FOODLAND

GUIDELINES ON IMPLEMENTATION AND MANAGEMENT OF BIO-BASED PACKAGING PROCESSES

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Task 4.5: Technological research for secondary processing

Subtask 4.5.4: Bio-based packaging

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Task 4.5: Technological research for secondary processing

Subtask 4.5.4: Bio-based packaging

Leader: UNIBO
Participants: INAT (TN), UoN (KE), SUA (TZ), NARO (UG), MAK (UG), and SMEs
Start-End: M10-M40
Status: *On time*

1. Introduction

1.1 Objectives of the subtask

Subtask 4.5.4 aims to develop and test #4 bio-based packaging materials able to preserve the quality of some food products during storage (and transport) and increase their sustainability and attractiveness.

The availability of environmentally friendly, affordable and optimal packaging materials (i.e. local bio-based materials from renewable plants and/or obtained by suitable food waste/by-products such as edible coatings and films together with primary and secondary packaging materials) and technologies were assessed, pre-selected and tested for different local food products (soft-dried eggplants and strawberries, tamarillo powder, quinoa snack, extruded and non-extruded composite flours, soft-smoked fish-fillets, deep-fried fish fingers, whole fresh eggplants).

The main technological properties of the materials obtained were tested and shelf-life studies were carried out to assess the quality and safety of the selected food products. The environmental (LCA) and economic (LCC) impacts of the bio-based packaging materials were also assessed.

1.2 Objectives and description of the innovations

The summary of the objectives, innovations and activities carried out by each partner in the field of Subtask 4.5.4 are reported in **Table 1**.

INAT (TN) developed a bio-based material from local agricultural waste such as wheat straw fibres (WSFs) and artichoke by-products as a sustainable packaging solution. The cellulose nanocrystals (CNCs) were obtained from WSFs using a chemical process, while the antioxidant extract was obtained by maceration of artichoke by-products in methanol. The bio-based material was produced using PBAT and TPS with CNCs and antioxidant extract by blown extrusion. The film was used for the packaging of mild-dried fruits and vegetables, such as eggplants and strawberries.

UoN (KE) produced a bio-based material from local cassava tubers and coconut oil as a sustainable packaging film. The cassava starch was extracted following a wet process and was added to distilled water, coconut oil and glycerol as ingredients to produce the bio-based film by casting method. The bio-based film was used for the packaging of local dry foods such as tree tomato fruit (tamarillo) powder and snack made with quinoa and wheat flours (mandazi).

SUA (TZ) developed a bio-based material from nanocellulose obtained from pineapple peels (PPs). The PPs cellulose was isolated by a chemical method and nanocellulose (PPnc) was obtained by an acid hydrolysis method and used as an ingredient with water, gellan gum and glycerol to produce a film by a casting process. The film was used for the packaging of extruded and non-extruded composite flours for porridge.

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NARO and **MAK (UG)** used cassava starch and chitosan to produce an edible bio-based coating and a bio-based film. The cassava starch was extracted by a wet process. Distilled water, chitosan and glycerol were added to the cassava starch to obtain the “film-forming dispersion”. The bio-based film and coating were used for the packaging of soft-smoked fish fillets/deep-fried fish fingers alone or in combination (NARO) and the coating for the packaging of fresh whole eggplants (MAK).

Table 1. Subtask objectives and contributions of each partner.

Expected Result (ER 6)	Specific Result SR 6.2	Country	Partner	Short description	Target food products
Methodological and technological innovations enhancing secondary processing	#4 Prototypes of bio-based packaging released	TN	INAT	Bio-based film produced from wheat straw fibres nanocellulose.	Fruits and vegetables (soft-dried strawberries and eggplants)
		KE	UoN, KEPC	Bio-based film produced from cassava starch and coconut oil.	Therapeutic food (tree tomato fruit powder and quinoa snacks)
		TZ	SUA, KTM	Bio-based film produced from pineapple peels nanocellulose.	Composite flours (extruded and non-extruded)
		UG	NARO	Bio-based film and coating produced from cassava starch and chitosan.	Fish products (soft-smoked fish fillets; deep-fried fish fingers)
		UG	MAK, NUT	Characterisation and shelf-life test of the coating developed by NARO.	Fruits and vegetables (fresh whole eggplants)

2. Description of small-scale tests and results

2.1 Wheat straw fibres cellulose nanocrystals-based film (INAT)

2.1.1 Selection of local bio-raw materials and extraction of polymers

Four agricultural wastes/by-products collected from local agricultural companies in the region of Jendouba (TN) were investigated as raw materials such as pea hulls, artichoke by-products, olive tree by-product, and wheat straw fibres (**Figure 1**). After assessing the percentage content of moisture, cellulose, hemicellulose and lignin, wheat straw fibres (WSFs) were selected for the extraction of polymers useful for making bio-based film as they are characterised by the lowest moisture content (~10%) and the highest cellulose content (~22.50%).

