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Dental calculus reveals diet habits and medicinal plant use in the Early Medieval Italian population of Colonna

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ABSTRACT

The community of Colonna (Rome, Central Italy), dated back to 8th–10th century CE, was characterized by a poor quality of life. In the present archaeobotanical investigation, we obtained a detailed qualitative reconstruction of the food habits of this Medieval population. To do it, light microscopy and GC–MS techniques were applied on the dental calculus of specimens exhumed from the cemetery of Colonna archaeological site, an approach never performed before on Italian Medieval samples. The identification of starch grains and other plant microfossils (e.g. pollen, phytoliths) showed a nutritional plan coherent with coeval poor classes, consisting of both C_3 (e.g. *Triticum* sp.) and C_4 (e.g. *Sorghum bicolor*) plant species, with a particular intake of acorns (Fagaceae) and legumes (Fabaceae) rich in proteins. Chromatographic analysis revealed the use of a large variety of foods: oil-rich seeds and fruits, aquatic resources (e.g. fish, molluscs, algae), animal derivatives (e.g. eggs, meat, milk, dairy products), Brassicaceae, Rosaceae fruits, aromatic herbs (Lamiaceae), mushrooms, wine and tea. In addition, specific markers of medicinal plants (e.g. *Digitalis* sp., *Hyssopus officinalis*, *Artemisia* sp., *Ephedra* sp.) evidenced the pharmaceutical culture of this historical period. Finally, the present work also amplified the knowledge about past environment, trade, plant uses and customs.

1. Introduction

The Middle Ages represents a long historical period characterized by deep political, religious, socio-economic and cultural changes which strongly influenced diet habits.

The current knowledge of Medieval foods essentially derives from ancient recipe books; however, such information is not fully exhaustive and should be integrated with other data reported in documents about breeding techniques, sale acts of lands, trades and archaeo-anthropological investigations.

In Medieval Italy, although several social realities existed, the common diet was mainly based on cereals and pulses, which were used to prepare breads or pottages. Sometimes, these foods were supplemented by other vegetables and, more rarely, by meat or fish, especially in wealthy classes. Meat was available to workers and peasantry but it was surely consumed in smaller quantities and lesser quality cuts than nobility (Giovannini, 1994; Montanari, 1979, 1988; Salvadori, 2003). Different cooking modalities were applied to meat; however, it was well known that boiling made this food sterile and much more digestible (Tuliani, 2001).

Milk and eggs were the most important protein sources for poor classes. In addition, dairy products were commonly employed and salted herring and some molluscs, being relatively cheap, represented accessible marine foodstuffs (Müldner and Richards, 2005). Other ordinary nourishments were collected from woods and forests, such as acorns, chestnuts, roots, mushrooms and wild fruits (Montanari and Brombert, 2015). Finally, wine consumption was predominant, not only for its energizing effect but also because water was often undrinkable and unhealthy (Piccinni, 2002).

Medieval daily life and even eating habits were subjected to the rigid rules that Church imposed on believers; it forced to eat fish instead of meat, use vegetable fats rather than animal ones and replace animal milk with almond juice, some days of the week and during the whole period of Lent (Montanari, 1988; Salamon et al., 2008; Tucci, 1985).

Detection and taxonomic identification of plant remains in ancient sites provide important ethnobotanical data. However, recently, several research groups demonstrated that the best way to study past diet is the direct analysis of the dental calculus found on human mandibular and maxillary rests (Cummings et al., 2016; Hardy et al., 2012; Henry et al., 2011; Radini et al., 2016). Indeed, mouth and teeth are the first organs

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to arrive in contact with food and the dental calculus, being a dense mineral deposit made up by inorganic salts and crystallized residues of oral microbiota, easily entraps in its matrix the organic molecules deriving from diet (Lindhe, 1984). Therefore, the dental calculus, persisting to archaeological contexts (Lieverse, 1999), represents a collection of compounds which are indisputable proofs of foods ingested during lifetime.

According to all this evidence, the main purpose of the present work was the determination, in qualitative terms, of ethnobotanical customs relative to a Medieval population who lived in Central Italy during the 8th–10th century CE. In particular, the investigations were carried out analysing the dental calculus of human remains found in the necropolis of Colonna (Rome, Latium). The original aspect of our research was the combined application of Light Microscopy (henceforth LM) and Gas-Chromatography Mass Spectrometry (henceforth GC–MS) on the ancient samples, approaches always performed separately in literature (Buckley et al., 2014; Cristiani et al., 2016; Cummings et al., 2016; Hardy et al., 2012; Henry et al., 2011). In conclusion, our study demonstrated a predominant usage of both Mediterranean and exotic plant species, as food and medicinal sources, in the Italian Early Medieval culture.

2. Materials and methods

2.1. Sample collection

Human remains found in the Medieval cemetery of the archaeological site of Colonna (Rome, Italy) were provided by the laboratory of Skeletal Biology and Forensic Anthropology (Dept. of Biology, University of Rome 'Tor Vergata') which performed the anthropological analyses of these samples (Baldoni et al., 2016). A total of 47 individuals, 34 adults and 13 sub-adults, were examined. Information about their estimated sex and age at death were obtained by Baldoni et al. (2016) and reported in Table 1. Between 1 and 30 mg of dark supragingival calculus per sample were collected and stored, at 4 °C, into Eppendorf tubes, until their analysis.

