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Research Article

Characterization of *Jatropha curcas* L. Protein Cast Films with respect to Packaging Relevant Properties

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There is increasing research ongoing towards the substitution of petrochemical based plastics by more sustainable raw materials, especially in the field of bioplastics. Proteins of different types such as whey, casein, gelatine, or zein show potential beyond the food and feed industry as, for instance, the application in packaging. Protein based coatings provide different packaging relevant properties such as barrier against permanent gases, certain water vapour barrier, and mechanical resistance. The aim of this study was to explore the potential for packaging applications of proteins from *Jatropha curcas* L. and to compare the performance with literature data on cast films from whey protein isolate. As a by-product from oil extraction, high amounts of *Jatropha* meal are obtained requiring a concept for its sustainable utilization. *Jatropha* seed cake includes up to 40% (w/w) of protein which is currently not utilized. The present study provides new data on the potential of *Jatropha* protein for packaging applications. It was shown that *Jatropha* protein cast films show suitable barrier and mechanical properties depending on the extraction and purification method as well as on the plasticiser content. Based on these findings *Jatropha* proteins own potential to be utilized as coating material for food packaging applications.

1. Introduction

Jatropha curcas L. is a tropical plant of the genus *Euphorbiaceae*. Being rather non-demanding, the plant grows under various climatic conditions and is able to survive in arid regions with poor and stony soils, not suitable for food cultivation [1, 2].

During the last two decades the interest in its exploitation increased since *Jatropha* seeds are rich in nonedible oil which can be processed to biodiesel [3, 4]. However, in order to ensure the sustainability of *Jatropha* cultivation there is an urgent need for additional processing routes taking into consideration the by-product from oil extraction, namely, the *Jatropha* meal. The meal offers a protein content of up to 40% [1]. Neither the meal nor the protein is directly suitable for feed or food applications, since most varieties of *Jatropha curcas* L. comprise several toxic and antinutritional factors such as phorbol esters, lectin, and phytate [5]. Thus, the utilization of *Jatropha* protein in technical applications (e.g., glues, film coatings, emulsifiers) is an interesting area of value creation, concurrently avoiding the competition with food production.

Technical protein applications are gaining more attention, due to an increasing awareness of the importance of an integrated and sustainable utilization of agroindustrial raw materials. This is supported by the quest of the chemical industry for environmentally friendly, biodegradable processes and products [6].

The extraction of protein from *Jatropha* meal has only recently been considered. Previous studies report protein yields of about 53% to 82% after extraction and recovery [7–9].

Protein isolation is most commonly performed by alkaline extraction and consecutive isoelectric precipitation, ultrafiltration, or spray drying of the extracts [10–12]. The most suitable method depends on factors such as the nature of the protein, its foreseen application, or the processing costs [13, 14].

The functionality of the proteins extracted from *Jatropha curcas* was investigated beforehand indicating good emulsifying properties comparable to the ones of soy protein. However, the technofunctional properties of proteins extracted from oil seeds are largely depending on the extraction process applied and on the pretreatment of the raw material [15, 16]. For *Jatropha* protein this was also observed for its foam forming ability. This property varies significantly depending on the respective protein extraction and recovery method [9, 17–19]. Therefore, the processing has to be taken into account when evaluating the potential of a protein for certain applications by investigating the relevant technofunctional properties. For utilization as protein film in packaging systems these are, for instance, the barrier, mechanical, and optical properties. Several studies have already proven the potential of plant and animal based proteins for their packaging relevant properties [20]. For instance, protein films made from whey protein isolate (WPI) are well studied in terms of edible film and packaging related applications [21–27]. However, most of the proteins characterized in these studies such as soy, whey, or wheat proteins are suitable for a use in food and feed. Therefore, their application in packaging is directly competing with food production. Here *Jatropha* protein might provide a suitable and sustainable alternative.

