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**Title:** Relief Food Subsistence Revealed by Microparticle and Proteomic Analyses of Dental Calculus From Victims of the Great Irish Famine

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**Author contributions:** J.Geber designed research. M.T., A.S., and J.H. performed experiments; J.Geber, M.T., A.S., C.W. analyzed the data; P.N., and J.Grossmann provided analytical support; A.B. and C.W. contributed material resources; J.Geber wrote the paper with input from the other coauthors.

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**Data deposition:** The protein spectra have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository (accession no. **XXXXXX**).

**Abstract:**

Food and diet were class markers in nineteenth-century Ireland, which became evident as nearly one million people, primarily the poor and destitute, died as a consequence of the notorious Great Famine of 1845–52. Famine took hold after a blight (*Phytophthora infestans*) destroyed the virtually only means of subsistence—the potato crop—for a significant proportion of the population. This study seeks to elucidate the variability of diet in mid-nineteenth-century Ireland through microparticle and proteomic analysis of human dental calculus samples ( $n = 42$ ) from victims of the Famine. The samples derive from remains of people who died between August 1847 and March 1851 while receiving poor relief as inmates in the union workhouse in the city of Kilkenny (52°39'N, -7°15'W). The results corroborate the historical accounts of food provisions before and during the Famine, with evidence of corn (maize), potato and cereal starch granules from the microparticle analysis and milk protein from the proteomic analysis. Unexpectedly, there is also evidence of egg protein—a food source generally reserved only for export and the better-off social classes—which highlights the variability of the pre-Famine experience for those who died. Through historical contextualisation, this study shows how the notoriously monotonous potato diet of the poor was opportunistically supplemented by other foodstuffs. While the Great Irish Famine was one of the worst subsistence crises in history, it was foremost a social disaster induced by the lack of access to food and not the lack of food availability.

**Keywords:** bioarchaeology; paleoethnobotany; microfossil; potato; poverty

**Significance:**

This study is the first to provide direct evidence of the dependency on relief food in Ireland around the time of the Great Famine (1845–52), through dental calculus analysis of archaeological human remains. The findings show a dominance of corn (maize) and milk from the identified foodstuffs and corroborate the contemporaneous historical accounts of diet and subsistence. It shows that microparticle and proteomic analyses, even when based on small archaeological samples, can provide a valid snapshot of dietary patterns and food consumption. The occurrence of egg protein, generally only included in the diet for the better-off social classes, also highlights how these analytical techniques can provide new and unanticipated insights into the variability of diet in historical populations.

Ireland in the nineteenth century was characterised by political turbulence, economic decline and drastic social and demographic changes (1). Poverty and destitution were widespread, and a highly stratified society denoted social class from a variety of factors, including diet. The potato (*Solanum tuberosum*), and especially the Irish lumper cultivar, was a staple for the poor and destitute (2). For numerous reasons, by the 1840s approximately 40% of the population of Ireland had become utterly dependent on the potato for subsistence. The dependency on a single food source exposed people to significant risks and culminated in a devastating famine between 1845 and 1852 when a fungal blight (*Phytophthora infestans*) caused widespread destruction of the potato crop (Figure 1). Nearly one million people died as a direct consequence of famine-induced starvation and disease. An estimated 200,000 of these famine-related deaths took place in the union workhouses (3), which were institutions for poverty relief that were introduced to Ireland following the Irish Poor Law Act of 1838 (4). When the potato harvest failed, vast quantities of food were imported as poor relief (5). This food comprised primarily of oatmeal from Britain and, so-called, “Indian meal” (maize) from the United States. The latter was to become an even more distinct social marker of poverty than the potato.

While the historical records of Victorian-period Ireland are vast, they generally do not reflect on the variability of the labouring classes’ diet on an individual level. The aim and objectives of this study are to elucidate aspects of food availability and consumption patterns amongst the socially marginalised and impoverished in mid-nineteenth-century Ireland through microparticle and proteomic analyses of dental calculus. The calculus derives from a subsample ( $n = 42$ ) of archaeological skeletons of individuals ( $n = 970$ ) who died while receiving poor relief in the union workhouse in Kilkenny City (Figure S1) between August 1847 and March 1851. The precise date of the remains—and the abundant historical resources pertaining to this period—enables a unique opportunity to use dietary analysis of dental calculus to evaluate both the historical and bioarchaeological sources and provide a holistic view of the condition of the mid-nineteenth-century poor and destitute.

