Characterization of semi-refined carrageenan film plasticized with glycerol incorporated with *Persicaria minor* extract as antioxidant additives

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Characterization of semi-refined carrageenan film plasticized with glycerol incorporated with *Persicaria minor* extract as antioxidant additives

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Abstract. Semi refined carrageenan (2%w/w) plasticized with 0.9% (w/w) glycerol (G) added with *Persicaria minor* (PM) extract and 0.4% (w/w) BHA as antioxidant additives for the development of active packaging films. The extraction of PM using 75% (v/v) ethanol: water ratio showed the highest polyphenol content with 176.80±4.37 Gallic Acid Equivalent (GAE)/L sample and 94.65±0.17% inhibition analyzed using DPPH antioxidant activity ($p<0.05$). The characterization of SRC-G based film containing 0.4, 1.0, 1.5 and 2.0% (w/w) PM and BHA were observed using FTIR, mechanical and physical properties of the treated films. FTIR spectrum band showed insight analysis of PM extract and BHA with carrageenan. SRC film plasticized with glycerol improved overall physical properties including thickness, barrier and flexibility and increased tensile strength and elongation at break compared to the SRC film only ($p<0.05$). The active films with PM extracts (2.0% w/w) exhibited good mechanical properties with tensile strength and elongation at break with 28.01±0.17 and 37.37±0.29 respectively ($p<0.05$). The addition of PM extract in SRC films increased the moisture content and opacity as proportionally to the concentration of PM extracts. Film treated with 2.0% PM showed lowest value of films solubility compare to all sample ($p<0.05$). Hence, the characterization measurement of SRC based films demonstrated great potential with natural extract formulation for the development of active film packaging for food products.

1. Introduction

The single use plastic in food packaging and storages received great attention towards the new polymers development with biodegradability properties. Chemical synthetic plastic which is practically non-biodegradable leads to an environmental concern and generates waste disposal problems cause pollution to the ocean and landfill. Biodegradable films offer alternative for packaging materials with competitive properties as conventional films in the market. Furthermore, the development of bioactive films containing biopolymer combined with antioxidant compound appears to be an interesting way not only provides the environmentally friendly packaging but also able to protect against oxidative reaction in the food [1].

Biopolymer are derived from many renewable and abundance agricultural resources consist of lipid, protein and polysaccharide. Polysaccharides such as starch, alginate, carrageenan, chitosan and pectin have excellent film forming characteristics of the biodegradable polymers and provide good barriers to gas, oils and lipids. Recently, semi-refined carrageenan (SRC) commonly use as biopolymers in the biodegradable products that can be discovered abundantly in some red seaweed families (*Rhodophyceae*). Addition of carrageenan based bio-polymer with natural plasticizers such as

glycerol exhibited low migration levels and low toxicity for the manufacturing of bio-based material which improves film flexibility during film handling, prevent pores and cracks in the polymeric matrix [2]. Previous study confirmed the addition of SRC plasticized with glycerol improves the mechanical and physical properties of the films [3]. In addition, natural plasticizers also play a role as an alternative additive to modify the polymer matrix that can act as a mass transport for the active agents in the active packaging films development [4].

The growing interest by potential buyers with healthier food options such as natural and no artificial additives showed promising discovery of natural antioxidants for food preservation. Active films formulation requires incorporation of active compounds such as natural antioxidants used in foods packaging are able to delay the oxidative deterioration in foods. The development active films could be an effective strategy to develop healthier food products without direct use synthetic preservative in foods. Many literatures have successfully performed the development active film incorporate natural antioxidants such as mulberry [5], rosemary [6], green tea [7] and etc. *Persicaria minor* (PM) is from the family Polygonaceae also locally known as daun kesum or laksa leaf. Previous literature reported that the PM leaves have high antioxidants that can help to reduce oxidative damage caused free radicals oxidation due to the leaf composition are riches with polyphenol compounds such as catechin, rutin, isorhamnetin, quercetin and kaempferol [8]. Recent study showed the development of active packaging film containing SRC plasticized with glycerol and incorporated with 0-2.0% w/w PM extract pronounced positive effect delaying the lipid oxidation in meat patties [9].

