Nutritional contribution of plant foods to human diet in evolution

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CHAPTER 2

IMPACT OF BRIEF ROASTING ON STARCH GELATINIZATION AND IMPLICATIONS FOR PLANT FOOD NUTRITIONAL ECOLOGY IN HUMAN EVOLUTION

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ABSTRACT I

Cooking is an important technology for its capacity to increase food digestibility, potentially playing a significant role in human evolution as a means to increase nutritional density of food. However, the contextual impetus for cooking is less well understood. One mode of cooking used by modern humans is to roast food directly in an open flame fire for a wide range of times. This technique may be a behavioral analogue to that of early human ancestors. Here, we provide the first experimental data on starch gelatinization patterns of USOs and starchy fruit as a result of brief fire roasting. We employed a count-based method using light microscopy and a semi-quantitative photometric strategy to observe the kinetic process of gelatinization. We find that brief, five-minute roasting over an open fire results in low levels of starch gelatinization in the parenchymal tissue, which persists even after longer roasting durations, exceeding ten minutes. However, at fifteen and twenty minutes, the majority of starch throughout each specimen is sufficiently gelatinized to enable alpha-amylase activity. These findings suggest that instances of very brief roasting, up to ten minutes, may not necessarily benefit a consumer through changes to starch structure and susceptibility to alpha-amylase activity alone.

2.1 Introduction

Cooking is widely recognized as a highly significant step in human evolution, in part because it can increase the nutritional value of food, especially for starch-rich foods (Boback et al., 2007; Carmody et al., 2011). Cooking causes mechanical and chemical changes to food, which increases digestibility through a reduction in molecular structure, softening of tissues, or facilitating exposure of nutrients to digestive enzymes (Englyst and Cummings, 1986; Holm et al., 1988; Kataria and Chauhan, 1988; Bornet et al., 1989; Zink et al., 2014). Cooking also helps deactivate anti-nutrients that often inhibit nutrient uptake (Greenhouse, 1981; Johns, 1996; Wandsnider, 1997). The extra energy derived from cooking could be used to build other "expensive tissues" in the human body (Aiello and Wheeler, 1995), and it has been proposed that the increased brain size seen in *Homo erectus* was a direct result of the calories gained from cooking (Wrangham and Conklin-Brittain, 2003; Carmody and Wrangham, 2009; Carmody et al., 2011; Organ et al., 2011). While earlier work has explored patterns of starch gelatinization and detoxification as a result of differential degrees of cooking, the actual effect of direct brief fire roasting on starch gelatinization in whole, intact foods has not, to our knowledge, been thoroughly investigated, particularly within the context of studying human evolution (Holm et al., 1988; Wollstonecroft et al., 2008; Henry et al., 2009; Messner and Schindler, 2010; Crowther, 2012). Furthermore, previous research that looks at the effect of cooking on caloric gain by irradiating food in a microwave does not account for the differential degree of change to food properties as a result of variation in intensity, duration, and type of thermal treatment (Carmody et al., 2011). Here, we aim to address this lacuna by investigating the pattern of starch gelatinization as a result of brief roasting on a high flame open fire.

Cooking facilitates caloric acquisition for most foods, yet recent evolutionary models have focused on the contribution of starchy underground storage organs (USOs) as a major source of calories that potentially led to hominin brain growth (Wrangham et al., 1999). Several lines of evidence support the importance of USOs, including observations of modern hunter-gatherers, research on thermal processing and nutrition, studies on wild ape feeding ecology, microfossil analysis, amylase gene copy variation, and even isotope analysis (Perry et al., 2007; Marlowe and Berbesque, 2009; Carmody et al., 2011; Henry et al., 2011).

Though we expect cooked USOs and other plants were important in human history, there is very little archaeological evidence for their consumption or early use of fire for cooking. Fire definitively enters the archaeological record around 300-400 thousand years ago in Europe, although earlier dates are proposed, they remain less well supported and a consistent presence over time is notably absent (Roebroeks and Villa, 2011). African sites contain evidence of fire presence between 1.0-1.5 million years ago, however these are open-air sites that lack anthropogenic association and only in Wonderwerk Cave do we find unambiguous evidence of fire in the Acheulean strata 1.0 million years ago, though the source of the fire is debatable (Berna et al., 2012). Plants, and the tools used to harvest

and process them, are rarely preserved in the archaeological record (Marean, 1997; O'Connell et al., 1999; Richards, 2002; Lemorini et al., 2014). Only in the last two decades has the ability to find and identify microscopic plant artifacts such as starches and phytoliths been rigorously applied to archaeological materials (Cortella and Pochettino, 1994; Lamb and Loy, 2005; Piperno et al., 2009; Henry et al., 2011; Hardy et al., 2012). Due to this lack of information from the archaeological record, we must heavily rely on modern experimental simulations to test the significance of plant consumption and associated processing techniques (Zink et al., 2014).

One of the most important effects of cooking plant foods is the breakdown of structural components, such as cellulose, and nutritional components, such as starch, which helps to soften the food and expose nutritional elements to digestive enzymes (Lee et al., 1985; Holm et al., 1988; Kataria and Chauhan, 1988; Bornet et al., 1989; Muir et al., 1995; Wandsnider, 1997; Dona et al., 2010; Carmody et al., 2011). Cooking practices vary by ecological and ethnographic environment depending on a variety of factors such as food type, resource availability, cultural precedence, and consumer preference. Cooking of a single food type can also vary both in frequency and in duration. In the context of cooking techniques that may be attributable to early hominins, we focus on "brief" roasting characterized by short duration roasting (five to 20 minutes) on an open-flame surface fire. This technique represents a basic method of cooking that is employed by extant hunter-gatherers, particularly in arid tropical environments (see Table 1) (Silberbauer, 1981; O'Connell et al., 1983; Malaisse and Parent, 1985; Youngblood, 2004; Marlowe, 2010; Leonard et al., 2015), and may fall within the behavioral and cognitive capacity of early hominins. Therefore, this body of research helps fill a gap in our knowledge about the effect of a specific type of cooking, namely open fire roasting, on starch gelatinization in various whole, intact, starchy plant foods.

Table 1. Summary of USOs and preparation technique for extant foragers from arid tropical regions; NS not specified.

			Preparation technique								
Family	**Plant**	**Type**	**Raw**	**Roast**	**Boil**	**Bake**	**NS**	**Notes**	**Region**	**Group**	**Reference**
Agavaceae	*Sansevieria scabrifolia*	bulb	X					chewed raw for fluid, high use	Central Kalahari	G/wi	Silberbauer 1981
Apiaceae	*Chamarea capensis*	tuber					X	aromatic, like fennel	Cape, SA	?	Youngblood 2004
Apocynaceae	*Leichhardtia australis* R. Br.	taproot	X	X				emergency food	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Apocynaceae	*Ceropegia sp.*	tuber	X	X				food and fluid, favored, rare	Central Kalahari	G/wi	Silberbauer 1981
Apocynaceae	*Brachystelma thunbergii*	tuber	X				X	juicy	Zeekoe Valley, SA	?	Youngblood 2004
Apocynaceae	*Fockea angustifolia* K. Schum	tuber	X						NW Namibia	Twe	Leonard et al. 2015
Apocynaceae	*Brachystelma barberiae*	tuber	X	X				food and fluid, favored, rare	Central Kalahari	G/wi	Silberbauer 1981
Apocynaceae	*Caralluma lutea*	root	X					pounded and eaten raw	Central Kalahari	G/wi	Silberbauer 1981
Apocynaceae	*Raphionacme burkei*	tuber	X					fluid source, shredded pulp	Central Kalahari	G/wi	Silberbauer 1981
Asparagaceae	*Albuca sp.*	bulb					X	eaten by children	Zeekoe Valley, SA	?	Youngblood 2004
Asparagaceae	*Scilla sp.*	bulb		X				peeled and eaten, high use	Central Kalahari	G/wi	Silberbauer 1981
Burseraceae	*Commiphora pyracanthoides*	root	X					fluid, moderate use	Central Kalahari	G/wi	Silberbauer 1981
Convolvulaceae	*Ipomoea transvaalensis*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010
Convolvulaceae	*Ipomoea costata* F. Muell. ex Benth.	tuber				X		pit hearth; staple	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Convolvulaceae	*Ipomoea transvaalensis*	tuber	X	X				food and fluid, moderate use	Central Kalahari	G/wi	Silberbauer 1981
Cucurbitaceae	*Coccinea surantiaca*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010
Cucurbitaceae	*Coccinia rehmanni, C. sessifolia*	tuber		X				food and fluid, staple, poisonous	Central Kalahari	G/wi	Silberbauer 1981
Cyperaceae	*Cyanella hyacinthoides*	bulb/corm		X					Zeekoe Valley, SA	?	Youngblood 2004
Cyperaceae	*Cyperus esculentus*	sedge					X		Zeekoe Valley, SA	?	Youngblood 2004
Cyperaceae	*Cyperus usitatus/fulgens*	sedge		X				slightly roasted	Zeekoe Valley, SA	Bushmen	Youngblood 2004
Cyperaceae	*Cyperus bulbosus* Vahl	sedge	X	X				raw or lightly roasted; staple	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Fabaceae	*Eminia entennulifa*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010
Fabaceae	*Rhynchosia comosa*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010
Fabaceae	*Vatovaea pseudolablab*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010
Fabaceae	*Vigna frutescens*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010