## Results

**Microparticles.** Microparticle analysis was conducted on all samples, of which 41 contained a total of 383 starch granules ( $\geq 1$  granule per sample), most of which were identified to six probable taxa (Table 1). Any granules measuring less than 10  $\mu\text{m}$  were classified as unknown due to the difficulty in clearly distinguishing diagnostic features at this scale using light microscopy. The most dominant plant species identified was likely corn (*Zea mays*), which amounted to a total of 164 starch granules present in 38 samples (92.7%). There were 41 starch granules identified as probable oat (*Avena* spp.) present in 22 samples (53.7%). Probable wheat (*Triticum* spp.) granules ( $n = 21$ ) were identified in 16 samples (39.0%). Five granules were identified as potato (*Solanum tuberosum*) and five as probable malted barley (*Hordeum* spp.) (Figure 2).

In addition to the identified taxa described above, there were seven granules in six samples (14.6%) that derive from unidentified cereals (Poaceae), and there were a total of 140 granules across 37 samples (90.2%) that are of unknown origin, less than 10  $\mu\text{m}$  or displayed non-diagnostic features. Of these unidentified granules, the majority are likely to derive from Poaceae starch. Also included amongst these are damaged starch microparticles (in all cases, they were not damaged to the point of losing visible extinction crosses). While it is well known that heat damages starch granules, studies have shown that it is still possible for some granules to survive unless the foods were boiled for more than 60 minutes (6-8), and these damaged granules may show the effects of cooking and food processing (6, 7).

Other findings include fungal material, hair, fibres, insect parts and other unidentified plant and organic material (Dataset S1). Some of the unidentified organic material may be chaff from unprocessed or poorly processed cereals, and possibly reflects food adulteration. It may also be microparticles that derive from non-dietary sources (9), such as from beddings that for the poor comprised of nothing more than chaff and straw both before and during institutionalisation (10). The hair and fibres were present in nine samples, with a notably high concentration in dental calculus from a 26–35-year-old female (CCCXXI).

**Proteomics.** Dental calculus was extracted from 14 individuals, which were selected based on having deposits of dental calculus  $\geq 1$  mg for potential analysis. Seven samples contained animal (milk or egg) proteins (Figure S4), and six samples contained glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a ubiquitous cellular protein, of probable plant origin (Table 2). Modifications expected for ancient/historical samples, such as deamidation (N, Q), were observed (Dataset S2). Peptides from the milk whey protein beta-lactoglobulin (BLG) were positively identified in seven of the samples, originating from five males and two females. Taxonomically diagnostic sequence variants in the BLG peptides confirmed the specific consumption of cattle (*Bos*;  $n = 5$ ) and goat (*Capra*;  $n = 1$ ) milk, and three individuals consumed bovid milk of ambiguous origin (cattle/sheep or sheep/goat). Cattle milk appears to have been the dominant milk source, and it is likely that all seven individuals consumed cattle milk. Peptides from the egg protein ovalbumin, originating from domestic chicken (*Gallus*), were also identified in two males and one female, and each of these individuals also consumed milk. The cellular protein GAPDH has been previously found to be the most frequently recovered plant protein in archaeological dental calculus (11), and GAPDH peptides of probable plant origin were identified in six individuals (three males, three females). Unfortunately, the peptide sequences are highly conserved among green plants (Viridiplantae), as well as a small number of bacterial species, and are therefore not specific enough to identify individual taxa.

## Discussion

While the potato was the staple diet for the poor throughout Ireland, there were regional differences regarding agricultural and horticultural produce and dietary patterns across the island. In the west, where the social conditions were most deprived, oatmeal was rarely a dietary component while the opposite was true in the north-east. Bread was mostly consumed in the east and milk was a common component in the diet in the south-west, south-east and the north (12). Local agriculture in Kilkenny was dominated by grain and cattle production, one of the foundations of the relative prosperity of the city (13). Other than cereals and vegetables, markets were well-supplied with sea fish, meat, and reportedly even exotic fruits such as peaches, melons, grapes and pineapple (14). Much of the agricultural produce, however, was destined for the export market, and the diet of the average labourer in Kilkenny would corroborate what was seen elsewhere in Ireland. An account from the early 1840s stated that occupants on even sizable County Kilkenny farms of 70 to 100 acres would generally only have “potatoes and milk, stirabout<sup>1</sup>, or oaten-bread and milk for breakfast and supper, and generally potatoes and butter, but once or twice herrings or butcher-meat, for dinner” (15).

