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Biofuel, Bioenergy and Feed Valorization of By-Products and Residues from *Hevea brasiliensis* Cultivation to Enhance Sustainability

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Received: 6 August 2020; Accepted: 15 September 2020; Published: 17 September 2020

Abstract: In the latex production chain, rubber tree seeds (*Hevea brasiliensis*) represent an underutilized fraction with high potentialities, which can increase the sustainability of the whole process if rightly valorized. In the present study, the quality of all the fractions obtained from the rubber fruit were evaluated, with the aim to identify possible applications for their valorization with a circular economy perspective. Seeds from five different varieties of rubber tree were analyzed. Furthermore, a whole mass and energy balance was defined, which has allowed us to define hypothetical production scenarios. The obtained results show negligible differences among varieties. Shells and capsules have shown a composition similar to woody biomass, with high heating values (more than 16.5 MJ kg^{-1}), low nitrogen content (below 0.5% on weight basis (*w/w*)) and reduced ash content (0.51% *w/w* and 1.90% *w/w*, respectively). Kernels were chemically extracted comparing two different solvents: n-hexane and ethanol. Both solvents showed similar extraction yields, i.e., 49% *w/w* and 46% *w/w* for n-hexane and ethanol, respectively. The resulting extraction flour was characterized by a high protein content (around 40% *w/w*) making it suitable for animal feeding. The rubber seed oil could be used in blends of different vegetable oils for biodiesel production. All this information is useful for improving the sustainability of the latex production chain and to assess the sustainability of possible bioenergy value chains.

Keywords: vegetable oil extraction; circular economy; biorefinery; rubber seed variety; biodiesel

1. Introduction

Recently, several actions have been taken worldwide to support the move to a more circular economy. These activities cover the full life cycle from production and use to waste management. In a circular economy, the value of products and secondary raw materials should be maintained for as long as possible and waste and resource use should be minimized, contributing to innovation, growth and job creation. The valorization of production residues or even wastes is thus fundamental to improve the sustainability of the production chains.

Natural rubber is a widely employed polymer, which can be found in different fields. Its high diffusion is due to excellent mechanical properties, i.e., high elasticity, low hysteresis, high durability, excellent toughness [1]. The starting raw material to produce natural rubber is a milky emulsion extracted from tree's stem by bark scratching, which contains cis-1,4-isoprene particles, called latex. This one is obtained mainly from the *Hevea brasiliensis* tree, commonly called the rubber tree [2–4]. Although the *Hevea brasiliensis* tree is native to the Amazon rainforest, today it is mainly cultivated in South East Asia regions, which produce 95% of the world's latex [4,5].

Since latex is the principal product of the natural rubber production chain, seeds are currently not considered as worthy material. Less than 25% of seed production is employed for seeding [2], while the remaining part is left underutilized in the field [6] or disposed as a waste [5], losing its potential as organic matter or energy.

The rubber tree produces lustrous, mottled brown seeds with a length of 2.5–3 cm and a weight of 2–4 g, which are enclosed in a three-ellipsoidal pod (capsule), each of which contains three seeds. The capsule disperses the seeds on the ground by a loud mechanical explosion [7,8]. Each rubber seed is composed of a kernel and a shell, which account for around 60% w/w_{wb} and 40% w/w_{wb} of the whole seed weight, respectively [4,9–11].

According to literature, the production of rubber seeds can reach 2060 kg ha^{-1} $year^{-1}$ [2], even though values of 150–200 kg ha^{-1} $year^{-1}$ are more realistic considering that cultivation conditions make collection and rot prevention difficult; moreover, the employment of a non-dedicated collection system is highly probable [4,12,13]. The main producer countries of rubber seeds are Thailand (3,172,394 ton), Indonesia (5,367,980 ton) and Malaysia (1,735,522 ton), which account for two-thirds of the World's cultivated area [2,10]. Nigeria leads the harvested area of rubber plantation among African states (377,733 ha), followed by the Ivory Coast (189,937 ha) [12,14].

The involved quantities, the residual nature and the annual availability [5] of the rubber seeds make them an interesting feedstock for bioenergies, especially where they are managed as a waste. It is also a non-edible oil source, entailing no competition with existing agricultural resources for food production.

The kernel contains 40–50% of non-edible vegetable oil and 19–23% of crude protein [2,12].

Due to its high protein content and amino acids composition, defatted kernel/meal can be employed as feed for animal nutrition (i.e., fish, pigs, ruminants, poultry) [5,12,15]. However, the presence of different anti-nutritional factors in rubber seed, especially cyanogenic glycoside, limits its exploitation for feeding applications [12,16,17].

The rubber seed oil (RSO) is highly unsaturated as contains mainly linoleic, linolenic and oleic acids [4,9,18]. RSO represents the highest worth product of the rubber seed so far and it is mainly employed for biodiesel production [18–20] and different other applications, such as biolubricant [21], fuel in compression engine [22].

Rubber seed shell is characterized by a low ash content (0.1–0.3%) and a heating value (19–24 MJ kg^{-1}) comparable to that of virgin wood, which are interesting features for a solid biofuel [10,23]. In scientific literature can be found different applications of the shell, such as starch foam addictive [24], pyrolysis [10], bio-oil production [23], gasification [25], co-liquefaction [26] and catalysis [6].

The use of natural rubber is becoming increasingly more important considering the global effort to reduce the production of plastic from fossil sources. Finding the best valorization of the related by-products, residues and wastes becomes an important aspect to maximize the sustainability. However, it is difficult to find in scientific literature a study focused on all the products, by-products and residues of this cultivation simultaneously. This lack makes it difficult to identify the valorization opportunities and to have the information needed to check the sustainability of the possible options. For example, some authors assessed the sustainability of biodiesel from RSO by Life Cycle Assessment, but a lot of information is estimated or taken from different sources because it is not available as direct measurements [27].