<u>Operative procedure for WSFs washing and characterization</u>:

- Clean the raw WSFs under running tap water and then soak it in distilled water for 1 h
- Dry the cleaned raw material in an oven at 40 °C to evaporate the distilled water
- Crush and sieve through a 0.280 mm mesh sieve to obtain a fine powder
- Moisture content by drying the powder in an oven at 105 °C until constant weight according to AOAC 1990 (925.10)
- Cellulose, hemicellulose and lignin contents of the powder following the methods of detergents according to Goering and Van Soest (1970).

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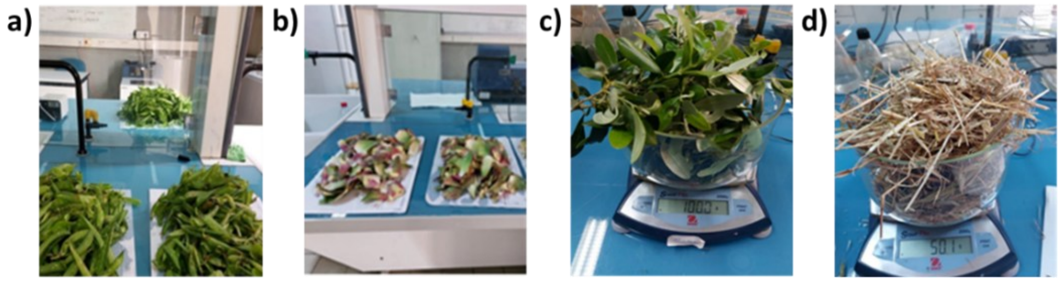

Figure 1. Examples of tested agricultural waste and by-products collected in the Jendouba (TN) region: **a)** pea hulls, **b**) artichoke by-products, **c**) olive tree by-products, **d**) wheat straw fibres.

Two methods for the extraction of cellulose from WSFs were investigated: the chemical method and the steam explosion method. The chemical method was chosen to extract the cellulose from the selected raw material as it gives a higher extraction yield (23.5 ± 2.0% vs 17.8 ± 1.8%).

Operative procedure for the extraction of cellulose from WSFs by chemical method (El Achaby et al., 2018):

- Treat ground fine powder of WSFs in distilled water for 1 hour at 60 °C
- Treat the resulted WSFs three times with 4% (w/w) of sodium hydroxide (NaOH) solution at 80 °C for 2 h under stirring
- Treat the resulted alkali WSFs three times by bleaching using a solution made up of equal parts (v/v) of acetate buffer (27 g NaOH and 75 mL glacial acetic acid, diluted to 1 L of distilled water) and of aqueous sodium chlorite ($NaClO_2$) (1.7%, w/w, in water)
- In all previous treatments the ratio of the WSFs to liquor was 1/20 (g/mL)
- Dry the resulting white cellulose extract in an oven at 40 °C for about 24 h
- Calculate cellulose extraction yield (Yield % = (m/M)·100), where m: mass of cellulose extracted and M: mass of raw WSFs used.

The main steps to extract cellulose from WSFs with chemical method are shown in **Figure 2**.

Operative procedure to obtain cellulose nanocrystals (CNC) from WSFs cellulose by acid hydrolysis (El Achaby et al., 2018):

- Treat WSFs cellulose with 64% (w/w) sulphuric acid (H_2SO_4) (pre-heated) at 45 °C for 45 min under mechanical stirring
- Stop the acid hydrolysis by adding cold distilled water (ice cubes) into hydrolysed solution
- Centrifuge three time at 9500 rpm and 10 °C for 10 min to remove the excess of sulphuric acid resulting in white CNC suspension
- Subject the white CNC suspension to a dialysis process against distilled water at 4 °C for 72 h to reach a neutral pH
- Homogenize the CNC with ultrasonic homogenizer
- Store the resulting CNC suspension in the refrigerator until its use.

Operative procedure to obtain antioxidant-rich extract from artichoke by-products by methanol maceration:

- Clean the inedible plant parts of artichoke (**Figure 1b**) under running tap water and then soak it in distilled water for 1 h

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- Dry the cleaned raw material in an oven at 37 °C to evaporate the distilled water
- Crush and sieve through a 0.280 mm mesh sieve to obtain a fine powder
- Plant extracts were obtained by maceration in methanol 80%. After filtration, the extract was transferred to vials and stored in the dark at 4 °C until use.

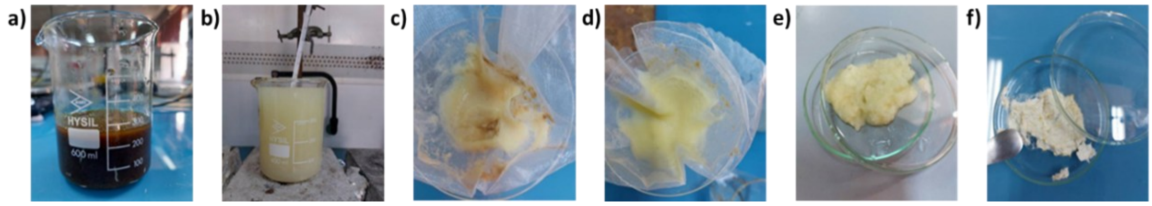

Figure 2. Main steps for extracting cellulose from wheat straw fibres (WSFs) by chemical method: **a**) final treatment with alkali solution, **b**), **c**), **d**) three bleaching treatments, **e**) white cellulose extract, **f**) dried white cellulose extract.