In accordance with the Poor Law, the composition of the Kilkenny Union Workhouse diet (Table S1) was intentionally dire. Before 1846, potatoes were a staple and supplemented with milk rations that would have reflected the diet for many of the inmates before admission into the institution. During the Famine, potatoes were chiefly replaced with Indian meal. In the latter years of the Famine, a vegetable soup was introduced as part of the adult food rations, although at rations of only half a pint for dinner. This soup consisted of five ounces of rice, five ounces of oatmeal, 12 lbs of turnips (swedes), 2 lbs of parsnips, and onions with pepper and salt as required per gallon (16). Some meat was provided as part of the medical treatment in the workhouse infirmary (17), and for some periods the minute books reveal the quantities of meat stored in the house. In the last week of February 1851, for instance, 390 lbs of meat were recorded in the food inventory. On those dates, the same records show that the union was providing indoor relief for 4,282 people! Other than flavour enhancers such as pepper and allspice, the minute books also reveal other food products such as arrowroot, sugar, broth, cocoa, port and sherry wine, whiskey, and porter. These are likely, just as the meat, to have been provisioned only for inmates treated in the workhouse infirmary.

**Historical contextualisation of results.** The microparticle and proteomic analyses of the dental calculus samples exhibit a dominance of foodstuffs that are consistent with the nineteenth-century Irish labourer’s diet and relief food consumed during the Famine, suggesting that the calculus build-up in the analysed sub-sample reflects tartar accumulations prior to and/or during life in the workhouse. This

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<sup>1</sup> porridge made from either oats or Indian meal or both.

notion is primarily argued from the dominance of corn deriving from Indian meal, which was a central component of the workhouse diet during the Famine, although it had also occasionally been distributed as poor relief in County Kilkenny on previous occasions (18). Stable isotope analyses conducted to date on a sub-sample of the skeletal remains have also indicated a C<sub>4</sub> dietary input (maize), which further reflects the relief food dependency for the poor before and during the Famine (19). The evidence of oat starch granules in the calculus samples could reflect both the relief food and/or the pre-Famine diet of these individuals.

The high occurrence of identifiable corn starch granules may also reflect the documented difficulty in processing the grain; Irish millers generally lacked the knowledge of how to sufficiently grind Indian corn and the coarse grind may have allowed for the starch granules to survive the boiling process. The fact that the starch granules from corn dominate the samples also highlights how the experience of the Famine was a prolonged period of suffering and struggle and that death was not instant. While Indian meal would have helped to combat hunger, it may ultimately also have caused further health deprivations. The corn was profoundly inferior in its nutritional value as it, at the time, was improperly prepared with a devastating effect on the health of the already weakened and frail. This was largely the result of a European rejection of adopting the traditional indigenous American method of preparing maize through the process of nixtamalization (alkaline cooking), which releases niacin from the grains (20). As a consequence, niacin deficiency—resulting in pellagra—is likely to have been an inadvertent consequence of the relief food distributed to the starving poor in Ireland during the Famine (21).

As stated above, the workhouse dietary included a soup but its vegetable ingredients were not identified by protein or microscopic analysis in the current study. This is likely due to the quick gelatinisation process of vegetable starches (tubers and grains) during cooking (22, 23) as well as incomplete proteomic reference databases and the lack of identifiable microparticle production of some plants (onions, swedes). Starch granules from rice (*Oryza sativa*), another ingredient in the soup, could potentially be identified from microparticle analysis as they gelatinise at much higher temperatures and have a higher lipid content (23). However, these granules would measure less than 10 µm in size (23) and so would all be present amongst the unidentifiable microparticles found in this study. The absence of any grass/grain phytoliths in the dental calculus suggests that the plant foods people ate were processed and not wild. Phytoliths are generally found in the husks and skin of grains, and in pre-Industrial archaeological dental calculus—where processing was minimal or done by hand—phytoliths are commonly found (24, 25).

