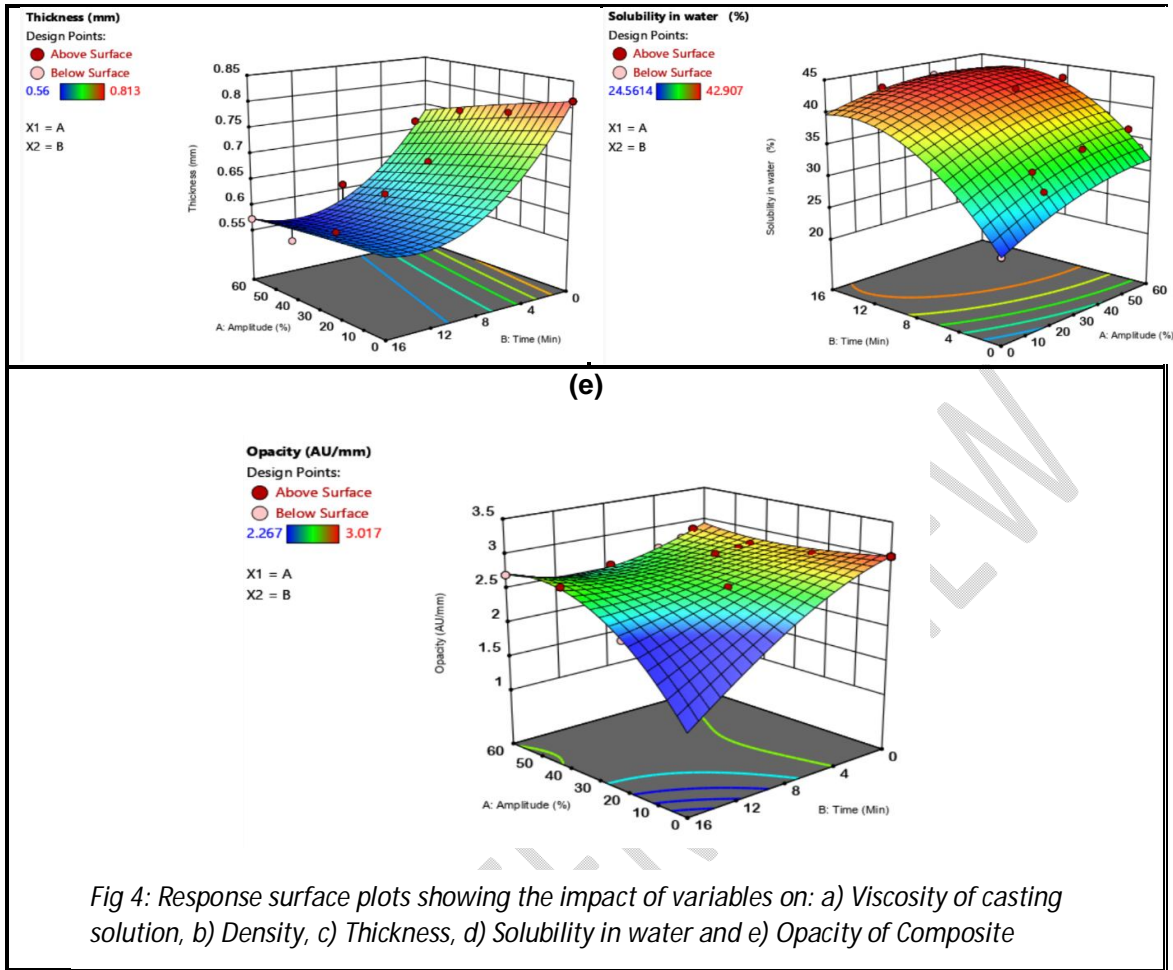


Fig 4: Response surface plots showing the impact of variables on: a) Viscosity of casting solution, b) Density, c) Thickness, d) Solubility in water and e) Opacity of Composite

4.12 Quadratic model Equations

After analyzing the experimental data, regression equations were formulated and presented below. Additionally, the Predicted R^2 closely aligns with the Adjusted R^2 , with a difference of less than 0.2 depicted in Table 5, indicating reasonable agreement. The Adequate Precision metric, which assesses the signal-to-noise ratio, reveals a ratio greater than 4, signifying a satisfactory signal. This model offers potential for navigating the design space effectively. Furthermore, the p-value associated with the regression model is less than 0.05, indicating that the model is significant at the 95% confidence level.