The aim of this study was to evaluate the quality of all the different material flows linked to rubber tree cultivation to identify the possible applications, mainly for energy, with the aim to improve the latex production sustainability by valorizing residues and wastes with a circular economy perspective. In fact, the knowledge of the quantity and the properties of products, by-products and residues represents the basis to identify the opportunity to use a certain material to produce renewable energy, biofertilizers and feeding materials within the production chain. To this aim, many different analytical procedures have to be carried out simultaneously.

This comprehensive approach focused on this subject, also considering different varieties of *Hevea brasiliensis*, is missing in the scientific literature and could represent the basis for performing sustainability assessment of these productions based on primary data.

2. Materials and Methods

2.1. Introduction

Samples of rubber seed coming from Africa were supplied by Green Dragon Environmental Ltd. (Chichester, United Kingdom), a UK company providing professional engineering services to renewable energy companies. Biomass Lab of Università Politecnica delle Marche carried out several analyses on the rubber seed samples with the aim to verify mass balances of the first processing steps and the quality of products and residues. Different varieties of rubber seed were tested for solvent extraction to understand the variability of the extracted rubber seed oil (RSO) in terms of quantity and quality considering a possible application for biodiesel production, due to the interest of different stakeholders in this possibility. A test of mechanical extraction was carried out to assess the differences in terms of oil yield and have a comparison with data reported in scientific literature. The by-products of cultivation, seed preprocessing and solvent extraction were analyzed as well to understand possible valorization options with the aim to increase the sustainability of the biodiesel production chain and indirectly to increase also the sustainability of the latex production chain, currently the main driver of rubber tree cultivation. Materials, treatments and analyses are detailed in the following paragraphs.

2.2. Materials

Rubber seeds were handpicked in Alépé, Ivory Coast, during September 2019 and shipped to Biomass Lab in Italy. Seed samples from five varieties of rubber tree were evaluated, in particular:

- PB 260
- IRCA 331
- IRCA 109
- GT1
- IRCA 41

All samples, around 5–10 kg for each variety, were shipped and stored in a plastic bag until analysis. An additional sample of empty capsules was sent for evaluation.

2.3. Pre-Treatment

The samples received in the Biomass Lab were sealed in plastic bag and there was no evidence of impurities such as dirt, mud, sand and stones. For each sample of rubber seed, kernel and shell were split by manual dehulling. Rotten rubber seeds were removed from the not degraded one. The share of rotten seeds was always below 5% *w/w*. Masses of all the fractions were recorded after each step to define an overall mass balance. No drying step was needed as a pretreatment because of the relatively low moisture content of the received samples.

2.4. Chemical Oil Extraction

Two solvents were tested for the chemical extractions: hexane and ethanol, which represent the common extraction solvent used in industry and a possible alternative, respectively. Although ethanol is not so common as seed oil extraction solvent, it can be biogenic and can be easily obtained from a large variety of biological materials [28] also in developing countries (e.g., by fermentation) and could be employed for rubber seed oil extraction at local level to further improve sustainability. Furthermore, hexane shows toxic properties and originates from non-renewable sources [29].

For each extraction test, 250 g of kernels were ground in a bench-top mill (mod. A11 basic analytical mill, IKA, Staufen im Breisgau, Germany) just before extraction. Afterwards, a representative

portion of 25 g was extracted with 400 mL of n-hexane (Sigma Aldrich, purity > 95%) or ethanol (Baker, purity 96%) in a standard Soxhlet apparatus for 24 h.

The extraction solvent was removed from the rubber seed (RSO) oil at 70 °C or 85 °C depending on the solvent. The solvents were recovered by condensation. A following treatment at about 100 °C under vacuum in a rotavapor apparatus (mod. Laborota4000, Heidolph, Schwabach, Germany) was performed to remove water from the oil sample. The RSO obtained was stored in the dark at 4 °C until analysis.

The solid residue (flour) was dried at 85 °C to remove residual solvents before storing it in plastic bag until analysis. The mass balance of the whole extraction process was evaluated. Chemical extraction tests were performed in triplicate for each solvent. The results were statistically evaluated with the Tukey–Kramer test at the 0.05 level of significance.

2.5. Mechanical Oil Extraction

Preliminary tests were carried out to tune the mechanical extraction of the rubber seeds on a single screw expeller for vegetable oil extraction (mod. 205 S, COTER, Bracco Srl, Bagnatica, Italy). Dried seed results are unsuitable for mechanical extraction with the used device, causing the block of the system. The successful tests were conducted by using not dried and not dehulled seed. A test was carried out with the kernel but the dehulled material was not able to reach the high pressures required for mechanical extraction (data not shown), so the extraction test was conducted on the whole seed, in line with literature [13].

Mechanical extraction yield was assessed on GT1 variety due to the mass of sample required.

A portion of around 5 kg of whole seeds was extracted with the expeller at 80 °C and with an 8 mm diameter compression ring with three extraction cycles.

The raw RSO was clarified by decantation and centrifugation and the sludge removed. The solid residue (oil cake) was stored in plastic bag until analysis.

2.6. Chemical-Physical Analysis of Rubber Seed Oil and By-Products/Residues

A list of the analytical parameters evaluated is reported in Table 1, describing references of standard methods employed, principle of analysis and fractions analyzed (see standard test methods for further details).

Except for RSO, all fractions were initially characterized by a proximate analysis (i.e., moisture content, higher and lower heating values, ash content) necessary to define mass balances and to understand which are the most promising applications for such materials.