However, WPI will, due to its good and well-known properties, serve as benchmark material for comparison with the cast films characterized in this study. Unplasticized *Jatropha* films just as WPI-based films are very brittle and thus not directly applicable. This brittleness is due to intermolecular chain interactions such as disulphide bonding, hydrogen bonding, hydrophobic interactions as well as electrostatic forces between protein chains [25]. Plasticisers like glycerol are most commonly used to overcome film brittleness. They reduce protein chain-to-chain interactions, increase chain mobility, and, thus, improve the flexibility of the protein films [24]. However, these interactions are important in order to obtain the desired oxygen and water vapour barrier as well as for the mechanical performance [22, 24, 25, 28]. Therefore, as a result of adding plasticizers, the permeability values usually increase when no additional formulation optimization is performed [29]. Such formulation optimizations or adaptions could be among others a chemical modification [29] and/or a biochemical modification by enzymes [28] and/or a molecular weight adoption of the protein [24] leading to improved performance of the protein based films towards packaging applications.

The objective of this work was to characterize cast films from *Jatropha curcas* L. protein with respect to packaging relevant attributes such as barrier, mechanical, and optical properties. In order to evaluate whether these new cast films are suitable for packaging applications or not their properties are compared to the properties of WPI-based films which have been intensively studied and confirmed to be suitable for food packaging applications [22, 24–49]. This approach is most reasonable since similar processing routes and cast film productions were used. To take the effect or raw material pretreatment into account, proteins extracted from two different by-products of *Jatropha* oil extraction, namely, screw-pressed (SPJR) and aqueous deoiled *Jatropha* residue (ADJR), were compared. In addition, the relationships between the protein recovery method and the protein properties were evaluated.

2. Materials and Methods

2.1. Raw Materials and Chemicals. Jatropha seeds were obtained from Rajasthan (India). The seeds were stored at 14°C until further processing.

2 N NaOH, 1 N HCl, and glycerol anhydrous puriss. were obtained from Th. Geyer GmbH & Co. KG (Renningen, Germany).

2.2. Preparation of Jatropha Raw Material. Deoiling of the *Jatropha* seeds was achieved by screw-pressing (50 Hz; max. 70°C) of the whole seed. The press cake obtained (SPJR) was stored at 14°C for about two months.

Additionally, a water-based extraction process developed by GEA Westfalia Separator AG (Oelde, Germany) was performed after removal of the shells and grinding of the kernels. Water was added to the grinded kernels yielding a solid-to-liquid ratio of about 1 : 2. The fluid was heated and subsequently oil, water, and solids were separated utilizing a two-phase decanter and a separator (GEA Westfalia Separator AG, Oelde, Germany). The extraction residue (ADJR) obtained in this process was kindly provided by GEA Westfalia Separator AG. The residue was stored at −20°C for about two months.

2.3. Protein Extraction. Parameters applied in aqueous protein extraction of screw-pressed (SPJR) and aqueous-extracted (ADJR) residue from *Jatropha curcas* L. are given in the following. 50 g of SPJR and ADJR, respectively, were suspended in 500 g of water in a double-wall reactor (Gebr. Rettberg GmbH, Göttingen, Germany). The solid-to-liquid ratio was thus 1 : 10. While heating to 60°C, the mixture was stirred with 200 rpm and pH was adjusted to 11 using 2 M NaOH. After 30 min of extraction, the solid was separated from the liquid by centrifugation (Thermo Scientific, Waltham, MA, USA) (4000 g, 10 min).

2.4. Protein Recovery from the Extracts of Aqueous and Enzyme-Assisted Protein Extraction

Isoelectric Precipitation. Isoelectric precipitation of aqueous-extracted protein from ADJR and SPJR was investigated utilizing 250 g of the protein extracts. The extract was stirred with 200 rpm at 22°C. Protein was precipitated by lowering the pH value to 4 utilizing 1 M HCl. After reaching the pH value, the mixture was stirred another 5 min in order to guarantee maximum protein yield. Subsequently, the solid was separated from the liquid by centrifugation (4000 g, 10 min). The precipitates were freeze-dried and analyzed according to Section 2.5.

