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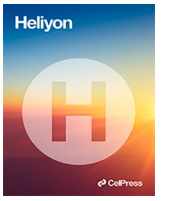

Research article

Evaluation of the responsiveness pattern to caffeine through a smart data-driven ECG non-linear multi-band analysis

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ABSTRACT

This study aimed to explore more efficient ways of administering caffeine to the body by investigating the impact of caffeine on the modulation of the nervous system's activity through the analysis of electrocardiographic signals (ECG). An ECG non-linear multi-band analysis using Discrete Wavelet Transform (DWT) was employed to extract various features from healthy individuals exposed to different caffeine consumption methods: expresso coffee (EC), decaffeinated coffee (ED), Caffeine Oral Films (OF_caffeine), and placebo OF (OF_placebo). Non-linear feature distributions representing every ECG minute time series have been selected by PCA with different variance percentages to serve as inputs for 23 machine learning models in a leave-one-out cross-validation process for analyzing the behavior differences between ED/EC and OF_placebo/OF_caffeine groups, respectively, over time. The study generated 50-point accuracy curves per model, representing the discrimination power between groups throughout the 50 min. The best model accuracies for ED/EC varied between 30 and 70 %, (using the decision tree classifier) and OF_placebo/OF_caffeine ranged from 62 to 84 % (using Fine Gaussian). Notably, caffeine delivery through OFs demonstrated effective capacity compared to its placebo counterpart, as evidenced by significant differences in accuracy curves between OF_placebo/OF_caffeine. Caffeine delivery via OFs also exhibited rapid dissolution efficiency and controlled release rate over time, distinguishing it from EC. The study supports the potential of caffeine delivery through Caffeine OFs as a superior technology compared to traditional methods by means of ECG analysis. It highlights the efficiency of OFs in controlling the release of caffeine and underscores their promise for future caffeine delivery systems.

1. Introduction

Discovered in 1819 by German chemist Friedlieb Ferdinand Runge, caffeine, the common name for 1,3,7-trimethyl xanthine, is the most consumed psychoactive substance worldwide [1,2]. Today, approximately 80 % of the adult population worldwide consumes one caffeinated product daily, and as opposed to other drugs, the consumption happens at every socioeconomic level [2,3]. In addition to

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the cost-benefit, caffeine acts as a powerful stimulant to the central nervous system, causing physiological effects that are very appealing to the consumer, specifically on a cognitive level and in terms of physical performance [4]. The psychostimulant effect of caffeine is so powerful that just the expectation of ingesting it can, by itself, potentiate the action of caffeine in the human organism, even if no amount is consumed [5]. The physiological impact of caffeine includes the promotion of alertness, concentration, cognition and memory, excitement, energy, and elevated mood [6–8], increased adrenergic neurotransmission responsible for stimulation of the peripheral nervous system [6,9,10] and reduction of general fatigue [11]. Several studies have demonstrated significant connections between caffeine intake and neuropsychological and cognitive outcomes. For instance, a study by Maric et al. [12] has shown that caffeine metabolism plays a crucial role in predisposing pregnant women to preeclampsia. This research underscores the broader physiological implications of caffeine and motivates our investigation into its effects on the cardiovascular system. Caffeine's ability to modulate neurotransmitter release and vascular tone suggests its impact might extend beyond simple stimulation, affecting vascular health and stress responses. Exploring cognitive domains, Kim and colleagues have identified how regular coffee consumption modifies brain network connectivity [13]. Their findings suggest that these alterations may enhance cognitive efficiency due to improved functional connectivity within the brain network. Additionally, using advanced analytics, research conducted by Huang A.A. and Huang S.Y [14]. employed machine learning techniques to analyze nutritional data, revealing that caffeine and other nutritional factors contributed significantly to the predictive accuracy of the XBoosted model, accounting for 37 % of its overall prediction. In cardiovascular research, Stevens and colleagues [15] have utilized machine learning techniques to identify potential risk factors for major diseases such as coronary heart disease, stroke, and heart failure, with higher coffee consumption emerging as a protective factor against heart failure. This insight is supported by further machine learning studies that have identified coffee intake as a prominent predictor in the risk assessments for Parkinson's disease [16]. Another study [17] investigated the impact of circulating caffeine levels by analyzing genetically predicted variations in caffeine metabolism. The findings indicated that higher genetically predicted circulating caffeine levels among caffeine consumers were associated with a reduced risk of obesity, osteoarthrosis, and osteoarthritis. These findings highlight the extensive impact of caffeine on health and illustrate the advantages of employing machine learning approaches to enhance our understanding of how nutrition, particularly caffeine consumption, influences disease and brain activity.

The results in the scientific community could be clearer, particularly regarding the form of caffeine administration that seems to influence the body's response [18,19]. Most consumers choose the oral route of substance administration (namely caffeine) because of its simplicity and convenience. Nevertheless, the bioavailability of substances via the oral route varies greatly, not only because of their physicochemical properties but also because of first-pass metabolism caused by the physiological environment of the gastrointestinal system, leading to drastically reduced bioavailability [20]. Consequently, alternative routes of substance administration have gained increasing attention in recent years due to their potential to enhance drug delivery efficiency and bypass the constraints of the oral way.

Oral Films (OF) emerged as innovative breath freshening formulations and quickly evolved to respond to different market needs, having increasingly received attention from researchers from the pharmaceutical and nutraceutical industries, namely as a promising new drug release system easy-to-carry and easy-to-swallow [21]. Furthermore, the high vascularization of the oral mucosa allows a rapid disintegration and absorption of the active substance, improving bioavailability and anticipating the onset of action and the manifestation of physiological effects, not needing water, or chewing [22,23]. These release systems can transport the active ingredients according to the desired load and release rate and may also incorporate inactive components to design the final product according to the target profile. These substances include plasticizers, sweeteners, flavorings, colorants, and saliva-stimulating agents [24]. The versatility of such systems makes them very attractive compared to traditional routes of administration. For all these benefits mentioned, this new technology proves to be an efficient solution for caffeine administration. Even based on preliminary results, studies indicate that the presence of caffeine in the oral mucosa can stimulate sensory nerves, inducing brain responses even before any caffeine uptake [25]. However, the delivery of caffeine via these release systems is challenging for researchers. On the one hand, the current legislation on OF development and production is still unclear. On the other hand, although caffeine has been widely studied (its pharmacokinetics and dynamics profile are already well known), its other physiological effects, especially when consumed through OF, deserve further study [26,27].

The physiological measures are essential parameters to characterize neuromodulator molecule delivery systems and can complement the guidelines to ensure the safety and quality of this new delivery system, being relatively simple to perform, non-invasive, and having accurate real-time results [28]. Furthermore, it is possible to take advantage of caffeine's influence on modulating nervous system activity by tracking the variability of cardiac activity [9]. Cardiac activity generates an electric current detectable by an electrocardiograph, resulting in an electrocardiographic signal (ECG). Each deflection in this signal correlates with a specific cardiac electrical event, translating the heart's activity level into signal complexity [29]. ECG signals often display rapid oscillations over brief periods or gradual changes over extended times [30]. Traditional analysis tools, such as the Discrete Fourier Transform and linear metrics, may not adequately address this signal's non-linear and chaotic nature [31,32], as they rely on assumptions that could lead to incorrect interpretations when applied to ECG signals. In contrast, emerging techniques like the Discrete Wavelet Transform (DWT) are gaining traction in ECG analysis due to their ability to offer adaptable resolution across the time-frequency spectrum. This adaptability allows for high temporal resolution in capturing rapid changes at high frequencies while offering high spectral resolution to discern subtle variations at lower frequencies. By leveraging the DWT, researchers aim to overcome the limitations of traditional methods and achieve more accurate insights into the complex dynamics of ECG signals [33,34]. Thus, to maximize the discriminative capability for evaluating the physiological impact of caffeine consumed as expresso coffee (EC) or OF over time through ECG analysis in healthy subjects, this work proposes to.