Fabaceae	*Vigna macrorhyncha*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010
Fabaceae	*Vigna sp.*	tuber	X	X				briefly roasted ~5-15min	NW Tanzania	Hadza	Marlowe 2010
Fabaceae	*Tylosema esculentum* A. Schreib	rhizome		X				roasted >1hr	NW Namibia	Twe	Leonard et al. 2015
Fabaceae	*Erythrina vespertilio* Benth.	roots		X				emergency food	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Fabaceae	*Vigna lanceolata* Benth.	taproot	X	X				raw or lightly roasted; staple	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Fabaceae	*Bauhinia esculenta*	tuber	X					fluid source, moderate use	Central Kalahari	G/wi	Silberbauer 1981
Fabaceae	*Bauhinia macrantha*	root	X					fluid source, chewed, slight use	Central Kalahari	G/wi	Silberbauer 1981
Fabaceae	*Vigna dinteri*	root	X	X				food and fluid, favored, high use	Central Kalahari	G/wi	Silberbauer 1981
Fabaceae	*Vigna sp. (triloba?)*	root		X				food and fluid, moderate use	Central Kalahari	G/wi	Silberbauer 1981
Geraniaceae	*Pelargonium antidysentericum*	tuber	X					eaten by children	Zeekoe Valley, SA	?	Youngblood 2004
Hyacinthaceae	*Dipcadi sp.*	bulb		X				food and fluid, slight use	Central Kalahari	G/wi	Silberbauer 1981
Iridaceae	*Babiana dregei, B. nana*	corm					X		Zeekoe Valley, SA	San	Youngblood 2004
Iridaceae	*Babiana hypogea*	bulb/corm				X			Zeekoe Valley, SA	Bushmen	Youngblood 2004
Iridaceae	*Lapeirousia sp.*	corm		X				roasted ~5min to remove tunic	NW Namibia	Twe	Leonard et al. 2015
Iridaceae	*Lapeirousia*	corm		X				food, slight use	Central Kalahari	G/wi	Silberbauer 1981
Lamiaceae	*Clerodendrum floribundum* R. Br.	roots		X				emergency food	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Lobeliaceae	*Cyphia sp.*	tuber	X			X		watery, sweet	Zeekoe Valley, SA	Bushmen	Youngblood 2004
Lobeliaceae	*Cyphia stenopetala*	tuber		X				fluid, slight use, small tuber	Central Kalahari	G/wi	Silberbauer 1981
Nyctaginaceae	*Commicarpus pentandrus*	tuber					X	overcollected and rare	South Africa	?	Youngblood 2004
Nyctaginaceae	*Boerhavia diffusa* L.	taproot		X				emergency food	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Oxalidaceae	*Oxalis sp.*	bulb/corm	X						Zeekoe Valley, SA	Bushmen	Youngblood 2004
Pedaliaceae	*Harpagophytum procumbens*	root	X					fluid, chewed	Central Kalahari	G/wi	Silberbauer 1981
Pezizaceae	*Elderia arenivaga* (Cooke) McLennon	desert truffle					X	rare but favored	Northern Territory, AUS	Alyawara	O'Connell et al. 1983
Portulacaceae	*Talinum caffrum*	taproot stock	X					medicine, food, moisture	Kalahari Desert	Bushmen	Youngblood 2004
Tecophilaeaceae	*Walleria nutans*	bulb	X	X				food and fluid, slight use	Central Kalahari	G/wi	Silberbauer 1981
Unknown	*Unknown [ozonduvi]*	corm		X				roasted ~5min	NW Namibia	Twe	Leonard et al. 2015
Unknown	*Unknown [otjihakariwa]*	tuber	X						NW Namibia	Twe	Leonard et al. 2015

The diets of contemporary hunting and gathering populations are important informants about the nutritional ecology of early human ancestors (Eaton and Konner, 1985). Hunter-gatherers should not be mistaken as Paleolithic emulations, but rather referents to the behavioral and nutritional adaptations necessary for foraging subsistence regimes common throughout human evolution (Marlowe, 2005). USOs and starchy plants are an important resource for many extant forager groups, and their exploitation in evolutionary history is tentatively evidenced by comparative C_4 isotope signatures in early robust hominins (Yeakel et al., 2007; Ungar and Sponheimer, 2011), sedge phytoliths found in *Australopithecus sediba* (Henry et al., 2012), and a climate induced shift to more USO producing arid and grassland ecosystems in the Pleistocene (Andersen, 1987; Cerling, 1992; deMenocal, 2004). Most wild USOs are rarely targeted by other primates unless they can be found close to the ground surface, which would have made them a relatively uncontested food resource for early hominins (Peters and O'Brien, 1981; Hernandez-Aguilar et al., 2007). More recent human adaptations to starch consumption highlight the extent to which starch is also a valuable component of the modern human diet (Perry et al., 2007). Wild and domestic starchy plant foods, which normally include USOs, nuts, seeds, sedges, and fruits, are widely exploited by many different hunter-gatherers ranging from tropical to temperate climates in both humid and arid environments around the world including: rainforest dwelling African hunter-gatherers (Coursey, 1976; Milton, 1985; Hladik et al., 1993; Bahuchet, 1999); inhabitants of southern Shaba and tropical African grasslands (Malaisse and Parent, 1985; Marean, 1997); Hadza of Tanzania (Vincent, 1985); Twe of Namibia (Leonard et al., 2015); Batek of Malaysia (Endicott and Bellwood, 1991); Ainu of Hokkaido (Watanabe, 1972); Australian Aborigines (Gott, 1982, 2008; O'Connell et al., 1983; O'Dea, 1991); Hiwi of Venezuela (Hurtado and Hill, 1990); prehistoric groups from Northern Chile (Holden, 1991); and indigenous peoples of northwestern North America (Couture et al., 1986; Thoms, 1989; Turner and Davis, 1993).

Modern human foragers, especially groups in tropical regions such as sub-Saharan Africa and the Northwest Territories of Australia, which we summarize in Table 1, consume starchy plant foods often for major portions of their diet and may constitute a representative model for plant food exploitation by ancient hunter-gatherers. Some well-known and studied forager groups include (but are certainly not limited to): the Hadza of Northern Tanzania (Marlowe, 2010), the Mbuti pygmies of Eastern Zaire (Hart and Hart, 1986), the Baka pygmies of Southern Cameroon (Dounias, 2001), Twe of Namibia (Vashro, 2014; Leonard et al., 2015), the G/wi, San and ǃKung of the Kalahari Desert (Lee, 1979; Silberbauer, 1981; Youngblood, 2004), ancient and contemporary Native Americans (Couture et al., 1986; Thoms, 1989), and Australian Aborigines (O'Connell et al., 1983; O'Dea, 1991). Common resources are typically non-cultivated wild species that come from larger plant families such as Fabaceae (legumes), Dioscoreaceae (yams), Typhaceae (wetland rhizomes), Apiaceae (aromatic herbs and taproots), Liliaceae (flowering bulbs), Apocynaceae ('dogbane' family, or milkweeds), Convolvulaceae (morning glories and herbaceous vines), and Cucurbitaceae (squashes, melons, and gourds). Hadza women in particular roast wild tubers over a quick-burning open-flame fire, lasting

approximately 15 minutes, with roasting times that range between three to 30 minutes – with most accounts settling on an average of five minutes (Tomita, 1966; Woodburn, 1966; Schoeninger et al., 2001; Mallol et al., 2007; Dominy et al., 2008; Marlowe and Berbesque, 2009; Schnorr et al., 2015). Woodburn (1966) described Hadza tubers as "not really cooked" and "lightly charred" as a result of this brief roasting (Woodburn, 1966). Species of *Vigna* that the Hadza consume are very fibrous and are chewed and wadged in the cheek to expel the starchy intercellular matrix. A similar starchy fibrous parenchymatous matrix is described for tubers consumed raw and roasted in the central Kalahari Desert (Silberbauer, 1981; Malaisse and Parent, 1985; Youngblood, 2004). It must be noted, however, that not all human consumed USOs contain starch, and a great many contain fructose polymers called fructan or inulin (especially members of Cyperaceae, Liliaceae and the amaryllids such as onions), which cannot be digested by mammalian enzymes and are instead fermented by the microbiota in the colon. For many such fructan containing USOs, long-duration roasting in hot coals or in earth ovens is more commonly utilized in order to hydrolyze fructans into their constituent simple sugars (Thoms, 1989; Wandsnider, 1997; Kubiak-Martens, 2002).