2.1.2 Formulation and preparation of bio-based film

The bio-based film, enriched with CNCs and antioxidant extract was prepared using a pilot production with twin-screw and blown extruders.

<u>Operative procedure for the preparation of wheat straw cellulose-based film material (PBAT + TPS + CNCs + artichoke by-products extract) (**Figure 3**)</u>:

- Preparation of a "master-batch" (4 kg) in the form of granules by mixing:
    - 5% TPS (thermoplastic starch, consisting of 70% starch and 30% glycerol),
    - 58% PBAT (polybutylene adipate terephthalate),
    - 3% PBAT grafted maleic anhydride,
    - 4% CNCs obtained from wheat straw fibres and 10 mL artichoke extract by a twin-screw extrusion process (**Figure 3a**).
- Production of a thin film by blown extrusion from the «master-batch» using a temperature of 110 °C (**Figure 3b-c**).

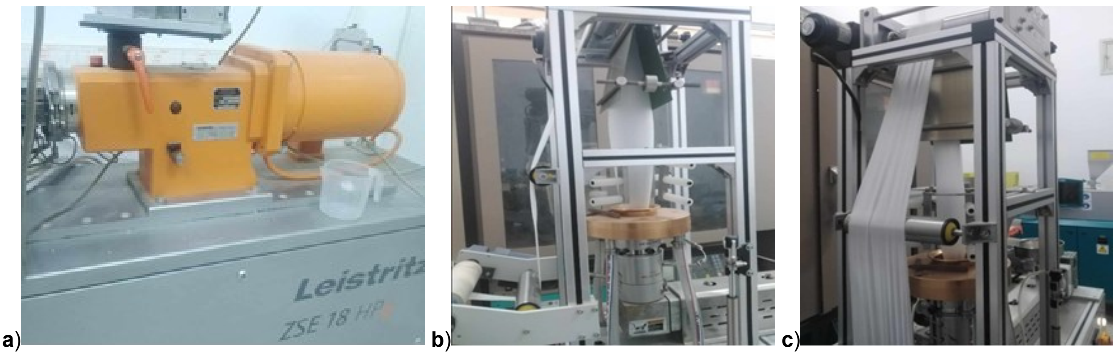

Figure 3. **a**) Twin screw extrusion machine and **b-c**) blown film extrusion process.

The obtained film was evaluated for the main physico-chemical properties such as thickness (μm), moisture (%), water solubility (%), biodegradability (%), elongation (%), tensile strength (MPa), tear resistance (N), coefficient of friction (μs static and dynamic), overall migration (mg/dm²) and water vapor transmission rate - WVTR (g/m^2 × day) (**Table 2**).

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Table 2. Physical-chemical properties of the PBAT + TPS + CNCs + artichoke by-products extract film.

Property (SI)	Value (Mean ± SD)
Thickness (µm)	50.50 ± 1.80
Moisture (%)	7.93 ± 0.30
Water solubility (%)	1.38 ± 0.40
Biodegradation (%) after 18 weeks	34.86
Elongation (%) - SM*	643.2 ± 45.6
Elongation (%) - ST*	136.2 ± 16.8
Tensile strength (MPa) - SM*	9.60 ± 1.06
Tensile strength (MPa) - ST*	4.03 ± 1.09
Tractive force (N)	6.30 ± 0.06
Tear resistance (N) - SM*	3.54 ± 2.02
Tear resistance (N) - ST*	8.28 ± 2.58
Coefficient of friction (µs static)	0.645 ± 0.001
Coefficient of friction (µd dynamic)	0.729 ± 0.001
Overall migration (mg/dm²): Ethanol 10% Acetic acid 3% Ethanol 95% Isooctane	 1.1 ± 0.17 2.6 ± 0.15 4.4 ± 0.3 0.8 ± 0.0
WVTR (g/m^2 × day)	16.88 ± 2.17

SM*: Machine direction which is the direction of alignment of the molecular chains versus to the machine. ST*: Cross direction is perpendicular to the direction of the machine.

2.2 Cassava starch- and coconut oil-based film (UoN)

2.2.1 Selection of local bio-raw materials and extraction of polymers

Cassava tubers (*Manihot esculenta*) purchased at the Marigiti fresh produce market in Nairobi (KE) was selected as a local raw material rich in starch to produce the bio-based packaging material after characterisation (**Table 3**).

<u>Operative procedure for cassava tubers characterization</u>:

- Peel, rinse and mill the cassava tubers
- Moisture content according to AOAC 2010 (920:151)
- Starch content according to AOAC 2010 (996.1)
- Crude protein content according to AOAC 2010 (2001:11)
- Crude fat content according to AOAC 2010 (996.06)
- Crude fibre content according to AOAC 2010 (2011.25)
- Ash content according to AOAC 2010 (940:26).

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Table 3. Results of the characterisation of the raw cassava tubers (*Manihot esculenta*).

