EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF GUAR GUM- ANTIOXIDANTS EMULSION COATINGS

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ABSTRACT

Coatings and films with different functional additives for the packaging of food commodities are appealing consumers due to their remarkable benefits. In the present research, total 27 emulsions (E1-E27) were formulated using guar gum (0.25, 0.5 and 0.75 %) with different antioxidants for instance garlic oil (0.125, 0.5 and 1%), α -tocopherol (0.2, 0.4 and 0.6 %) and basil oil (1, 2 and 3%). The emulsions, GGOE (guar gum-garlic-oil emulsion), GBOE (guar gum-basil oil emulsion), GATE (guar gum- α -tocopherol emulsion) were analyzed for physicochemical characteristics such as density, surface tension and antioxidant activities by four different methods such as DPPH, ABTS, FRAP and CUPRAC. Lowest surface tension was offered by E1 (0.25 % guar gum + 0.125% garlic essential oil) which improves spreadability and enhances the adhesion between solution and fruit's surface. E2 (0.25 % guar gum + 0.25% garlic essential oil), E11 (0.25 % guar gum + 2 % basil essential oil) and E19 (0.25% guar gum + 0.2% α -tocopherol) showed better results for density. DPPH and ABTS assays showed highest antioxidant activities possessed by E1 and E8 (0.75 % guar gum + 0.5% garlic essential oil) respectively. FRAP and CUPRAC assays showed maximum antioxidant activity by E27 (0.75% guar gum + 0.6% α -tocopherol) and E24 (0.5% guar gum + 0.6% α -tocopherol) respectively. The results suggested that the garlic oil emulsion acquired the best results followed by α -tocopherol emulsion, proved as efficient coatings in terms of physicochemical attributes.

KEYWORDS: Coating, Guar Gum, Antioxidants, Physicochemical Characteristics

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INTRODUCTION

After China, India is the second-largest producer of fruit and vegetables. However, number of different post-harvest events takes place while conveying of the produce from grower to consumer. Post-harvest losses directly impacts farmers, retailers and customers who pay higher prices due to large losses in the distribution chain. The degradation in quality of these commodities during transport from growing area to consumers is quite severe (Staff, 2018). Even though India is the principal producer of fruits and vegetables, the country suffers from an extreme degree of malnutrition as it is wasting a significant portion of farm produce owing to a poor system of the cold chain and 16 per cent of fruit and vegetables being wasted every year (Sharma, 2019). After harvesting, the biochemical changes in fresh fruits and vegetables leads to loss of moisture, exposure to pathogenic attack, loss of essential bioactive compounds occurs. To reduce these losses, numbers of chemical pesticides have been used by growers on field to prevent the deterioration commencement. On the other hand, owing to chemical nature of those pesticides, they might be harmful to human, animals and also become costly for farmers leads to their economic

loss. To compensate these issues, there is need of active packaging of these fresh commodities which should be convenient, bio-based, compatible and nonhazardous to living beings. Edible films and coatings are already in demand to fulfill the said requirements, attracting the growers and spreading awareness in society. Fresh fruits and vegetables are going through the packaging systems using biodegradable films and edible coatings which offer unbelievable benefits. Moreover, the emulsions can be reinforced with various additives such as antimicrobials, antioxidants, and many more functional ingredients to form composite films and coatings. To make the efficient coating emulsions, physicochemical analysis of the solution has the greater importance to fulfill the desired requirements. Therefore, current research was especially emphasis on the characterization of coating emulsions in terms of physicochemical analysis.

MATERIALS AND METHODS

Different antioxidant such as Garlic oil (0.125, 0.5 and 1%), α -tocopherol (0.2, 0.4 and 0.6 %) and Basil oil (1, 2 and 3%) with different guar gum concentrations (0.25, 0.5 and 0.75 %) were prepared. Each solution was added with 0.4% glycerol which acts as plasticizer. Total 27 emulsions were analyzed for various physicochemical properties. All chemicals and reagents used in this study were of analytical grade.