Shells, kernels and capsules were further characterized as required or evaluating a possible application in the solid biofuel sector, i.e., chlorine and sulfur contents, elemental analysis (CHNO).

The RSO extracted was analyzed for the most important quality parameters: acid number, iodine number, saponification number. Moreover, RSO was investigated analyzing the fatty acid methyl ester (FAME) composition, important for a possible application for biodiesel production. Kinematic viscosity and density were determined only on the oil extracted mechanically due to the significant amount needed for the analysis. The flours were also analyzed for cyanide content and other parameters useful for a possible use for animal feeding. Analyses were performed in triplicate except for RSO analyses. The results were statistically evaluated with the Tukey–Kramer test at the 0.05 level of significance.

Table 1. Standard methods used for the analyses.

Parameter	Unit of Measure [1]	Reference Method	Principle	Fractions Analyzed [2]
Proximate analysis				
Moisture content	% a.r.	ISO 18134-2	Drying at 105 ± 2 °C in a forced ventilation oven (mod. M120-VF, MPM Instruments, Bernareggio, Italy)	Cap, Sh, Ker, Flo, Sd
Ash content	% d.m.	ISO 18122	Ignition at 550 ± 10 °C in a thermogravimetric analyzer (mod. TGA 701, LECO, St Joseph, MI, USA)	Cap, Sh, Ker, Flo
HHV/LHV	MJ kg^{-1}	ISO 18125	Combustion in a bomb calorimeter (mod.C2000 basic, IKA, Staufen im Breisgau, Germany), LHV calculated considering H content	Cap, Sh, Ker, Flo
Ultimate analysis				
Chlorine and Sulfur content	% d.m.	ISO 16994	Acid combustion gases absorption (calorimetric bomb) and measuring by ion chromatography (mod. 761 IC, Metrohm, Formello, Roma, Italy)	Cap, Sh, Ker, VO
Elemental analysis (CHN/O)	% d.m.	ISO 16948	Analysis with an elemental analyzer (mod. 2400 Series II CHNS/O, Perkin Elmer, Milano, Italy). Oxygen content calculated by difference	Cap, Sh, Ker
Feed analysis				
Crude fiber	% d.m.	ISO 6865	Gravimetric determination of residue obtained after acid and alkaline digestion	Flo
Ether extract	% d.m.	ISO 6492	Solvent extraction (ether) with a Soxhlet apparatus	Flo
Crude protein	% d.m.	ISO 16634-1	Nitrogen content determination (mod. 2400 Series II CHNS/O, Perkin Elmer, Milano, Italy) and calculation by conversion factor	Flo
Nitrogen free extractives	% d.m.	calculated	calculated by difference	Flo
Cyanides	ppm	ISO 2164	Steam distillation and titration with silver nitrate	Flo

Table 1. *Cont.*

Parameter	Unit of Measure [1]	Reference Method	Principle	Fractions Analyzed [2]
RSO analysis				
Kinematic viscosity	cSt	ISO 3104	Determination with a Cannon-Fenske viscometer tube at 40 °C	VO
Density	g cm^{-3}	ISO 12185	Determination with oscillating U-tube density meter (mod. Minivis 445, Grabner Instruments Messtechnik, Wien, Austria)	VO
Iodine number	gI2 100·g^{-1}	ISO 3961	Iodometric titration (Wijs reagent, potassium iodide and sodium thiosulfate as titrant)	VO
Acid number	mg KOH g^{-1}	ISO 660	Titration with KOH	VO
Saponification number	mg KOH g^{-1}	ISO 3657	Saponification (potassium hydroxide) and titration with HCl	VO
Fatty acid composition	%	ISO 12966	FAME production by transmethylation/methylation with boron trifluoride and GC-FID analysis	VO

[1] a.r: as received; d.m.: dry matter. [2] Cap: Capsule; Sh: Shell; Ker: Kernel; Flo: Flour; VO: Vegetable oil; Sd: Seed.

2.7. Mass and Energy Balances and Possible Production Scenarios

Taking into account all results obtained in this study (primary results) and some others taken from the literature (secondary results), it was possible to define a production scenario for latex by-products, in order to appreciate the positive contribution to the latex production chain.

The following data were taken from literature or estimated (secondary data):

- Mass yield of rubber seed referred to 1 hectare equal to 150 kg ha^{-1} [2,4,12,13]. Higher yields are reported by other authors [27] but in the present study the lower yield was used to take into account that part of the seeds could be difficult to harvest and should be used to help preserve the ecological balance in the soil.
- According to the authors' knowledge, there are no data in literature related to capsule yield. The value was estimated from the small quantity obtained and it should be considered as a preliminary indication. This value was also employed to estimate the rubber tree fruit yield.
- Energy content of rubber seed was calculated by energy contents and mass balances of both shell and kernel fractions.
- In the chemical extraction step ethanol was considered as solvent since it represents a more environmentally sustainable scenario.

Mass and energy balances for each residue were evaluated taking into account some considerations:

- Masses were expressed on dry matter basis since moisture content is a parameter strongly dependent by logistic variables.
- Energies were expressed as lower heating value (LHV) to obtain a more realistic estimation of the energetic performances, keeping the dry basis as mentioned above.
- Mass and energy balance were referred to 1 ha of rubber tree cultivation
- Mass percent values were referred on whole seed basis since represents the main residue of the latex chain.