Test	Content (%)
Moisture	67.86 ± 0.37
Starch	25.60 ± 0.42
Crude protein	1.04 ± 0.04
Crude fat	0.35 ± 0.07
Crude fibre	1.08 ± 0.1
Ash	0.80 ± 0.08

Operative procedure for the extraction of starch from cassava tubers by wet method (Noorfarahzilah et al., 2020):

- Suspend the milled cassava tubers in distilled water at 25 °C
- Sieve the pulp using a cheese cloth and wash the residual on the screen cloth 3-4 times with distilled water
- Discard the fibre on the cheese cloth
- Let the extracted starch to sediment
- Filter the leftover material with the cheese cloth and wash with distilled water
- Dry the extracted starch in an oven at 45 °C for 6 h and in a solar tunnel dryer for 4 h
- Mill the dried starch
- Store the milled starch in an airtight container to avoid contamination and moisture gain.

Operative procedure for the characterisation of starch extracted from cassava tubers (**Table 4**):

- Starch content according to AOAC 2010 (996.1)
- Starch extraction yield (Yield % = (m/M)·100), where m: mass of starch extracted and M: mass of raw peeled cassava used, according to Mamat et al. (2021)
- pH according to AOAC 2010 (981.21)
- Moisture content according to AOAC 2010 (920:151)
- Crude fibre content according to AOAC 2010 (2011.25)
- Ash content according to AOAC 2010 (940:26).

Table 4. Results of the characterisation of the extracted cassava starch.

Test	Value (Mean ± SD)
Starch (%)	60.24 ± 0.2
Starch extraction yield (%)	12.64 ± 0.20
pH	5.49 ± 0.13
Moisture (%)	13.12 ± 0.06
Crude fibre (%)	0.18 ± 0.07
Ash (%)	0.62 ± 0.04

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2.2.2 Formulation and preparation of bio-based film

Operative procedure for the preparation of cassava starch- and coconut oil-based film (Costa et al., 2014 with modifications):

- Add cassava starch (4%), coconut oil (0.5%) and glycerol (0.5%) to distilled water
- Stir the mixed solution with a magnetic stirrer for 1 h in a water bath at 90 °C
- Cast the viscous solution obtained into plastic Petri dishes (inner diameter 138 mm)
- Dry the filled Petri dishes under renewable circulating air at 35 °C for 12 h
- Condition the dried film for 10 days at 23 °C and 75% relative humidity to acclimatise it to the environment until characterisation.

The main steps for the preparation of the bio-based film from cassava starch are shown in **Figure 4**.

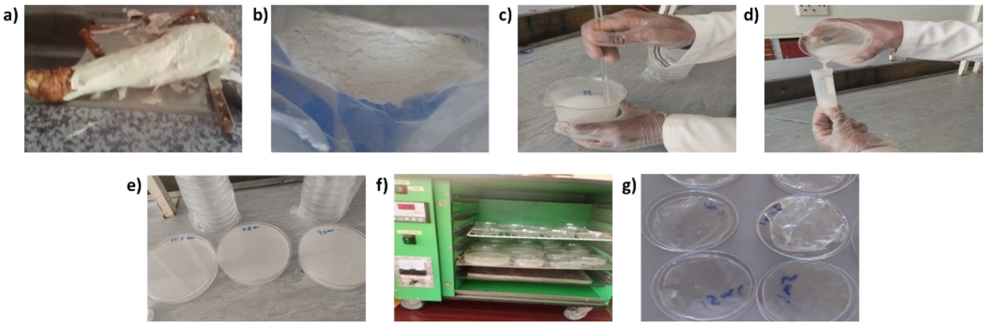

Figure 4. Main steps to produce bio-based film from cassava starch by casting method: **a**) raw cassava tuber for starch extraction, **b**) dried cassava starch, **c**) mixing of bio-based film ingredients **d**) measuring the different solution amounts **e**) Petri dish filled with the solution, **f**) film solution drying process **g**) final film samples.

The obtained film was evaluated for the main physic-chemical properties such as thickness (µm), moisture (%), water solubility (%), biodegradation (%), transparency (%), tensile strength (MPa), tear strength (N), elongation at break (%), Young's modulus (MPa), water vapor transmission rate - WVTR (g/m^2 × day) (**Table 5**).

Table 5. Physical-chemical properties of the cassava starch- and coconut oil-based film.

Property (SI)	Value (Mean ± SD)
Thickness (µm)	200.00 ± 0.10
Moisture (%)	10.16 ± 0.19
Water solubility (%)	39.08 ± 2.71
Biodegradation (%) after 4 months	89.82 ± 0.06
Transparency (%)	4.45 ± 0.01
Tensile strength (MPa)	18.03 ± 3.25
Tear strength (N)	0.58 ± 0.02
Elongation at break (%)	3.00 ± 0.04

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Young's modulus (MPa)	317.33
WVTR (g/m^2 × day)	1.95 ± 0.08

2.3 Pineapple peels nanocellulose-based film (SUA)

2.3.1 Selection of local bio-raw materials and extraction of polymers

Pineapple is one of the most common and popular fruit in tropical countries, including Tanzania (TZ). Pineapple peels (PPs), which constitute about 35% of the total weight of pineapple, are discarded as waste, although they are a good source of biomass that can be used for various purposes. Since PPs are rich in cellulose, they were selected as the main raw material and source of cellulose and nanocellulose for the production of a bio-based film.