Plant cooking technique and duration varies widely across culture and geography: brief fire-roasting may last three to five minutes for some high moisture, small, or non-toxic species (Tomita, 1966; Woodburn, 1966; Youngblood, 2004; Marlowe and Berbesque, 2009; Leonard et al., 2015), whereas wild varieties of Dioscoreaceae can require extensive preparation, such as boiling and leaching over many days, for detoxification (Couture et al., 1986; Hart and Hart, 1986; Headland, 1987; Raj Bhandari and Kawabata, 2006). The ethnographic literature on tropical foragers that we summarized in Table 1 is dominated by depictions of USO consumption in either raw or roasted forms, compared to boiled or baked (Table 2). Roasting, as opposed to boiling or baking, may be a more valuable cooking method in arid regions where water is scarce or seasonal (Silberbauer, 1981), and when the plant carbohydrates are more easily digested simple sugars and starch rather than fructose polymers (Wandsnider, 1997). From the food science literature, we know that different cooking techniques, such as frying, boiling, or dry roasting, can greatly affect digestibility and glycemic load in isolated starch and starch-containing foods (Lee et al., 1985; Wandsnider, 1997). However, if experimental models are to have explanatory power as to how adoption of cooking accommodated significant physiological adaptations in human evolution, then we must attempt to replicate putative early cooking technologies (Wollstonecroft et al., 2008; Messner and Schindler, 2010; Zink et al., 2014). In this way, we can begin to clarify the varying effects that different cooking techniques inevitably have on the physical and chemical properties of food that may affect the overall caloric accessibility to a consumer.

Table 2. Tabulations of all preparation techniques by plant family from Table 1; NS not specified[1]

Family	Species	Raw	Roast	Boil	Bake	NS
Agavaceae	1	1	0	0	0	0
Apiaceae	1	0	0	0	0	1
Apocynaceae	7	7	3	0	0	1
Asparagaceae	2	0	1	0	0	1
Burseraceae	1	1	0	0	0	0
Convolvulaceae	3	2	2	0	1	0
Cucurbitaceae	2	1	2	0	0	0
Cyperaceae	4	1	3	0	0	1
Fabaceae	13	10	11	0	0	0
Geraniaceae	1	1	0	0	0	0
Hyacinthaceae	1	0	1	0	0	0
Iridaceae	4	0	2	0	1	1
Lamiaceae	1	0	1	0	0	0
Lobeliaceae	2	1	1	0	1	0
Nyctaginaceae	2	0	1	0	0	1
Oxalidaceae	1	1	0	0	0	0
Pedaliaceae	1	1	0	0	0	0
Pezizaceae	1	0	0	0	0	1
Portulacaceae	1	1	0	0	0	0
Tecophilaeaceae	1	1	1	0	0	0
Unknown	2	1	1	0	0	0
Total	**50**	**30**	**30**	**0**	**3**	**7**

[1]Note that there are multiple entries for single specimens from Table 1 when more than one preparation technique is listed.

In the current experiments, we processed four varieties of domestic tubers, one tap root, and one starchy gourd to quantify the extent of starch gelatinization resulting from different durations of direct fire-roasting between five and 20 minutes. We focus primarily on the five minute duration, which represents the briefest fire-roasting duration that has been regularly observed primarily among the Hadza of Tanzania, but is also recorded for the Twe of Namibia (Tomita, 1966; Woodburn, 1968; Mallol et al., 2007; Dominy et al., 2008; Leonard et al., 2015). Additionally, "light roasting" has been used to describe the cooking practice for some resources frequently exploited by the San, !Kung, and G/wi Kalahari desert, and the Alyawara Aborigines from Australia (Table 1) (Lee, 1979; Silberbauer, 1981; O'Connell et al., 1983; Youngblood, 2004), and accounts of raw and roasted USO consumption far exceed that of boiled or baked modes of cookery (Table 2). We use these results to make inferences about starch gelatinization kinematics *in-situ* in whole, unprocessed, starchy plant foods submitted to brief open fire roasting. We can then answer whether brief roasting acts to improve digestibility by breaking down the semi-crystalline starch structure, or whether brief thermal treatment serves an alternative purpose that otherwise facilitates consumption, such as softening cell-wall structure.

Domestic cultivars, while highly divergent from wild species in their nutritional composition (O'Dea, 1991), can still inform us as to the propensity for heat transfer across a high-moisture starchy organ and address the likelihood that the starch molecular structure is affected. Wild plants are highly

variable in their nutritional content and often contain pronounced resistances against consumers by either mechanical or chemical means (Johns, 1996; Wandsnider, 1997). Intensification of plant food exploitation is evidenced by the expansion of dietary breadth and concomitant plant processing techniques in the Epipaleolithic (Hillman et al., 1989; Wollstonecroft et al., 2008), and by the gradual cultivation and domestication of more compliant taxa (Fuller, 2007). The effect of long-term plant domestication leads to morphological and physiological changes that reduce refractory structures, increase palatability, increase caloric density and digestibility, and reduce plant chemical defenses (which includes phytohormones, phytates, phenols, tannins, vitamins and minerals) (O'Dea, 1991; Johns, 1996; Purugganan and Fuller, 2009). Domesticated species, such as those we analyzed in the current study, would therefore be expected to overestimate the effect of thermal treatment on starch gelatinization. This would, therefore, bias our conclusions to determine that gelatinization happens more rapidly and at lower temperatures than what might actually occur in wild species. We provide some images and comparisons from preliminary work with roasting wild tubers to expand on this important topic in our discussion. Overall, our results suggest that starch gelatinization is not a driving factor for brief roasting as a cooking strategy, particularly at the briefest durations lasting approximately five to ten minutes (Schnorr et al., 2015). Alternatively, other factors such as improved taste or ease of peeling and chewing may better explain why brief roasting is practiced. Future work to interpret processing techniques in specific contexts should endeavor to use the wild foods in question when they are available.

2.2 Methods

2.2.1 Experimental setup

To characterize tuber starch gelatinization, we used domestic cultivars taxonomically related to wild tuber species commonly consumed by extant foragers as a simple proxy. All specimens, time trials, and fire measurements are listed in Table 3. In particular, *Cucurbita pepo* (summer squash) and *Ipomoea batatas* (sweet potato) were selected for this study because of their close taxonomic relation to the wild East African tubers, *Coccinea surantiaca* and *Ipomoea transvaalensis*, at the family and genus level respectively. In addition, *Dioscorea rotundata* (yam) and *Manihot esculenta* (manioc) are prominent cultivars in Africa and South America, the former related to important wild-types found in rain forest habitats, and the latter of which has sweet varieties that can sometimes be consumed raw. *Daucus carota* (carrot), the only tap root, was chosen due to its smaller size and cylindrical shape, which we expect may change heat distribution and rate of gelatinization. Finally, *Solanum tuberosum* (potato) was included in this study because it represents one of the universal starch standard specimens (Finglas

and Faulks, 1984; Zeeman et al., 2010; Nguyen Do Trong et al., 2011; Parada and Aguilera, 2011; Crowther, 2012). Together, these plant species share in common the production of insoluble starch in above and below ground fruit and storage organs (Reichert, 1913).

We conducted fire-roasting cooking trials at five minutes in triplicate (a, b, and c specimens) for each species and compared the outcome against a "d" specimen, which was roasted for 20 minutes. We then selected *I. batatas* (sweet potato) and *S. tuberosum* (potato), the two tuber species that can be reliably found year-round in local markets, for a second roasting trial at 10 and 15 minutes on single specimens. This second trial fills the time gap between five and 20 minutes, serving as a proxy assessment of gelatinization kinematics across four total time points. Gelatinization was measured quantitatively from the center (C), middle (M), and edge (E) of cross-sectioned specimens using light microscopy to spot count gelatinized starches with Congo Red (CR) staining (Fig.1). We opted to use non-enzymatic methods because the extra processing required by enzyme-based assays (freeze-drying and grinding) can affect enzyme susceptibility of starch and confound the effects from thermal treatment alone.

Fig. 1 Example of a cross-section of a recently roasted *M. esculenta* and the location of the three sampling points used throughout the study: center (C), middle (M), and edge (E).