Hence, the potential of SRC plasticized with glycerol and incorporated with PM extract (SRC+G+PM) in delaying meat oxidation as food active films was successfully determined. However, there no studies done of the SRC+G with different concentration of PM extracts on the physical and mechanical and physical properties of the films. Thus, the objective is to determine the characteristic of active packaging film formulation SRC+G films incorporated with PM extracts as antioxidant additives using FTIR and the mechanical and physical properties through tensile strength, % elongation at break, thickness, opacity, water solubility and moisture content.

2. Material and Methods

2.1. Materials

Persicaria minor (PM) was bought from local market (Kuantan, Pahang). Semi-refined carrageenan (SRC) was bought from Taneka Sdn. Bhd. Kunak, Sabah. Reagents were used are ethanol (purity 96%), glycerol (purity 99%), butylated-hydroxyqanisole (BHA) as synthetic antioxidant were supplied by Sigma-Aldrich.

2.2. Extraction of Persicaria minor (PM)

PM was dried in the room temperature and finely grounded using a standard kitchen food processor (Panasonic dry mill blender). PM was extracted into three concentration of ethanol: water solvent (50%, 75% and 90% v/v) with 1:10 (w/v) dry weigh of ground plant. The extractions were performed for 4 days in the room temperature [10]. The solutions of PM extract were centrifuged at 10 °C and 30000 rpm. The extract was concentrated using rotary evaporator at temperature 40 °C to remove the ethanol in the solution. Finally, the PM extract was kept frozen at -20 °C until further used.

2.3. Determination of total Phenolic Content and Antioxidant activity

2.3.1. Total Phenolic Content (TPC).The Folin-Ciocalteu method was used to identify the total phenolic content with minor modification [11]. Method of Folin-Ciocalteu: The mixture of final concentration (v/v) was prepared with 90% ethanol of food simulant, 10% Folin reagent, 17% sodium carbonate solution and diluted with 790 µL MiliQ water accordingly. The mixture was measured its absorbance at 765 nm using UV-visible spectrophotometer. The results were reported as g of Gallic acid equivalents/L sample (g GAE/ L sample). The concentration Gallic Acid calibration curves obtaines as Y= 0.018X-0.0586 with R = 0.998.

2.3.2. DPPH Assays. The scavenging of DPPH radicals of PM method was prepared according Azman et al. with slight modifications [11]. Appropriate dilution of ethanol dissolved in 0.6 mM DPPH to enable the level of DPPH to be between 10 and 90% (v/v). A diluted aliquot, 0.025 ml sample (90% ethanol of food simulant) was dissolved in 0.975 ml of 0.6 mM DPPH. The mixture was put into the plastic cuvette and after 4 hours the absorbance was determined using UV-visible spectrophotometer at 585 nm. The results were expressed as percentage of inhibition of DPPH radical of PM concentration.

2.4. Preparation of semi-refined carrageenan films

Semi-refined carrageenan (SRC) films preparation were adapted by Farhan & Hani with slightly modification [3]. SRC powder was dissolved in miliQ water (2% w/w) and mixed at temperature of 60 °C and stirring continuously. Then, plasticizers glycerol (0.9% w/w) was added under continuous stirring heated up to 80 °C to obtain gelation. Then, the homogenous film solution was cooled until it reached a temperature of 50 °C prior to add PM extracts and BHA. The solutions were mixed and homogenized with Ultra Turrax T25 (IKA, Germany) at 1300 rpm for 20 min to obtain good dispersion and homogenized film solution. The final film solutions were casted on acrylic casting plate (20 cm × 3.5 cm) and dried for 24 hours at temperature 40±2 °C. Then, all samples were kept in desiccators at 50% relative humidity (RH). Table 1 showed the film samples prepared for this studies.

Table 1. The classification SRC based films. All samples are prepared in 2% w/w SRC.

Sample	Materials (% w/w)	Concentration of antioxidant (% w/w)
Control	SRC	0
SRC +G	SRC + 0.9%G	0
SRC + G + BHA0.4%	SRC+ 0.9%G + BHA0.4%	0.4
SRC + G + PM0.4%	SRC + 0.9%G + PM0.4%	0.4
SRC + G + PM1.0%	SRC + 0.9%G + PM1.0%	1.0
SRC + G + PM1.5%	SRC + 0.9%G + PM1.5%	1.5
SRC + G + PM2.0%	SRC + 0.9%G + PM2.0%	2.0

2.5. Characterization and mechanical properties of films

2.5.1. Fourier Transform Infrared (FTIR) Spectroscopy. Fourier-transform infrared (FTIR) spectroscopy was performed to observe the structural interaction between semi-refined carrageenan based film incorporated with PM extract. The films were placed in the beam and performed in the spectrum range of 400 – 4000 cm^{-1} at resolution 4 cm^{-1} by using FTIR spectrophotometer [12].