$$\text{Viscosity} = 2347.3 + (-4.64735) \times A + (-305.004) \times B + -0.0458915 \times AB + 0.0012366 \times A^2 + 13.203 \times B^2$$

$$\text{Thickness} = 0.613622 + (-0.00377914) \times A + (0.00485272) \times B + (-0.0194412) \times AB + (0.00216653) \times A^2 + (0.0952147) \times B^2 + 0.052621 \times A^2B + (-0.0364023) \times AB^2 + (-0.00382681) \times A^3 + -0.134903 \times B^3$$

$$\text{Density} = 0.0735375 + -0.000135968 \times A + 0.00445466 \times B + 6.10849e-06 \times AB + 6.93473e-06 \times A^2 + (-0.000155444) \times B^2$$

$$\text{Solubility in water} = 41.2931 + 2.57143 \times A + 6.36896 \times B + (-1.19777) \times AB + (-1.58064) \times A^2 + (-5.00949) \times B^2$$

Table 5: Regression co-efficient of viscosity, density, thickness and solubility

Regression co-efficient	Viscosity	Density	Thickness	Solubility in water
Adjusted R ²	0.8697	0.9628	0.9922	0.9916
Predicted R ²	0.8399	0.9242	0.8924	0.9861
R ²	0.8980	0.9709	0.9953	0.9934
Adequate precision	13.7324	32.4786	46.7617	55.2853

Conclusion

This study explored the impact of ultrasonication treatment on biodegradable cup production, focusing on its influence on various cup properties such as viscosity, thickness, solubility, opacity, and density. Ultrasonication treatment notably reduced the viscosity of the casting solution, resulting in thinner cups. For instance, viscosity decreased from 2326 ± 14.8 to 536 ± 12.32 cP following treatment. The viscosity reduction ranged from 20-30% with increasing intensity and duration of ultrasonication, leading to a thickness reduction in cups by up to 31% and denser cups with less material, bolstering their suitability for biodegradable packaging. The solubility of cups notably increased with higher ultrasonication intensity, while density and opacity were influenced by ultrasonication parameters. For instance, the solubility of the control sample increased from $24.561 \pm 1.166\%$ to $42.889 \pm 0.59\%$ post-treatment. Despite a notable increase in solubility in water, up to 40%, which may present challenges for packaging, it offers advantages for specific applications such as food encapsulation. Moreover, ultrasonication treatment decreased cup opacity by up to 35%, enhancing material transparency. These findings underscore ultrasonication's potential to enhance biodegradable packaging materials, possibly broadening their utility in food technology and environmental contexts.

Visual representations through 3D surface response graphs vividly depicted trends for each variable, revealing the intricate interplay between amplitude and time on measured responses. Notably, viscosity and thickness exhibited noticeable decreases, while solubility in water increased. Density showed an upward trend with increasing complexity, and opacity slightly decreased with rising factors. These findings highlight ultrasonication as a promising technique to optimize fabrication processes and enhance the physical characteristics of biodegradable cups, paving the way for further exploration in sustainable packaging solutions.