Table 1: Composition of Guar Gum-Antioxidant Emulsions

Guar gum (%)	Antioxidants Emulsions					
	Garlic Oil (%)	Emulsion	Basil Oil (%)	Emulsion	α -tocopherol (%)	Emulsion
0.25	0.125	E1	1	E10	0.2	E19
0.25	0.5	E2	2	E11	0.4	E20
0.25	1	E3	3	E12	0.6	E21
0.5	0.125	E4	1	E13	0.2	E22
0.5	0.5	E5	2	E14	0.4	E23
0.5	1	E6	3	E15	0.6	E24
0.75	0.125	E7	1	E16	0.2	E25
0.75	0.5	E8	2	E17	0.4	E26
0.75	1	E9	3	E18	0.6	E27

Total 27 emulsions were formulated using guar gum and antioxidants, each at different concentration (Table 1). After glycerol addition, they stirred on magnetic stirrer and subjected to analysis of various physicochemical properties.

Physicochemical Properties of Emulsions

Density

Density is defined as the mass per unit volume, expressed in g/ml. By observing the mass of liquid divided by its volume, the density of fluid (Emulsion) was calculated.

Surface Tension

Surface tension of liquids was analyzed by using Du Nouy Ring Tensiometer following the procedure given by Khanna et al., (2017). The Du Nouy ring method is the technique by which the surface of liquids can be measured. This technique was developed by French Physicist Pierre Lecomte du Nouy in 1925. It is one of the oldest four common methods available for determining the surface tension. In the Du Nouy ring method a platinum ring is used to measure surface tension. The ring is dipped completely into the liquid to be analyzed. It is then slowly withdrawn from the liquid until maximum force is attained and the reading can be noted from the Dial and Vernear, it is a circular dial consisting of a scale

from 0 -90 which coin-side with a vernier. The point coin-sides with the dial give the measurement of surface tension in Dynes /cm.

Antioxidant Activity

Four different methods such as DPPH (2,2-diphenyl-1-picryl-hydrazyl), ABTS(2,2'-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid), FRAP (Ferric ion reducing antioxidant power) and CUPRAC (Cupric Reducing Antioxidant Capacity) to accurately assess the antioxidant activities of emulsions, four distinct antioxidant activity measurements were performed.

DPPH Assay

The DPPH assay of the essential oil was carried out as previously described (Okoh et al., 2011). A solution of 0.135 Mm DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of the emulsion. The reaction mixture was vortexed thoroughly, left in the dark at 25 °C for 30 min and measured at 517 nm. The ability of the essential oil to scavenge DPPH radical was calculated as % inhibition by the following equation:

$$\% \text{ inhibition} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \times 100$$

Where Abs control is the absorbance of the DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + Emulsion.

ABTS Assay

The ABTS free radical assay given by Witayapan et al., (2007) was used. The pre-formed radical mono cation of ABTS was generated by oxidation of ABTS solution (7 mM) with 2.45 mM potassium persulfate solution in equal amount. The mixture was allowed to react for 12 h in the dark at 25 °C. 1 mL of the resulting solution was diluted in 60 mL of methanol to obtain an absorbance of 0.706 ± 0.001 at 734 nm. 1 mL of the ABTS radical cation solution was added to emulsions and absorbance measured at 734 nm. The percentage inhibition of ABTS radical by the oils was calculated using the equation described in the DPPH assay.

FRAP

This method is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} ions. At low pH, in the presence of TPTZ (Sigma Aldrich, India), ferric-tripyridyltriazine (Fe^{3+} TPTZ) complex is reduced to the ferrous (Fe^{2+} -TPTZ) form with the formation of an intense blue colour having an absorption maximum at 593nm. The method described by Benzie and strain (1996) was followed. 2.3mL of the FRAP reagent was mixed with 0.7 mL of the emulsion. The mixture was then incubated at 37°C for 30 min in the dark. The absorbance was measured at 593 nm against a blank having all the reagents excluding the sample using spectrophotometer. Increased absorbance of the reaction mixture indicates an increase of reduction capability. Trolox (Merck, India) was used as the standard. Standard curve of Trolox solution was prepared using the similar procedure from which the regression formula was derived. Results were expressed in μg of Trolox equivalents /ml of extract.