3. Results and Discussion

Table 2 lists seed moisture contents and mass balances of kernel and shell. The average seed moisture value is about 16.1% *w/w*, ranging from 11.9% *w/w* (GT1) to 20.5% *w/w* (PB 260). Literature reports several values for seed moisture content from around 3% *w/w* [7,30] up to around 25–30% *w/w* for collected seed [13]. Since rubber tree capsules explode when ripe, releasing the seeds on the ground, moisture content is strictly related to the collection methods employed, i.e., collection time after the seed dispersal, the amount of rain form the dispersal, storage conditions [2], more than the variety typology.

Table 2. Mass balance of seed fractions.

Variety	Seed Moisture (% *w* w^{-1} a.r.)	Kernel (% *w* w^{-1} d.m.)	Shell (% *w* w^{-1} d.m.)
GT1	11.9 a	51.1 a	48.9 b
IRCA 109	14.3 a	50.5 a	49.5 b
IRCA 331	19.4 b	56.8 b	43.2 a
IRCA 41	14.6 a	51.2 a	48.8 b
PB 260	20.5 b	51.8 a	48.2 b
Mean	**16.1**	**52.3**	**47.7**
St.Dev.	**3.7**	**2.6**	**2.6**

Note: values in the same column that do not share a letter are significantly different at *p*-value 0.05.

High fungal contamination could be probably related to the high seed moisture content, which promotes the mold formation during the storage as observed by Widyarani et al. [2].

Shell and kernel represent 47.5% w/w_{db} and 52.3% w/w_{db} of the whole seed, respectively, showing a low data variation among the tested varieties. The obtained results are in line with literature [2,13,31].

Table 3 reports characterization results for kernel, shell and capsule.

Table 3. Characterization of shell, kernel and capsule.

Variety	Fraction	Moisture (% $w\ w^{-1}_{a.r.}$)	Ash (% $w\ w^{-1}_{d.b.}$)	HHV (kJ $g^{-1}_{d.m.}$)	LHV (kJ $g^{-1}_{a.r.}$)	C	H	N	O	Cl	S
								(% $w\ w^{-1}_{d.b.}$)			
GT1	shell	13.4 b	0.38 a	21.07 d	17.23 b	51.4	5.1	0.4 b	42.7	<0.01	0.01
	kernel	10.5 ab	3.23 c	29.22 fg	25.34 f	60.7	5.7	3.3 e	26.9	0.02	0.17
IRCA 109	shell	14.8 b	0.45 a	20.54 bc	16.44 a	50.7	5.6	0.9 c	42.3	0.01	0.01
	kernel	13.7 b	3.28 c	28.82 e	24.14 e	60.6	5.0	3.6 fg	27.4	0.02	0.11
IRCA 331	shell	16.7 b	0.41 a	20.47 bc	16.12 a	51.5	5.6	0.4 b	42.1	0.01	0.01
	kernel	21.3 c	3.40 c	29.11 ef	22.87 d	63.2	5.2	3.7 g	24.4	0.02	0.10
IRCA 41	shell	14.4 b	0.48 a	20.64 c	16.78 ab	51.2	5.6	0.4 b	42.3	<0.01	0.01
	kernel	14.7 b	3.14 c	28.91 e	24.46 e	52.5	4.9	2.8 d	36.6	0.01	0.10
PB 260	shell	16.8 b	0.83 a	20.28 ab	16.18 a	50.3	5.6	0.5 b	42.8	0.01	0.02
	kernel	23.6 c	3.19 c	29.48 g	23.81 e	60.2	4.8	3.4 ef	30.3	0.02	0.11
Capsule	Capsule	8.0 a	1.90 b	20.04 a	18.87 c	50.2	5.5	<0.1 a	42.3	0.01	0.03
Mean		**16.0**	**1.88**	**24.86**	**20.34**	**55.2**	**5.3**	**1.9**	**35.8**	**0.02**	**0.06**
St.Dev.		**3.5**	**0.14**	**0.28**	**0.68**	**2.3**	**0.3**	**0.3**	**2.5**	**<0.01**	**0.02**
ISO 17225-2	A1 limit	10	0.7	16.5 ≤ LHV ≤ 19.0		-	-	0.3	-	0.02	0.03
	A2 limit	10	1.5	16.3 ≤ LHV ≤ 19.0		-	-	0.5	-	0.02	0.03
ISO/TS 17225-9	I1 limit	45	3.0	-		-	-	0.5	-	0.05	0.05
	I4 linit	60	7.0	-		-	-	1.5	-	0.10	0.10

Note: values in the same column that do not share a letter are significantly different at *p*-value 0.05 (e.g., "fg" and "ef" are not significantly different because sharing "f". In addition, "fg" is also not significantly different from "g", while "ef" is significantly different from "g").

It is clear that kernel and shell show a very different composition. Due to the higher protein content, kernel contains much more nitrogen (3.3% *w/w*) and sulfur (0.12% *w/w*) than shell (0.50% *w/w* of ash and 0.01% *w/w* of sulfur). For the same reason, kernel exhibits a higher ash content than shell, 3.25% and 0.51%, respectively.

Ishak et al. [26] found similar results for ultimate analysis, highlighting higher values for C (64.45%), N (3.63%) and S (0.32%) in kernel with respect to shell. However, shell shows a higher N content (1.5%) with respect to this work. Rubber seed shell characterized by Reshad et al. [10] showed an N content of 3.13%.

Although both fractions are characterized by high heating values, the RSO contained in kernel gives to it a higher energy content with respect to shell, 24 MJ/kg against 16.5 MJ/kg of LHV, respectively. The same trend is evident for the respective carbon contents, since vegetable oil is mainly composed of carbon (above 70% *w/w*). Chlorine contents are low for both fractions, i.e., less than 0.02% *w/w*.

Similar results for heating values can be found in literature [10,26].

