Fingerprinting Starch Degradation Products: Chemical Species of Interest to Ancient Starch Research

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Introduction

Recent field reviews of Archaeological Starch (e.g. Copeland and Hardy, 2018; Mercader et al., 2018a) have asked if one might expect ancient starch granules to retain chemical functionality such as that seen in native starch or if we should be looking for remnants of degraded starch not visible through microscopy. Ancient starch researchers face challenges in elucidating taphonomic pathways to explain the appearance of native starch granules in archaeological contexts (e.g. Haslam, 2004; Barton and Matthews 2006; Barton 2009; Henry 2015; see recent discussion in Mercader et al. 2018a), and currently lack evidence that starch granules undergo fossilization. It is important to recognize that well-preserved physical structure does not imply chemical conservation (Briggs 2003; Briggs and Summons 2014). Moreover, organic species in physically structured fossils is often exogenous, and proof of origin is difficult to obtain, as shown in silicified or pyritised fossils that preserve excellent physical structure while not conserving endogenous biomass (Stach and Murchison, 1982; Saitta et al. 2019).

Different biochemical precursors have variable resistance to alteration: Lipids, lignins, and proteins are recalcitrant (Gupta et al., 2007, 2009), while carbohydrates rapidly decompose (Briggs 2003; Gupta and Briggs 2011). One pathway to organic matter preservation that has received little attention in Archaeology is the formation of humic and kerogeneous materials. They have high molecular weights and are less accessible to microorganisms (Tissot and Welte 1984; Gupta 2015). High molecular weight mixtures are diagenetically stable, for instance in the Maillard browning reaction leading to melanoidins (Maillard, 1912; McWeeny et al. 1974). With amino acids and carbohydrates as the source, this is the prime reaction for kerogen formation in soils and sediments (Hoering 1973; Larter and Douglas 1980). Melanoidins react with and bind a variety of functionalized species (Larter et al. 1983), while on thermal alteration they acquire some of the properties from kerogens. This paper investigates whether hydrolyzed polysaccharides can generate by-products that retain chemical signals amenable to identification through Fourier Transform Ion Cyclotron Resonance Mass Spectrometry that could be used by archaeologists.

Materials and Methods

The starch molecule is a polymer linking glucose monomers: linear amylose and branched, crystalline amylopectin (Pérez et al. 2009). Ratios of these two components vary between species, affecting crystallinity (Gallant et al., 1992; Jarvis 1993; Hoover 2000; Xie et al., 2009; Zou 2012). We selected starch samples from a collection at the University of Calgary <https://olduvaigorgesds.com/botanical-reference-collection/> on Africanist, ethnobotanical criteria. Sixteen taxa across phylogenetic orders (n=8) and genera (n=15), with varied crystallinity (Table 1: Type A = 5, B = 3, C = 8) and granule morphometry (Mercader et al 2018b). From this total, four non-African crops (potato, wheat, maize, and taro) were included because of their universal availability and well understood chemical and molecular structure (e.g. Jane et al., 1994, 2009; Shi et al 1998; Tomasik 2009). To impart the thermal and pressure effect of diagenesis, starch was

first mixed with glycine ($\text{C}_2\text{H}_5\text{NO}_2$) in a ratio of 0.75/1 by weight in a test tube (10 ml). We added reverse osmosis deionized water (RODI: 7 ml) and maintained pH at ~ 7 . This mixture was sealed in a pressure vessel, in triplicate; one vessel at room temperature, another one at 70°C , and another at 150°C for 24 h. Each treatment was centrifuged (3000 rpm), collecting an aliquot (300 μl) from the aqueous phase which received ammonium hydroxide (NH_4OH : 20 μl).

The extracts were analyzed in a 12T Bruker Solarix Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR-MS). Our protocol was modified from Martins et al. (2001) and Hwang et al. (2010). Samples were processed by electrospray ionization in negative ion mode (ESI-N) to investigate species with deprotonation potential, as described in Oldenburg et al. (2014, 2017). Analyte flow rate was 200 $\mu\text{L/h}$, capillary voltage 4 kV, and nebulizer pressure at 1.0 bar. Spectra were collected in absorption mode, using Kilgour et al. (2013)'s algorithm. Two hundred transients of 8 million points were collected and summed to improve our experimental signal/noise ratio (SNR).