Operative procedure for obtaining PPs material (**Figure 5**):

- Clean ripe pineapple under running tap water
- Peel the pineapple and save the peels (inedible fruit parts)
- Cut the PPs in small size
- Dry the PPs in an oven at 60 °C for 24 h
- Crush to obtain a fine powder.

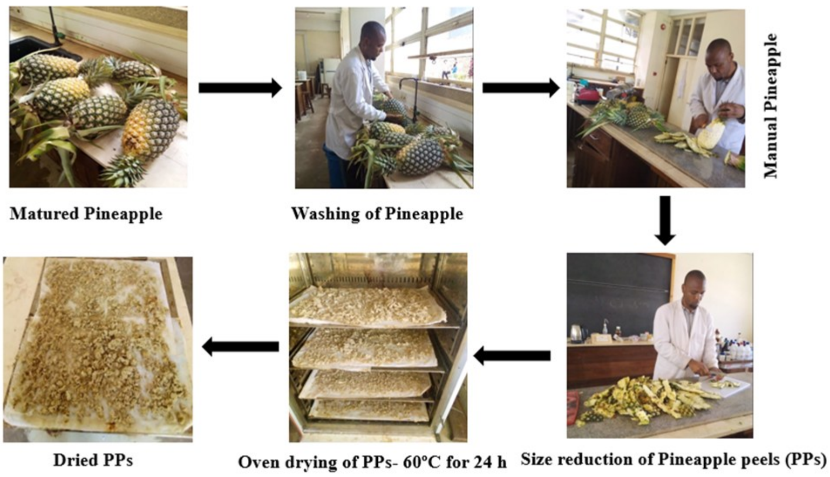

Figure 5. Main steps for the preparation of dried pineapple peels (PPs) for the extraction of cellulose.

The PPs were characterised for moisture, density, water absorption, water solubility, ash, lignin, cellulose and hemicellulose. The results reported in **Table 6** show that PPs are a potential biomaterial for the production of bio-based films.

Operative procedure for the extraction of cellulose from PPs powder (Dai et al., 2018):

- Dissolve the PPs powder in distilled water at 80 °C for 2 h
- Treat the residue with 7.5% sodium chlorite ($NaClO_2$) solution, followed by the addition of hydrochloric acid (HCl) solution at 75 °C for 2 h

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- After filtration wash the residue with distilled water until the washing liquid turn colourless
- Mix the residue (bleach treated PPs) with a 10% sodium hydroxide (NaOH) solution at room temperature (~25 °C) for 10 h to remove the hemicellulose
- Wash with distilled water and 95% ethanol until the filtrate turn neutral
- Dry the residue in an oven at 50 °C until a constant weight
- Grind the pineapple peel cellulose (PPc) into fine particles (150 to 200 micrometres).

Table 6. Physical-chemical properties of the pineapple peels (PPs).

Property (SI)	Value (Mean ± SD)
Moisture (%)	3.39 ± 0.31
Density (g/ml)	0.27 ± 0.06
Water absorption (%)	389.00 ± 97.82
Water solubility index (%)	15.48 ± 1.29
Ash (%)	6.51 ± 0.05
Lignin (%)	11.32 ± 0.26
Cellulose (%)	19.58 ± 1.62
Hemicellulose (%)	24.72 ± 2.28

Operative procedure to obtain nanocellulose from PPc by acid hydrolysis (Dai et al., 2018; Madureira et al., 2018):

- Hydrolyse the PP cellulose powder (about 10 g) in 15% (w/w) sulfuric acid at a ratio of 1:20 g/mL at 50 °C for 45 min with vigorous and constant stirring
- Dilute the hydrolysis by adding ten times cold distilled water and centrifuge at 5000 rpm for 10 min
- After discarding the supernatant, collect the precipitate, wash continuously with cold distilled water and centrifuge again. Repeat this wash-centrifuge procedure until the supernatant has become turbid (approx. 4 wash-centrifuge cycles)
- Subject the collected suspension to a dialysis process against distilled water at 4 °C for 72 h in order to achieve a neutral pH value
- Sonicate the resulting suspension for 30 min and store at 4 °C for film preparation or freeze-dry the resulting pineapple peel nanocellulose (PPnc) at –50 °C for 24 h.

2.3.2 Formulation and preparation of bio-based film

Operative procedure protocol for the preparation of pineapple nanocellulose-based film:

- Prepare 2% (w/w) of gellan gum and 12% (gellan gum, w/w) of PPnc using distilled water as a solvent
- Add glycerol (30% of gellan gum, w/w) as a plasticizer to the solution
- After complete dissolution and dispersion, sonicate the mixed solutions for 10 min
- Cast the solution on clean glass plate

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- Dry the filled glass plates at room temperature (~25 °C) to fully evaporate the water.

The obtained film was evaluated for the main physico-chemical properties such as thickness (µm), moisture (%), water solubility (%), acid solubility (%), alkaline solubility (%), biodegradation (%), transparency (%), opacity (%), L* (lightness), a* (redness), b* (yellowness), tensile strength (MPa), elongation at break (%), WVTR (g/m^2 × day) (**Table 7**).

