Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Valorization of papaya fruit waste through low-cost fractionation and microbial conversion of both juice and seed lipid

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METHODS

Constructing a *Y. lipolytica* strain expressing mCherry-GFP nanobody fusion protein

To construct pINA69-MNG, anti-GFP nanobody-His_6 coding sequence was PCR amplified from pCTNG[1] using forward primer NGB: 5'-CGCGGATCCCAGGTTCAACTGGTGGAAAG-3' (BamHI site underlined), and reverse primer XGR: 5'-CGCCTCGAGTTAGTGATGGTGAT-3' (XhoI site underlined), digested with BamHI and XhoI and then ligated to BamHI-XhoI restricted pETMD [1] to form pETMNG. Then, the mCherry-GFP nanobody-His_6 coding sequence was amplified using forward primer MAF 5'-AATGGTGAGCAAGGGCGAGGAGGA-3', and reverse primer: HKR: 5'- CGCGGTACCTTAGTGGTGGTGGTGGTGG -3' (KpnI site underlined), digested with KpnI and ligated to PmlI–KpnI restricted pINA1269 to generate pINA69-MNG. Transformation of *Y. lipolytica* Polg and selection of transformants were performed as described previously.[2]

GFP-Rsn2 protein expression and purification

GFP-Rsn2 expressing *E. coli* strain was grown in 50 mL of LB media at 37°C, shaking at 250 rpm. Once the culture reached an OD_{600} of 0.5, isopropyl-β-D-thiogalactopyranoside (IPTG) was added at a final concentration of 0.1 mM to induce the culture overnight. Cells were collected via centrifugation at 7,500 rpm for 3 min, then resuspended in 5 mL of Tris-Buffered Saline (TBS) with 50 µL of 0.1 M phenylmethylsulfonyl fluoride (PMSF), placed on ice and lysed via ultrasonication at 5 watts for 3 cycles of 30 sec on and 30 sec off using a Qsonica Q125 ultrasonicator. The supernatant was collected via centrifugation at 7,500 rpm for 5 min, followed by centrifugation at 12,000 rpm for 30 min. The supernatant was then collected and filtered using a 0.2 µm polyethersulfone syringe filter. GFP-Rsn2 (containing a hexa-histidine tag) was purified from the crude protein extracts using a HiTrap 5 mL immobilized metal affinity chromatography column and a BioRad BioLogic DuoFlow liquid chromatography system. The purified protein was eluded in TBS consisting of 50 mM Tris and 137 mM NaCl.

References

1. Z. Han, B. Zhang, Y. E. Wang, Y. Y. Zuo and W. W. Su, *Appl Environ Microbiol*, 2012, **78**, 3249-3255.
2. Z. Han, C. Madzak and W. W. Su, *Biotechnol Bioeng*, 2013, **110**, 702-710.