CR is a water soluble chemical dye and is routinely used as a contrast stain for cellulose, amyloid fibrils, and starch (Carroll and Van Dyk, 1953; Carroll and Cheung, 1960; Collison and Chilton, 1974; Valetudie et al., 1995; Lamb and Loy, 2005; Weston, 2005). CR is a good indicator of gelatinization due to its affinity for damaged rather than undamaged starch and characteristic red staining under brightfield illumination and polarized light (Adler et al., 1994). Starch granules are semicrystalline structures made from layers of amylose and amylopectin chains (Jane et al., 1994; Wang et al., 1998;

Mcpherson and Jane, 1999; Hoover, 2001; Yoo and Jane, 2002; Stevenson et al., 2005; Zeeman et al., 2010; Parada and Aguilera, 2011). When starches are heated or damaged, the crystalline structure breaks down and absorbs water, allowing CR to react with the amylose. Therefore, CR staining can only occur when starch damage is sufficient to expose amylose within the confines of the highly branched amylopectin structure (Carroll and Cheung, 1960). The reduction in the granule integrity and crystallinity similarly helps increase human salivary α-amylase activity on the starch by increasing the potential binding sites for the enzyme (Muir et al., 1995; Butterworth et al., 2011). Therefore, the same starch modifications that enable CR staining also greatly increase α-amylase activity such that a positive stain can infer digestibility of differentially gelatinized starch. Studies showing an increase in starch digestibility after cooking indicate the reliance of amylase activity on initial starch damage, lending further support to the importance of interrupting the starch structural integrity to increase binding sites on the amylose chain (Carroll and Van Dyk, 1953; Englyst and Cummings, 1986; Muir et al., 1995).

Finally, we conducted a third roasting trial using only *I. batatas* tubers to witness the kinematics of starch gelatinization across four time points from five to 20 minutes of fire roasting. We fire-roasted whole *I. batatas* tubers in triplicate (n = 12 tubers), and took cross-sectional samples from the C, M and E of each specimen. Gelatinization was inferred from photometric measurement of amylose-iodine complex formation with iodine potassium iodide solution (IKI) and amylose chains dispersed using a potassium hydroxide solution (KOH). This final trial, termed the kinetics of gelatinization, provided a secondary, semi-quantitative, and objective approach to help validate the results derived from the previous, slightly more subjective, histological methods. Therefore, the results represent an initial assessment of the kinetic properties of gelatinization in whole tubers as a result of fire roasting. The gelatinization kinetics method was also advantageous for the ability to rapidly analyze a high number of samples and technical replicates, which was not possible using the manual histological count-based method.

Table 3. Specimen, cooking time, diameter, fire temperatures (before [i] and after [o] roasting), and specimen surface temperature (after [o] roasting) for all experimental trials

Trial: Sample ID	Common name	Species name	Family	Time (min)	Diam (cm)	Fire Temp °C (i)	Fire Temp °C (o)	Surface Temp °C (o)
1:1a	Potato	*Solanum tuberosum*	Solanaceae	5	5.0	550	600	88
1:1b	Potato	*Solanum tuberosum*	Solanaceae	5	5.5	550	600	100
1:1c	Potato	*Solanum tuberosum*	Solanaceae	5	5.5	550	600	90
1:1d	Potato	*Solanum tuberosum*	Solanaceae	20	4.5	500	750	112
1:5a	Cassava	*Manihot esculenta*	Euphorbiaceae	5	4.5	550	600	75
1:5b	Cassava	*Manihot esculenta*	Euphorbiaceae	5	5.0	550	600	71
1:5c	Cassava	*Manihot esculenta*	Euphorbiaceae	5	5.0	550	600	66
1:5d	Cassava	*Manihot esculenta*	Euphorbiaceae	20	5.0	500	750	120
1:6a	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	5	4.5	500	600	71
1:6b	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	5	5.5	500	600	70
1:6c	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	5	5.5	500	600	66
1:6d	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	20	5.0	500	750	113
1:7a	Yam	*Dioscorea rotundata*	Dioscoreaceae	5	5.0	500	590	71
1:7b	Yam	*Dioscorea rotundata*	Dioscoreaceae	20	5.0	500	750	97
1:8a	Carrot	*Daucus carota*	Apiaceae	5	2.0	500	590	97
1:8b	Carrot	*Daucus carota*	Apiaceae	5	2.5	500	590	88
1:8c	Carrot	*Daucus carota*	Apiaceae	5	2.0	500	590	46
1:8d	Carrot	*Daucus carota*	Apiaceae	20	1.5	600	800	165
1:9a	Zucchini	*Cucurbita pepo*	Cucurbitaceae	5	3.5	600	700	94
1:9b	Zucchini	*Cucurbita pepo*	Cucurbitaceae	5	4.0	500	700	75
1:9c	Zucchini	*Cucurbita pepo*	Cucurbitaceae	5	3.0	550	700	99
1:9d	Zucchini	*Cucurbita pepo*	Cucurbitaceae	20	3.5	550	750	79
2:sp10	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	10	5	200	312	105
2:sp15	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	15	5	450	560	106
2:pot10	Potato	*Solanum tuberosum*	Solanaceae	10	7	200	312	100
2:pot15	Potato	*Solanum tuberosum*	Solanaceae	15	6.5	450	560	104
3:5A	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	5	6.7	200	300	69
3:5B	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	5	8.4	200	300	72
3:5C	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	5	8.0	200	300	74
3:10A	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	10	4.9	400	400	94
3:10B	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	10	7.5	400	400	78
3:10C	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	10	9.7	400	400	84
3:15A	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	15	6.2	350	400	87
3:15B	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	15	8.6	350	400	98
3:15C	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	15	9.2	350	400	106
3:20A	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	20	6.2	350	580	121
3:20B	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	20	9.2	350	580	81
3:20C	Sweet Potato	*Ipomoea batatas*	Convolvulaceae	20	9.7	350	580	276

2.2.2 Preparation and controls

S. tuberosum, *M. esculenta*, *D. rotundata*, *I. batatas*, *D. carota,* and *C. pepo* were purchased from various markets in Leipzig, Germany. Each species was tested in triplicate (a, b, c) for the five minute roasting time, and singly (d) for the 20 minute roasting time, except for *D. rotundata*, for which only two specimens could be obtained. Raw control samples were taken from each of the test species before cooking in order to confirm presence of starch and ensure that the CR dye did not stain any completely raw starches. To do this, we removed a small slice containing about one cubic centimeter of material from the distal end of each specimen and then mounted starch samples onto a standard glass microscope slide as stained and unstained raw controls (following methods of Matthews, Robertson and Loy (Torrence and Barton, 2006)). Slides were observed under brightfield illumination and cross polarization to confirm starch presence and non-activity of CR.

2.2.3 Fire and cooking

We modeled our fires on Hadza tuber cooking fires, as they represent a basic and well documented cooking fire used by extant foragers. Hadza cooking fires are described in detail by Mallol and colleagues (2007) and tuber roasting fires, in particular, have been observed and anecdotally reported on in many additional studies (Tomita, 1966; Woodburn, 1968; Vincent, 1985; Hawkes, 1996; Schoeninger et al., 2001; Marlowe and Berbesque, 2009; Marlowe, 2010). These sources consistently describe the tuber roasting fire as an above ground, high-flame, and short-lived fire, using only small to medium sized sticks from surrounding brush. Tubers are positioned in the middle of the brush pile and turned regularly during roasting. Using this as a model, we created our own replica roasting fire using a low-standing metal fire pit comprising a shallow basin to contain the flames. This basin was filled with sand to about three centimeters and pine firewood was layered to form a small platform on which the tubers could be placed (Fig. 2).

Fig. 2 Representative tuber-roasting fires documented from Hadza bush camps (**a**) and the experimental fire pit used for roasting trials to emulate a short duration open-fire suitable for roasting USOs (**b**). Photos by authors S.L.S. and A.N.C.

Once fire was established, the temperature was measured with a Voltcraft IR 800-20D infrared thermometer on the top of the platform, and then again after cooked tubers were removed (Table 3). Average fire temperatures for each trial fall in range of moderate to high intensity fires (>350° C), similar to what has been reported for Hadza tuber roasting fires (Mallol et al., 2007; Dominy et al., 2008). Wood was added if needed between cooking trials to maintain a high flame. Tubers were roasted by species for five minutes for specimens a, b, and c, and were turned 90° every 75 seconds to ensure even roasting on all sides. D specimens for each species were roasted together for 20 minutes to obtain fully gelatinized reference specimens. We elected not to snap freeze or cool specimens on ice after roasting because this can affect the starch structure, often causing cracks or breaks in the starch as a result of freezing. We felt these changes could potentially damage native (intact) starches and increase the likelihood of false positives when staining with CR. We recognize that when starch is cooled slowly, recrystallization, or retrogradation, occurs, causing the amylose and amylopectin chains to realign, forming a gel of even higher crystallinity than the native starch. To rule out that this would negatively impact CR staining of actual gelatinized starch, we conducted preliminary tests with whole potatoes cooked in a variety of ways (boiling, baking, and roasting) at different times (5, 10, and 20 minutes) and allowed to cool to room temperature over a period of two to 24 hours. We spot sampled these specimens at two, five, 12, and 24 hours with our CR staining protocol, and found that retrogradation did not impact the efficacy of CR to stain gelatinized starch.