1. Introduce the novel utilization of five non-linear features (Shannon entropy, Energy, Higuchi fractal dimension, Hurst exponent, and Lyapunov exponent) extracted every 30 s under a DWT multi-band ECG signal analysis. This approach aims to characterize cardiac responsiveness pattern during 50-min observation periods, uncovering nuanced temporal variations in cardiac activity in response to caffeine ingestion and clarifying its physiological effects on the cardiovascular system.
2. Evaluate the discriminatory performance of these features between two group pairs (EC vs decaffeinated coffee (ED) and Caffeine OF vs placebo OF), employing an extensive set of Machine-Learning models.
3. Highlight the adaptability of extracted features in contributing to robust ML models which can aid in accurately evaluating the human body's response to caffeine consumed in two different forms.
4. Assess the efficiency of as a vehicle for caffeine delivery by comparing the cardiac behavior of subjects who consumed caffeine in the forms of EC and OF.

2. Materials

2.1. Database

The ECG signals were collected in the Human Neurobehavioral Laboratory (HNL) facilities at the Universidade Católica Portuguesa in Porto, Portugal. A stratified random sample with 13 participants was used in this study, with an age range from 18 to 46 years old (mean age of 24.15 years old with a standard deviation of 7.71 years, consisting of 61.5 % females and 38.5 % males). All participants who met the inclusion criteria were healthy individuals above 18 years of age who consumed coffee or other caffeinated products daily. Additionally, they had no history of cardiovascular disease or implant, substance abuse disorders, medication usage affecting cardiac activity or endocrine function, physical or mental conditions that could compromise physiological measurements, pregnancy or breastfeeding, or smoking habits. All participants gave their prior consent before participating in this study, whose protocol number May 2018 was approved and authorized on May 14, 2018 by the "Ethics Lab" of the "Institute of Bioethics - Universidade Católica Portuguesa. Concerning the self-report data, the applied instrument included a socio-demographic section and a caffeine consumption profile section. These data are presented in Tables 1 and 2, respectively. All electrocardiographic signals were acquired at a sampling rate of 100Hz, using the Biopac MP-160 data acquisition system with the ECG100C amplifier and Acknowledge software 5.December 0, 2017 (Biopac System Inc., Santa Barbara, CA, USA) connected to a computer. The ECG was monitored with a standard configuration of disposable Ag–AgCl electrodes placed on the right clavicle and at the V6 precordial site after cleaning the skin with alcohol to minimize impedance and promote good contact between the electrode and the skin [35,36].

2.2. Experimental procedures

The experimental procedure used an intra-subject comparison model and consisted of the following four phases: (1) welcoming, informed consent, instructions, application of the questionnaire and equipment setup; (2) baseline task; (3) consumption of the modality under study; and (4) monitoring phase. Thus, after an eligibility screening, participants were appointed to four laboratory sessions held on consecutive days during the morning, to avoid the diurnal variability in ECG parameters. It was ensured that the study took place in a well-controlled laboratory environment. Days before the laboratory appointment, participants were requested to keep the same caffeine intake pattern until the data collection, avoiding psychostimulants such as chocolate, cola, or even coffee 2 h before the session [35]. At each laboratory visit, each participant was randomly assigned to one of the four conditions under analysis: coffee, decaffeinated, OF with caffeine, OF without caffeine (these OFs were produced in the Centro de Biotecnologia e Química Fina of the Universidade Católica Portuguesa - Porto by an experienced research team in the field [37,38]). It should be noted that the produced OF employed an innovative methodology that distinguishes it from commercial OFs. Their approach involves incorporating

Table 1
Socio-demographic characteristics of the participants ($n = 13$).

Categorical Measure	%
Gender	
Females	61.5
Males	38.5
Marital status	
Single	92.3
Married/in a relationship	0
Divorced/separated	7.7
Widower	0
Education levels	
Elementary School	7.7
Secondary	61.5
Higher education (degree)	23.1
Higher education (master's, doctorate, and post-doc)	7.7
Profession	
Students	84.6
Other professions	15.4

Table 2
Caffeine consumption profile *(n = 13)*.

Categorical Measure	%
Enjoy coffee	
Yes	84.6
No	15.4
Frequency of coffee drinking per day	
Up to once a day	38.5
2 times a day	7.7
2 to 3 times a day	23.1
More than 3 times a day	7.7
Rarely	15.4
A few times a week	7.6
Reasons for drinking coffee	
Wake up	15.4
Socially	7.7
Health	7.7
Several (no specific)	69.2

microparticles into the composition, enabling the retention of caffeine. As a result, in addition to dispersing caffeine in the film, like commercial OFs, encapsulated caffeine is present within these OFs. Consequently, the procedure was fully blinded for the participants and the researcher assistants in charge of the data collection [35].

After 5 min of resting in the sitting position, while the participants filled out a questionnaire and became familiar with the laboratory environment, electrodes were placed on the participants in suitable positions for signal collection. Subsequently, and before the consumption of any modality, the baseline task was performed. For this step, participants were asked to perform a low cognitive demanding task and evaluate whether pairs of images were the same or different. A behavioral task was also performed to assess the level of cognitive performance before caffeine ingestion. Then, after consuming the randomly assigned modality, the cardiac activity of the participants was monitored for 50 min. For this monitoring phase, participants were predisposed to a set of specific tasks: rate their alertness using the Visual Analog Scales (VAS), choosing among "alert/able to concentrate", "anxious", "energetic", "feel confident", "irritable", "jittery/nervous", "sleepy" and "talkative"; perform the Attentional Network Test (ANT), a computer-based test to measure participants" performance in three separate components of attention: alerting, orienting, and executive control; and a continuous performance test that assesses attention and impulsivity (Test of variables of attention, T.O.V.A.) [35].

3. Methods

This topic explains the methodology adopted to attain the objective of this work. It was fully developed and codded within the MATLAB® R2019a software environment.

3.1. Methodology structure

The data analysis strategy is subdivided into three main steps: (1) Loading and pre-processing (filtering), (2) Signal processing: wavelet multi-band decomposition, non-linear analysis, and features extraction, and (3) Classification per 1min time-series length.

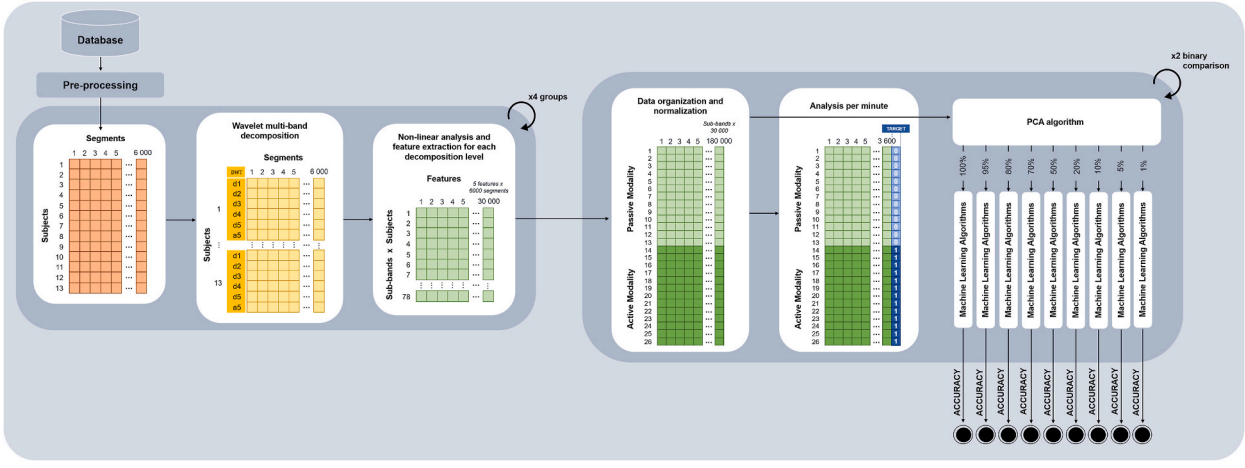

Fig. 1. Methodology workflow.