Ultrafiltration. Ultrafiltration was performed at 22°C with capillary membranes (Pall Corporation, Port Washington, New York, USA) with a pore size of 10 kDa inserting 5 L of aqueous-extracted protein from ADJR and SPJR, respectively. Protein extracts were concentrated from an initial dry matter

Table 1: Composition of the *Jatropha* protein products obtained after extraction of aqueous extracted (ADJR) and screw-pressed *Jatropha* residue (SPJR) applying different methods for protein recovery (unpublished data Fraunhofer IVV).

Composition	ADJR			SPJR		
	UF	IEP	Lyo	UF	IEP	Lyo
Dry matter (%)	94.8	97.0	95.1	91.6	86.6	93.7
Ash (%)	5.6	8.1	13.7	10.2	2.2	13.5
Protein (%)	72.9	62.8	45.4	72.4	82.4	61.2
Fat (%)	16.9	21.6	16.3	11.0	14.0	9.5

UF: ultrafiltration; IEP: isoelectric precipitation; Lyo: lyophilization.

Table 2: Measurements performed to the cast films obtained from the different *Jatropha* protein products (see Table 1).

		OP	WVTR	TS	E	YM	Surface Energy	Light transmission
ADJR UF	20% glycerol	X	X	X	X	X	X	X
	30% glycerol	X	X	X	X	X	X	X
SPJR UF	20% glycerol	X	X	X	X	X	X	X
	30% glycerol	X	X	X	X	X	X	X
ADJR IEP	30% glycerol	X					X	X
SPJR IEP	30% glycerol	X					X	X
ADJR Lyo	20% glycerol	X					X	X
	30% glycerol	X					X	X
SPJR Lyo	20% glycerol	X		X	X	X	X	X
	30% glycerol	X		X	X	X	X	X

OP: oxygen permeability; WVTR: water vapour transmission rate, TS: tensile strength; E: elongation at break; YM: Young's modulus.

of 4% to a dry matter of approx. 12%. Afterwards the concentrates were freeze-dried and analyzed according to Section 2.5.

Lyophilisation. Protein extracts and precipitates from ADJR and SPJR were freeze-dried utilizing a lyophilisator BETA 1–8 (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). For this the samples were frozen to −50°C and lyophilized at a pressure of 1 mbar, increasing shelf temperature continuously from 5 to 40°C. Temperature of the ice condenser was −55°C. Lyophilized samples were analyzed according to Section 2.5.

2.5. Analysis of the Chemical Composition of Jatropha Raw Material and Protein Extracts. Chemical composition of the raw material (protein, ash, dry matter) was analyzed in duplicate. Protein content was measured by Dumas combustion method [50], using 6.25 as the conversion factor. Dry matter and ash content were analyzed in a thermogravimetrical system (TGA 601, Leco Corporation, St. Joseph, MI, USA) at 105°C and 950°C, respectively.

Composition of the samples is given in Table 1.

2.6. Production of Cast Films. A 10% (w/w) *Jatropha* protein solution was stirred with 400 rpm at ambient temperature for 10 min. Subsequently, 20 or 30% of glycerol in relation to the protein dry matter was added. The mixture was stirred for another 30 min and afterwards 23 g of the solution was casted into 10 × 10 cm petri dishes and dried in a climate room (23°C, 50% r.h.) until the films reached there equilibrium moisture content (EMC) before analysis.

The cast films obtained from the *Jatropha* protein products were analyzed according to Section 2.7. Due to the limited availability of the samples, the measurements to be undertaken were conducted as shown in Table 2.

2.7. Analysis of the Properties Relevant to Packaging Materials

Film Thickness. The film thicknesses of *Jatropha*-based films were measured after they reached their respective EMC at 23°C and 50% r.h. by the Precision Thickness Gauge FT3 (Rhopoint Instruments, Bexhill on Sea, UK) at five different positions. The measurement conditions were 23°C and 50% r.h. The arithmetic mean of the film thicknesses was used to normalize the oxygen permeability (OP) and water vapour transmission rate (WVTR) to a thickness of 100 μm for comparison reasons and to calculate the mechanical film properties.