2.2.4 Histological methods

After cooking, a transverse slice was made at the midpoint of each tuber and photographed. Starch samples were taken twice from the C, M, and E positions of each specimen: one for the CR stain and one for the NS sample slide. Slides were prepared following the methods of Torrence and Barton (2006) for starch isolation, and Lamb and Loy (2005) for CR staining. A small amount of specimen was finely diced with a scalpel on a dish and allowed to dry. A pipette with 40 µl of water was used to agitate the sample and distribute the starch granules and then 20 µl was drawn off and ejected onto a slide and allowed to dry. To stain, 40 µl of CR was applied to the dried sample on the slide and the cover slip placed on top. After 15 minutes of staining, 20 µl of 1 M NaCl solution was applied at one edge of the slide and drawn across using filter paper to wash the stain. Clear nail polish was applied at each corner of the cover slip to hold it in place. Slides were rehydrated for microscope analysis and observed under brightfield and cross polarized light to quantify the starch concentration. All observations were conducted using the Zeiss Axio A.1 compound light microscope under brightfield illumination at x200 and x400 magnification with cross polarization capability. Images were captured using the AxioCam MRm and accessed with AxioVision release version 4.8.2.0. Visual fields were selected using a randomized coordinate generator that produced ten points along ten concentric squares (Supplementary

Figure 1), totaling ten observation points per slide. This provided a balanced representation of the entire 22 mm^2 slide cover-slip area containing the specimen. For stained slides, the number of stained starches and the number of total starches within the entire visual field were counted and recorded per observation point. For view of fully gelatinized starches where the starch grain boundary is fully degraded, we used Paint.net v3.4.9 to detect color contiguity across a stained area in order to overcome the inability to count individual starches. However this is less relevant as our model uses only the percent of stained to total starch.

2.2.5 Gelatinization kinetics methods

To measure the starch gelatinization kinetics in roasted *I. batatas* (sweet potato), we adapted a previous method developed by Birch and Priestley (1973), and validated against newer techniques by Baks *et al.* (2006), which involves measuring the amylose-iodine complex formation in alkali dispersed starch (Birch and Priestley, 1973; Baks et al., 2007). In this, we were able to take advantage of a unique property of *I. batatas* in which once the tuber starch is sufficiently heated (approximately 60° C), it is susceptible to endogenous diastase such that most of the starch is rapidly hydrolyzed into dextrin and maltose (Gore, 1920, 1923; Losh et al., 1981). Therefore, we can expect that gelatinized sweet potato no longer contains amylose, but instead, oligosaccharides, which cannot complex with iodine to form the distinctive violet-blue color change. Our assay thus follows the reduction of amylose from hydrolysis, signaling gelatinization, by comparing cross sectioned samples of roasted tuber against individual raw and gelatinized controls. The process of starch hydrolysis from endogenous diastase does not occur above boiling point, presumably because this temperature deactivates the enzymatic activity, but starch gelatinization still proceeds rapidly (Gore, 1923). Therefore, the starches in the "edge" position, just underneath the peel, did not undergo complete hydrolysis due to extreme temperature of the fire, though in all specimens this starch was fully gelatinized, and is therefore not included in subsequent analysis.

We roasted *I. batatas* tubers in triplicate for four separate time trials: 5, 10, 15, and 20 minutes, totaling 12 individual tuber specimens. Just prior to the roasting trial, we peeled and removed a small slice, ~4 cm^3, from one distal end of each tuber to serve as a raw and gelatinized reference. The raw references were frozen immediately at -20° C in individual 15 ml tubes. The gelatinized reference samples were each finely diced and gelatinized in a 10 w/w % tuber-water ratio in a water bath held constant at 90° C for 60 minutes. After 60 minutes in the water bath, gelatinized references were placed immediately on dry ice for 30 minutes, and then frozen for 24 hours at -20° C. The roasting fire was set up and established in exactly the same way as for the previous two roasting trials. Temperatures of the fire before and after roasting as well as the surface temperature of each tuber after roasting are likewise reported in Table 3. Upon removal from the fire pit, tubers were immediately put on dry ice to stop further gelatinization and enzymatic activity. After approximately 10 minutes in dry ice, tubers were

cross-sectioned in the middle and approximately 3-5 grams of fresh weight samples were removed from the center, middle and edge of each cross-sectioned half. These samples were put in individual 15 ml tubes and frozen at -20° C for 24 hours. Upon freezing, all samples, including the references, were lyophilized and then ground to a fine powder using an IKA tube mill (IKA®-Werke GmbH & CO. KG, Staufen, Germany) at 2000 rpm for six cycles of 20 second intervals.

To disperse and isolate the amylose in each sample, triplicate measures of each powdered sample (0.012 g) were dissolved in 15 ml of 0.60 M KOH and shaken vigorously for 20 minutes. The resulting solution was centrifuged (3000 rpm, 10 min, acc. 3, dec. 9) to separate the insoluble part of the sample. After centrifugation, 1 ml of the supernatant was removed and neutralized with 9 ml 0.066 M HCl. Afterwards, 100 µl IKI reagent (1 g iodine, 4 g potassium iodide in 100 ml water) was added to each neutralized sample to form the blue amylose-iodine complex. Samples were pipetted in triplicate for technical replicates in a 96 well plate and the absorbance measured at 620 nm. Degree of gelatinization is expressed as a fraction of the raw reference measurement after subtracting out the fully gelatinized reference measurement, which is depicted in the following equation:

$$\text{Degree of Gelatinization} = 1 - \frac{(A_e - A_g)}{(A_r - A_g)}$$

where A_e stands for absorbance measurement of the experimental sample, A_r is the absorbance measurement of the raw reference, and A_g is the absorbance measurement of the gelatinized reference, subtracted from 1 to give the fraction of gelatinization.

2.2.6 Statistical analysis

Statistics were conducted using the R statistical package free software, version 2.15.1 (2012-06-22) and the *lme4* package for fitting linear and generalized linear mixed-effects models (GLMM) (2012-06-23) (R Core Team 2013). Due to non-normal distribution, repeat measures, and the influence of fixed and random effects on the response, we performed a linear mixed model using the function [lmer] to analyze the relationship between species, position sampled, and the percent of starch gelatinization for our five minute roasting trial (Baayen et al., 2008; Bolker et al., 2009; Winter, 2013). The response was selected as the percentage of total starch that was stained per observation square (because of non-convergence errors, the continuous count of stained and unstained starch as a response could not be used). Species and position were interacting fixed effects while the individual specimens (that is, the "subjects" of our experiment) and the observation number were random intercepts. We also included by-individual and by-observation-number random slopes for the effect of diameter measurements. Since the presence of starch within the sampling area of the microscope slide was biased towards the center of the square area, we weighted the model by the total starch counted per observation. A step-wise comparison between the full and the null model using likelihood ratio testing with the function

[anova] informed us of the significance of the effect and interactions between species and position. Visual inspection of residual plots did not reveal deviations from homoscedasticity, and a histogram and Q-Q plot of residuals did not indicate any violation of normality (Supplementary Figure 2). To look for overly influential observations, we ran a loop to perform "leave-one-out" diagnostics, which reruns the model with each observation point excluded one by one and reports the impact of that observation on the model outcome. Visual inspection of the resulting values against their intercepts indicated that the model was a good fit.

2.3 Results

2.3.1 Visual assessment

After five minutes of cooking, all specimens exhibited charred exteriors and some flaking of the outer skin. We noticed that it was difficult to remove specimens from the fire using cooking tongs because the outer skin was too soft and broke apart easily. Once specimens were brought back to the lab for handling and sampling, all exhibited this same softening and ease of peeling. Much care had to be taken to disturb the outer layers as little as possible while cutting and sampling the starch. Upon cutting into the middle of the five and 10 minute specimens, the interior flesh was firm, juicy, and largely indistinguishable from the raw control cuts. Only in some specimens was a ring of faint discoloration noticeable, which seemed to mark the boundary between raw and gelatinized starch. The specimens cooked for 15 and 20 minutes all had charred exteriors, crumbling interior tissue, and a considerable reduction in moisture. Fiber content of the domestic specimens used is notably much less and starch content much greater than what is often found in wild plants (O'Dea, 1991). However, the main factors affecting starch gelatinization are water and temperature, and as long as the moisture content is greater than 60 percent, then there is very little interference from the presence or absence of non-starch components such as fiber or protein (Wang and Kim, 1998).

2.3.2 Histological results

Example histological views of native, partially gelatinized, and fully gelatinized starch with Congo Red staining are shown in Figure 3. The result of the final linear mixed model using only the five minute roasting data from the first roasting trial is summarized in Table 4 and the average and standard deviation of gelatinization fraction are reported in Table 5. The interaction between species and position sampled significantly influence the amount of gelatinization after five minute roasting (χ^2

(17) = 268.45, $p < 0.0001$). An effect of species alone is present; however the effect of position is still significant independent of species. Therefore, we can expect that the gelatinization pattern of any starchy USO or fruit after five minutes will be largely raw in the center with increasing gelatinization towards the edge. Each five minute observation point for each position, and their average, is plotted in Figure 4, along with the 20 minute gelatinization fraction. Trend lines all corroborate the increase in gelatinization predicted by the linear model as observations traversed from C to E of each specimen. However, the spread of data is very large, particularly for *C. pepo* and *D. carota*, which is also apparent from the standard deviation values reported in Table 5.