CUPRAC

Cupric ion reducing capacity was measured in accordance to the method of Apak et al., (2004). 1 ml 10 mM cupric chloride, 1 ml 7.5 mMneocuproine and 1 ml 1 M ammonium acetate buffer of pH 7 solutions were added to test tubes

containing 2 ml of distilled water. Emulsions were added to each test tube separately. These mixtures were incubated for half an hour at room temperature and measured against blank at 450 nm. Trolox was used as positive reference standard. Results were expressed in mMol/ml_trolox equivalent.

RESULTS AND DISCUSSIONS

Surface Tension

Table 2: Surface Tension of Emulsions

GGOE	Dyne/cm	GGOE	Dyne/cm	GATE	Dyne/cm
E1	46.63h	E10	46.13f	E19	54.20i
E2	47.70e	E11	49.20e	E20	56.40e
E3	48.90g	E12	46.20g	E21	57.10c
E4	49.30d	E13	51.13c	E22	54.30h
E5	47.33f	E14	50.43d	E23	56.76d
E6	47.60e	E15	52.60b	E24	57.33b
E7	51.66a	E16	53.46a	E25	55.63g
E8	52.33b	E17	53.50a	E26	55.70f
E9	52.43c	E18	50.43d	E27	63.53a
L.S.D.	0.0738	L.S.D.	0.0934	L.S.D.	0.066

The table 2 depicts the values of surface tension of emulsions in Dyne/cm. In case of all the emulsions, as the concentration of oil increased, level of surface tension also increased. These results were in accordance with Homayoonfal et al., (2014) who revealed that an increase in oil concentration increases the values of surface tension. The emulsions E1 (0.25 % guar gum + 0.125% garlic essential oil) has shown the least value of surface tension which is 46.63 Dynes/cm followed by E10 (0.25 % guar gum + 1% basil essential oil) and E19 (0.25 % guar gum + 0.2 % α -tocopherol) with the values of 46.13 Dynes/cm and 54.20 Dynes/cm respectively. These emulsions could offer improved wet ability and thus more efficient coating. It has been previously mentioned that the reduced values of surface tension improve the wet ability of the solution (Choi et al., 2002 and Ribeiro et al., 2007). In addition, it was also revealed as decreased surface tension of the liquid leads to improved spread ability and enhances the adhesion between solution and fruits surface (Moncayo et al., 2013).

Density

Table 3: Density of Emulsions

GGOE	g/ml	GBOE	g/ml	GATE	g/ml
E1	0.99d	E10	0.99d	E19	0.99d
E2	0.97f	E11	0.97c	E20	0.99d
E3	0.95e	E12	0.97c	E21	0.99d
E4	1.02c	E13	0.99d	E22	0.99d
E5	1.00b	E14	0.97c	E23	0.99d
E6	1.00a	E15	0.97c	E24	1.01c
E7	1.03b	E16	1.00a	E25	1.08a
E8	1.00a	E17	0.99d	E26	1.03b
E9	1.00a	E18	0.97c	E27	1.01c
L.S.D.	1E-9	L.S.D.	0.0066	L.S.D.	0.0198

Table 3 demonstrates the density of each emulsion in g/ml. Densities of all the emulsions were ranged from 0.96 g/ml to 1.03 g/ml. It was found that in all emulsions, the density of solution decreased as the oil concentration increased. The results are similar to the findings of Homayoonfal et al., (2014) who optimized density, surface tension

characterization of walnut oil nano-emulsion. They revealed that, density of solution decreased as the oil concentration increased.

Moreover it was found from the above table, as concentration of guar gum increases, thickness and density of solution increases. These results are in agreement with the statement given by Lindner et al., (2017) who told that when the thickness increases, the density of the solution also increases. On the other hand, from the previous trails of the present research it was found that emulsions containing more that 0.75% guar gum, leads to mould growth at ambient storage conditions. Therefore the low density emulsions with low thickness viz.E2 (0.25 % guar gum + 0.5% garlic essential oil), E11 (0.25 % guar gum + 2% basil essential oil) and E19 (0.25 % guar gum + 0.2 % α -tocopherol) showed better results.