Fig. 1 illustrates the methodology implementation steps.

3.1.1. Loading and signal pre-processing

The raw data contains contaminations that are usually large enough to camouflage the small amplitude features of the signal that are of physiological or clinical interest [39]. After loading the database in a MATLAB® environment, a set of preprocessing techniques were applied to the 52 signals (13 subjects x 4 modalities of study) to remove discrepancies and scale the resources to an equivalent range. All raw signals were digitally filtered by a Butterworth filter with order 5 and 2–49Hz cutoff frequencies. Then, the amplitude of each signal was normalized according with,

$$x(n) = \frac{x(n)}{\sum_{n=0}^{N-1} x^2(n)} \tag{1}$$

where N represents the signal size, and the mean value was then removed.

3.1.2. Signal analysis and feature extraction

This subsection describes the set of features extracted from each ECG signal.

3.1.2.1. Wavelet multi-band decomposition. The DWT of a finite-energy signal in discrete time refers to breaking it down into a set of fundamental functions derived from a limited number of prototype sequences and their time-shifted versions [40]. As suggested by Vetterli and Kovacevic in 1995 [40], this approach is highly effective for analyzing signals in the time-frequency domain. The process of structured expansion is executed by employing a critically decimated filter bank with octave-band spacing, proposed by Malvar in 1992 and further developed by Vetterli and Kovacevic in 1995 [40,41]. Considering only the positive frequencies, the m-th sub-band is confined to [41].

$$W_m = \begin{cases} \left[0, \frac{\pi}{2^S}\right], m = 0, \\ \left[\frac{\pi}{2^{S-m+1}}, \frac{\pi}{2^{S-m}}\right], m = 1, 2, \ldots, S, \end{cases} \tag{2}$$

where S is the number of sub-bands or levels of decomposition, S+1 is the number of sub-bands and π is the normalized angular frequency which is equivalent to half the sampling rate.

The DWT uses an analysis scale function $\widetilde{\varphi}_1(\mathrm{n})$ and an analysis wavelet function $\widetilde{\psi}_1(\mathrm{n})$ defined as

$$\widetilde{\varphi}_1(\mathrm{n}) = h_{LP}(n) \tag{3}$$

and

$$\widetilde{\psi}_1(\mathrm{n}) = h_{HP}(n), \tag{4}$$

where $h_{LP}(\mathrm{n})$ and $h_{HP}(\mathrm{n})$ are the impulse responses of the half-band low-pass and high-pass filters, respectively. Defining the following recursion formulas

$$\widetilde{\varphi}_{i+1}(\mathrm{n}) = \widetilde{\varphi}_i\left(\frac{n}{2}\right) * \widetilde{\varphi}_1(n), \tag{5}$$

$$\widetilde{\psi}_{i+1}(\mathrm{n}) = \widetilde{\varphi}_i(n) * \widetilde{\psi}_1\left(\frac{n}{2^i}\right), \tag{6}$$

The equivalent analysis filter of the m-th sub-band is given by

$$h_m(n) = \begin{cases} \widetilde{\varphi}_S(n), m = 0 \\ \widetilde{\psi}_{S+1-m}(n), m = 1, 2, \ldots, S. \end{cases} \tag{7}$$

The m-th sub-band signal of $x(\mathrm{n})$ is given by

$$x_m(n) = \begin{cases} \sum_{k=-\infty}^{\infty} x(k) h_m\left(2^S n - k\right), m = 0, \\ \sum_{k=-\infty}^{\infty} x(k) h_m\left(2^{S-m+1} n - k\right), m = 1, 2, \ldots, S. \end{cases} \tag{8}$$

Each sub-band signal is resampled to the original sampling rate frequency using the wavelet interpolation method described in Rioul & Vetterli (1991) [30].

3.1.2.2. Non-linear analysis. According to the non-linear dynamic theory, a complex system (such as the heart) is characterized by

non-linear dynamic properties. Due to physiological events, the heart's environment is constantly changing over time. Consequently, ECG signal exhibit non-linear and chaotic behavior. Additionally, the degree of complexity of the heart represents the time series randomness. Depending on the intensity of the heart activity, the ECG signal can be more or less complex depending on the information complexity per signal segment [42,43] Evidence shows that ECG signal analysis using non-linear techniques improves the feature extraction and classification process, with each measure reflecting complexity with different approaches [43]. Although many non-linear models have been proposed in recent years, this dissertation is limited to the most common ones for a first approach.

3.1.2.2.1. Chaos theory. The correct analysis of the evolution of dynamic systems implies the spatial representation of their states over time, in which a system defined by *n*-variables can be represented by a point in *n*-dimensional space, which represents the value of all its variables at a given time [44]. The consecutive states represented in this phase space define trajectories, which, when observed over long periods, tend to converge to a specific geometric structure independent of the starting point, called an attractor [45]. Thus, the system evolves towards extremely irregular, complex, and non-periodic behaviors – called chaotic behaviors – in which, although there is no predictability, they converge toward a structurally stable behavior [46].

The evolution of system dynamics is typically investigated by extracting non-linear features from the attractor, which analyzes concepts related to stability, variability, complexity, and similarity [47]. The accuracy of the attractor reconstruction is critically important for the application of these methods, as such two parameters should be considered: the delay (τ) and the embedding dimension (d). Thus, the attractor reconstruction is achieved using a single state variable ($x(i)$) [48]:

$$x(i) = (x(i), x(i+\tau), x(i+2\tau), \ldots, x(i+(d-1)\tau)) \tag{9}$$

- Lyapunov Exponent: The trajectories in a chaotic attractor evolve in two ways in particular: expansion, in which the trajectories of the points considered diverge exponentially from the initial conditions (close points in phase space), and convergence, in which the trajectories converge with each other over time [49]. From the dynamical point of view, the Lyapunov exponent (λ) measures the average rate of expansion and convergence of trajectories in phase space, thus characterizing the predictability of the dynamical system [50,51]. For a space with dimension N, there are N Lyapunov exponents. However, it is common to determine only the largest Lyapunov exponent because it is simpler to calculate and provides a more comprehensive view of the dynamics of the system [52]. If the largest Lyapunov exponent is positive then the attractor is chaotic, with the magnitude of the exponent reflecting the time scale at which this behavior is visible [51]. This measure can be estimated using the following mathematical equation:

$$\lambda(\mathrm{i}) = \frac{1}{(\mathrm{K_{max}} - \mathrm{K_{min}} + 1)\mathrm{dt}} \sum_{\mathrm{K=K_{min}}}^{\mathrm{K_{max}}} \frac{1}{\mathrm{K}} \bullet \ln \frac{\| \mathrm{Y_{i+K}} - \mathrm{Y_{i^*+K}} \|}{\| \mathrm{Y_i} - \mathrm{Y_{i^*}} \|} \tag{10}$$

where, K_{min} and K_{max} represent the expansion interval, dt is the sampling time, and Y_i represents the reconstruction value of the signal for the considered dimension and delay [53].