Water Vapour Transmission Rate Measurement. WVTR was measured according to the standard DIN 53 122-1. The WVTR (water vapor transmission rate) Q of the cast films was measured at 23°C and 50% → 0% r.h. The WVTR is calculated by the following equation:

$$\mathrm{WVTR} = Q = \frac{24}{t} \times \frac{\Delta m}{A} \times 10^4, \quad (1)$$

where t is the period of time between two weight measurements, Δm represents the mass difference between two weight measurements, and A is the film area in cm^2. The WVTR values, Q, are given in the unit, g·m^{-2}·d^{-1}, and normalized to the thickness d of 100 μm (Q_{100}) using the following

equation to allow direct comparison of different materials independently of the cast film thicknesses (d):

$$Q_{100} = Q \cdot \frac{d}{100}. \quad (2)$$

A fourfold determination was performed.

Oxygen Permeability Measurement. The OP (oxygen permeability) measurement was performed according to the standard DIN 53380-3 at 23°C and 50% r.h. The instrument Mocon Twin (Mocon Inc., Minneapolis, Minnesota, MN, USA) was used. The *Jatropha* films were masked using aluminum films in order to stabilize the samples. The OP values, Q, are given in the unit, cm^3 (STP)$m^{-2} \cdot d^{-1} \cdot bar^{-1}$, and were converted to the thickness, d, of 100 μm (Q_{100}), according to the above-mentioned equation.

Mechanical Characterization. The tensile strength (TS) of *Jatropha*-based films was measured by the universal compression-tension machine, RM 50 (Doli GmbH Industrie Electronic, Munich, Germany) according to the standard ISO 527.

The cast films were cut into strips of 15 mm width and a length of 70 mm. A load cell of 50 N was used and the specimen was stretched using a testing speed of 100 mm/min. A tenfold determination was performed for each sample at 23°C and 50% r.h.

Young's Modulus or the elastic modulus (EM) of *Jatropha* films was also measured by the universal compression-tension testing machine, RM 50 (Doli GmbH Industrie Electronic, Munich, Germany), in a separate measurement next to the tensile test according to ISO 527 with the same sample preparation and measurement conditions except for the testing speed which was 0.5 mm/min for the Young's Modulus measurement.

Surface Energy Measurement. The surface energy of *Jatropha*-based films was measured by the contact angle measuring system, G2 (Krüss GmbH, Stephanskirchen/Rosenheim, Germany). The sessile drop method was used. The following testing liquids were used: double distilled water, diiodomethane, ethylene-glycol, and dimethyl phthalate. The samples were cut into pieces of 7.5 mm × 7.5 mm. A testing drop of 3 μL was induced using a dosing rate of 20 μm/min. After an ideal screen drop was set, the screen of the inducing liquid drop on the solid surface was frozen after exactly 20 s to measure the contact angle between the baseline and to calculate the surface energy according to Young's equation as well as according to the Owens, Wendt, Rabel, and Kaelble method, in order to divide the surface energy into their respective disperse and polar fractions.

Light Transmission. The light transmission is measured with a spectrophotometer TMQ of Carl Zeiss. A detector measures the light intensity. For every sample a twofold determination was performed. The value of transmittance was given be read off in percent. The testing range was from 250 to 1000 nm.