Table 7. Physical-chemical properties of the pineapple peels nanocellulose-based film.

Property (SI)	Value (Mean ± SD)
Thickness (µm)	60 ±1.53
Moisture (%)	10.86 ± 1.73
Water solubility (%)	25.29 ± 1.03
Acid solubility (%)	30.85 ± 0.32
Alkaline solubility (%)	9.25 ± 0.50
Biodegradation (%) after 4 weeks	91.14 ± 1.41
Transparency (%)	24.52 ± 0.86
Opacity (%)	7.47 ± 1.03
L* (lightness)	58.71 ± 1.68
a* (redness)	1.44 ± 0.11
b* (yellowness)	11.73 ± 0.37
Tensile strength (MPa)	69.6 ± 4.95
Elongation at break (%)	8.5
WVTR (g/m^2 × day)	69.66 ± 0.08

2.4 Cassava starch- and chitosan-based film and coating (NARO)

2.4.1 Selection of local bio-raw materials and extraction of polymers

Cassava tubers purchased at the Kawempe market in Kampala (UG) were selected as local, starch-rich raw materials to produce the bio-based packaging materials (film and coating) in combination with chitosan.

Operative procedure for the extraction of starch from cassava tubers by wet method (**Figure 6**):

- Wash, peel and grate the fresh cassava tubers
- Grind in a high-speed blender for 5 min
- Suspend the pulp in ten times its volume of water and stir for 5 min
- Filter with a double folded cheese cloth
- Leave the filtrate to stand for 2 h to allow the starch to settle

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- Decant and discard the top liquid
- Add water to the sediment and stir the mixture for 5 min
- Filter with a double folded cheese cloth
- Allow the starch to settle
- Decant the top liquid
- Dry the sediment (starch) at 55 °C for 24 h
- Grind the dried starch.

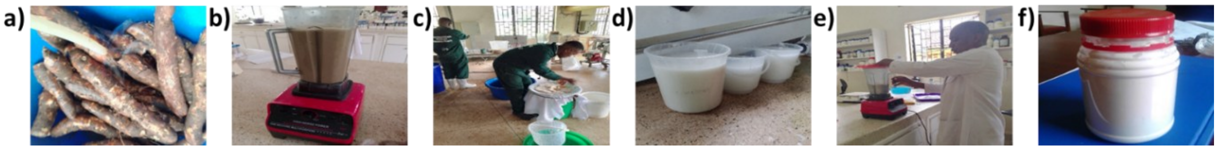

Figure 6. Main steps for the extraction of starch from cassava tubers: **a**) raw material washing, **b**) grind cassava with water, **c**) pulp filtration, **d**) filtered pulp sedimentation, **e**) dried cassava starch grinding, **f**) storage of dried and ground cassava starch.

2.4.2 Formulation and preparation of bio-based film and coating

Operative procedure for the preparation of cassava starch- and chitosan-based film (**Figure 7**):

- In a 500 mL beaker disperse 1 g of chitosan (Sigma) in 100 mL of distilled water (1%, w/w)
- Add 1 mL glacial acetic acid (1%, v/v) and stir with a magnetic stirrer at 300 rpm for 2 h to dissolve most of the chitosan
- Filter the suspension through cheesecloth to remove the undissolved chitosan and keep the supernatant for later use
- Add 3 g of cassava starch (3%, w/v) and 0.9 g of glycerol (30%, w/w starch) to the chitosan solution
- Stir at 300 rpm for 2 min to obtain a homogeneous mixture. Increase the temperature to 80 °C until completely gelatinised (the solution becomes viscous and almost transparent)
- Maintain this temperature for a further 10 min (this will reduce the bubbles in the solution).
- Cool and pour 500 mL of the solution onto a 0.1 m² plastic tray, spreading evenly on the surface
- Dry the film in an oven at 45 °C for 8-10 h
- Remove the film from the trays
- Rehydrate the film by spraying a mist of water droplets to improve its flexibility as drying makes the films brittle.

Operative procedure for the preparation of cassava starch- and chitosan-based coating (**Figure 8**):

- Same above 6 steps procedure by mixing 3% starch, 0.3% chitosan and 1% glycerol
- Let the solution cool to about 40 °C and use it as a coating solution.

The film was evaluated for key physicochemical properties such as thickness (µm), moisture (%), water activity (a_w), water solubility (%), biodegradation (%), transparency (%), L* (lightness), a* (redness), b* (yellowness), tensile strength (MPa), elongation at break (mm), Young's modulus (MPa), overall migration limits - OML (mg/dm^2); WVTR (g/m^2 × day) (**Table 8**).

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Figure 7. Preparation of cassava starch/chitosan-based film.

Figure 8. Preparation of cassava starch/chitosan-based coating.

Table 8. Physical-chemical properties of the cassava starch- and chitosan-based film.