- Hurst Exponent: The Hurst exponent (H) is a dimensionless measure used to assess the self-similarity and long-range correlation properties of time series [54]. The calculation of this exponent is defined in terms of the asymptotic behavior of the rescaled interval (a statistical measure of the variability of a time series) as a function of the time series period, as follows:

$$H = \frac{\log\left(\frac{\mathrm{R}}{\mathrm{S}}\right)}{\log(\mathrm{T})} \tag{11}$$

where T is the sample length, R/S is the value corresponding to the rescaled interval, R is the difference between the maximum and minimum deviation from the mean, and S represents the standard deviation [55,56].

When $H = 0.5$, there is no correlation in the time series (random behavior). If $0 < H < 0.5$, the time series exhibits long-term alternation, i.e., a high value is likely to be followed by a low one and vice-versa. On the other hand, if $0.5 < H < 1$ then the time series is defined as positive long-run autocorrelation. In the theoretical limit, when $H = 1$, the time series presents a perfect correlation [55,57].

3.1.2.2.2. Fractal dimension. Fractal is a mathematical model used to describe scale-invariant random processes and it is characterized by its fractional dimensions [58]. This unique characteristic makes it possible to recognize similar geometric patterns that repeat infinitely many times at any scale of magnification [59]. There are several methods for estimating fractal dimensions. Among them, Higuchi's algorithm is known to be the most accurate and efficient, aiming to quantify the self-similarity and complexity of the signal [60].

Higuchi Fractal Dimension (Df_H) is based on a length measure $L(k)$ of the curve representing the time series. If scaled as $L(k) \sim k^{-Df}$, the curve is believed to show the fractal dimension Df. To do this, from a given time series the algorithm constructs k new time series, $x_{m,k} = x(m), x(m+k), x(m+2k), \ldots, x\left(m + int\left(\frac{N-m}{k}\right)k\right)$, where m represents the initial value of time, k is the interval time and $int(r)$ represents the integer part of a real number r. The length of $L_m(k)$ of each curve is then calculated by:

$$L_m(k) = \frac{\sum_{i=1}^{int\left(\frac{N-m}{k}\right)} |x(m+i\bullet k) - x(m+(i+1)k)|}{int\left(\frac{N-m}{k}\right)k} \tag{12}$$

where, N is the number of samples and *int* represents the number rounded to the nearest integer. This process is repeated for each k, from 1 to k_{max}, with k_{max} being experimentally determined. Finally, the Df_H value is calculated using least squares, with the value Df_H being the slope of the linear regression between $\ln(L_m(k))$ e $\ln(1/k)$ [61,62].

3.1.2.2.3. Energy. Energy can be seen as a measure of signal strength and can be obtained from equation (13), where g represents the signal [63,64].

$$E = \sum_i |g(n)|^2 \tag{13}$$

3.1.2.2.4. Entropy. Entropy quantifies a system's disorder and uncertainty level [65]. Entropy assesses the predictability of future amplitude values based on the probability distribution of previously observed amplitude values, i.e., present information can be explained by past information history. Along this line of reasoning, high entropy values are associated with data that is uncertain (too much information) and more difficult to predict. In contrast, if all observations are unanimous, there is no uncertainty and the entropy value is zero [66–68]. Shannon entropy can be estimated by equation (14), which g represents the signal [68].

$$E_s = -\sum_i |g(n)|^2 \bullet \log\left[|g(n)|^2\right] \tag{14}$$

3.1.2.3. Feature extraction, selection, and classification. For each windowing signal analysis of 0.5s, five non-linear features (λ, H, Df_H, E, and E_S) have been extracted from each sub-band computed by DWT decomposition until level 5 using symlet5. This Wavelet also prove to be a good choice for ECG signal analysis in Refs. [69,70]. It implies 36000 non-linear analyses per each signal subject participant (6 sub-bands x 6000 windows analysis, 120 windows analysis per min x 50 min), as illustrated in Fig. 1. As five features have been extracted per each sub-band windowing analysis, at the end of the process, a time-series vector of 180000 features was collected per each signal participant. The data has been organized for the binary comparisons decaffeinated/coffee and OF_placebo/OF_caffeine, resulting in two independent data matrixes of 26x180000. Per binary comparison, data have been normalized by the z-score algorithm [71,72]. Each matrix has been divided into sequential 50 time-series matrices of 26x3600 allowing us to focus our analysis per minute. Finally, as we will apply in this study supervised machine learning models, the last step concern labeling each entry according to the different group's modalities. In other words, each entry related to an active substance is labeled with a value of 1 and the placebo with a value of 0. The normalized matrixes per minute were applied as inputs to a cascade of one Principal Component Analysis (PCA) algorithm and one classical machine learning algorithm. Per minute, different features combinations have been selected by PCA based on 100 % (no application), 95 %, 80 %, 70 %, 50 %, 20 %, 10 %, 5 %, and 1 % of the variance and different machine learning models (check Table 3) have been used for discrimination between group pairs. For every classifier model, accuracy

Table 3
Used classifiers and default parameters.

Classification Models	Classifier	Default Parameters
Decision Trees	Fine Tree	Maximum number of splits = 4
	Medium Tree	Maximum number of splits = 20
	Coarse Tree	Maximum number of splits = 100
Discriminant Analysis	Linear Discriminant	Covariance structure: complete
Logistic Regression	Logistic Regression	Covariance structure: complete
Naïve Bayes	Gaussian Naïve Bayes	–
Support Vector Machines (SVM)	Linear SVM	Box constraint level = 3
	Quadratic SVM	Box constraint level = 3
	Cubic SVM	Box constraint level = 4
	Fine Gaussian	Box constraint level = 3
	Medium Gaussian	Box constraint level = 3
	Coarse Gaussian	Box constraint level = 1
K-Nearest-Neighbors (KNN)	Fine KNN	Number of neighbors = 1
	Medium KNN	Number of neighbors = 10
	Coarse KNN	Number of neighbors = 100
	Cosine KNN	Number of neighbors = 10
	Cubic KNN	Number of neighbors = 10
	Weighted KNN	Number of neighbors = 10
Ensembles	Boosted Trees	Maximum number of splits = 10
	Bagged Trees	Maximum number of splits = n-1 (n = number of observations in the training sample)
	Subspace Discriminant	Covariance structure: complete
	Subspace KNN	Number of neighbors = 3
	RUSBoosted Trees	Maximum number of splits = 150

ratios have been attained per minute allowing us to trace accuracy curves of 50 points, representing the discrimination power of each model along the 50 min for both comparison groups, respectively.

4. Results and discussion

Given the large volume of data results obtained, the focus of this section is on the 50-point accuracy curve average values. The results of the classification for each comparison case are shown in Fig. 2. It is noticeable that the accuracy percentages of each classifier were similar by using various levels of PCA variances, and it can be inferred that there is redundant information between the selected metrics. Scrutiny Fig. 2, among the selected results, the decision tree classifiers - PCA 100 % for the decaffeinated/coffee comparison and Fine Gaussian - PCA 95 % for the OF_placebo/OF_caffeine comparison stand out as the best ones within groups comparison. It is important to mention that with this kind of analysis, there are accuracy peaks camouflaged by the average.