FTICR-MS raw data were processed using the calibration and peak assignment software CaPA v.2.2 (Aphorist Inc.). Peaks with $\text{SNR} > 5$ were assigned on m/z measurements and stable isotopic pattern, to the third most intense isotopolog. The elemental composition boundaries were $^{12}\text{C}_{4-95}$, $^1\text{H}_{0-200}$, $^{16}\text{O}_{0-30}$, $^{14}\text{N}_{0-8}$, $^{32}\text{S}_{0-2}$, $^{23}\text{Na}_{0-2}$, $^{15}\text{P}_{0-2}$, and $^{35}\text{Cl}_{0-2}$, and only errors < 300 ppb were accepted. The mass spectra were internally calibrated using homologous peak series. Controls in solvent blanks recognised the peaks not to consider.

Identified peaks were produced from single-charged ions, with $< 10\%$ of the total spectral intensity being left unassigned. Ragnarök v.2.4 (Aphorist Inc.) was used for quantitative processing, visualization, and interpretation of multidimensional / multi-sample FTICR-MS data. Molecular masses and ring number/ unsaturation levels for each chemical species were determined. We assessed double bond equivalents (DBE) ($\text{CcHhNnSsOo} = c-h/2+n/2+1$). In addition, we derived lumped, summed abundances of whole heteroatom classes, DBE group distributions within a heteroatom class, carbon number distributions of an individual group of pseudo-homolog compounds, and plots for related groups.

Results

To highlight the complexity of compounds that can derive from the reaction of glycine with starch consider the $> 5,300$ molecular formulae in the melanoidins from a single model species (*Ipomoea longituba*, Fig. 1). The heteroatom group distribution (Fig. 2) indicates minor changes in the glycine-starch mix at room temperature. Conversely, after heating it at 70°C for 24 h, the complexity and dominance of mixed nitrogen and oxygen species increase, to peak at 150°C for 24 h. The products' molecular weights reached 1,200 Daltons for individual heteroatom compounds. Double bond equivalents (DBEs) for the species (1 - 22) demonstrate extensive cyclisation and unsaturation compared to glycine and starch ($\text{DBE} = 1$).

The compound class distribution of melanoidins from 16 starch taxa appears in Fig. 3. The number of heteroatom compound classes ranges from 20 (*Zea mays*) to 51 (*Sorghum bicolor*). While starch has oxygen as its heteroatom, the products derived contain mixed nitrogen and oxygen species. The diversity of melanoidins is manifest when all 16 starches overlap in modified Kendrick (Fig. 4) and Van Krevelen diagrams (Fig. 5, 6). From these, we observe the lowest number of melanoidins in *Ensete ventricosum*, *Euphorbia quadrangularis*, *Zea mays*, and *Solanum tuberosum*. In the middle, there is *Colocasia esculenta*, *Cyperus rotundus*, *Cyphostemma serpens*, *Lablab purpureus*, *Senna didymobotria*, and *Triticum aestivum*. The highest number is in

Dioscorea esculenta, *Dioscorea schimperiana*, *Erythrococca bongensis*, *Ipomoea longituba*, *Mucuna pruriens*, and *Sorghum bicolor*.

Relative distribution and intensity of heteroatom class groups are variable (Fig. 7): The number of NyOx classes is higher than Ox in all melanoidins, except for three taxa. However, when weighted by intensity, oxygen-only chemical species show higher summed abundances in nine out of 16 starches. The Ox species reflecting altered moieties of starch are noticeable when looking at the carbon number distribution (Fig. 8). Figure 9A shows a predominance of species with carbons multiples of 6, and many species in O₆ DBE=1, C#₆, O₁₁ DBE=2 C#₁₂, O₁₆ DBE=3 C#₁₈, O₂₁ DBE=4 C#₂₄, which becomes even higher for the Ox species (Fig. 9B). When focused on the nitrogen atom only (NOx; Fig. 9C), we see chemical species with two additional carbons, reflecting a combination of starch related moieties with one glycine backbone component. At higher nitrogen numbers (e.g. for N₂Ox species in Fig. 9D), the complexity of reactions and diversity of products makes relationships between precursors and products speculative.