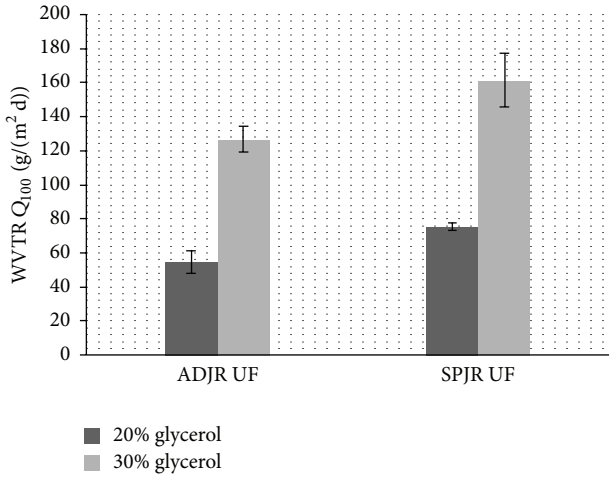

Figure 1: WVTR 50% → 0% r.h. (Q_{100}) of ultrafiltrated (UF) ADJR and SPJR protein films with 20% and 30% (w/w) plasticizer content based on protein dry matter.

2.8. Statistical Analysis. Extreme values of a sample were removed from the data series by the Grubbs outlier test or when they were negative. For the analyses the online outlier calculator (GraphPad Software, Inc., La Jolla, Cam USA) was used. OP measurements were calculated from a twofold determination. Those data were not tested, but the minimum and maximum values were given accordingly as error bars.

3. Results and Discussion

The characterization and comparison of *Jatropha* protein films casted from protein solutions obtained from different processing routes was the aim of this study and reported hereafter accordingly.

3.1. Water Vapour Transmission Rate. The WVTR (Q_{100}) of *Jatropha*-based films ranges from approx. 55 to 161 g/(m^2 d) (Figure 1) which is in the area of WPI-based films. According to Schmid [25], and Schmid et al. [24, 28] the WVTR (Q_{100}) of WPI-based films range from approx. 2–400 g/(m^2 d) depending on the processing method, modification, molecular weight, and plasticizer concentration. More details about the respective results are given in the corresponding literature [24, 25]. However, literature indicates that especially highly cross-linked formulations and/or formulations with low plasticizer concentrations provide low WVTR values suitable for packaging applications.

WVTR of Jatropha protein films increases when the plasticizer content increases. These results are consistent with the results from Sothornvit and Krochta [49] on the effect of glycerol concentrations on the WVTR of WPI-based films. It is known that plasticizers decrease intermolecular interactions and increase the free volume in the polymer matrix, leading to increased transmission rates [25]. The higher WVTR of SPJR UF in comparison to ADJR UF could be explained by the higher fat content of ADJR as shown in Table 1. This most probably leads to a higher

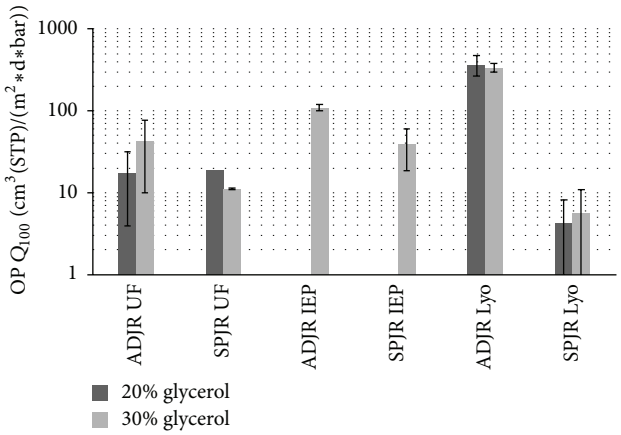

Figure 2: OP of *Jatropha* protein based films measured at 50% r.h. and 23°C.

hydrophobicity of the cast film and therefore decreases the solubility coefficient. Since the permeation coefficient is the product of solubility coefficient and diffusion coefficient the permeation decreases when hydrophobic components increase in the polymer matrix and consequently the water vapour transmission rate decreases [51].

3.2. Oxygen Permeability. OP of *Jatropha*-based films (Figure 2) range from approx. 4 to 368 cm^3 (STP)/(m^2 d bar) which is also in the range of WPI-based films. The OP of WPI-based films varies between 1.5 and 200 cm^3 (STP)/(m^2 d bar) at comparable measurement conditions depending on the processing method, the formulation recipe, the kind of modification, molecular weight, and plasticizer concentration [24–29]. In particular, SPJR Lyo showed very low OP values and is thus most suitable to act as barrier material in food packaging laminates, comparable to WPI-based films and coatings.