REFERENCE

1. Abdorreza, M. N., Cheng, L. H., & Karim, A. A. (2011). Effects of plasticizers on thermal properties and heat sealability of sago starch films. *Food Hydrocolloids*, 25(1), 56–60. <https://doi.org/10.1016/j.foodhyd.2010.05.005>
2. Abhishaben, M. S., Chandegara, V. K., Jithender, B., & Pankajkumar, M. S. (2019). Whey Protein Isolate based Biodegradable Food Packaging Film as affected by Protein to Glycerol Ratio, pH and Sonication Amplitude. *International Journal of Current Microbiology and Applied Sciences*, 8(03), 895–909. <https://doi.org/10.20546/ijcmas.2019.803.107>
3. Abral, H., Atmajaya, A., Mahardika, M., Hafizulhaq, F., Kadriadi, Handayani, D., Sapuan, S. M., & Ilyas, R. A. (2020). Effect of ultrasonication duration of polyvinyl alcohol (PVA) gel on characterizations of PVA film. *Journal of Materials Research and Technology*, 9(2), 2477–2486. <https://doi.org/10.1016/j.jmrt.2019.12.078>
4. Adebowale, K. O., Afolabi, T. A., & Lawal, O. S. (2002). Isolation, chemical modification and physicochemical characterisation of Bambarra groundnut (*Voandzeia subterranean*) starch and flour. *Food Chemistry*, 78, 305–311.
5. Battezzore, D., Bocchini, S., Nicola, G., Martini, E., & Frache, A. (2015). Isosorbide, a green plasticizer for thermoplastic starch that does not retrograde. *Carbohydrate Polymers*, 119, 78–84. <https://doi.org/10.1016/j.carbpol.2014.11.030>
6. Bertuzzi, M. A., Armada, M., & Gottifredi, J. C. (2007). Physicochemical characterization of starch based films. *Journal of Food Engineering*, 82(1), 17–25. <https://doi.org/10.1016/j.jfoodeng.2006.12.016>
7. Brodnjak, V., & Todorova, D. (2018). CHITOSAN AND RICE STARCH FILMS AS PACKAGING MATERIALS. *Proceedings of 9th International Symposium on Graphic Engineering and Design*, 275–280. <https://doi.org/10.24867/GRID-2018-p34>

8. Cheng, W., Chen, J., Liu, D., Ye, X., & Ke, F. (2010). Impact of ultrasonic treatment on properties of starch film-forming dispersion and the resulting films. *Carbohydrate Polymers*, 81(3), 707–711. <https://doi.org/10.1016/j.carbpol.2010.03.043>
9. Cruz-Díaz, K., Cobos, Á., Fernández-Valle, M. E., Díaz, O., & Cambero, M. I. (2019). Characterization of edible films from whey proteins treated with heat, ultrasounds and/or transglutaminase. Application in cheese slices packaging. *Food Packaging and Shelf Life*, 22, 100397. <https://doi.org/10.1016/j.fpsl.2019.100397>
10. Debet, M. R., & Gidley, M. J. (2007). Why Do Gelatinized Starch Granules Not Dissolve Completely? Roles for Amylose, Protein, and Lipid in Granule “Ghost” Integrity. *Journal of Agricultural and Food Chemistry*, 55(12), 4752–4760. <https://doi.org/10.1021/jf070004o>
11. Deshmukh, A. K., Talwar, G., & Chandla, N. K. (2022). Effect of ultrasonication on mechanical properties, biodegradability, and morphological behavior of composite biodegradable cup. *Journal of Food Processing and Preservation*, 46(7). <https://doi.org/10.1111/jfpp.16706>
12. Fuentes-Alonso, S., & Martínez-Pañeda, E. (2020). *Fracture in distortion gradient plasticity* (arXiv:2008.0289). arXiv. <http://arxiv.org/abs/2008.00289>
13. Ghanbarzadeh, B., Almasi, H., & Entezami, A. A. (2010). Physical properties of edible modified starch/carboxymethyl cellulose films. *Innovative Food Science & Emerging Technologies*, 11(4), 697–702. <https://doi.org/10.1016/j.ifset.2010.06.001>
14. Han, X.-Z., & Hamaker, B. R. (2002). Association of Starch Granule Proteins with Starch Ghosts and Remnants Revealed by Confocal Laser Scanning Microscopy. *Cereal Chemistry Journal*, 79(6), 892–896. <https://doi.org/10.1094/CCHEM.2002.79.6.892>
15. Iida, Y., Tuziuti, T., Yasui, K., Towata, A., & Kozuka, T. (2008). Control of viscosity in starch and polysaccharide solutions with ultrasound after gelatinization. *Innovative Food Science & Emerging Technologies*, 9(2), 140–146. <https://doi.org/10.1016/j.ifset.2007.03.029>