2.5.2. Tensile strength and elongation at break. The mechanical properties of the film samples were performed using a universal testing machine (AG-Xplus Series, Shimadzu, Japan). The cuts of film samples (10 × 1.5 cm) were clamped between tensile grips with a 50 mm/min crosshead speed setting. The tested film strips were equilibrated at 25 °C and 50% relative humidity (RH) in desiccators for 48 h prior to testing. Each sample and the measurement was carried out in triplicates. The following equation 1 and equation 2 were performed to calculate the tensile strength elongation at break of the films respectively:

$$TS(MPa) = \frac{F_{\max}}{\phi} \quad (1)$$

where F_{max} is the maximum load and ϕ is the cross-sectional area.

$$EAB(\%) = (\frac{\Delta_1}{l_0}) \times 100 \quad (2)$$

where Δ1 is the extension of film and l_0 is the film sample initial length.

2.6. Physical Properties

2.6.1. Thickness measurement. The film thickness was determined by using a micrometer (Mitutoyo Co., Tokyo, Japan). Five random reading were taken at diffrent position within the film samples and the average values were calculated [3].

2.6.2. Opacity measurement. The film opacity was measured to determine the transparency of the film. The films then were cut into a rectangular shape (3 cm x 0.3 cm) and placed in the spectrophotometer cell. The film opacity was determined at 600 nm using an UV spectrophotometer. The following equation has been performed to determine opacity value [13].

$$Opacity = \frac{Abs600}{b} \quad (3)$$

Where Abs 600 is the absorbance value at 600 nm and b is the film thickness (mm).

2.6.3. Solubility in water. The film samples were uniformly cut (2 cm × 2 cm) and the samples were dried at 100 °C in an oven for 24 hours. After that, the samples were weighed to determine initial dry weight. Then, the film were added into screw capped centrifuge tube with 30 ml of distilled water and placed in a water bath (Memmert Waterbath, WNE14, Tokyo, Japan) for 24 hours at 25 °C under constant shaking. After that, filter paper was used to filter undissolved films using filter paper (Whatman No. 1) and dried for 24 hours at 100 °C to measure final dry weight [3]. Each sample and the measurement was carried out in triplicates. By using the following equation, water solubility was calculated as a percentage:

$$WS\% = (\frac{Wo - Wf}{Wo}) \times 100 \quad (4)$$

Where Wo is the film initial dry weight and Wf is the dried undissolved film final weight.

2.6.4. *Moisture content*. The film moisture content were determined by calculating the film weight loss (2 cm x 2 cm) before and after drying for 24 hours in an oven at 100 °C [14]. Each sample and the measurement was carried out in triplicates. The moisture content was calculated in percentage according following equation:

$$MC\% = (\frac{Wwet - Wdry}{Wwet}) \times 100 \quad (5)$$

where *W* is the film sample's weight.

3. Result and discussion

3.1. Total Phenolic Content and Antioxidant Activity of Persicaria minor extracts

Many of polyphenol compounds are insoluble in water such as flavonoid thus, bipolar solvent; ethanol aqueous are considered to be used solvent for the extraction. The value of total phenolic compounds allowed the estimation of all polyphenol compounds including flavonoids, rutin, catechin, quercetin, isorhamnetin and kaempferol compounds present in the extract [8]. Moreover, ethanol are selected as a solvent because it consider as GRAS (Generally Recognized as Safe) for safer applications in the food products.

Table 2. Comparison of DPPH and TPC value of different solvent concentration

Concentration EtOH:H_2O (v/v)	Analysis	
	TPC (mg Gallic Acid Equivalent (GAE)/ L sample)	DPPH (%inhibition)
90%	129.23±3.15[a]	88.49±1.09[a]
75%	176.80±4.37[b]	94.65±0.17[b]
50%	143.09±4.00[c]	13.47±3.33[c]

Values are mean ± standard deviation.
GAE: Gallic acid equivalent.
Different letters in the same column indicate significantly different ($p<0.05$).