Antioxidant Activity

DPPH Radical Scavenging Activity (%)

Table 4: Antioxidant Activity of Emulsions with DPPH Assay

Antioxidant Activity of Emulsions					
Emulsion	% inhibition of DPPH	Emulsion	% inhibition of DPPH	Emulsion	% inhibition of DPPH
E1	94.74a	E10	90.25a	E19	89.69a
E2	91.38b	E11	85.28d	E20	89.65a
E3	86.36d	E12	85.24d	E21	89.53a
E4	82.83f	E13	84.15e	E22	87.36b
E5	85.80e	E14	83.71f	E23	85.80b
E6	86.32d	E15	86.20c	E24	82.83c
E7	86.48d	E16	86.68b	E25	80.79d
E8	86.32d	E17	86.28c	E26	79.19d
E9	88.65c	E18	86.24c	E27	80.94d
L.S.D.	0.4058	L.S.D.	0.3606	L.S.D.	1.8156

Table 4 showed % inhibition of DPPH at the level of 94.74% (GGOE), 90.25% (GBOE) and 89.69 % (GATE) by the emulsions. It can be said that garlic oil emulsion possess highest antioxidant activity which was 94.74% (E1: 0.25 % guar gum + 0.125% garlic essential oil). Preliminary studies on the garlic essential oil mentioned its higher antioxidant activity. Shang et al., (2019) revealed that garlic exhibited stronger antioxidant activity evaluated by 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical scavenging assay, 2,2'-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay, and ferric ion reducing antioxidant power (FRAP) assay. Also, Lawrence et al., (2011) imparted from their study as garlic essential oil is effective in scavenging free radical and has the potential to be powerful antioxidant. Additionally, Ariviani et al., (2019) concluded as the incorporation of chitosan-garlic EO into beef meatballs edible coatings provide an alternative antioxidant-based functional food development, especially as the radical scavenger.

Antioxidant Activity of Emulsions with ABTS Assay**Table 5: ABTS Radical Scavenging Activity (%)**

Antioxidant Activity of Emulsions					
GGOE	% inhibition of ABTS	GBOE	% inhibition of ABTS	GATE	% inhibition of ABTS
E1	14.41c	E10	18.12d	E19	9.34f
E2	21.45c	E11	16.66e	E20	19.53bc
E3	17.32c	E12	15.82f	E21	17.46cde
E4	27.93bc	E13	20.28c	E22	13.17ef
E5	39.81b	E14	23.61a	E23	17.04cde
E6	19.48c	E15	17.98d	E24	31.92a
E7	87.04a	E16	21.27b	E25	22.20b
E8	96.80a	E17	14.36g	E26	18.87bcd
E9	95.25a	E18	13.28h	E27	14.27de
L.S.D.	13.773	L.S.D.	0.3941	L.S.D.	4.6357

Table 5 represents the ABTS radical scavenging activity (%) measured in terms of % inhibition of ABTS. It was ranged from 14.41 to 96.80 % by GGOE, from 13.28 to 23.61 % and from 9.34 to 31.92 % by GATE. The results demonstrate as % inhibition of ABTS was highest in GGOE (E8: 0.75 % guar gum + 0.5% garlic essential oil) which was 96.80%. The statement can be justified by the findings of researchers given for the results of DPPH assay which showed maximum antioxidant activity by GGOE.