Discussion

We characterized the products of starch-amino acid reactions to evaluate whether individual starch taxa can transfer distinctive information to their respective melanoidins. The Maillard reaction produces complex mixtures from simple biochemical precursors (Figure 10): Described in detail by Hodge et al. (1953) and Martins et al. (2001), during this browning reaction glucose and amino acids produce glycosylamine, forming an Amadori rearrangement product known as ketose. The subsequent degradation of this component is pH-dependent. At pH < 7, it undergoes 1, 2-enolisation with the formation of furfural or hydroxymethylfurfural (C₆H₆O₃). At pH > 7, the degradation of Amadori compounds involves 2, 3- enolisation, in which reductones such as 4-hydroxy-5-methyl-3-furanone (C₅H₆O₃), as well as acetol (C₃H₆O₂), pyruvaldehyde (C₃H₄O₂), and diacetyl (CH₃CO)₂ are created. Later, cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations, and further condensations occur, leading to complex brown nitrogenous polymers and co-polymers. Starch-derived melanoidins have potential for diagenetic resistance, as suggested by bulk compositions combining both relict and neoformation species. Moreover, there are systematic differences in melanoidin character among starch taxa. Outside Archaeology, complex, heterogeneous mixtures such as these are found in marine dissolved organic matter (Hansell, 2013; Hansell et al. 2009).

The long-term survival of organic chemical species depends on many variables. Sometimes organics are found at concentrations below the metabolic threshold for biodegradation (Arrieta et al., 2015). Another path is their inclusion in organic (Mann et al. 2014) and inorganic compounds such as calcium phosphate (Mercader et al. 2018a, Soto et al. 2019) that inhibit microbial activity. In other cases, we see functionalization in heteroatoms of oxygen, nitrogen, and sulphur, as well as biotic and abiotic parameters in depositional environments that favor preservation (oxygen, temperature, light, microbial modulation). Lastly, adsorption onto mineral phases provides a shelter (Cheshire 1977; Adu and Oades 1978, Collins et al. 1995; Hedges and Keil 1995; Baldock and Skjemstad 2000; Kislenko 2002; Xie et al. 2004; Collins and Copeland 2011; Hawkes et al. 2015; Demarchi et al. 2016; Jessen et al. 2017; Eigenbrode et al. 2018).

Conclusions

Molecular degradation of heteroatom rich biopolymers such as polysaccharides is to be expected in archaeological samples, and these must show damage proportional to the taphonomic environment where they belong. The exceptional preservation of native starch granules extensively reported in archaeological sciences remains unexplained. Amino acids and starch react to create complex mixtures of heteroatom-containing products known as melanoidins. Their compounds have a wide range of molecular weights and geochemical species. Some of them are refractory and, if found in archaeological contexts, they could be utilized as proxies of the burial environment and starch precursors. While all the melanoidins show the same basic distribution of heteroatom groups and/or DBE distributions, clear differences among starch taxa do emerge. After the endogenous starch structures and chemistries from native starch granules have been lost to degradation, FTICR-MS is an analytical window to characterize starchy residue. Thus, there is potential for recognizing taxonomic signals in the Maillard reaction degradation products that remain on archaeological residues from stone tools, ceramics, dental calculus, and sediment.

Author Contributions

Melanoidin experimental work was carried out by Jamie Inwood and Melisa Brown. Thomas Oldenburg, Melisa Brown, Jago Radovic and Steve Larter interpreted FTMS data. Julio Mercader and Jamie Inwood selected and provided starch samples. Julio Mercader and Steve Larter conceived of and supervised the project. All authors contributed to the manuscript.