Table 2 illustrates the effect of solvent concentration on the total phenolic content (TPC) and antioxidant activity of PM extracts. Overall, the leaf extracted with 75% ethanol showed the highest polyphenol contents compare to 95% v/v and 50% v/v ethanol aqueous significantly ($p<0.05$). DPPH scavenging activity showed similar observation for the highest antioxidant activity is PM extracts using 75% v/v ethanol: water solvent. Chunli et al. reported the use of 75% v/v ethanol aqueous in extraction showed higher polyphenol recovery and antioxidant properties in many plants extract [15]. The similar observation also demonstrated by Azman et al. with the highest antioxidant activity found in *Betula alba* and *Convolvulus arvensis* using 75% v/v ethanol aqueous extracts [11]. From Table 1, the result showed proportionally correlation between the phenolic content and antioxidant activity in the DPPH assay which contributes to many polyphenols content such as rutin, catechin, and quercetin in the PM extract [8]. Similar observation found by Qader et al. that showed the ethanol aqueous extract of PM has the highest antioxidant activity with comparison to water extract [16]. Therefore, 75% v/v ethanol:aqueous extract demonstrated the highest phenolic content recovery and antioxidant activity of PM was to be used in the formulation of active packaging films.

3.2. Fourier Transform Infrared (FTIR) Spectroscopy

Figure 1 displays the functional groups between semi-refined carrageenan, glycerol, and *Persicaria minor* (PM) extract at 4000–400 cm^{-1} in the wavenumber region. The broad band observed at 3340 cm^{-1} corresponds to the O–H stretching that was created by the hydroxyl group of carrageenan and water [3]. Film plasticized with glycerol incorporated with PM extract and BHA into the SRC based films, the band shifted to a lower wavenumber of 3309 cm^{-1} and 3298 cm^{-1}. Besides, asymmetric stretching (CH_2) vibration was determined at 2916 cm^{-1}, 2922 cm^{-1} and 2940 cm^{-1}intense band. The major peaks show SRC based film FTIR spectrum at 1033 cm^{-1}, 1034 cm^{-1} and 1035 cm^{-1} were correspond to glycosidic linkages of carrageenan [3]. Moreover, the interaction between SRC with glycerol and PM extract, BHA can be determined from shifted band to a lower wavenumber. On the other hand, figure 1 illustrates the behaviour, at the wavenumber region between 800 and 880 cm^{-1}. According to previous literature, the peaks observed at ~850 cm^{-1} was often associated with S–O− stretching vibration of OSO^{3-} in carrageenan. Small and sharp peak was observed at 847 cm−1 at control sample [17]. However, SRC+G, SRC+G+PM 2.0% and SRC+G+BHA show no significant change of functional groups due to the homogeneous film solution. Generally, the peak intensity was increased when the glycerol and PM extract incorporate SRC compared SRC-control film. Similar

findings of FTIR were detected for carrageenan-based films reinforce nanofillers of silver nanoparticles (AgNPs) and clay mineral where the intensity of peaks did not change clay mineral when and AgNPs were included in the carrageen films [18].