2.2. Sterilization and decontamination procedures

To decontaminate the whole working area from airborne modern plant material, detergents (starch-free soap), 5% sodium hypochlorite and UV light were employed to treat all surfaces, instruments, supplies and tools. First of all, fragments of masticatory apparatus were gently dry-brushed with a single-use toothbrush. After dental calculus collection, in order to eliminate any past and modern environmental contaminants from calculus surface, each sample was UV-treated for 10 min on each side, soaked in 5% sodium hypochlorite for 15 min, washed twice in sterile bidistilled water (40 °C), rinsed in 100% ethanol, to remove the aqueous component, and let it dry, at 37 °C, in a new sterile Eppendorf tube. All these steps were obtained by joining several literature guidelines (Cristiani et al., 2016; Crowther, 2012; Cummings et al., 2016; Gismondi et al., 2016; Hardy et al., 2016; Henry et al., 2014; Weyrich et al., 2017). In addition, all procedures were carried out under a sterile vertical laminar flow hood (Heraeus HERAsafe HS12 Type). To validate the sterilization and decontamination protocols, five dental calculus were randomly selected and subjected to the following analysis. In particular, samples were washed with 200 μL of water which was partially (100 μL) observed by light microscopy. Only rare plant microremains, such as fungal spores, were detected. Then, the same samples were treated according to our previous cleaning protocols and washed again with 200 μL of water. In this last case, the microscopic investigation of the washing solution did never show any plant microremains, demonstrating that these procedures were necessary to remove all external impurities. Similarly, these controls were also performed by GC–MS. Indeed, the remaining 100 μL of both washings were completely dryed out, resuspended in 100% hexane and treated and analysed as reported in the relative paragraph (GC–MS analysis). No organic molecule was revealed after the decontamination.

Table 1
Biological profile of the Medieval specimens. All individuals exhumed from Colonna necropolis were associated to a code, reporting tomb (T) and stratigraphic unit (US) number, according to Baldoni et al. (2016). For each specimen, estimated sex (F, female; M, male; NDA, adults with not determined sex; NDS, sub-adults with not determined sex because of their sexual immaturity), estimated age at death (GA, generic adult individual; GS, generic sub-adult individual) and district of the masticatory apparatus used for dental calculus sampling were reported. In particular, sampled teeth were codified according to Universal Teeth Numbering System (American Dental Association, 1999).

Samples	Sex	Age (years)	Calculus location	
			Teeth	Surface
4	F	36–50	11,12,26,27	Lingual
6 US 63	NDS	2–3	S,T	Buccal
8 US 64	F	36–50	24,25	Lingual
12 US 74	NDS	5–8	S	Buccal
13 US 73	NDS	3–9	K	Lingual
14 US 75	M	36–50	19	Buccal
18 US 79	M	36–50	3,4,25,26	Lingual
19 US 81	M	18–35	20,21,22	Lingual
22 US 84	F	36–50	14,21,22	Buccal
25 US 87	F	18–35	23,24,25,26	Lingual
26 US 88 1A	F	30–35	7,8	Lingual
27 US 89	F	18–35	14,26	Buccal
30 US 95	F	36–50	2,3,19	Buccal and lingual
31 US 96	F	18–35	2,17,18,19	Buccal
32 US 97 1A	M	20–25	24,25	Lingual
33 US 98	NDA	18–25	2,5,11,17,18,19,22	Buccal
37 US 104	M	23–25	13,24,25	Lingual
39 US 106 A	M	15–18	4,10,30,31	Buccal and lingual
40 US 107 A	NDA	33–55	31,32	Buccal and lingual
40 US 107 B	NDA	GA	16,32	Buccal and lingual
42 US 109	NDS	GS	M,30	Lingual
44 US 114	NDS	12	14,27	Lingual
48 US 124	F	18–35	2,30,31	Buccal
49 US 125	M	35–50	13,14,25,26	Lingual
51 US 127	M	36–50	4,5,19	Buccal
52 US 134	NDS	10–12	8,25	Lingual
53 US 135	F	36–50	6,7,27	Lingual
54 US 140	NDS	13–16	3	Buccal
57 US 152	NDS	5–7	L,T	Buccal
63 US 185	F	36–50	19	Buccal and lingual
65 US 186	F	36–50	5,7,21,22	Lingual
67 US 188	NDS	14–16	7,8,9,30,31	Buccal and lingual
68 US 200	NDS	GS	19,20	Buccal and lingual
69 US 204	M	36–50	12,13,30,31,32	Lingual
71 US 216	F	25–35	18,19,20	Lingual
71 US 247	M	36–50	5,6,18,21	Buccal
80 US 354 1E	NDS	8–10	3	Lingual
80 US 354 2D	NDS	7–9	I,J	Buccal and lingual
80 US 354 3A	F	36–50	8,27,30	Lingual
80 US 354 4B	NDA	36–50	8,10,11,21	Buccal and lingual
80 US 354 5C	M	35–50	9,10,28,30,31	Buccal and lingual
81 US 247 1A	M	36–50	12,13,14,15,18,20,21	Buccal
83 US 73 A	M	35–50	26,27	Lingual
83 US 179 1A	M	30–40	18,20,30	Buccal
83 US 179 B	F	36–50	18,19	Buccal and lingual
83 US 179 C	NDS	14–17	3,15	Buccal and lingual
84 US 211	F	18–20	14,30	Buccal

2.3. LM analysis

To extract starch granules and other microremains from the dental calculus, a protocol based on Hardy et al. (2009) method was used, after appropriate modifications. From 1 to 20 mg of sample, where available, were resuspended with 500 μL of 1 M HCl, sonicated for 10 min (Falc Instruments MOD: LBS1 34) and left in agitation for 24 h. After centrifugation at 13.000 rpm for 10 min, the pellet was subjected

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