References

- Adu, J.K., Oades, J.M. 1978. Utilization of organic materials in soil aggregates by bacteria and fungi, *Soil Biology and Biochemistry* 10, 117-122.
- Arrieta, J.M., Mayol, E., Hansman, R.L., Herndl, G.J., Dittmar, T., Duarte, C.M. 2015. Dilution limits dissolved organic carbon utilization in the deep ocean. *Science* 348, 331-333.
- Baldock, J., Skjemstad, J. 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry* 31, 697-710.
- Barton, H., Matthews, P. 2006. Taphonomy, in: Torrence, R., Barton, H. (Eds.), *Ancient Starch Research*. Left Coast Press, Walnut Creek, pp. 75-94.
- Barton, H. 2009. Starch Granule Taphonomy: The Results of A Two Year Field Experiment, in: Haslam, M., Robertson, G., Crowther, A., Nugent, S., Kirkwood, L. (Eds.), *Archaeological Science Under a Microscope: Studies in Residue and Ancient DNA Analysis in Honour of Thomas H. Loy*. ANU Press, Australia, pp. 119-130.
- Briggs, D.E.G. 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annual Review of Earth and Planetary Sciences* 31, 275-301.
- Briggs, D.E.G., Summons, R.E. 2014. Ancient biomolecules: Their origins, fossilization, and role in revealing the history of life. *BioEssays* 36, 482-490.

- Cheshire, M.V. 1977. Origins and stability of soil polysaccharide. *European Journal of Soil Science* 28, 1-10.
- Collins, M.J., Bishop, A., Farrimond, P. 1995. Sorption by mineral surfaces: Rebirth of the classical condensation pathway for kerogen formation? *Geochimica et Cosmochimica Acta* 59, 2387-2391.
- Collins, M.J., Copeland, L. 2011. Ancient starch: Cooked or just old? *Proceedings of the National Academy of Science* 108, E145.
- Copeland, L., Hardy, K. 2018. Archaeological Starch, *Agronomy* 8, 4.
- Demarchi, B., Hall, S., Roncal-Herrero, T., Freeman, C.L., Woolley, J., Crisp, M.K., Wilson, J., Fotakis, A., Fischer, R., Kessler, B.M., Jersie-Christensen, R.R., Olsen, J.V., Haile, J., Thomas, J., Curtis, W.M., Parkington, J., Presslee, S., Lee-Thorp, J.A., Ditchfield, P., Hamilton, J.F., Ward, M.W., Wang, C.M., Shaw, M.D., Harrison, T., Domínguez-Rodrigo, M., MacPhee, R.D.E., Kwekason, A., Ecker, M., Horwitz, L.K., Chazan, M., Kröger, R., Thomas-Oates, J., Harding, J.H., Cappellini, E., Penkman, K., Collins, M.J. 2016. Protein sequences bound to mineral surfaces persist into deep time, *eLife* 5, 1-50.
- Eigenbrode, J.L., Summons, R.E., Steele, A., Freissinet, C., Millan, M., Navarro-González, R., Sutter, B., McAdam, A.C., Franz, H.B., Glavin, D.P., Archer, P.D., Mahaffy, P.R., Conrad, P.G., Hurowitz, J.A., Grotzinger, J.P., Gupta, S., Ming, D.W., Sumner, D.Y., Szopa, C., Malespin, C., Buch, A., Coll, P. 2018. Organic matter preserved in 3-billion-year-old mudstones at Gale crater. *Mars*, pp. 1096-1101.
- Gallant, D.J., Bouchet, B., Buelon, A., Perez, S., 1992. Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition* 46, S3-S16.
- Gupta, N.S., Michels, R., Briggs, D.E.G., Collinson, M.E., Evershed, R.P., Pancost, R.D. 2007. Experimental evidence for the formation of geomacromolecules from plant leaf lipids. *Organic Geochemistry* 38, 28-36.
- Gupta, N.S., Yang, H., Leng, Q., Briggs, D.E.G., Cody, G.D., Summons, R.E. 2009. Diagenesis of plant biopolymers: Decay and macromolecular preservation of *Metasequoia*. *Organic Geochemistry* 40, 802-809.
- Gupta, N.S., Briggs, D.E.G., 2011. Taphonomy of animal organic skeletons through time, in: Allison, P., Bottjer, D.J. (Eds.), *Taphonomy: Process and Bias Through Time*, 2 ed., Springer, Dordrecht, Netherlands, pp. 199-221.
- Gupta, N.S. 2015. Plant biopolymer-geopolymer: Organic diagenesis and kerogen formation. *Frontiers in Materials*. 2, 61. doi: 10.3389/fmats.2015.00061.