Except for SPJR UF and ADJR Lyo the OP slightly increases when the plasticizer concentration is increased (Figure 3). For ADJR Lyo the OP change is within the standard deviation and thus not considered to be significant. For SPJR UF, however, the decrease in OP with increasing glycerol content is higher than standard deviation. This is neither in accordance with the observations made for the other *Jatropha* proteins nor with the literature cited above. Therefore, a more detailed investigation of this effect is required.

In case of ADJR and SPJR IEP no OP measurement was possible since the films cracked during the measurement leading to free diffusion. Thus, the obtained values did not reflect typical permeability behaviour and are therefore not plotted in Figure 2.

In Figure 2 it can be seen that the OP values of *Jatropha* films from SPJR UF and ADJR UF are similar which goes along with their similar protein content. The same, namely, a correlation of protein content and OP, was observed for the precipitates and the lyophilized samples. Low protein content goes along with high OP of the films.

3.3. Mechanical Properties. The tensile strengths (TS) of *Jatropha*-based films varies from 2 to 12 MPa which is in the range of WPI-based films [24, 25] and therefore also suitable for packaging applications.

The TS decreases and elongation at break increases as expected when the plasticizer concentration is increased (Figure 3). Young's modulus values of *Jatropha*-based cast films (Figure 4) as another indicator of the stiffness of a material are also in the range of WPI-based films [24, 25]. As expected, Young's modulus is decreased when the plasticizer concentration is increased.

The discrepancy of the elongation at break observed for protein films with 20 or 30% of glycerol is larger for the films casted from SPJR than for the one casted from ADJR. Former studies also reported that the degree of denaturation affects the technofunctional properties of protein films [52]. According to Schmid et al. [52] it is necessary to gain a certain degree of denaturation in order to achieve lower permeability values towards oxygen and water vapour on the example of whey protein isolate based films.

The differences between the samples could be explained by their different composition with regard to fat and protein concentration. Cast films from SPJR UF were stiffer than films from ADJR UF which can be attributed to the higher fat content of ADJR UF. The protein content of SPJR Lyo is 61% and thus significantly lower than the protein content of the ultrafiltrated products ranging at 72 or 73%. In addition the molecular weight of the ultrafiltrated products is above 10 kDa, while the lyophilized protein products also contain smaller fragments of proteins. Therefore, it is likely to assume that the lower values obtained for SPJR Lyo can be attributed to the protein purification technique.

Lestari et al. [18] also investigated TS, elongation at break, and Young's modulus of *Jatropha* protein films. The films were obtained after extraction and isoelectric precipitation of the proteins from screw-pressed (and hexane deoiled) *Jatropha* cake. However, the values reported were significantly lower than the ones obtained in this study. This overall deviation could be explained by different preparation and processing methods used in this study.

3.4. Surface Energy. The overall surface energy values of *Jatropha*-based films range from approx. 44 to 64 mN/m (Figure 5), thus slightly exceeding the surface energy values reported for WPI-based films which are in the range of approx. 48 to 62 mN/m measured at similar conditions [25]. High surface energy values (>40 mN/m) are a prerequisite for further processing such as additional wet coating or glue lamination. The surface energy values of *Jatropha*-based cast films provide in all cases a sufficient high surface energy in order to be further processed to laminate structures for packaging applications.

With the exception of ADJR Lyo the polar part of the surface energy increases when the plasticizer concentration is increased. This can be explained by the chemical structure of the plasticizer glycerol. Glycerol is a hydrophilic plasticizer with 55.4% hydrophilic groups and nine hydrogen bonds [53], thus increasing the polar part of the surface energy when its concentration is increased.