After analyzing all results, it can be concluded that when the system was trained with PCA 100 %, meaning no feature selection applied, for both binary classifications, the Decision Tree classifiers were those that showed the best discriminative average power, with an average accuracy rounding of 50.2 % for decaffeinated/coffee and 65.5 % for OF_placebo/OF_caffeine. This family of classifiers was also provided to attain the best performance with PCA 95 %, 80 %, 70 %, and 50 %, for the comparison decaffeinated/coffee, with average accuracy values ranging from 44.9 % to 46.8 %. However, for these same PCA variances, in the case of_placebo/OF_caffeine comparison, the Fine Gaussian classifier showed the best classification accuracy with average values ranging between 67.3 % and 72 %. Regarding the system training with PCA 20 %, 10 %, and 5 % of the variance, for the OF_placebo/OF_caffeine binary classification, the Quadratic SVM, Medium KNN, and Cubic KNN classifiers showed, respectively, the best classification performance with average accuracy values ranging from 68.2 % to 69.7 %. And for the decaffeinated/coffee comparison, with PCA 20 % the Bagged Trees and Fine KNN classifiers show the best results with an average performance of 47.9 % and 47.5 %, respectively. In this same classification, for training with PCA 10 % and 5 % variance, the best average accuracy manifested itself in the Subspace KNN and Fine KNN classifiers with values between 47.2 % and 47.6 %. Finally, when the system was trained with PCA 1 %, for the decaffeinated/coffee binary comparison, the Bagged Trees, Fine KNN, and Subspace KNN classifiers showed the best discriminative power with a performance of 47.2 %. And for the OF_placebo/OF_caffeine comparison, the Quadratic SVM classifier showed the best performance with 68.6 % of average accuracy.

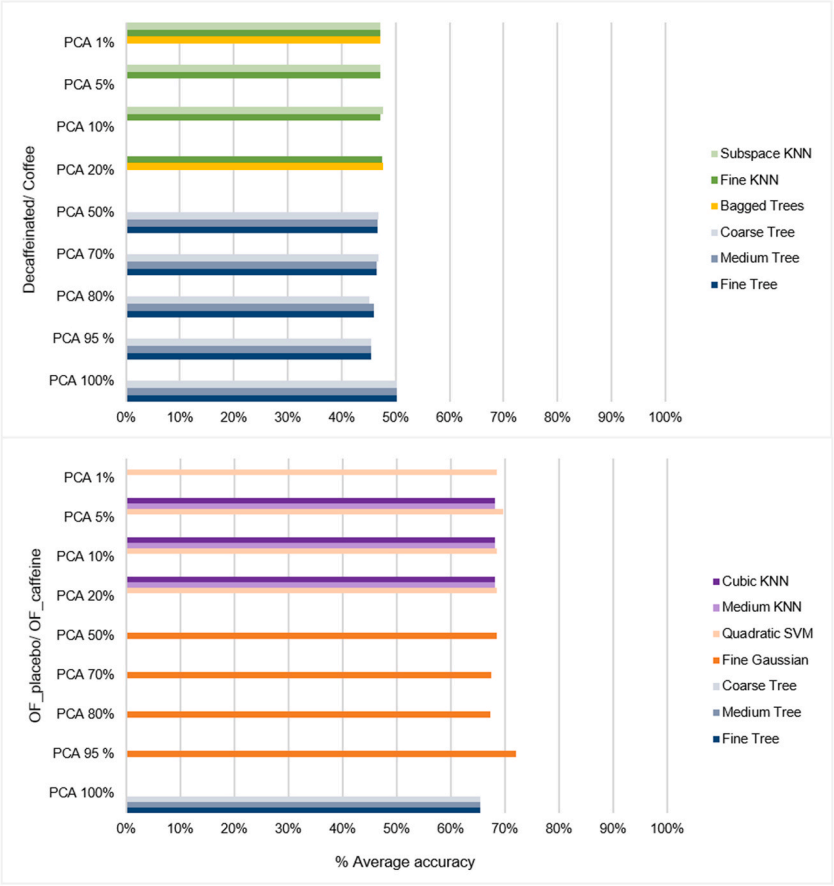

Fig. 2. Comparison of the best-selected results for each binary classification.

The remaining unmentioned results obtained clarify that these classifiers did not prove to be efficient in their classification process. Thus, it is of special interest to understand the accuracy curves of the classifiers over time that showed the best performance. Figs. 3–11 illustrate these results.

First, a superficial analysis of the accuracy curves shows that the amplitude of the classifiers' performance is within the expected range. The ability to detect physiological changes induced by caffeine demonstrates the discriminative power of ECG signals across both active and placebo modalities, highlighting the effectiveness of the DWT approach as documented in the literature [73]. Although the data extracted from these signals confirm that caffeine influences cardiac activity, the results do not precisely delineate the exact morphological changes in the ECG characteristics. This alignment with previous studies reinforces the validity of our methods and situates our findings within a broader research context that links caffeine consumption with specific cardiac responses. At this point, it is important to reinforce the idea that the main objective of this study is to verify the effect of caffeine over time by analyzing the classifier's discriminative capacity. Thus, higher accuracy rates obtained by a classifier indicate more significant differences between binary comparison groups, allowing to evaluation of proximity or divergence of cardiac behavior among various study groups over 50 min following consumption. Furthermore, the physiological characteristics intrinsic to everyone reduce the possibility of finding outstanding accuracy rates (close to 100 %) over time, and it is also expected that accuracy rates will decay as caffeine is metabolized in the body, limiting the classifier's discrimination power.

Examining individually the 50-point accuracy curves, the discrimination accuracy values obtained in the group decaffeinated/coffee were much lower than those obtained for the group OF_placebo/OF_caffeine. These accuracy values indicate that the differences between expresso coffee and, its placebo, decaffeinated, consumed through the same vehicle, are not as significant as the ones found by using OF as an administration vehicle.

While previous research [74] utilizing headspace solid-phase microextraction/gas chromatography–mass spectrometry has revealed variances in aroma-related compounds—such as aldehydes, ketones, acids, and alcohols—between decaffeinated and regular espresso coffee, our study presents a novel insight. We found that when individuals consume these two substances, minimal variations in cardiac behavior, as indicated by ML accuracy curves over an ECG span of 50 min, were observed. Notably, the accuracy values depicted relatively low amplitudes. The relatively low amplitudes in accuracy values suggest that the observed disparities might be attributed to: (1) the low sensitivity to the doses of caffeine administered through coffee, (2) the psychological effect (or conditioned from previous consumption), since the aroma, the smell, and the act of drinking may awaken behaviors associated with the real ingestion of the substance by activating the nervous system, or (3) the fact that both modalities were served from the same machine and caffeine residues may have passed to the decaffeinated beverage. However, in the first few minutes of analysis, it would be expected that in the most sensitive individuals, the accuracy values would be noticeably lower than in the rest of the signal collection period. This was found very subtly in some of the accuracy curves and can be attributed to two main factors: (1) psychological effects, since, according to a scientific study [5] the caffeine psychostimulant effect is so potent that merely anticipating its consumption can