- Hansell, D.A. 2013. Recalcitrant dissolved organic carbon fractions. *Annual Review of Marine Science* 5, 421-445.
- Haslam, M., 2004. The decomposition of starch grains in soils: implications for archaeological residue analyses, *Journal of Archaeological Science* 31, 1715-1734.
- Hawkes, J.A., Rossel, P.E., Stubbins, A., Butterfield, D., Connelly, D.P., Achterberg, E.P., Koschinsky, A., Chavagnac, V., Hansen, C.T., Bach, W., Dittmar, T. 2015. Efficient removal of recalcitrant deep-ocean dissolved organic matter during hydrothermal circulation. *Geoscience*, 856.
- Hedges, J.I., Keil, R.G. 1995. Sedimentary organic matter preservation: An assessment and speculative synthesis. *Marine Chemistry* 49, 81-115.
- Henry, A.G. 2015. Formation and taphonomic processes affecting starch granules. in: Marston, J.M., D'Alpoim Guedes, J., Warriner, C. (Eds.), *Method and Theory in Paleoethnobotany*. University Press of Colorado, Boulder, pp. 35-50.
- Hodge, J.E. 1953. Dehydrated foods, chemistry of browning reactions in model systems, *Journal of Agricultural Food Chemistry* 1, 928-943.
- Hoering, T.C. 1973. A comparison of melanoidin and humic acid, *Annual report of the director of the geophysical laboratory, Carnegie Institution of Washington Year Book* 72, 682-690.
- Hoover, R. 2000. Acid treated starches. *Food Reviews International* 16, 369-396.
- Hwang, C.-S., Shemorry, A., Varshavsky, A. 2010. N-terminal acetylation of cellular proteins creates specific degradation signals. *Science* 327, 973-977.
- Jane, J.-L., Kasemsuwan, T., Leas, S., Zobel, H., Robyt, J.F. 1994. Anthology of starch granule morphology by Scanning Electron Microscopy, *Starch-Stärke* 46.
- Jane, J.-L. 2009. Structural features of Starch Granules II, in: Whistler, R. (Ed.), *Food science and technology: Starch*, Academic Press, New York, pp. 193-236.
- Jarvis, S.C. 1993. Nitrogen cycling and losses from dairy farms. *Soil Use and Management* 9, 99-104.
- Jessen, G.L., Lichtschlag, A., Ramette, A., Pantoja, S., Rossel, P.E., Schubert, C.J., Struck, U., Boetius, A. 2017. Hypoxia causes preservation of labile organic matter and changes seafloor microbial community composition (black sea). *Science Advances*. 3, e1601897.
- Kilgour, D.P., Wills, R., Qi, Y., O'Connor, P.B. 2013. Autophaser: an algorithm for automated generation of absorption mode spectra for FT-ICR MS, *Analytical chemistry* 85, 3903-3911.