16. Jambrak, A. R., Mason, T. J., Lelas, V., Herceg, Z., & Herceg, I. L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering*, *86*(2), 281–287.
<https://doi.org/10.1016/j.jfoodeng.2007.10.004>
17. Kardos, N., & Luche, J.-L. (2001). Sonochemistry of carbohydrate compounds. *Carbohydrate Research*, *332*(2), 115–131. [https://doi.org/10.1016/S0008-6215\(01\)00081-7](https://doi.org/10.1016/S0008-6215(01)00081-7)
18. Li, R., Wang, Y., Xu, J., Ahmed, S., & Liu, Y. (2019). Preparation and Characterization of Ultrasound Treated Polyvinyl Alcohol/Chitosan/DMC Antimicrobial Films. *Coatings*, *9*(9), 582. <https://doi.org/10.3390/coatings9090582>
19. Li, X., Ji, N., Qiu, C., Xia, M., Xiong, L., & Sun, Q. (2015). The effect of peanut protein nanoparticles on characteristics of protein- and starch-based nanocomposite films: A comparative study. *Industrial Crops and Products*, *77*, 565–574.
<https://doi.org/10.1016/j.indcrop.2015.09.026>
20. Lionetto, F., Maffezzoli, A., Ottenhof, M.-A., Farhat, I. A., & Mitchell, J. R. (2006). Ultrasonic investigation of wheat starch retrogradation. *Journal of Food Engineering*, *75*(2), 258–266. <https://doi.org/10.1016/j.jfoodeng.2005.04.015>
21. Muthuraj, R., Misra, M., & Mohanty, A. K. (2018). Biodegradable compatibilized polymer blends for packaging applications: A literature review. *Journal of Applied Polymer Science*, *135*(24), 45726. <https://doi.org/10.1002/app.45726>
22. Romero-Bastida, C. A., Bello-Pérez, L. A., García, M. A., Martino, M. N., Solorza-Feria, J., & Zaritzky, N. E. (2005). Physicochemical and microstructural characterization of films prepared by thermal and cold gelatinization from non-conventional sources of starches. *Carbohydrate Polymers*, *60*(2), 235–244.
<https://doi.org/10.1016/j.carbpol.2005.01.004>
23. Seguchi, M., Higasa, T., & Mori, T. (1994). Study of wheat starch structures by sonication treatment. *Cereal Chemistry*. *Cereal Chemistry*, *71*(6), 636–639.

24. Uthumporn, U., Zaidul, I. S. M., & Karim, A. A. (2010). Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes. *Food and Bioproducts Processing*, 88(1), 47–54. <https://doi.org/10.1016/j.fbp.2009.10.001>
25. Wang, L. Z., Liu, L., Holmes, J., Kerry, J. F., & Kerry, J. P. (2007). Assessment of film-forming potential and properties of protein and polysaccharide-based biopolymer films. *International Journal of Food Science & Technology*, 42(9), 1128–1138. <https://doi.org/10.1111/j.1365-2621.2006.01440.x>
26. Wu, F., Misra, M., & Mohanty, A. K. (2021). Challenges and new opportunities on barrier performance of biodegradable polymers for sustainable packaging. *Progress in Polymer Science*, 117, 101395. <https://doi.org/10.1016/j.progpolymsci.2021.101395>
27. Zhang, Y., Cui, L., Xu, H., Feng, X., Wang, B., Pukánszky, B., Mao, Z., & Sui, X. (2019). Poly(lactic acid)/cellulose nanocrystal composites via the Pickering emulsion approach: Rheological, thermal and mechanical properties. *International Journal of Biological Macromolecules*, 137, 197–204. <https://doi.org/10.1016/j.ijbiomac.2019.06.204>
28. Zhao, B., Zhang, Y., & Ren, H. (2020). Effects of microcrystalline cellulose surface modification on the mechanical and thermal properties of polylactic acid composite films. *Plastics, Rubber and Composites*, 49(10), 450–455. <https://doi.org/10.1080/14658011.2020.1801231>
29. Zisu, B., Bhaskaracharya, R., Kentish, S., & Ashokkumar, M. (2010). Ultrasonic processing of dairy systems in large scale reactors. *Ultrasonics Sonochemistry*, 17(6), 1075–1081. <https://doi.org/10.1016/j.ultsonch.2009.10.014>

UNDER PEER REVIEW