From the results reported in Table 3, it can be noticed that shell has a composition similar to woody biomass, with some characteristics that comply even with solid biofuel standard limits for high quality pellet classes, i.e., ash content, heating value and Cl/S contents for class A1 and nitrogen content for class A2 [32]. Similar considerations can be applied to capsule fraction, which presents a lower N content (<0.1% *w/w*) than shell but a higher ash (1.90% *w/w*) and S (0.03% *w/w*) contents, making it a material suitable for energy applications complying with the recent hogfuel requirements [33].

Given the small quantity of capsule sample analyzed, the results should be considered just as a preliminary indication, needing a further in-depth analysis for understanding the possible quality variability.

Different authors have found low ash and moisture contents for the shell fraction [10,26,31].

The results obtained from characterization analysis show negligible differences among varieties, suggesting a quite standardized feedstock.

High protein and oil contents make kernel an unsuitable material for energy valorization, from both environmental and economic point of views. In fact, a high amount of pollutants can be emitted during combustion of kernel, conversely more valuable products can be obtained after oil extraction, i.e., biodiesel from RSO and feed from extracted flour.

Table 4 reports results for the chemical oil extraction. Hexane extracts more RSO than ethanol, until 8.0% *w/w* more, although differences in yields on an average basis are limited, i.e., 49.0% *w/w* against 46.2% *w/w*, respectively.

Table 4. Oil yields of chemical extraction (kernel basis).

Variety	Solvent	Oil [1] (% $w\ w^{-1}_{db}$)	Flour (% $w\ w^{-1}_{db}$)	Residue (% $w\ w^{-1}_{db}$)
GT1	Ethanol	43.6 a	45.0 b	11.4
	Hexane	48.8 de	51.2 de	-
IRCA 109	Ethanol	47.0 c	43.4 a	9.6
	Hexane	47.9 cd	52.1 e	-
IRCA 331	Ethanol	47.6 cd	42.8 a	9.6
	Hexane	49.5 ef	50.5 cd	-
IRCA 41	Ethanol	45.1 b	43.4 a	11.4
	Hexane	48.6 de	51.4 de	-
PB 260	Ethanol	47.9 cd	42.5 a	9.6
	Hexane	50.2 f	49.8 c	-
Mean		**47.6**	**47.2**	**10.3**
St.Dev.		**1.5**	**1.0**	**1.0**

[1] yield without residue for ethanol extraction. Note: values in the same column that do not share a letter are significantly different at *p*-value 0.05 (e.g., "de" and "ef" are not significantly different because sharing "e". In addition, "de" is also not significantly different from "cd", while "ef" is significantly different from "cd").

Most of the papers found in literature about the oil chemical extraction of rubber seed use hexane as a solvent; few evaluate other solvents with a different dielectric constant [8,9,18,34–36]. To the authors' knowledge, papers focused on employing ethanol are limited [34,36].

The oil content yields obtained are in line with literature, the values of which range from around 33% to 55% for hexane extraction [26,31,34,37].

Mohd-Setapar et al. [34] have extracted around 45% of RSO, employing ethanol against around 55% obtained with hexane. Wildan et al. [36] found that hexane extracts around 12% more RSO from waste rubber seed with respect to ethanol.

After the solvent removal step from the ethanol/oil mixture, a white solid residue remains in the flask which accounts for around 10.3% on kernel basis.

Other authors [34] have observed white particles in both water and ethanol/water (70:30 v/v) after rubber seed extraction. Same authors describe the oily residues obtained as too viscous and not transparent as hexane extracted one. In the present work RSO-ethanol was easily separated from the solid residue after a mild warming of the mixture at 40 °C. At these conditions the RSO has become more fluid and it was separated by pouring it off from the flask while the residue remained stuck on flask wall.

Since the residue was highly water soluble, it could be composed mainly by sugars as it is known that ethanol exhibits a good ability to extract these compounds [38].

Differences in extraction yields and extract compositions are linked to solvent and compounds polarities. The higher ethanol polarity with respect to hexane allows for a co-extraction of compounds other than triglycerides, which are insoluble in non-polar solvent [34]. For the same reason hexane left more exhaust kernel (flour) at the end of the extraction process than ethanol, with a 51% and 43.4% mass balance, respectively.

Visually, the RSO extracted with ethanol was orange and turbid with respect to the hexane extracted RSO, yellowish and more transparent.

In Figure 1 are plotted the results of mechanical oil extraction for GT1 variety. Mechanical extraction produces 15.9% *w/w* of RSO on whole seed and as received basis, equal to 35.4% *w/w* on kernel dry basis, which accounts for 73% *w/w* of the total RSO contained (referred to hexane extraction).