- Kislenko, V. 2002. Polymer adsorption at solid surfaces, Adsorption: Theory, modeling, and analysis, Marcel Dekker Inc., New York, pp. 743-800.
- Larter, S.R., Douglas, A.G. 1980. Melanoidins - Kerogen Precursors and Geochemical Lipid Sinks - A Study Using Pyrolysis-Gas Chromatography (PGC), *Geochimica et Cosmochimica Acta* 44, 2087-2095.
- Larter, S.R., Solli, H., Douglas, A.G. 1983. Phytol containing melanoidins and their bearing on the fate of isoprenoid structures in sediments. in: *Advances in Organic Geochemistry*. Wiley, Chichester, pp. 513-523.
- Maillard, L.C. 1912. Action of amino acids on sugars. Formation of melanoidins in a methodical way. *Compte-Rendu de l'Academie des Sciences*. 154, 66-68.
- Mann, P.J., Sobczak, W.V., LaRue, M.M., Bulygina, E., Davydova, A., Vonk, J.E., Schade, J., Davydov, S., Zimov, N., Holmes, R.M., Spencer, R.G.M. 2014. Evidence for key enzymatic controls on metabolism of Arctic river organic matter., pp. 1089-1100.
- Martins, S.I.F.S., Jongen, W.M.F. 2001. A review of Maillard reaction in food and implications to kinetic modelling. *Trends in food science & technology*. 11, 364 -373.
- McWeeny, D.J., Knowles, M.E., Hearne, J.F. 1974. The chemistry of non-enzymic browning in foods and its control by sulphites, *Journal of the Science of Food and Agriculture* 25, 735-746.
- Mercader, J., Akeju, T., Brown, M., Bundala, M., Collins, M.J., Copeland, L., Crowther, A., Dunfield, P., Henry, A., Inwood, J., Itambu, I., Kim, J.-J., Larter, S., Longo, L., Oldenburg, T., Patalano, R., Sammynaiken, R., Soto, M., Tyler, R., Xhaufclair, H. 2018a. Exaggerated expectations in ancient starch research and the need for new taphonomic and authenticity criteria, *Facets* 30, 777-798.
- Mercader, J., Abtosway, M., Bird, R., Bundala, M., Clarke, S., Favreau, J., Inwood, J., Itambu, M., Larter, F., Lee, P., Patalano, R., Soto, M., Tucker, L., Walde, D. 2018b. Morphometrics of Starch Granules From Sub-Saharan Plants and the Taxonomic Identification of Ancient Starch, *Frontiers in Earth Science* 6.
- Oldenburg, T., Brown, M., Bennet, B., Larter, S.R. 2014. The impact of thermal maturity level on the composition of crude oils, assessed using ultra-high resolution mass spectrometry, *Organic Geochemistry* 75, 151-168.
- Oldenburg, T.B., Jones, M., Huang, H., Bennett, B., Shafiee, N.S., Head, I., Larter, S.R. 2017. The controls on the composition of biodegraded oils in the deep subsurface–Part 4. Destruction and production of high molecular weight non-hydrocarbon species and destruction of aromatic hydrocarbons during progressive in-reservoir biodegradation, *Organic Geochemistry* 114, 57-80.

- Perez, S., Baldwin, P.M., Gallant, D.J., 2009. Structural Features of Starch Granules, in: BeMiller, J., Whistler, R. (Eds.), *Starch*, Third ed., Academic Press, Burlington, MA, pp. 149-192.
- Soto, M., Inwood, J., Clarke, S., Crowther, A., Covelli, D., Favreau, J., Itambu, M., Larter, S., Lee, P., Lozano, M., Maley, J., Mwambwiga, A., Patalano, R., Sammynaiken, R., Vergès, J.M., Zhu, J., Mercader, J., 2019. Structural characterization and decontamination of dental calculus for ancient starch research, *Archaeol. Anthropol. Sci.* 11, 4847-4872.
- Stach, E., Murchison, D., 1982. *Stach's Textbook of Coal Petrology*, Borntraeger, Berlin.
- Saitta, E.T., Kaye, T.G., Vinther, J., 2019. Sediment-encased maturation: a novel method for simulating diagenesis in organic fossil preservation, *Palaeontology* 62, 135-150.
- Shi, Y-C., Capitani, T., Trzasko, P., Jeffcoat, R., 1998. Molecular structure of a low-amylopectin starch and other high-amylose maize starches, *Journal of Cereal Science* 27, 289-299.
- Tissot, B.P., Welte, D.H., 2013. *Petroleum formation and occurrence*, Springer Science & Business Media, New York.
- Tomasik, P., 2009. Specific physical and chemical properties of potato starch, *Food* 3, 45-56.
- Xie, F., Yu, L., Su, B., Liu, P., Wang, J., Liu, H., Chen, L., 2009. Rheological properties of starches with different amylose/amylopectin ratios, *Journal of Cereal Science* 49, 371-377.
- Xie, H., Zafiriou, O.C., Cai, W.-J., Zepp, R.G., Wang, Y., 2004. Photooxidation and its effects on the carboxyl content of dissolved organic matter in two coastal rivers in the southeastern United States, *Environmental science & technology* 38, 4113-4119.
- Zou, W., Yu, L., Liu, X., Chen, L., Zhang, X., Qiao, D., Zhang, R., 2012. Effects of amylose/amylopectin ratio on starch-based superabsorbent polymers, *Carbohydrate Polymers* 87, 1583-1588.