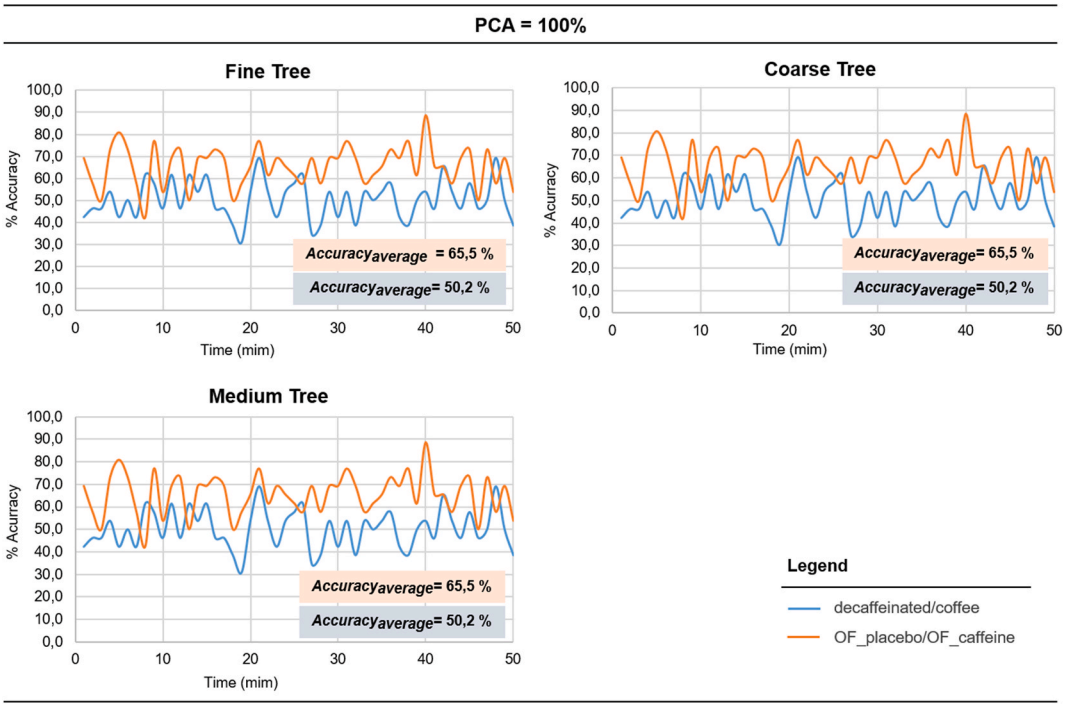

Fig. 3. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained without feature selection (PCA = 100 %).

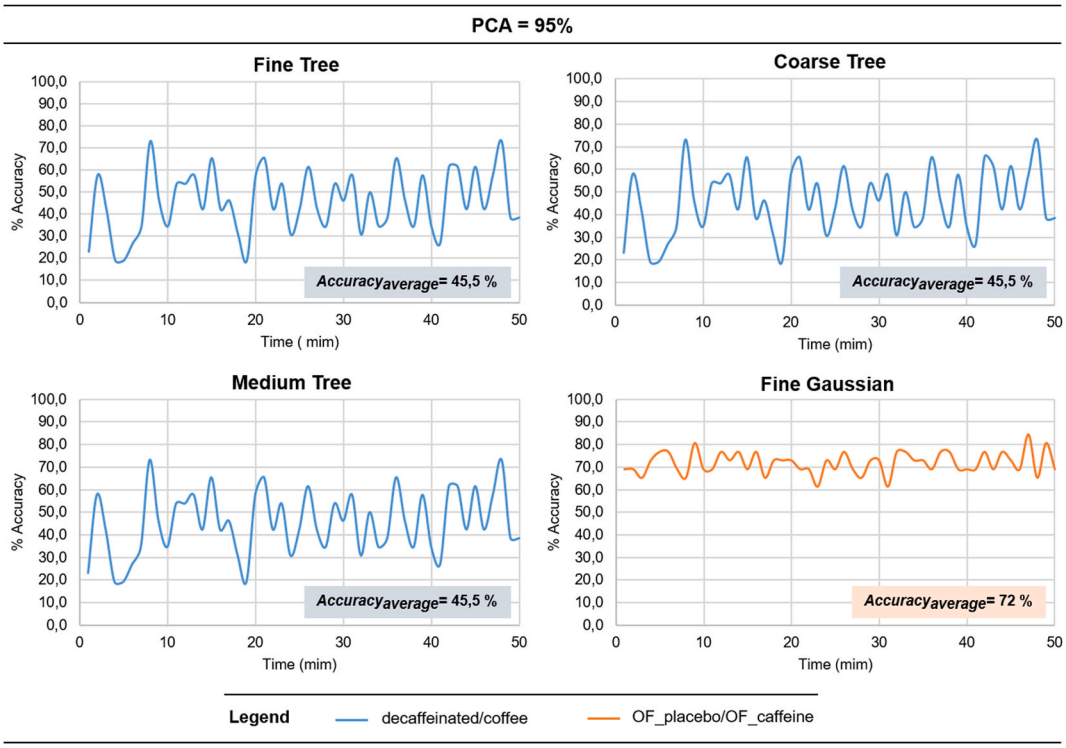

Fig. 4. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 95 %.

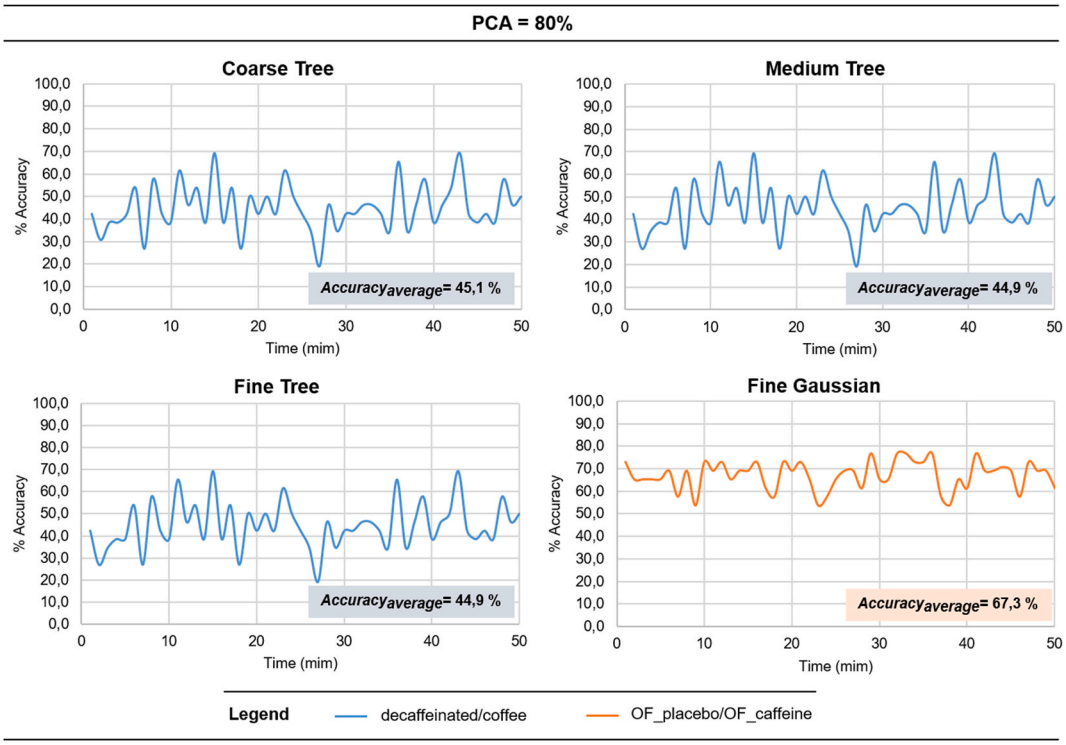

Fig. 5. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 80 %.