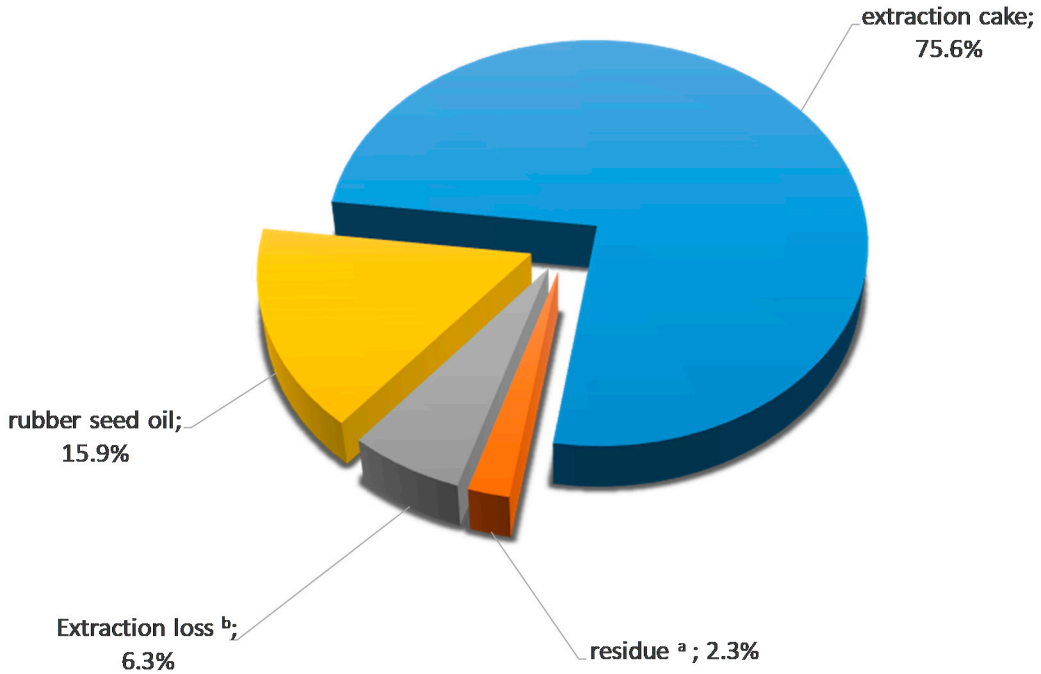

Figure 1. Oil yield of mechanical extraction (whole seed) for GT-1 (mass on whole seed as received basis): (a) residue separated after decantation/centrifugation; (b) mass loss and water evaporated (moisture).

Besides extraction cake, a solid residue (sludge) was obtained after the RSO clarification process (i.e., decantation and centrifugation), which has retained part of the oil. Since in the industrial extraction process sludge is re-extracted by addition to the initial feedstock (i.e., rubber seed), the oil contribution associated with this portion is not considered in the overall mass balance. Similar results were reported also by other authors. Ebewele et al. [37] have extracted RSO mechanically using a hydraulic press obtaining a yield of 28.5% *w/w* of kernel weight at 8 MPa and 70 °C. Lower pressure yields less RSO, as reported by Sabarish [39], which has obtained a yield of RSO about 16.5% w/w_{db} of kernel weight with 3.5 MPa pressure. Aigbodion et al. [40] have extracted 23% *w/w* RSO of whole seed using a pilot mill. Solid fraction produced after mechanical extraction (extraction cake) was higher than the one obtained from solvent extraction (flour) due to the presence of residual oil and the shell fraction.

Mechanical extraction with continuous screw press produces RSO without solvent employment and with relatively low operating costs; although, this solution does not allow one to separate and valorize the shell fraction before the extraction process. Moreover, from the animal nutrition point of view, the presence of residual oil in the extraction cake could make it difficult to manage fat balance in animal feeding applications and can significantly reduce its storage time (i.e., oil rancidification).

Table 5 reports characterization results for the chemical extraction flours. The comparison between flours obtained with two extraction solvents does not highlight important differences in composition. Flour from ethanol extraction shows a higher protein content than hexane, 43.7% *w/w* against 37.4% *w/w*, respectively. The difference could be attributable to the solid residue extracted by ethanol which returns a flour richer in protein. To the author knowledge in literature there are no papers on characterization of ethanol extracted flours. The obtained results are in line with Oluodo [12], which reviewed different papers on nutritional composition of rubber seed founding a content of moisture 3.99%, crude protein 23%, crude fat 68.5% and ash 4.3%.

Cyanide is recognized as a possible drawback of using this material for feeding and a specific analysis was carried out. Cyanide content was tested in GT1 flour extracted with hexane and ethanol and resulted 36 and 19 ppm, respectively. In both cases the cyanide amount was less than 500 ppm, which is considered the general safe level for animal forages [41]. Differences in cyanide contents of meals, higher for hexane extraction than ethanol, could be due to a partial removal of cyanogenic glycoside (called linamarin). Since linamarin is a water soluble compound [42], it could be partly removed by a polar solvent like ethanol during RSO extraction.

The results of oil characterization are shown in Table 6. High and variable values of acid number were obtained for RSO but are in line with literature [8,18,35]. According to several authors, acid number can also be significantly affected by logistics factors (i.e., collection time and method, storage) [11,31,43,44]. Abdulkadir [7] found an increase in RSO acid number from an initial value of 34 mg KOH/g for fresh seeds up to around 80 mg KOH/g for RSO extracted (with hexane) after two months of storage due to enzyme activities [31]. The obtained results agree with literature since around three months passed from seeds collection to laboratory analyses. The results show a higher acid number for RSO extracted with hexane than ethanol, as found by Attah [35], which compared FFA extraction for different solvents with similarly different dielectric constants. Thus, ethanol seems to extract fewer acid compounds than hexane in aged seeds. Iodine number and saponification number are in line with literature [17,22] and not affected by solvent polarity [35].

Table 5. Flour characterization.