Property (SI)	**Value (Mean ± SD)**
Thickness (μm)	0.23 ± 0.01
Moisture (%)	16.7 ± 0.0
Water activity (a_w)	0.66 ± 0.01
Water solubility (%)	80.5 ± 0.3
Biodegradation (%) after 12 days	97.7 ± 4.0
Transparency (%)	42.7 ± 2.3
L* (lightness)	83.3 ± 0.5
a* (redness)	0.4 ± 0.1
b* (yellowness)	5.2 ± 0.5
Tensile strength (MPa)	4.8 ± 0.8
Elongation at break (mm)	37.5 ± 15.3

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Young's modulus (MPa)	67.3 ± 49.5
OML (mg/dm^2)	0.12 ± 0.01
WVTR (g/m^2 × day)	19.7 ± 7.4

The coating solution obtained was analysed for its viscosity, which was measured using a Brookfield DVE series viscometer. The solution provided viscosity results consistent with the literature (113-225 CPS, Budiman, 2011) and was therefore optimal for the application of the coating on fish products.

2.5 Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) (all)

Compared to conventional low-density polyethylene (LDPE) packaging material, which was used as the standard reference, the bio-based packaging prototypes developed had a consistently lower environmental impact according to the life cycle assessment (LCA) results. This indicates that bio-based packaging materials are a more sustainable alternative to conventional LDPE and reduce environmental impacts such as greenhouse gas emissions. In particular, the percentages reduction in kg CO_2 equivalent per kilogram (kg CO_2-eq/kg) emissions between the LDPE packaging material and the wheat straw nanocellulose-based film (INAT), the cassava starch/chitosan-based film and coating (NARO, MAK), the cassava starch/coconut oil-based film (UoN) and the pineapple peels nanocellulose-based film (SUA) are 40.9%, 71.2% and 74.9%, 83.8% and 75.2%, respectively.

In terms of life cycle costing (LCC) results, the production costs of the new bio-based films were generally higher than those of the conventional LDPE film, with the exception of INAT's wheat straw nanocellulose-based film. Specifically, the costs for 500 cm² of cassava starch/chitosan-based film (NARO, MAK), cassava starch/coconut oil-based film (UoN) and pineapple peel nanocellulose-based film (SUA) are 873%, 14% and 923% higher than LDPE, respectively. In contrast, the cost of wheat straw nanocellulose-based film (INAT) is approximately 73% lower than LDPE film.

Specific results on LCA and LCC analysis are reported in the Zenodo database at the following DOI: https://doi.org/10.5281/zenodo.14918187

3. Validation activities

To assess the suitability of the bio-based material prototypes, they were tested in shelf-life trials with various local foods by comparing them to the performance of conventional materials commonly used to package the same foods.

The **INAT** team conducted studies on the shelf-life of soft-dried eggplants and strawberries packaged in a bio-based film prototype (PBAT + TPS + CNCs + artichoke extract) compared to a non-enriched bio-based material (PBAT + TPS) and conventional packaging (polypropylene film). The food products were stored for four months at room temperature (21 ± 2 °C) and at 35 ± 2 °C and analysed at regular intervals (0, 10, 20, 40, 60, 80, 100 and 120 days) for some quality parameters. The parameters assessed were weight loss, total phenolic content (TPC) and antioxidant activity (DPPH).

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The **NARO** team conducted studies on the shelf-life of soft-smoked fish fillets and deep-fried fish fingers packaged using the two bio-based packaging materials developed (cassava starch/chitosan/glycerol film and coating) compared to conventional packaging. Four packaging solutions were used for both fish products: (i) uncoated fish wrapped with LDPE film, (ii) coated fish wrapped with LDPE film, (iii) uncoated fish wrapped with bio-based film and (iv) coated fish wrapped with bio-based film. The fish products were stored at refrigerated temperature (5.6 ± 1.2 °C) for 15 days and analyses were performed at regular intervals (0, 3, 6, 9, 12 and 15 days). The quality parameters analysed were: moisture content, water activity, pH, peroxide value, total bacterial count, yeast and mould count and sensory evaluation (appearance, taste, aroma, texture, general acceptability).

The **MAK** team conducted studies on the shelf-life of whole fresh eggplants coated with NARO's optimised prototype coating. The harvested whole eggplants were sorted, washed and divided into two groups: coated and uncoated placed in a perforated plastic basket. Half of the samples were stored for 20 days at refrigerated temperature (7 °C) and the other half at room temperature (25-27 °C). The samples were analysed every 4 days for weight loss, moisture content, texture, pH, vitamin C, total soluble solids and microbial counts (total plate count, yeasts and moulds).

The **UoN** team conducted studies on the shelf-life of tree tomato fruit powder (tamarillo) and quinoa snacks (mandazi) packaged in the bio-based cassava starch/coconut oil film compared to conventional packaging materials. As a control, the tree tomato powder was packaged in laminated Kraft paper, while the mandazi snacks were packaged in 3 control pouches: LDPE laminated Kraft paper, Kraft paper and metallised LDPE. The tree tomato powder samples were stored at 25 ± 2 °C for 150 days and analysed for water activity, moisture content, pH, colour, vitamins A and C, sensory properties and microbial growth. The quinoa snack samples were stored at 25 ± 2 °C for 5 days and analysed for moisture content, free fatty acids (oleic acid) and microbial growth.