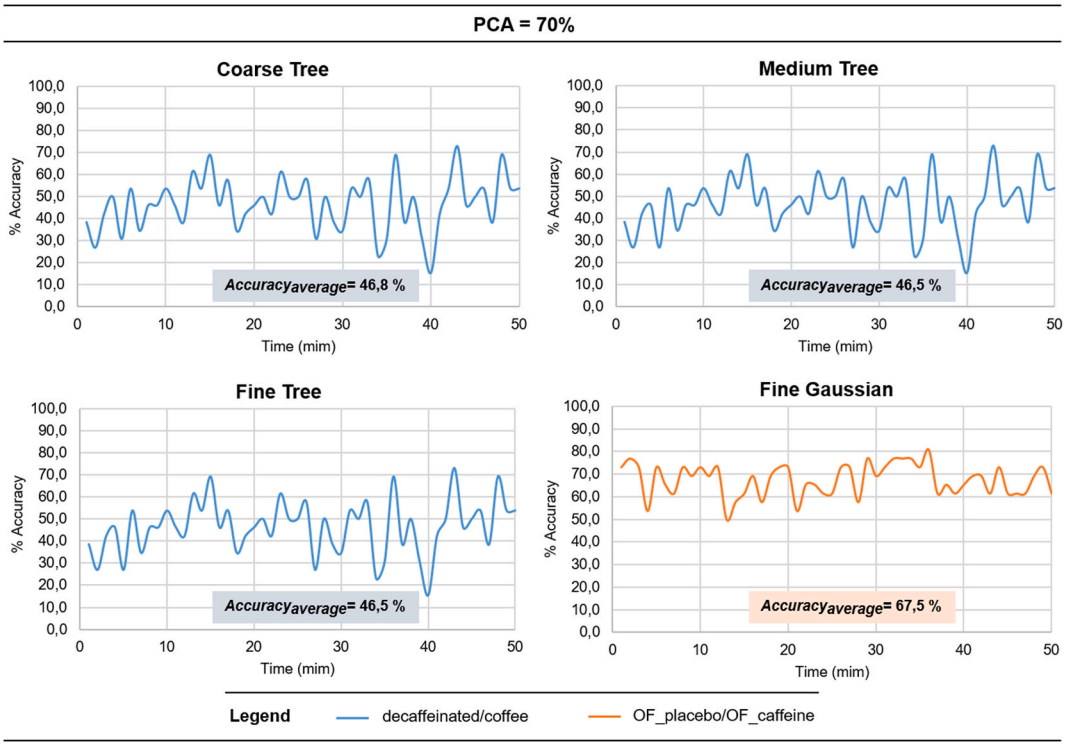

Fig. 6. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 70 %.

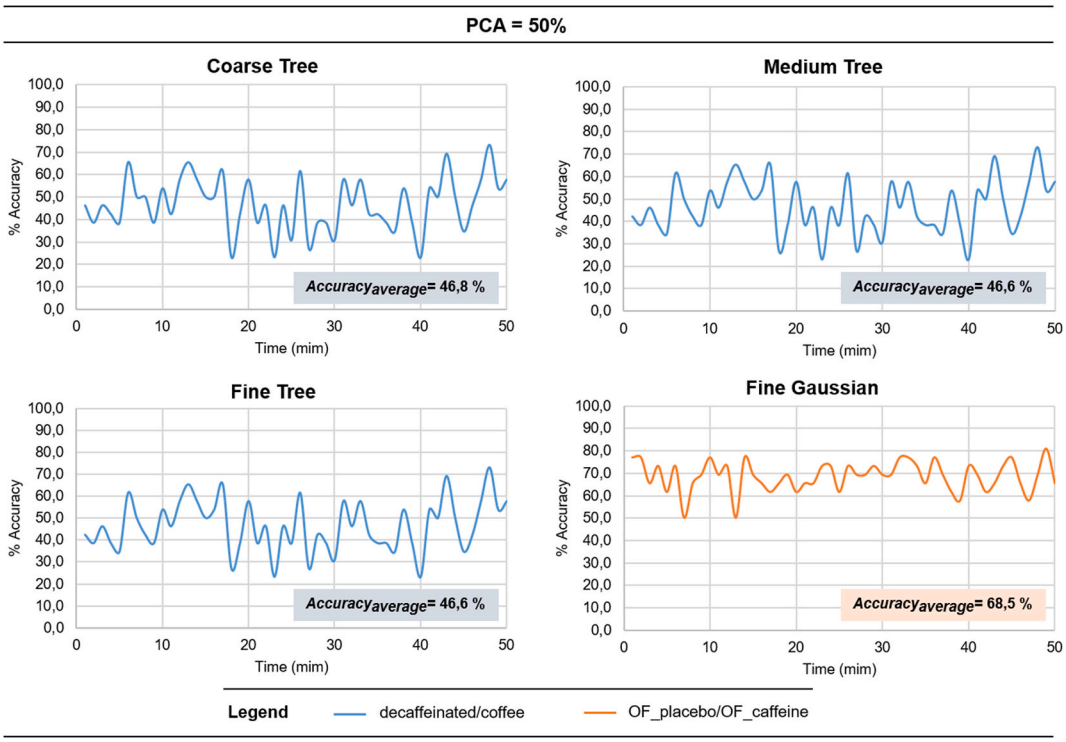

Fig. 7. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 50 %.

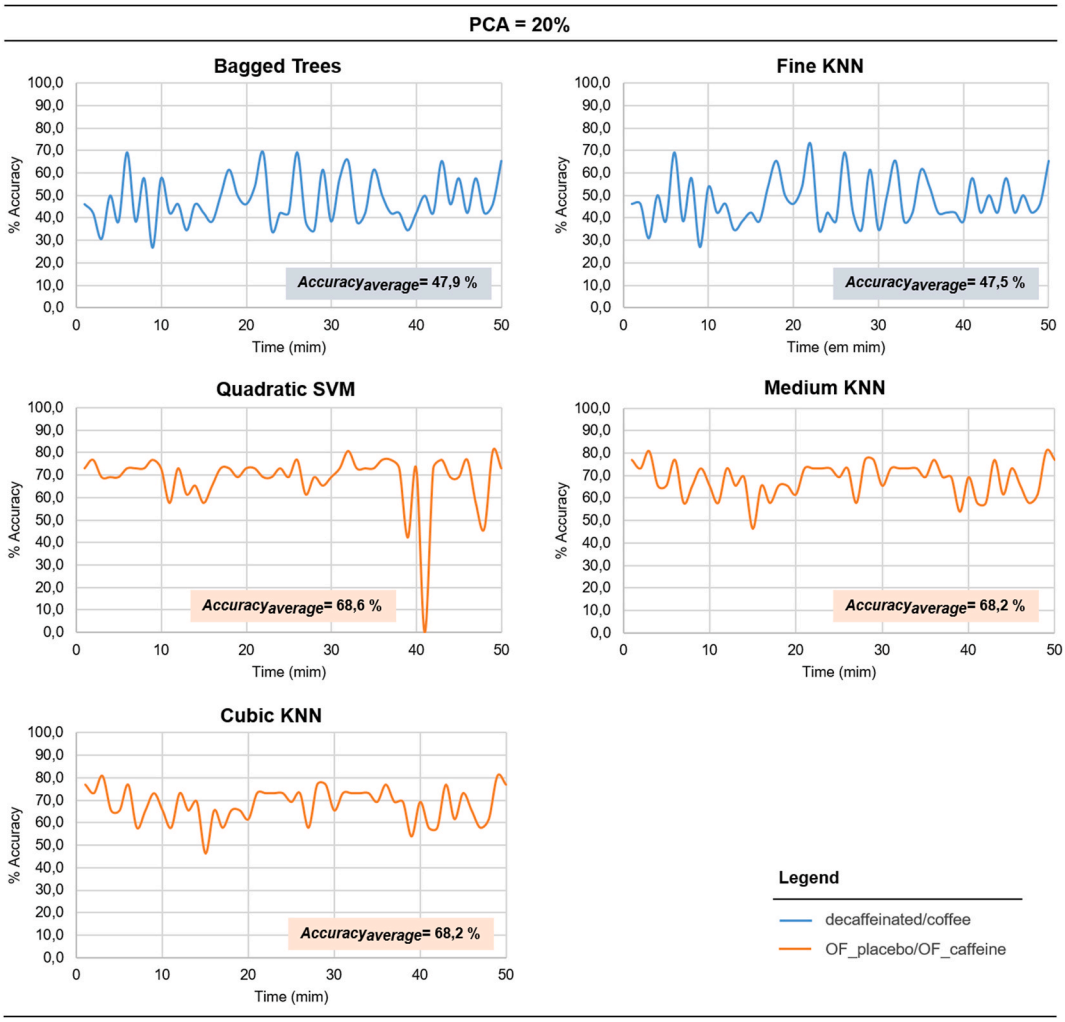

Fig. 8. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 20 %.