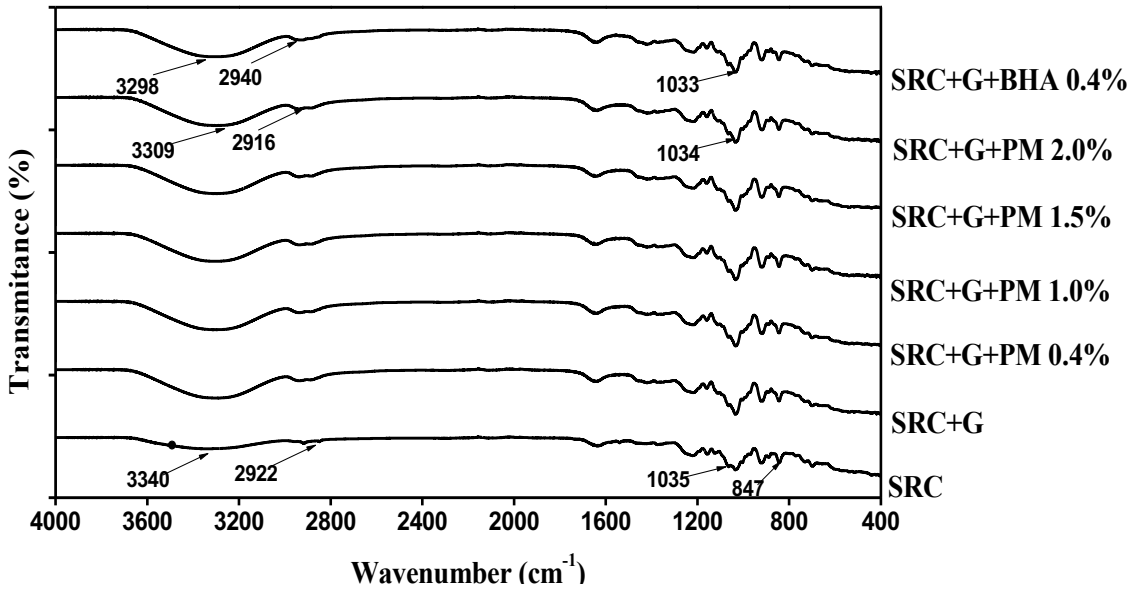

Figure 1. Fourier Transform infrared (FTIR) pattern for SRC based film.

3.3. Mechanical properties of films

Table 3 shows tensile strength (TS) and % elongation at break (EAB%) values of SRC films as control, SRC with glycerol films (SRC+G) and SRC films incorporate with PM extracts and BHA. Tensile strength measurement indicates the ability to sustain at maximum tensile stress applied to the film. SRC+G+PM (1.0, 1.5% and 2.0% w/w) experienced no significant different to each other ($p>0.05$) with improves TS value compre to other samples ($p<0.05$). Addition of glycerol 0.9% as plasticizer improved TS and EAB% for all treated samples significantly ($p<0.05$), whereas, SRC +G and SRC+G+BHA showed no significant different of TS value with average of 24 MPa ($p>0.05$). Adding natural plasticizer such as glycerol improves the mechanical properties of SRC based film due to the strong polymer-plasticizer interaction form via hydrogen bonding between SRC matrix chains and glycerol molecules [19].

Percentage elongation at break (%EAB) is to demonstrate the % value that allows the fills to elongate, extend and stretch. PM extracts (2.0% w/w) incorporate to the films formulation demonstrated the highest value EAB% where SRC film showed the lowest %EAB value. PM extract not only act as active antioxidant agent but also improves the film stretchability due to the interaction of PM extract modifies the polymeric matrix chains of SRC and glycerol by creating interfacial bonding between the hydrophilic SRC matrix and plasticizer [20]. Appropriate mechanical properties value that classifies as good TS/%EAB for biopolymer films are fall under the range of 10–100 Mpa of TS and EAB >10% . All plasticize film are fall under range of 10–100 MPa of TS and EAB >10% which demonstrated having good mechanical properties that can be further develop as active film formulation.

Table 3. Result on mechanical strength of films.

	Mechanical properties	
Sample	TS (Mpa)	EAB (%)
SRC	12.57±0.10[a]	5.06±0.01[a]
SRC + G	24.68±0.04[b]	23.85±0.13[b]
SRC + G + BHA0.4%	24.40±0.16[b]	28.13±0.20[c]
SRC + G + PM0.4%	25.10±0.21[c]	33.35±0.17[d]
SRC + G + PM1.0%	28.18±0.19[d]	31.80±0.11[e]
SRC + G + PM1.5%	27.93±0.03[d]	30.85±0.13[e]
SRC + G + PM2.0%	28.01±0.17[d]	37.37±0.29[f]

Values are mean ± standard deviation.
Different letters in the same column indicate significantly different ($p<0.05$).