Variety	Solvent	Moisture (% $w\ w^{-1}_{a.r.}$)	Dry Matter (% $w\ w^{-1}_{a.r.}$)	Gross Energy (kJ $g^{-1}_{d.m.}$)	Ash (% $w\ w^{-1}_{d.b.}$)	Organic Matter (% $w\ w^{-1}_{d.b.}$)	Crude Protein (% $w\ w^{-1}_{d.b.}$)	Ether Extract (% $w\ w^{-1}_{d.b.}$)	Crude Fiber (% $w\ w^{-1}_{d.b.}$)	Non-Nitrogen Extractives (% $w\ w^{-1}_{d.b.}$)
GT1	Ethanol	9.8 de	90.3 ab	19.94 bc	6.1 bc	93.9 c	40.7 bc	1.1 cd	4.2 bd	48.0 bc
	Hexane	6.8 bc	93.3 cd	18.72 ab	6.0 ab	94.0 cd	38.3 b	0.9 ab	4.3 cd	50.5 bc
IRCA 109	Ethanol	10.5 e	89.5 a	19.10 b	7.2 e	92.8 a	44.4 d	1.2 d	4.5 cd	42.7 a
	Hexane	6.8 bc	93.3 cd	19.70 bc	6.2 bc	93.8 c	38.9 b	0.8 a	3.8 a	50.4 bc
IRCA 331	Ethanol	10.3 e	89.7 a	18.03 ab	6.9 de	93.1 ab	42.2 cd	0.9 ab	4.1 ac	46.0 ab
	Hexane	8.2 cd	91.8 bc	18.48 ab	6.3 bc	93.7 c	32.1 a	1.0 bc	4.2 bd	56.4 d
IRCA 41	Ethanol	6.9 bc	93.1 cd	20.01 c	6.7 cd	93.3 b	45.4 d	0.9 ab	4.9 e	50.7 c
	Hexane	2.8 a	97.2 e	19.86 bc	5.7 a	94.3 d	38.4 b	0.9 ab	4.4 cd	42.3 a
PB 260	Ethanol	7.8 c	92.2 c	18.40 ab	6.9 de	93.1 ab	45.6 d	0.9 ab	4.5 cd	49.6 bc
	Hexane	5.6 b	94.4 d	17.87 a	6.4 c	93.6 bc	39.2 b	1.1 cd	3.9 ab	42.0 a
Mean		7.5	92.5	19.02	6.5	93.5	40.1	1.0	4.3	47.8
St.Dev.		1.8	1.8	0.87	0.4	0.4	2.6	0.1	0.3	4.6

Note: values in the same column that do not share a letter are significantly different at *p*-value 0.05 (e.g., "cd" and "de" are not significantly different because sharing "d". In addition, "cd" is also not significantly different from "bc", while "cd" is significantly different from "e").

Table 6. Oil characterization and composition.

Variety	Solvent	Acid Number	Iodine Number	Saponification Number	Fatty Acids Composition (%)						
		mg KOH g^{-1}	g I2 100 g^{-1}	mg KOH g^{-1}	C16:0	C18:0	C18:1 n−9	C18:1 n−7	C18:2 n−6	C18:3 n−3	C20:0
GT1	Ethanol	39.2	129.3	185.6	10.0	8.8	23.4	<0.1	36.9	20.7	0.2
	Hexane	76.2	126.4	191.2	11.0	9.3	22.1	<0.1	38.0	19.5	0.1
IRCA 109	Ethanol	28.1	127.3	191.7	12.9	12.0	36.2	<0.1	24.4	14.5	<0.1
	Hexane	105.7	127.9	188.4	13.0	12.3	36.0	<0.1	25.2	13.5	<0.1
IRCA 331	Ethanol	32.2	125.2	188.4	13.5	13.4	35.8	0.4	23.6	13.3	<0.1
	Hexane	84.9	129.5	192.3	14.0	12.4	34.6	<0.1	27.1	11.8	<0.1
IRCA 41	Ethanol	29.0	126.4	194.3	11.5	9.8	32.1	0.3	31.0	15.3	<0.1
	Hexane	100.4	125.2	189.7	11.9	10.2	27.3	0.5	37.6	12.4	0.1
PB 260	Ethanol	25.3	124.6	189.9	13.6	12.8	35.7	<0.1	26.5	11.3	0.1
	Hexane	66.0	131.9	191.3	10.1	8.1	37.2	0.4	32.3	11.9	<0.1
Mean	Ethanol	30.8	126.6	190.0	12.3	11.4	32.6	0.2	28.5	15.0	0.1
St.dev.		5.3	1.9	3.8	1.5	2.0	5.4	0.1	5.5	3.5	0.1
Mean	Hexane	86.6	128.2	190.4	12.0	10.5	31.4	0.2	32.0	13.8	0.1
St.dev.		16.5	2.6	1.7	1.6	1.9	6.5	0.1	5.9	3.2	<0.1

The results of fatty acid composition are in line with the range reported in scientific literature [14,18,22,45]. The different solvents used for extraction do not seem to influence the fatty acid composition. With respect to a standard vegetable oil used for biodiesel production (EN 14214) [46], it seems there is a higher amount of polyunsaturated fatty acids in RSO, which could make the biodiesel produced unstable, suggesting the use in blends of different vegetable oils.

The kinematic viscosity (40 °C) of RSO obtained by mechanical extraction was 36.5 cSt for GT1. The density of oil (at 30 °C) was about 0.88–0.89 g/cm^3, in line with scientific literature [8,9,19,31].

Mass and Energy Balances and Possible Production Scenarios

Figure 2 shows an overall mass and energy balance reported on a hectare basis for by-product and residues obtained from the latex production chain.