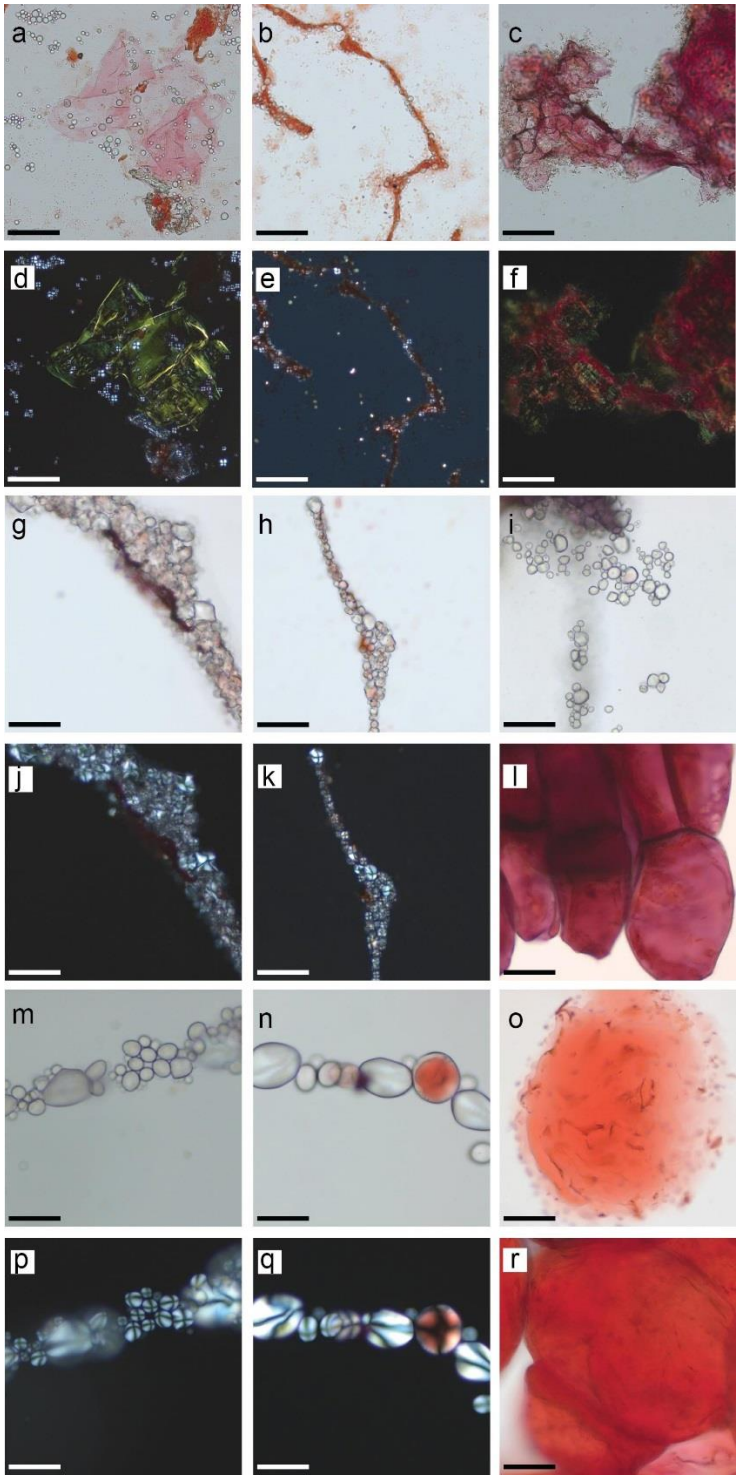

Fig. 3 Starch images of control (raw) and experimental (roasted) specimens show that the Congo Red stain reacts with partially and fully gelatinized starch, but not native starch. Images depict brightfield and polarized views of: *C. pepo* raw (**a**, **d**), partially gelatinized (**b**, **e**), and fully gelatinized (**c**, **f**); *I. batatas* raw and partially gelatinized (**g**-**k**), and fully gelatinized (**l**); and *S. tuberosum* raw (**m**, **p**), partially gelatinized (**n**, **q**), and fully gelatinized (**o**, **r**). Scale bars at 50µm.

Table 4. GLMM intercept estimates for degree of variable influence after five minutes of roasting[1]

Factor	Estimate	Std. Error	t value
(Intercept)	0.190	0.084	2.26
posE	0.555	0.068	8.22
posM	0.103	0.065	1.59
speciesD.carota	0.548	0.233	2.35
speciesD.rotundata	-0.197	0.115	-1.72
speciesI.batatas	-0.326	0.117	-2.78
speciesM.esculenta	-0.131	0.089	-1.47
speciesS. tuberosum	-0.199	0.107	-1.85
posE:speciesD.carota	-0.411	0.110	-3.75
posM:speciesD.carota	-0.171	0.105	-1.63
posE:speciesD.rotundata	-0.229	0.224	-1.02
posM:speciesD.rotundata	-0.098	0.119	-0.82
posE:speciesI.batatas	0.536	0.122	4.40
posM:speciesI.batatas	0.303	0.098	3.09
posE:speciesM.esculenta	0.119	0.089	1.34
posM:speciesM.esculenta	0.263	0.084	3.11
posE:speciesS.tuberosum	0.390	0.162	2.41
posM:speciesS.tuberosum	-0.088	0.090	-0.98

[1]Base levels represented here by (Intercept) are "posC" and "speciesC.pepo".

The average percentage of stained starches across all species remains low for both C and M positions during the five minute cooking and increases rapidly at the E position (Table 5, Fig. 4). In the 20 minute cooking treatment, all species except *I. batatas* are more than 50% gelatinized in the center, with gelatinization increasing precipitously to the edge position. The additional 10 and 15 minute time points that we obtained with a second roasting trial for *I. batatas* and *S. tuberosum* (Table 5) show that gelatinization increases slowly over these time periods. For both, gelatinization is on average below 40% in C positions after 10 and 15 minutes of roasting, and remains so at the M position for *I. batatas*. In *S. tuberosum* the percent of gelatinization jumps to between 40% and 60% after 10 and 15 minutes of roasting. Again, in both species, E positions were near or fully gelatinized.

Table 5. Mean ± standard deviation of gelatinized starch fraction for the CR experiment roasting time trials.

Species	C	M	E
C. pepo			
5 min	0.377 (± 0.39)	0.412 (± 0.35)	0.891 (± 0.24)
20 min	1.0 (± 0.43)	1.0 (± 0.39)	1.0 (± 0.20)
D. carota			
5 min	0.629 (± 0.48)	0.462 (± 0.52)	0.982 (± 0.06)
20 min	1.0 (± 0.42)	1.0 (± 0.50)	1.0 (± 0.05)
D. rotundata			
5 min	0 (± 0)	0 (± 0)	0.143 (± 0.38)
20 min	0.508 (± 0.33)	0.824 (± 0.47)	0.867 (± 0.50)
I. batatas			
5 min	0.005 (± 0.02)	0.105 (± 0.27)	0.970 (± 0.11)
10 min	0.091 (± 0.17)	0.349 (± 0.36)	1.0 (± 0)
15 min	0.146 (± 0.23)	0.209 (± 0.24)	0.971 (± 0.08)
20 min	0.318 (± 0.29)	0.400 (± 0.49)	1.0 (± 0)
M. esculenta			
5 min	0.093 (± 0.15)	0.324 (± 0.25)	0.528 (± 0.47)
20 min	1.0 (± 0.42)	1.0 (± 0.37)	0.981 (± 0.08)
S. tuberosum			
5 min	0.109 (± 0.29)	0.104 (± 0.24)	0.899 (± 0.30)
10 min	0.143 (± 0.20)	0.459 (± 0.36)	1.0 (± 0)
15 min	0.376 (± 0.39)	0.635 (± 0.48)	1.0 (± 0)
20 min	0.625 (± 0)	1.0 (± 0)	0.667 (± 0.58)