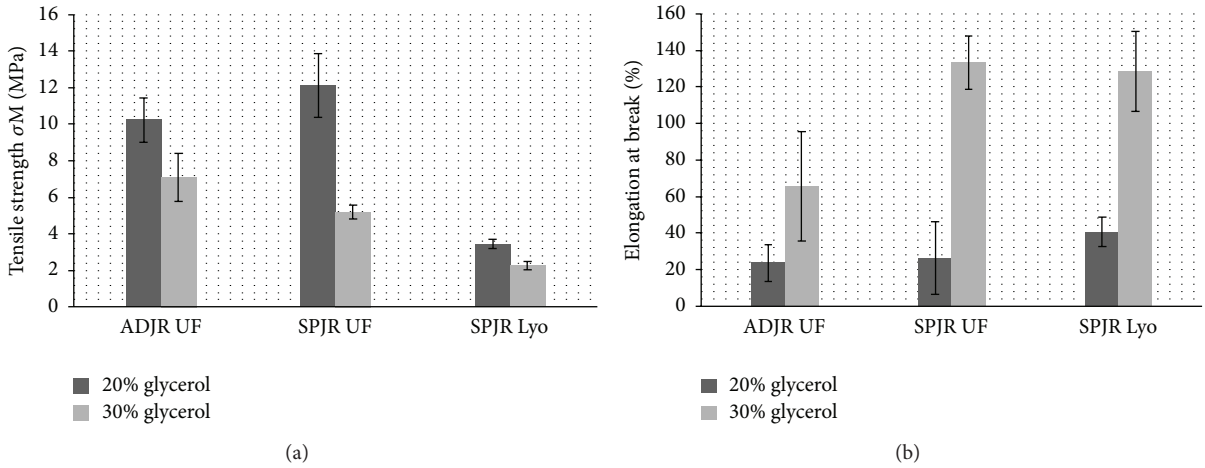

Figure 3: Tensile strength (a) and elongation at break (b) of *Jatropha*-based cast films.

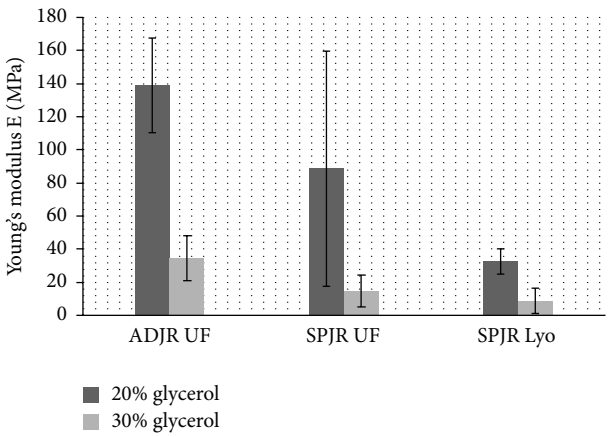

Figure 4: Young's modulus of *Jatropha*-based cast films.

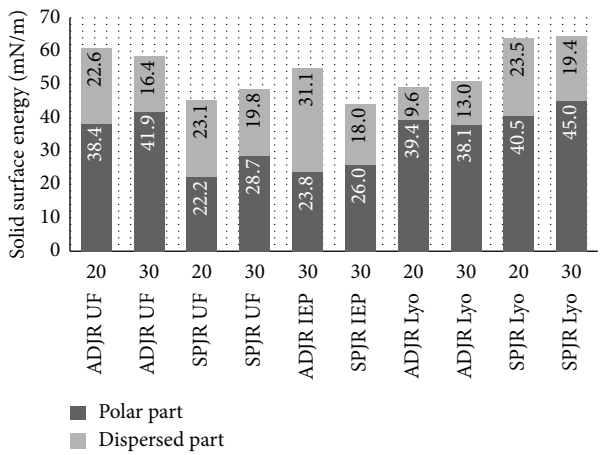

Figure 5: Surface energy values of *Jatropha*-based cast films. The numbers 20 and 30 indicate the percentage of glycerol added in relation to the dry matter.