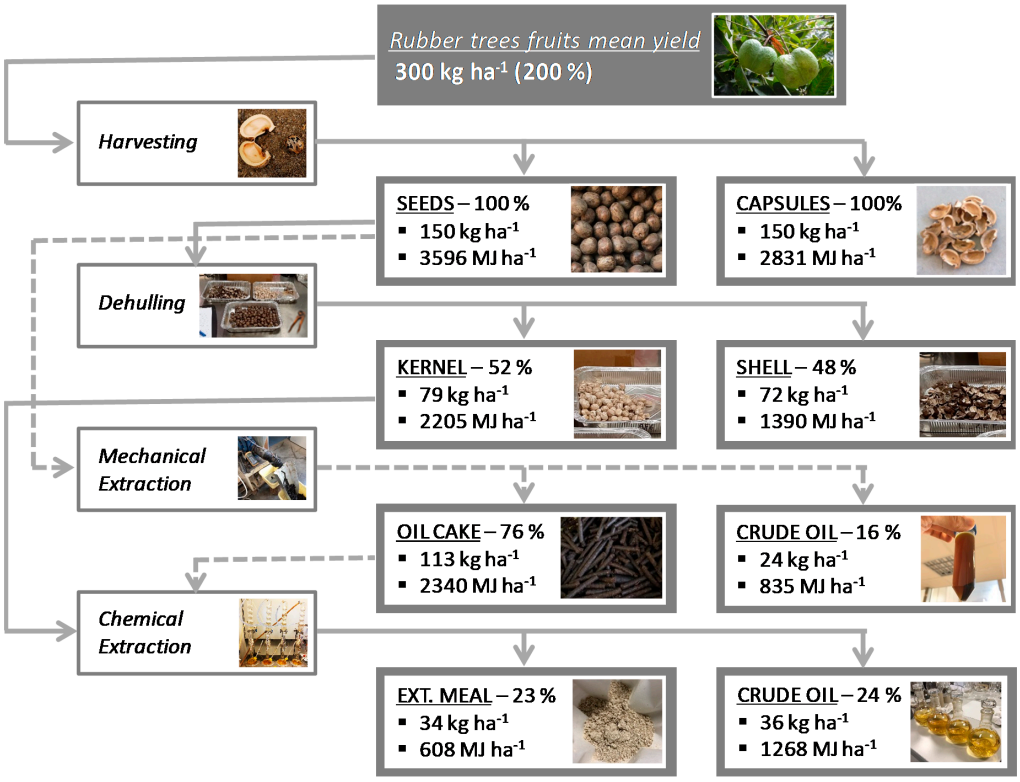

Figure 2. Mass and energy balance of the rubber seed oil (RSO) chain referring to 1 ha of rubber tree cultivation (percent values represent mass referred on whole seed basis, energy content was obtained using LHV on dry basis).

Since there were no substantial differences among tested varieties, averaged data were employed to make calculations.

The percentages represent the mass fraction with respect to the rubber seed mass (100%). Consequently, the rubber tree fruits are reported as 200% because the capsules were also included.

It can be noticed that a preliminary separation of shell and kernel, followed by a chemical extraction, enables a recovery of 74% of the initial energy content (seed) only considering an energy valorization of shell and RSO. Extraction meal instead, due to the high protein content, can be addressed to animal feeding, adding value to this fraction. On the other hand, a mechanical extraction needs the whole seed (according to results of this study), which entails a lower RSO extraction and a solid residue (oil cake) with high nitrogen content and low storage stability (oil rancidification).

From the results reported in Figure 2 and in previous tables, it is possible to define production scenarios to valorize residues and products. All the values reported below are referred to 1 ha cultivation of rubber seed.

Capsules can be burnt in specific devices to produce the thermal energy needed to dry about 5 ton of rubber seed (from 25 to 5% moisture content [13]) to improve seed stability. Shell can be used as well to dry about 4.5 ton of rubber seed. Considering these numbers, capsules and shells can widely satisfy the need of thermal energy for drying rubber seeds and also a significant quantity of other products.

As an alternative, capsules and shells could be used to produce electricity in biomass power plants. As an indication, a production of about 300 kWh_e can be estimated.

Another possibility for shell could be the use as feedstock for the production of pellet to be combusted in small domestic stoves for heating. It can be estimated that shell pellet can be used in an 11 kW stove for about 24 h at maximum power.

According to the RSO production calculated in this study, it could be possible to produce more than 30 kg of biodiesel, the same amount needed for driving a car for more than 450 km.

A last consideration can be made on the extraction meal. As an indication, it could be exploited to substitute feeding materials equivalent to about 30 kg of soybean meal. This possibility can be achieved by carefully including a limited amount of rubber seed meal in the animal diet. The manure can be then employed to return nutrients and organic matter to the soil closing the cycle. According to some authors, the extraction meal itself could be used as a weed controller or as a biofertilizer [47].

4. Conclusions

The study evaluated the quantity of residues of rubber tree cultivation and their quality to give useful indications for a possible valorization. From an energy point of view, there is a significant amount of biomass material linked to shell and capsules that could be exploited to provide heat for many applications also within the possible "RSO to biodiesel" chain. Capsules and shells can be used to produce enough thermal energy for drying rubber seeds and also a significant amount of other products.

From each hectare cultivated with rubber seed, about 30 kg of RSO biodiesel could be produced, substituting about 26 kg of fossil fuels.

The extraction meal could be used as a biofertilizer or for feeding purposes to further improve the sustainability. For each hectare of rubber seed cultivation, about 30 kg of soybean meal can be substituted with rubber seed meal.

This information is useful to improve the sustainability of the latex production chain and to assess the sustainability of the possible bioenergy value chains. The quantity and quality of the studied biomass materials are interesting, but the practical feasibility of these win–win solutions should be defined case by case considering logistical and technical issues.

Author Contributions: Conceptualization, A.P., D.D., G.T.; methodology, A.P., G.T.; validation, A.P., G.T.; formal analysis, A.P.; investigation, D.D., G.R., S.F.; resources, G.T.; data curation, A.P., G.T.; writing—original draft preparation, A.P., G.T., G.R., S.F.; writing—review and editing, A.P., D.D., G.T.; visualization, A.P.; supervision, G.T.; project administration, D.D., G.T.; funding acquisition, D.D., G.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Green Dragon Environmental under a specific research contract with Università Politecnica delle Marche.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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