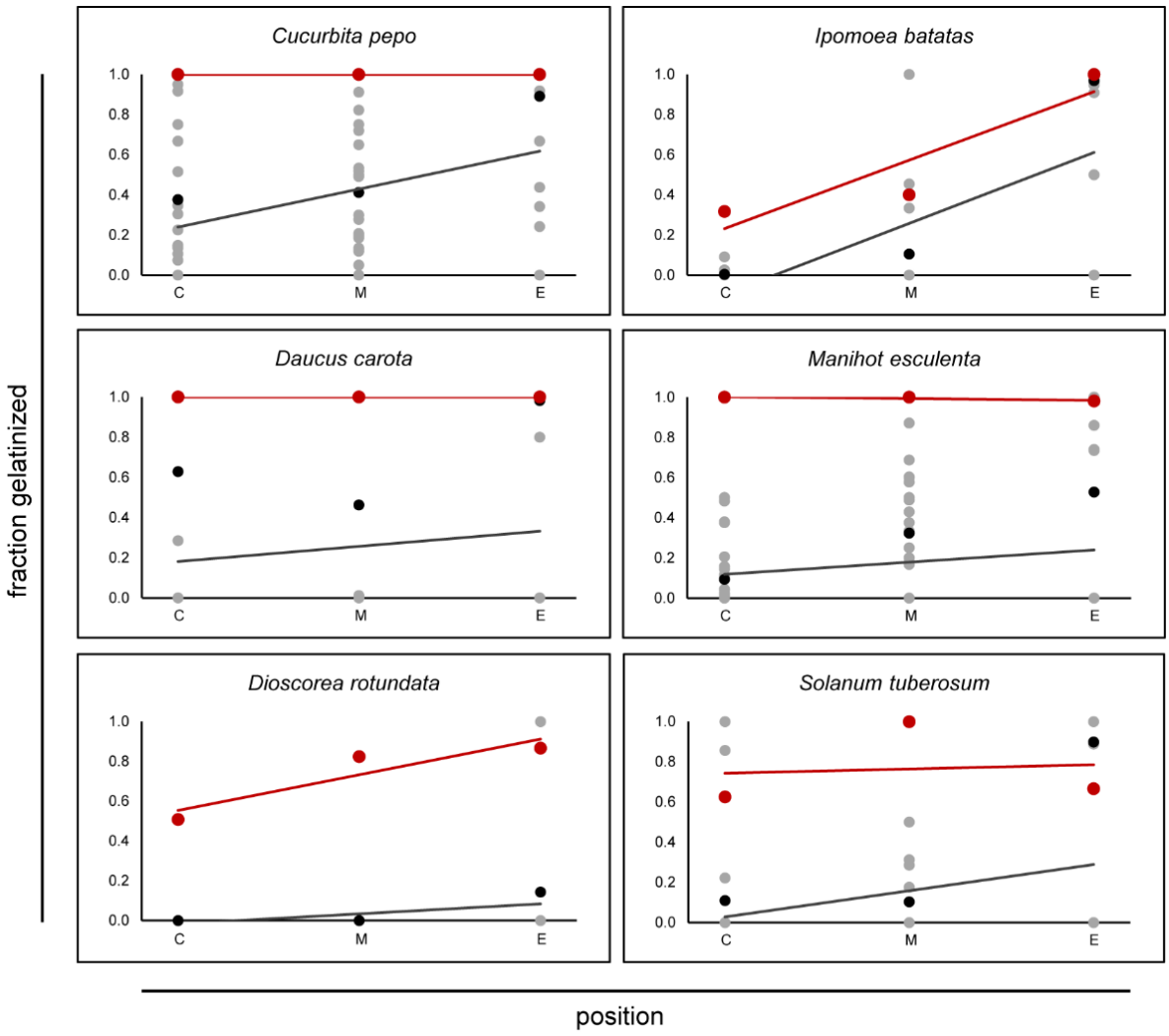

Fig. 4 Scatter plots of the observed gelatinization fraction in stained starch samples across C, M, and E positions for each species. Gray dots are individual observations for five minute roasting data, while black dots represent the mean of these observations. Red dots show the averaged fraction of gelatinization after 20 minutes of roasting. Gray and red trend lines indicate the linear response curve for both five and 20 minute roasting sessions respectively. Differences in number of data points are due to some slide observation coordinates containing no visible starch.

2.3.3 Gelatinization kinetics

The rather large standard deviation values and spread of data reported above indicate that histological staining methods are likely prone to a number of interpretive inconsistencies, such as unequal distribution of the stain across the slide, inability to distinguish gelatinized starches from other stained cellulose structures, and variation in starch susceptibility to Congo Red. Furthermore, staining and counting can easily miss starches buried in overlapping layers. Therefore, we elected to conduct a third trial using only *I. batatas* to validate our previous results in which gelatinization is inferred from a semi-quantitative and highly replicable photometric assay. The reduction in iodine binding affinity is indicative of the rapid diastase hydrolysis activity on damaged and gelatinized starch, and is our measure for gelatinization. The results of triplicate measures on triplicate specimens roasted at five, 10, 15, and 20 minutes are reported in Table 6 and Figure 5. Figure 5a shows the smoothed response curves predicting gelatinization across time-points based on the spread of observations, while Figure 5b displays the average and standard error of absorption measurements across time. Not surprisingly, the gelatinization kinetics found for *I. batatas* are in agreement with the general trend that gelatinization in the center of the specimen remains low, 26% on average, after only five minutes of roasting. Beyond that, gelatinization increases steadily to above 60% at 20 minutes. Measurements in the middle positions estimated a relatively higher level of gelatinization than did the histological staining methods, but average values held steady between 60% and 75% even after 15 minutes of roasting. We expect that perhaps the diastase activity is very efficient upon minor disturbances to the starch semi-crystalline structure, making the photometric assay much more sensitive to partial degradation of starch than CR staining.

Table 6. Percent and standard deviation of gelatinized starch from the kinetic experiment in the C and M position for all 12 specimens across all four time periods.

Time (mins)	Specimen	C	M
5			
	A	22.7 (± 1.7)	71.4 (± 4.8)
	B	23.7 (± 13.3)	77.1 (± 1.9)
	C	30.6 (± 2.2)	43.2 (± 4.4)
10			
	A	100.0 (± 0.2)	100.0 (± 0)
	B	44.4 (± 9.4)	70.4 (± 7.4)
	C	36.4 (± 3.7)	22.7 (± 14.2)
15			
	A	97.3 (± 4.8)	100.0 (± 0.8)
	B	28.9 (± 17.7)	71.1 (± 8.5)
	C	2.7 (± 1.0)	51.4 (± 2.5)
20			
	A	100.0 (± 0)	100.0 (± 0)
	B	50.9 (± 2.5)	78.9 (± 2.3)
	C	59.7 (± 5.3)	66.2 (± 2.2)

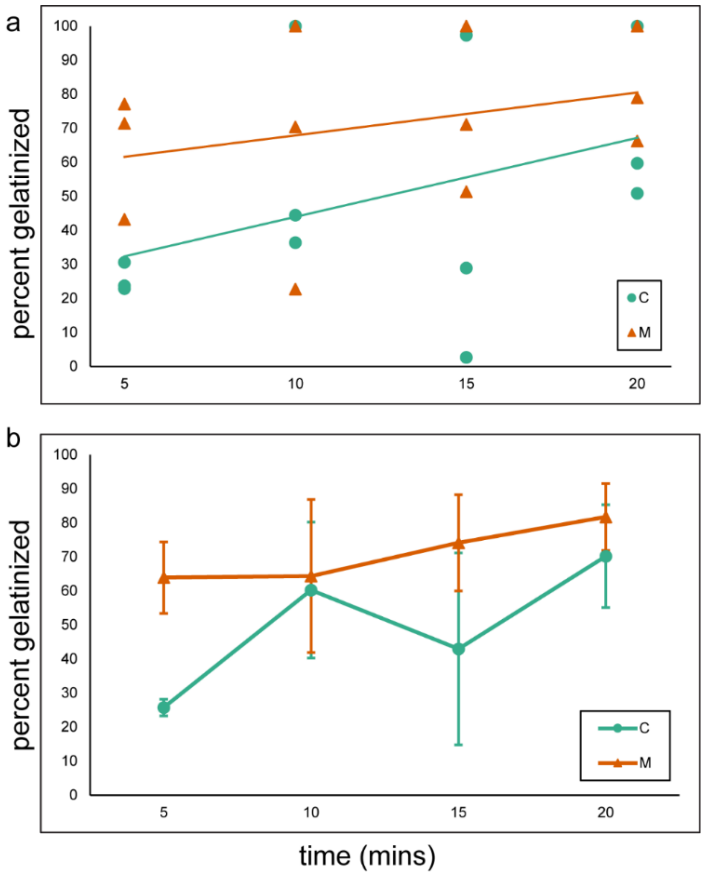

Fig. 5 Kinetics of gelatinization for *I. batatas* (experiment 3) across four time points showing the percent of gelatinization estimated by relative absorption values. (**a**) Individual gelatinization estimates for each of a, b, and c specimens at 5, 10, 15, and 20 minutes respectively. Smoothed trend lines allow an estimation of the gelatinization function by time. (**b**) Average overall gelatinization estimate and standard error per time point across all specimens by time. Green circles, C position; Orange triangles, M position.