The gelatinisation of starch granules during cooking may also explain the low frequency of potato and the high frequency of corn starch granules in the calculus samples, even though—naturally, in the context of this study—this may also reflect food availability. The gelatinisation process of potato starch begins around a temperature of c. 30°C and so boiling, which was the preferred method for cooking potatoes in nineteenth-century Ireland, would have completely gelatinized the potato starch granules (26). Corn, on the other hand, has a much higher gelatinisation temperature (27). The fact that some potato starch granules were nevertheless identified in a limited number of samples may reflect the way the poor prepared the vegetable:

“The cabin-boiled potato was dressed in two ways: with and without the *bone* or the *moon*, as it is universally called by the genuine Irish. In the latter form, the potato was done to the heart, equally mealy throughout, and bursting its skin with fatness. This was the supper when children and young persons were to partake of the meal; but when much work was to be done, or a long fast to be endured, the heart or central nucleus of the potato was allowed, by checking the boil at a particular period, to remain parboiled, hard and waxy; and when the rest of the potato had been masticated in the usual manner, this hard lump, about the size of a small walnut, was bolted; and in this manner nearly a stone of the root was taken into the stomach of the Irish labourer per diem.” (28)

The presence of the milk protein beta-lactoglobulin in the calculus of half of the tested individuals supports the historical records of milk being an important liquid component of the poor labourer’s diet. Somewhat surprising is that only one instance of beta-lactoglobulin from goat (*Capra*) and a second

from sheep/goat (*Caprinae*) was identified, given that the goat—generally referred to as the “poor man’s cow”—played a particularly important role in the Irish domestic economy due to, what was said, the “impoverished state of the country” (29). However, additional trypsin cut sites in caprine beta-lactoglobulin may lead to smaller peptides that are less likely to be identified through LC-MS/MS analysis; thus, an absence of these peptides does not necessarily indicate that goat milk was not widely consumed. If indeed the specific identification of cattle milk as opposed to other ruminant taxa is accurate, the fact that milk from cattle is dominating the identifications may also suggest that it derives primarily from the workhouse diet, as milk was purchased by the Kilkenny Union at large quantities every week from a limited number of suppliers, and this is likely to indicate it derived from a larger and more commercial production set-up.

**Diet variability and food access during the time of the Great Irish Famine.** The shame associated with poor relief in the form of food is mentioned in local folklore that tells of how receiving stirabout made from Indian meal was gravely humiliating for a great number of people, who prior to the blight had considered themselves as “respectable farmers” (30). For many others, however, the desperate struggle for subsistence—and the associated perceived stigma—had been a constant reality of their existence long before the Famine. A German visitor to Ireland gave one particularly evocative account from Kilkenny City where he arrived by coach in August 1837:

“At Kilkenny there is an old castle, and innumerable beggars. [...] The coach is besieged by them, and their cries resounds from all sides, and in all gradations of old and young voices. [...] I saw a mother pick up the gooseberry skins which one of the travellers had spit out, and put them into the mouth of her child. I never [before] saw any thing like this [...]” (31)

For obvious reasons, the situation for the poor and destitute had declined gravely a decade later, and folklore tells of how people in Kilkenny, just as elsewhere in Ireland, had resorted to eating grass and weeds during the Famine (32). Local accounts also state that while the poor and the labouring classes suffered greatly, the fertile lands in the county generated good crops of wheat during the years of the potato blight, but that this food was bought up by the local gentry who made profits by exporting it for sale at English markets (33). Other recorded narratives, on the other hand, tell of how locally produced oats and wheat had, indeed, been distributed in County Kilkenny as famine relief food (34).

While major aspects of the relief food provided by the Kilkenny Union is evidenced through this study, there were also dietary components indicated from the microparticle and proteomic analyses that show new aspects of the variability of the labourer’s diet in nineteenth-century Ireland. Proteomic analysis identified the egg protein ovalbumin in the dental calculus of three individuals. Eggs were regarded as “luxury food” for the working classes (12) and thereby also a dietary class marker. Although it was not uncommon for the peasantry to keep poultry, eggs were not eaten except for, perhaps, during celebratory occasions. Eggs were, just as much as the wheat crop, destined for the export market, or as one Irish labourer in the 1830s stated: “We eat none of them; they go to our rent, or to put a shoe on our foot, or a spade in our hands” (35).

The evidence of egg protein through this study is highlighting how the pre-Famine experience and living conditions might have varied for those people who were forced to resort to the Irish workhouses in the 1840s. The evidence of probable wheat in the dental calculus of some individuals from the Kilkenny Union Workhouse can also be viewed in the same light, although it is possible that the wheat derives from the occasional bread that was part of the workhouse diet or from an as yet unidentified local wild grass. Further references to the crop in Famine-period records from Kilkenny tell of how wheat was being “crop-lifted” by desperate people (36), and there were also several reports of “bread riots” occurring in the city during the Famine (37).