3.4. Physical properties of films

Table 4 showed effect of PM extracts and BHA on physical properties of the films including thickness, opacity, % moisture content, % water solubility. The thickness value of the films plasticized with glycerol increases compare to SRC film ($p<0.05$) whereas the addition different concentration of PM extracts showed no significant changes to the films thickness ($p>0.05$). Film samples plasticized with glycerol led to the thickest films due to the tendency of glycerol to absorb more moisture. Transparency characteristic of the film measured by the opacity value in Table 4. High value of opacity displays lower transparency appearance of the film which make the film shady and indistinct. The highest opacity value is SRC films incorporate natural antioxidant and films with glycerol reduced the opacity value significantly ($p<0.05$). Transparency of the films showed no significant different ($p<0.05$) with increase addition of PM extracts (1.0-2.0% w/w). Farhan and Hani reported the presence of glycerol improves the transparency characteristic in SRC film due to the action of glycerol hinders the ice formation by interposing itself within the network of water and hydrogen molecules [3]. This observation found similar to the SRC films plasticized with glycerol increase the light transmittance through the films resulted to the lower opacity compare to the SRC film without adding glycerol ($p<0.05$). Film solubility (%) measures the integrity of films in contact with water and the solubility value also attributes to the biodegradable properties of the films as packaging materials. The solubility of films was reduced with the addition of plasticizer and PM significantly ($p<0.05$) compare to SRC film only as shown in Table 4. The high percentage of solubility value of SRC film due to the hydrophilic nature of carrageenan that easily dissolve when in contact with water. Incorporating PM extract in the film samples displayed slight changes on the % film solubility value compare to glycerol. Addition of plasticizer such as oil into carrageenan polymer lead to significant decrease of films sample confirmed by previous literature [21]. The interaction between the hydroxyl groups of carrageenan chain with plant oil affected the availability of hydroxyl groups by reducing the polysaccharides-water interactions, thus decreasing water solubility of film. Percentage of moisture content in Table 4 displayed the significant increase of SRC film plasticized with glycerol compared to SRC control film ($p<0.05$). The % moisture content for SRC control film was less than 3% compare to the % moisture content films plasticized with glycerol that increase significantly almost 30 times higher than SRC film only ($P<0.05$) The high moisture content is due to the hydrophilic nature of glycerol affected the reorganization of the polysaccharide network led to the increase in the free volume and segmental motions that allows the water molecules to diffuse easily and result to the high % moisture content [3]. Meanwhile, the incorporation of different concentration *Persicaria minor* (PM) into the SRC film significantly increase the % moisture content by increase the moisture of the film. As concentration of PM increased, the % moisture content of the SRC films increased substantially. Film incorporated with 2.0% w/w of PM retained more water compared to other film. Some important characteristics has been analyzed including morphology and application in food

model for this film to become more competent for food packaging as it has proven to be an active film[9].

Table 4. Result on physical properties of films

Sample	Physical properties			
	Thickness (mm)	Opacity	Film Solubility (%)	Moisture Content (%)
SRC	0.248±0.11[a]	4.65±0.02[a]	60.52±0.41[a]	2.38±0.01[a]
SRC + G	0.252±0.16[b]	3.61±0.15b	60.00±1.10[a]	33.91±0.30[b]
SRC + G + BHA0.4%	0.252±0.01[b]	3.67±0.06[b]	67.57±1.10[b]	34.41±0.67[c]
SRC + G + PM0.4%	0.260±0.09[c]	3.85±0.06[c]	62.26±0.56[c]	38.95±1.50[d]
SRC + G + PM1.0%	0.264±0.04[c]	3.86±0.04[c]	51.32±0.70[d]	38.65±0.89[d]
SRC + G + PM1.5%	0.264±0.07[c]	3.86±0.10[c]	34.80±0.86[e]	39.19±0.73[e]
SRC + G + PM2.0%	0.268±0.09[c]	3.86±0.11[c]	31.21±0.36[e]	39.55±0.61[e]

Values are mean ± standard deviation.
Different letters in the same column indicate significantly different ($p<0.05$).

4. Conclusion

Incorporation of *Persicaria Minor* (PM) in SRC plasticized with glycerol improves the film characteristic by increased the value of tensile strength and the elongation at break %. Furthermore, physical characteristic of the films treated with antioxidant showed significant changes in all measurements including the solubility of film, opacity and % moisture content. SRC+G+PM2.0% demonstrated excellent resistance in contact to water that can be conclude as durable and robust film characteristic. Overall, the properties of SRC-based films were greatly affected by PM extract incorporation and the compounds may also act as antioxidant agent for food protection. In conclusion, the formulation of SRC incorporated with PM can exhibit extensively desirable good mechanical and physical properties value to maintain and to protect the packaged food integrity.

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