The **SUA** team conducted shelf-life studies of two composite flour products (one pre-cooked and extruded, the other non-extruded) packaged with a pineapple peel nanocellulose (PPnc)-based film. Samples of 250 g were packaged in three types of packaging: (i) PPnc-based pouch inserted in a paper pouch (secondary packaging); (ii) paper pouch and (iii) polyethylene pouch. The samples were stored for five months at room temperature (25 ± 3 °C) and under refrigerated conditions (4 ± 1 °C) and analysed for moisture content, water activity, colour (CIEL*a*b*) and total bacteria count, yeasts and moulds.

4. Technology and production key performance indicators

Food Hub	Innovation (new technology / system / tool / food product)	KPIs measuring the achievements
Jendouba (TN)	PBAT (polybutylene adipate terephthalate), TPS (plasticized starch) with CNC (cellulose nanocrystalline) and antioxidant extract-based film for mild-dried vegetable products (eggplants and strawberries).	- Improvement or similarity of total phenolic content (TPC) of mild-dried food products packaged in bio-based packaging compared to conventional packaging (Eggplants packaged in the new bio-based packaging had a 45.2% higher TPC content than those packaged in polypropylene (PP) after four

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		months of storage at 35 °C. Strawberries packaged in the new bio-based packaging had comparable TPC values to those packaged in PP after four months of storage at 35 °C). - Reduction of kg CO_2-eq/kg emissions by 40.9% compared to conventional LDPE packaging material.
Kajjansi / Masaka (UG) - NARO Nakaseke (UG) - MAK	Cassava starch- and chitosan-based film and coating for fish products (soft smoked fish fillets and deep fried fish fingers).	- Improvement of the microbiological quality of soft-smoked fish fillets coated with a bio-based packaging + LDPE film compared to the uncoated + LDPE film one (75.6% less total plate count and 41.2% less yeast and mould count in the coated sample). - Reduction of kg CO_2-eq/kg emissions by 71.2% and 74.9% compared to conventional LDPE packaging material for film and coating.
Kitui (KE)	Cassava starch- and coconut oil-based packaging film for tree tomato powder (tamarillo) and quinoa-based snack (mandazi) for children.	- Comparable quality in terms of VitA and VitC values between the samples of tree tomato powder packaged in the new and the conventional (LDPE-laminated Kraft paper) material after 150 days of storage at 25 °C. - Reduction of kg CO_2-eq/kg emissions by 83.8% compared to conventional LDPE packaging material.
Mvomero, Morogoro (TZ)	Pineapple peels nanocellulose-based packaging for composite flour (extruded and non-extruded).	- Similarity of microbiological quality of composite flours packaged in bio-based + paper packaging compared to conventional packaging (non-extruded flour had similar total bacterial and mould counts to polyethylene (PE) packaged flour after 150 days of storage at 25 °C and similar yeast and mould

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		counts after 150 days of storage at 4 °C. After 150 days of storage at 25 °C, extruded flour had a similar total bacterial count as flour packaged in PE). - Reduction of kg CO_2-eq/kg emissions by 75.2% compared to conventional LDPE packaging material.

5. References

- Association of Official Analytical Chemists - AOAC (1990). Official methods of analysis, 12th edn. AOAC, Washington (USA).

- Costa S. S., Druzian J. I., Machado B. A. S., de Souza C. O., Guimaraes A. G. (2014). Bi-functional bio-based packing of the cassava starch, glycerol, Licuri nanocellulose and red propolis. Plose One, 9(11), e112554.

- Dai H., Ou, S., Huang Y., Huang H. (2018). Utilization of pineapple peel for production of nanocellulose and film application. Cellulose, 25(3), 1743–1756.

- El Achaby M., Kassab Z., Barakat A., Aboulkas A. (2018). Alfa fibers as viable sustainable source for cellulose nanocrystals extraction: Application for improving the tensile properties of biopolymer nanocomposite films. Industrial Crops & Products, 112, 499–510.

- Goering H. K. & Van Soest P. J. (1970). Forage fiber analysis (apparatus, reagents, procedures and some applications). Agricultural hand book, 379, 1737–41.

- Madureira A. R., Atatoprak T., Çabuk D., Sousa F., Pullar R. C., Pintado M. (2018). Extraction and characterisation of cellulose nanocrystals from pineapple peel. International Journal of Food Studies, 7, 24–33.

- Mamat H., Lee J. S., Hamid M. A., Zainol K. (2021). Extraction and characterization of cassava starch cultivated in different locations in Sabah, Malaysia. Food Research, 5(3), 44–52.

- Noorfarahzilah M., Jau-Shya L., Mansoor A. H., Jahurul M. H. A., Umi Hartina M. R., Zainol M. K., Hasmadi M. (2020). Physicochemical properties of tarap (Artocarpus adoratisimus) starch. Food Research, 4(3), 602–611.

- Nsubuga, D., Chimoita, E., Mugisha, J., Gitau Magu, M., Kimani, J., Ben Rejeb, I., Tinyiro, S. E., Maitha, I., Okoth, M. W., Suileman, R., Schouten, M. A., Setti, M., & Romani, S. (2025). A comparative Life Cycle Assessment and Life Cycle Costing of selected bio-based packaging materials in comparison with an LDPE film. *Draft working paper*, *Zenodo*. https://doi.org/10.5281/zenodo.14918187

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