## **Conclusion**

The social, political and economic historical background of the Great Irish Famine is very complex, and the term “The Irish Potato Famine” (generally never used in Ireland) is problematic for that reason.

While the Famine was a subsistence crisis initiated by the destruction of the potato crop blight, it was much more than a natural disaster relating to a single vegetable. Prevailing *laissez-faire* economic convictions profoundly influenced the political response to the food crisis that occurred, and vast amounts of food products were exported from Ireland while the poor starved (38). There was also an undoubtedly colonial dimension to these economic policies where Ireland and its primarily Catholic population—which following the Act of Union in 1801 was governed directly by a British government from Westminster—were subjugated through various political means (39).

As made evident through this study, scientific evaluations using microparticle and proteomic analytical techniques of archaeological dental calculus can provide new and complimentary snapshots into overall diets even from exceptionally well-contextualised and well-recorded historical samples. The current study suggests that diet variability of the poor was limited and expressed equally across both sexes amongst the poor in Ireland during the time of the Great Famine. When interpreting the findings in their historical and cultural context, the evidence of eggs (and probably also wheat) also indicates how food subsistence pattern for the labouring classes was opportunistic. Contemporary nineteenth-century accounts would state that the potato was the food of choice for the poor (40), but this was a truth with modification. When commenting on whether the peasantry preferred potatoes over meal or bread, one labourer from the west of Ireland during the 1840s replied: “Why would we prefer that, that we feed our pigs on to better food? Don’t you like it better yourself, and why shouldn’t we? Never believe them that would want to make you think that we’d eat wet lumpers if we could get good bread” (41). This notion is further indicated from this study, which has provided new nuanced insights into how socially marginalised people sought to maintain their subsistence while enduring a life of constant hardship and unimaginable struggles.

## Material and Methods

A mass burial ground at the was excavated adjacent to the former Kilkenny Union Workhouse, Kilkenny City, Ireland, in 2006. A minimum of 970 individuals, from 63 burial pits, were recovered. The skeletal remains were analysed following standard osteological methods. Dental calculus was sampled from individual teeth extracted from a subsample of 42 skeletons prior to the reburial in 2010 (see *SI Appendix* for details).

Microparticles were extracted from 42 dental calculus samples (on average approximately 3×3 mm) using the EDTA extraction method described in Tromp et al. 2017 (42) and briefly reviewed in the *SI Appendix*. Starch granules were analysed primarily using published studies and descriptions (23, 43–49) as well as a reference collection; measurements and descriptions of relevant taxa are included in Dataset S3. Identifications were made based on the size (maximum width), shape and distinguishing features, such as visible lamellae, surface pitting, pressure facets, and visible and/or cracked hilum (for further details, see *SI Appendix* and Datasets S3; S5).

Proteins were extracted from decalcified dental calculus samples according to guidelines recommended by Hendy et al. (50). Extracted peptides were analysed by LC-MS/MS using a Q-Exactive HF mass spectrometer (Thermo Scientific, Bremen, Germany) coupled to an ACQUITY UPLC M-Class system (Waters AG, Baden-Dättwil, Switzerland) (see *SI Appendix* for further details). Tandem mass spectra were converted to Mascot generic files by MSConvert version 3.0.11781 with the 100 most intense peaks in each spectrum. All MS/MS samples were analysed using Mascot (Matrix Science, London, UK; version 2.6.0). Mascot was set up to search the databases SwissProt\_2017\_07.fasta and uniprot\_trembl\_2017\_07 database assuming the digestion enzyme trypsin and selecting the automatic decoy option (see *SI Appendix* for further details). Scaffold (version Scaffold\_4.8.9), Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications at a local FDR of less than 1.0% (Scaffold Local FDR algorithm) and protein identifications at an FDR of less than 5.0% and supported by at least three identified peptides were accepted (see *SI Appendix* for further details). Raw data files are available through the ProteomeXchange Consortium via the PRIDE partner repository (accession no. XXXXXX).



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## Figure captions

Figure 1. 'Destitution in Ireland. – Failure of the potato crop', illustration published in *The Pictorial Times*, 22 August 1846 (51).

Figure 2. Examples of starches observed in the microparticle analysis: (a–c) Corn (*Zea mays*); (d–e) Malted barley (*Hordeum* spp.); (f–h) Wheat (*Triticum* spp.); (i–j) Oat (*Avena* spp.); (k–l) Potato (*Solanum tuberosum*).