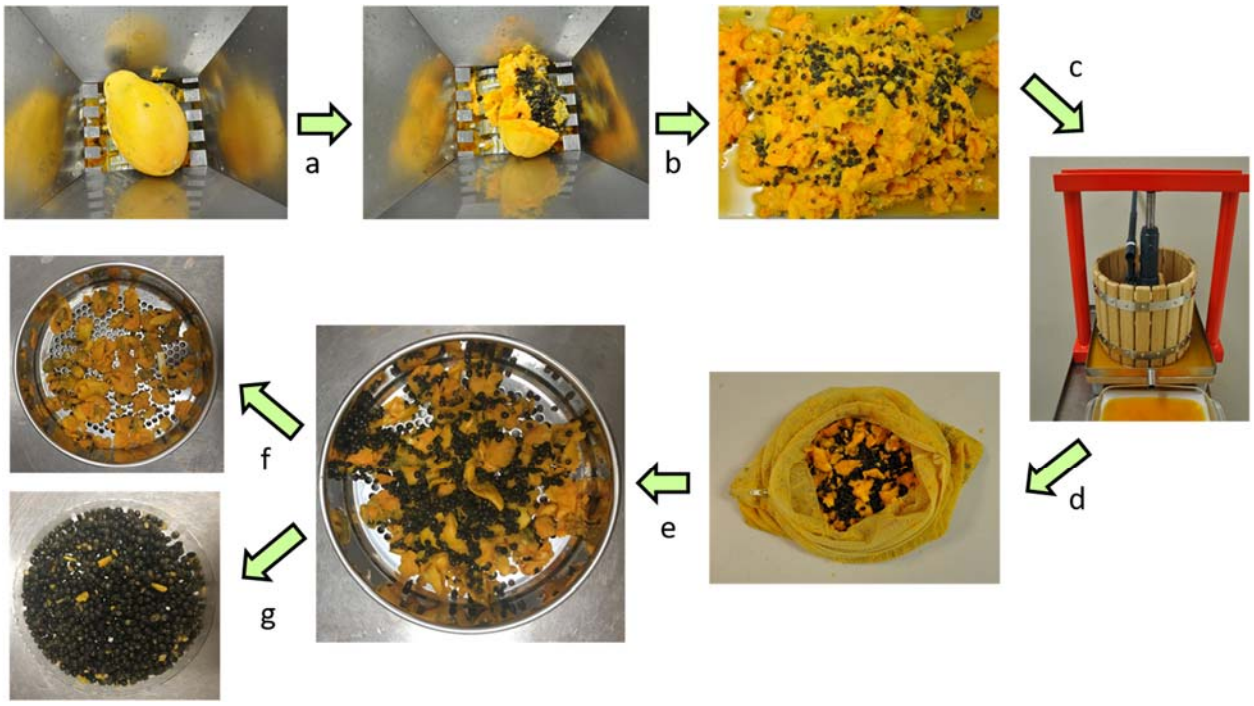

Figure S1. Fractionation of culled papaya fruit waste into puree, peel and unbroken seeds using only simple, low-cost and off-the-shelf fruit-processing equipment. (a, b) Culled papaya was crushed using a fruit crusher/grinder without damaging the seeds. (c, d) Crushed fruit was processed to extract puree/juice using a fruit hydraulic press. (e) The residual seeds and peels were then fractionated directly using a perforated plate sieve to generate fractions rich in peel (f) and unbroken seeds (g), respectively. The operation is particularly well suited for small producers or fruit packers typically do not own specialized puree processing equipment such as peeling machine and fruit pulper/finisher.

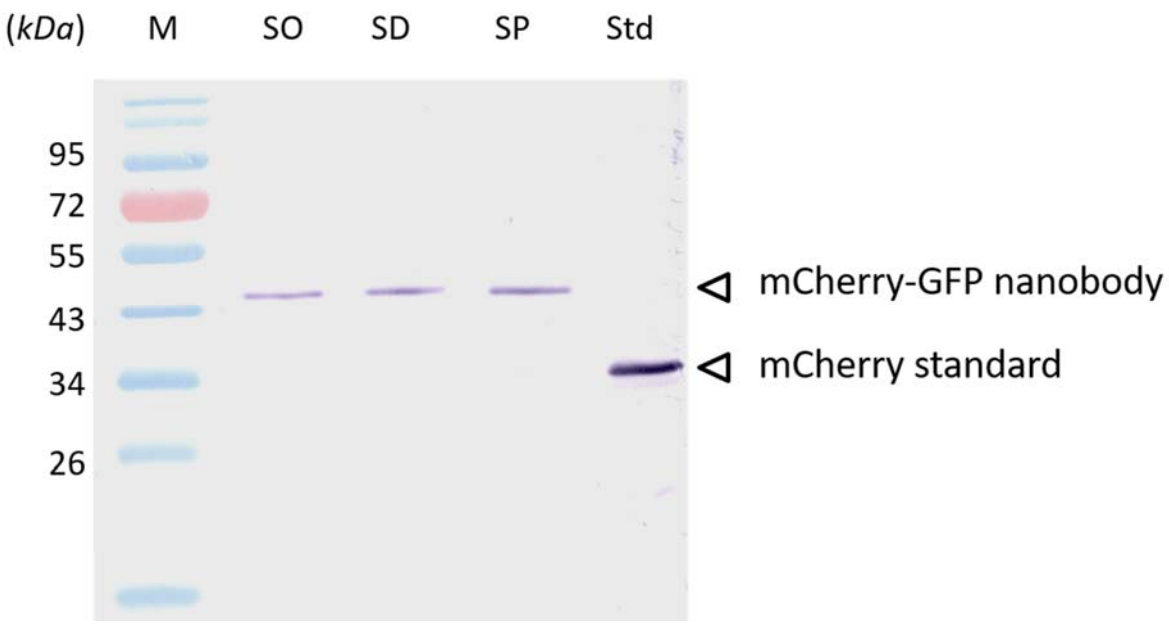

Figure S2. After 6 days of culture in SO (4% papaya oil), modified SD (1% glucose + 1% fructose), and SP (25% juice) media, respectively, mCherry-GFP nanobody fusion protein in cell extracts was analyzed using anti-His tag western blot (M: protein ladder).

Table S1 Proximate analysis of defatted papaya seed meal after extraction with hexane and methanol

	Content		Content
		Minerals:	
Dry matter (DM %)	95.35	Phosphorus (%)	0.80
Ash (%)	9.77	Potassium (%)	2.19
Crude protein (CP %)	38.89	Calcium (%)	1.07
Crude fat (EE%)	0.09	Magnesium (%)	0.69
Neutral detergent fiber (NDF%)	53.93	Sodium (%)	0.03
Acid Detergent Fiber (ADF%)	50.10	Boron (ppm)	18
Lignin (PMF%)	35.35	Copper (ppm)	16
Cellulose (%)	14.75	Iron (ppm)	105
		Manganese (ppm)	61
		Zinc (ppm)	99