2.4 Discussion and conclusions

Our experimental results indicate that brief five-minute fire roasting does not represent adequate cooking time to fully gelatinize native starch in five domestic USOs and one starchy fruit. Gelatinization of more than half of identified starch was on average only observed in the most exterior point sampled just below the peel, except for the center position of *D. carota*, which represents a unique case, likely due to the paucity of observable starch in this species. Gelatinization was rarely present in center (C) and middle (M) points and the red staining observed in these positions most often occurred on mildly damaged or swollen, but intact, starches. By comparison, the red stained starches in the edge (E) positions were largely gelatinized matrix. While significant differences in the gelatinization patterns did occur between species, the model as a whole indicated that the position sampled was the most significant factor in predicting gelatinization, indicating that gelatinization progresses quite slowly from exterior to the interior of the specimen. We acknowledge that this experiment should be repeated on non-cultivated starchy foods to further substantiate our claims. Recent work analyzing digestibility of raw and roasted wild tubers, however, also suggests that brief roasting has little effect on absorption of accessible glucose (Schnorr et al., 2015). Since the present experiment is a test of structural-molecular response to one particular method of thermal processing, we feel confident that our results are useful for helping to understand and predict the effect of brief roasting on any high moisture, intact, starchy organ or fruit.

Understanding the effects of different types of cooking methods on food properties is critical to our interpretations about the importance and necessity of cooking. Different toxic and nutritional properties of plant foods necessitate different processing techniques to navigate plant defense mechanisms and enable human consumption. Modern USO cultivars predominantly come from the families Dioscorea, Solanaceae, and Euphorbiaceae (*D. rotundata* [yam], *S. tuberosum* [potato], and *M. esculenta* [cassava] respectively) and often contain potent toxins such as alkaloids, saponins, glycosides, and raphides (Johns, 1996). Cooking the species of these families, then, is an important tool in the tradeoff between mitigating high toxicity and accessing concentrated starch. This is especially true for domestic varieties in which the lower allelochemical load is easily mitigated through heat treatment alone (Stahl, 1989; Johns, 1996). By comparison, wild plants often contain high amounts of oligosaccharides and phytochemicals (chemical compounds that come from plants such as carotenoids, carotenes, flavonoids, glucosinolates, and organic acids), likely because they have not been intensively selected for high starch content by human consumers (Johns, 1996; Fuller, 2007). Most plant toxins are stable at normal cooking temperatures (≥100 °C), making heating more useful for deactivating enzymes that liberate toxic compounds such as glucosinolates and cyanogenic glycosides (found in *M. esculenta*) or preventing protein activity by destroying the secondary molecular structure. Cooking, therefore, may be a less effective means for handling toxic plant compounds in wild plants. Instead, mechanical

processing or leaching are much more reliable strategies for disabling the majority of toxins found in wild USOs (Stahl, 1989; Johns, 1996).

If thermal processing is not intended to fully cook or gelatinize USO starch, then we must explore alternative desirable effects of fire treatment. Changes in mechanical properties as a result of brief roasting, proposed in Dominy et al. (2008), provide a salient alternative explanation for brief roasting. In particular, brief roasting can expedite removal of bark-like cortical tissue as well as reduce the fracture toughness of the interior parenchymatous tissue of certain wild tubers. In both cases, roasting facilitates improvements in mechanical processing, either from peeling or chewing, and may enable easier access and more thorough mastication (Dominy et al., 2008; Zink et al., 2014). Whether this confers a significant increase in calories alone is an interesting, yet still speculative, proposal. In fact, work by Wollstonecroft (2008) on the effect of thermal and mechanical processing on edibility of sea club-rush (*Bolboschoenus maritimus*) showed that when the plant cell walls and cell-cell adhesion are too strong, thermal treatment does not promote tissue softening despite gelatinizing interior starch. Instead, mechanical processing such as pounding or grinding are necessary to disrupt cell wall structure to soften the tissue and expose interior starch (Wollstonecroft et al., 2008). Therefore, fracturing, caused by tissue rupture (see Fig. 6), allows access to interior starch. Tissue fracture is instead a factor of cell wall structural integrity rather than starch gelatinization (Alvarez and Canet, 2001), and can be a greater factor to nutritional acquisition than gelatinization (Wollstonecroft et al., 2008; Tydeman et al., 2010). Since some plant tissue structures degrade from heat, then cooking can facilitate both access to cellular contents and an increase in their digestibility, but if cell-cell adhesion is weak, then tissue separation can occur before fracture (shown in Fig. 6), and severely limit nutritional acquisition (Tydeman et al., 2010).

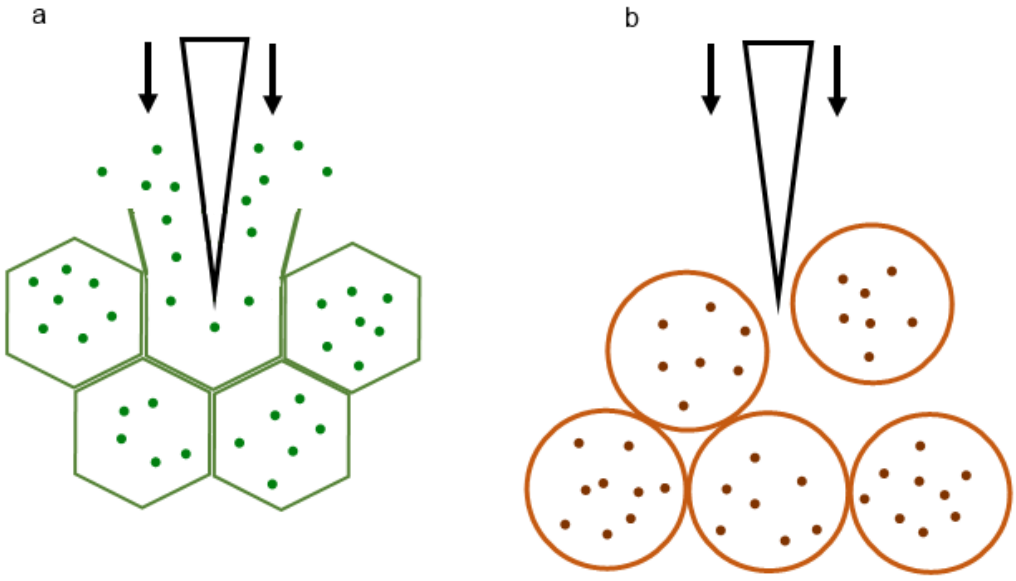

Fig. 6 Diagram representing cell rupture (**a**) resulting in tissue fracture where intercellular content is exposed, and (**b**) cell separation as a result of weak cell-cell adhesion where intercellular contents remain inaccessible, after Tydeman et al. 2010.

In the case of many of the tropical USOs, maximizing nutritional gain may be contingent upon brief roasting such that just enough internal turgor pressure exists to allow tissue fracture while the underlying cortical tissue softens just enough to separate the periderm and expedite consumption. Furthermore, the impact of cooking on other nutritional factors, such as water and micronutrients, is variable depending on the type of heat treatment and food type. Research suggests that cooking may actually be detrimental to vitamin retention and accessibility (Watson, 1976; Reddy et al., 1999; Mandalari et al., 2008; Wollstonecroft et al., 2008; Tydeman et al., 2010). Therefore, calorie content in plant foods may be ancillary to the selective acquisition of other vital nutrients such as carotenoids, ascorbic acid, and especially water (Silberbauer, 1981; Rothman et al., 2011; Jensen et al., 2012).

Brief fire roasting may enhance the culinary experience of foods in a number of ways that are not directly related to increasing caloric nutrition. Roasting may safeguard against pathogenic microorganisms, aid supplementation of particular minerals derived from wood ash (e.g. iron and calcium (Greenhouse, 1981)), or simply ease consumption and increase palatability, becoming a quality of life improvement. Since starchy plants are plentiful and reliable resources in a savanna-mosaic environment (Vincent, 1985), it would have been beneficial for human ancestors living in such regions to adopt strategies, either biological or technological, to enable plant food consumption. In light of our findings, the exploitation of starchy plant resources in and of itself, rather than cooking, may have been the answer to resource related selective pressures in environments occupied by early hominins (O'Connell et al., 1999). However, we recognize that cooking serves different functions in different environments, and it is necessary to model context-dependent variants of cooking technology to empirically test their value for human consumers in that particular environment.

The influence of cooking on starch food is well reported in the food science literature (Collison and Chilton, 1974; Valetudie et al., 1995; Hoover, 2001; Richardson et al., 2004; Stevenson et al., 2005; Nimsung et al., 2007; Sajeev et al., 2008; Singh et al., 2010; Nguyen Do Trong et al., 2011; Parada and Aguilera, 2011), however such research is inadequate for evolutionary-based hypothesis testing regarding the dietary ecology of human ancestors. Research models are needed that contextualize plant food cooking techniques with the archaeological evidence of early human and hominin technologies. We believe that a simple fire-roasting technique, which is regularly used by extant hunter-gatherers to cook USOs, is an appropriate analogy. Future experiments using wild plants are needed to improve our understanding of starch gelatinization properties under varied relevant thermal processing conditions. Such models can be powerful informants to how and why human ancestors experienced dietary diversification, especially with regards to plant foods, and exactly how cooking may have significantly influenced human evolution.

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