As expected, the lowest polar part of the surface energy of only 42% was found for ADJR IEP, the sample with the highest fat content, whereas the highest polarity was measured for ADJR Lyo exhibiting the highest concentration of highly polar substances of about 48% (residue + ash content) followed by SPJR Lyo with 29% (see Table 1).

In general, the interpretation and comparison of the surface energy of the different samples is difficult, since the interaction of all constituents of the sample exerts influence on the result.

3.5. Light Transmission. The light transmissions of *Jatropha*-based films strongly depend on the respective wavelength (Figure 6). In general, the cast films appear brownish and not transparent to the human eye. This contrasts with the behavior of WPI-based films which are highly transparent. Their light transmission is above 80% for the whole visible light range [27, 28].

The light transmission of *Jatropha* protein from ADJR is higher than the one of *Jatropha* protein from SPJR. This can probably be attributed to the different deoiling procedures (see Section 2.2) influencing the polyphenol concentration of the samples. At elevated temperatures polyphenols are oxidized very easily, thus accounting for the brownish colour of the deoiled residues. However, water-soluble polyphenols inherent to *Jatropha curcas* L. will probably be lost during aqueous deoiling. Therefore the protein products obtained from ADJR are expected to be less brownish and more transparent compared to the protein products from SPJR which is reflected by the light transmission behaviour.

The differences within the samples obtained from ADJR can be explained by the different protein contents of the products. As observed, the light transmission is increased with decreasing protein concentration. Besides, the light transmission slightly increases with the glycerol content. The same is true with respect to the different samples from SPJR.

4. Conclusions

The present study provides new data on the potential of *Jatropha* protein based films for packaging applications. It was

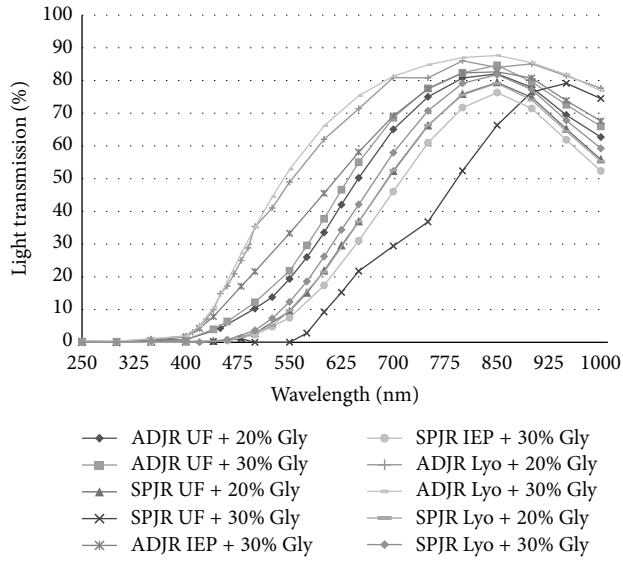

Figure 6: Light transmission of *Jatropha*-based cast films.

found that *Jatropha* protein cast films show suitable barrier and mechanical properties. However it was also proven that these attributes are depending on the raw material pretreatment, the extraction, and purification method as well as on the plasticizer content. The barrier, mechanical, and surface energy properties are in the range of whey protein based cast films which have been proven to be suitable for several food packaging applications in multilayer structures. Even though the colour and light transmission of the *Jatropha*-based cast films are not suitable for transparent packaging film application, the *Jatropha* protein based formulations could be very promising for the application in not transparent multilayer structures or in combination with paper and board. Based on these findings *Jatropha* proteins own the potential to be utilized as coating material for food packaging applications due to their technofunctional properties. However, as *Jatropha* protein concentrates and isolates contain toxic compounds such as phorbol esters, the material may not come in direct contact with food. It should rather be detoxicated and imbedded in multilayer structures. Final structures need then to be characterized if they meet food contact compliance.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Gabriele Gofferje and Markus Schmid contributed equally to this work.

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