	Order	Family	Species	Part	Cryst. Pattern	Color at 150°C
Primitive	Alismantales	Araceae	<i>Colocasia esculenta</i>	Tuber	A	dusky yellow
	Dioscoreales	Dioscoreaceae	<i>Dioscorea esculenta</i>	Tuber	C	dark yellowish orange
	Dioscoreales	Dioscoreaceae	<i>Dioscorea schimperiana</i>	Tuber	C	dark yellowish orange
	Poales	Poaceae	<i>Sorghum bicolor</i>	Grain	A	very pale orange
	Poales	Poaceae	<i>Triticum aestivum</i>	Grain	A (B)	light olive brown
	Poales	Poaceae	<i>Zea mays</i>	Grain	A	yellowish grey
	Poales	Cyperaceae	<i>Cyperus rotundus</i>	Tuber	C	greyish orange pink
	Zingiberales	Musaceae	<i>Ensete ventricosum</i>	Corm	B	moderate yellow brown
	Malpighiales	Euphorbiaceae	<i>Erythrococca bongensis</i>	Seed	A	moderate yellow brown
	Malpighiales	Euphorbiaceae	<i>Euphorbia quadrangularis</i>	Root	C	greyish orange
	Fabales	Fabaceae	<i>Lablab purpureus</i>	Legume	C	greyish brown
	Fabales	Fabaceae	<i>Mucuna pruriens</i>	Legume	C	olive gray
	Fabales	Fabaceae	<i>Senna didymobotria</i>	Legume	C	moderate yellow
	Vitales	Vitaceae	<i>Cyphostemma serpens</i>	Tuber	B	dark yellowish brown
	Solanales	Convolvulaceae	<i>Ipomoea longituba</i>	Tuber	C (A)	dark yellowish orange
Derived	Solanales	Solanaceae	<i>Solanum tuberosum</i>	Tuber	B	greyish orange

FIG 1.

(Modified) van Krevelen diagrams for ESI-N data from the melanoidins derived from *Ipomoea longituba* starch and glycine

Heteroatom class distributions in ESI-N mode spectra of *Ipomoea longituba* melanoidins

Fig. 3
Compound class distribution of water soluble melanoidin products of 16 different starch taxa (150°C 24h)

Dioscorea esculenta Classes

ESI-N Ragnarök

Dioscorea schimperiana Classes

ESI-N Ragnarök

Cyperus rotundus Classes

ESI-N Ragnarök

Euphorbia quadrangularis Classes

ESI-N Ragnarök

Lablab purpureus Classes

ESI-N Ragnarök

Mucuna pruriens Classes

ESI-N Ragnarök

Senna didymobotria Classes

ESI-N Ragnarök

Ipomoea longituba Classes

ESI-N Ragnarök

Fig. 4

Modified Kendrick plots of melanoidins from 16 starch taxa. Each plot contains all chemical species per sample analyzed in ESI-N ion mode

Fig. 5

Van Krevelen plots (H/C versus O/C) of the melanoidins from 16 starch taxa. Each plot contains all chemical species per sample analyzed in ESI-N ion mode

Fig. 6

Modified Van Krevelen plots (N/C versus N/O reflecting the distribution of NyOx species) of the 16 melanoidins. Each plot contains all chemical species per sample analyzed in ESI-N ion mode

Fig. 7

Compound class variation (nitrogen-containing NyOx and oxygen-only Ox species) across comparable melanoidins from all 16 starch taxa.

Fig. 8

Triangular plot illustrating the relative intensity distribution in classes O6, O11, and O16 [A] as well as O6, NO6 and N3O6 [B]

Fig. 9

Overall carbon number distributions of all species [A], species containing no nitrogen (Ox) [B], species containing one nitrogen (NOx) [C] and species with two nitrogen atoms (N2Ox) [D]. Some evidence of relict polysaccharides is evidence in component intensity peaks with carbon numbers of multiples of 6 (A, B). The same process is seen with an additional 2 carbon increment which may reflect relatively simple polysaccharide plus a single amino acid carbon skeleton derived but related product group (C).

Fig. 10

Chemical summary of the Maillard reaction modified from Martins et al (2001)