Antioxidant Activity of Emulsions with FRAP Assay**Table 6: Antioxidant Activity of Emulsions with FRAP Assay**

Antioxidant Activity of Emulsions					
GGOE	µg/ml	GBOE	µg/ml	GATE	µg/ml
E1	42.05h	E10	45.84c	E19	40.03e
E2	43.57g	E11	42.96e	E20	41.23d
E3	44.48e	E12	53.32a	E21	41.78d
E4	43.87f	E13	42.20f	E22	42.65c
E5	45.64d	E14	43.22d	E23	42.32c
E6	51.70a	E15	53.42a	E24	53.46a
E7	48.37c	E16	43.42d	E25	53.34b
E8	49.02b	E17	43.27d	E26	53.23b
E9	49.02b	E18	49.73b	E27	53.54a
L.S.D.	0.2125	L.S.D.	0.2409	L.S.D.	0.2056

The findings of FRAP assay (Table 6) showed the antioxidant activity of GGOE ranged from 42.05 to 51.70 µMol TE/ml for GGOE, from 42.20 to 53.42 µmol TE/ml for GBOE and from 40.03 to 53.54 µmol TE/ml for GATE. The highest antioxidant activity showed by E27 (0.75% guar gum + 0.6% α -tocopherol) at the level of 53.54 µmol TE/ml in GATE. It may be due to radical scavenging nature of alpha tocopherol (Sachdeva et al., 2014).

Antioxidant Activity of Emulsions with CUPRAC Assay

Table 7: Antioxidant Activity of Emulsions with CUPRAC Assay

Antioxidant Activity of Emulsions					
GGOE	mMol/ml_trolox equivalent	GBOE	mMol/ml_trolox equivalent	GATE	mMol/ml_trolox equivalent
E1	2461.88c	E10	2821.69bc	E19	2531.08b
E2	2493.44c	E11	2392.44e	E20	2503.34b
E3	2550.25b	E12	2897.44ab	E21	2503.21b
E4	2638.63a	E13	2947.94a	E22	2615.45a
E5	2651.25a	E14	2607.07d	E23	2631.67a
E6	2259.87e	E15	2613.38d	E24	2657.21a
E7	2108.37f	E16	2695.44d	E25	2038.47d
E8	2259.87e	E17	2872.19abc	E26	2026.76d
E9	2354.56d	E18	2790.12c	E27	2323.34c
L.S.D.	46.366	L.S.D.	42.517	L.S.D.	42.356

In CUPRAC assay a similar trend was observed (Table 7); value for antioxidant activity varied from 2108.37 to 2651.25 mMol TE/ml in GGOE, from 2392.44 to 2947.94 mMol TE/ml in GBOE and from 2026.76 to 2657.21 mMol TE/ml in GATE. The highest antioxidant activity (2657.31 mMol TE/ml) showed by emulsion containing alpha tocopherol i.e. E24 (0.5% guar gum + 0.6% α -tocopherol). Overall values of in the CUPRAC assay were significantly ($p < 0.0001$) higher than FRAP values. High antioxidant activity in CUPRAC may be attributed due to the combined effect of flavonoids such as flavanoids like kaempferol, myricetin guar gum and the phenolic compounds such as β -resorcylic acid, followed by pyrogallol, gallic acid, rutin, protocatechuic acid, as well as quercetin present in garlic essential oil (Shang et al., 2019). The antioxidant potency of flavonoids is roughly proportional to the total number of -OH groups and is positively affected by the presence of an o-dihydroxy moiety in the B-ring (Apak et al., 2008).

CONCLUSIONS

The emulsions containing 0.25 % guar gum and 0.125% garlic oil has shown the least value of surface tension among all emulsions while emulsion containing 0.25 % guar gum and 0.5% garlic oil showed the lowest density and found better for further study. DPPH and ABTS assays showed maximum antioxidant activities possessed by emulsion containing 0.25 % guar gum and 0.125% garlic oil and emulsion containing 0.75 % guar gum + 0.5% garlic essential oil respectively. FRAP and CUPRAC assays showed maximum antioxidant activity by emulsion formulated with 0.75% guar gum + 0.6% α -tocopherol and emulsion made with 0.5% guar gum + 0.6% α -tocopherol respectively. The findings reveal that the emulsion of garlic oil and guar gum had obtained the best outcomes followed by the emulsion of α -tocopherol has proved to be effective physicochemical attributed coatings.

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