trigger brain responses, even without actual intake. The presence of caffeine in the oral mucosa can enhance these effects. Also, (2) biochemical effects, since the high vascularization of the oral mucosa promotes rapid absorption of a small amount of caffeine while still in the mouth, accelerating the onset of physiological effects [21,25]. Furthermore, the high amplitude of the accuracy curves again reveals a contradiction with the literature since this oscillation represents successive instants of difference and similarity between the two modalities It is known that this does not correspond to reality, since the amount of caffeine administered through coffee is fully available for absorption at the same instant.

On the other hand, the OF_placebo/OF_caffeine accuracy curves show differences between the two modalities, which are manifested by changes at the cardiac level that cannot be identified with the resources used. That is, the classification methods only reveal that there are differences between the physiological signals by comparing the metrics of the active and placebo groups. In other words, one cannot conclude about the actual behavior of caffeine at the physiological level, i.e., for example, an increase or decrease in heart rate or the manifestation of arrhythmic events. However, it allows us to assess the efficiency of the OF as an administration caffeine vehicle. Regarding the dissolution power, the high accuracy values obtained show a high dissolution power of in the first minutes, because there is free caffeine in the OF, and it can be inferred that the amount of caffeine remained high over time because there is caffeine encapsulated within microparticles, which will allow a controlled release over time.

After analyzing and discussing the accuracy curves individually, it becomes convenient to understand the interaction between the two active modalities. By analyzing the graphic presented in Fig. 12, the behavior of the trajectories affected by the administration of caffeine by coffee and OF_caffeine is very similar.

Even though the results were not as satisfactory in the decaffeinated/coffee analysis, it was possible to prove that the benefits stated about OF, especially in controlling the release rate of the active substance, effectively work with caffeine, and proving the innovative character of these OF. Caffeine OFs become extremely promising and versatile, in addition to the rapid action of caffeine in the initial

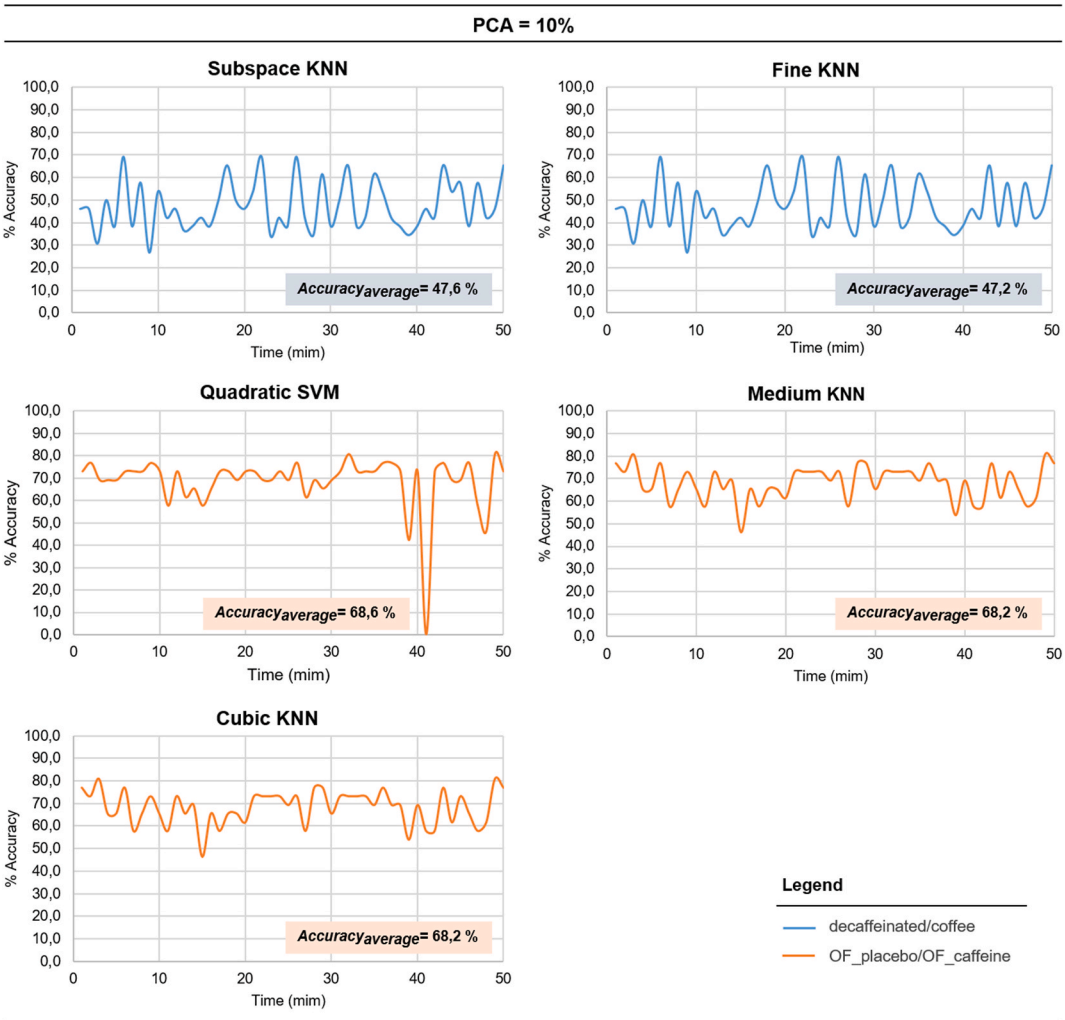

Fig. 9. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 10 %.

phase, due to the caffeine dispersed in the OF, the gradual release of the caffeine present in the microparticles will subsequently allow a gradual release of caffeine, increasing the permanence of the substance in the body for longer and avoiding its side effects. OFs can keep the organism at peak performance so they can help for instance health professionals mainly on night shifts; medical surgeons during long hours of surgery; emergency medical professionals; police, military, and firefighters during long hours of service mainly in disaster situations; top athletes; and pilots. However, it can even be interesting in other situations, such as esthetics, in the case where the person does not enjoy the sensations that drinking coffee provides in detriment to the coloring of the teeth; or even for those who do not enjoy this beverage but need its psycho-physiological effects.

5. Conclusion and future perspectives

There is a wide availability of caffeinated products in the market, stimulating the interest of the scientific community in the ways of using caffeine. OF are a pharmaceutical/nutraceutical form, which works as a delivery vehicle where several substances can be incorporated, with easy administration, fast dissolution and absorption, and high bioavailability being, therefore, a very attractive technology for caffeine delivery.

Hence, we took advantage of the influence of caffeine on the modulation of nervous system activity by tracking the variability of cardiac activity. Considering the scientific-technological age that we live within, robust signal processing methods and machine learning models were used to study binary comparisons to understand the behavior between decaffeinated/coffee and OF_placebo/OF_caffeine.

50-point accuracy curves have been traced per model, representing the model discrimination power between groups along the 50 min. The best model average accuracies obtained per each comparison group were: 50.2 % for Decaffeinated Coffee/Coffee with the

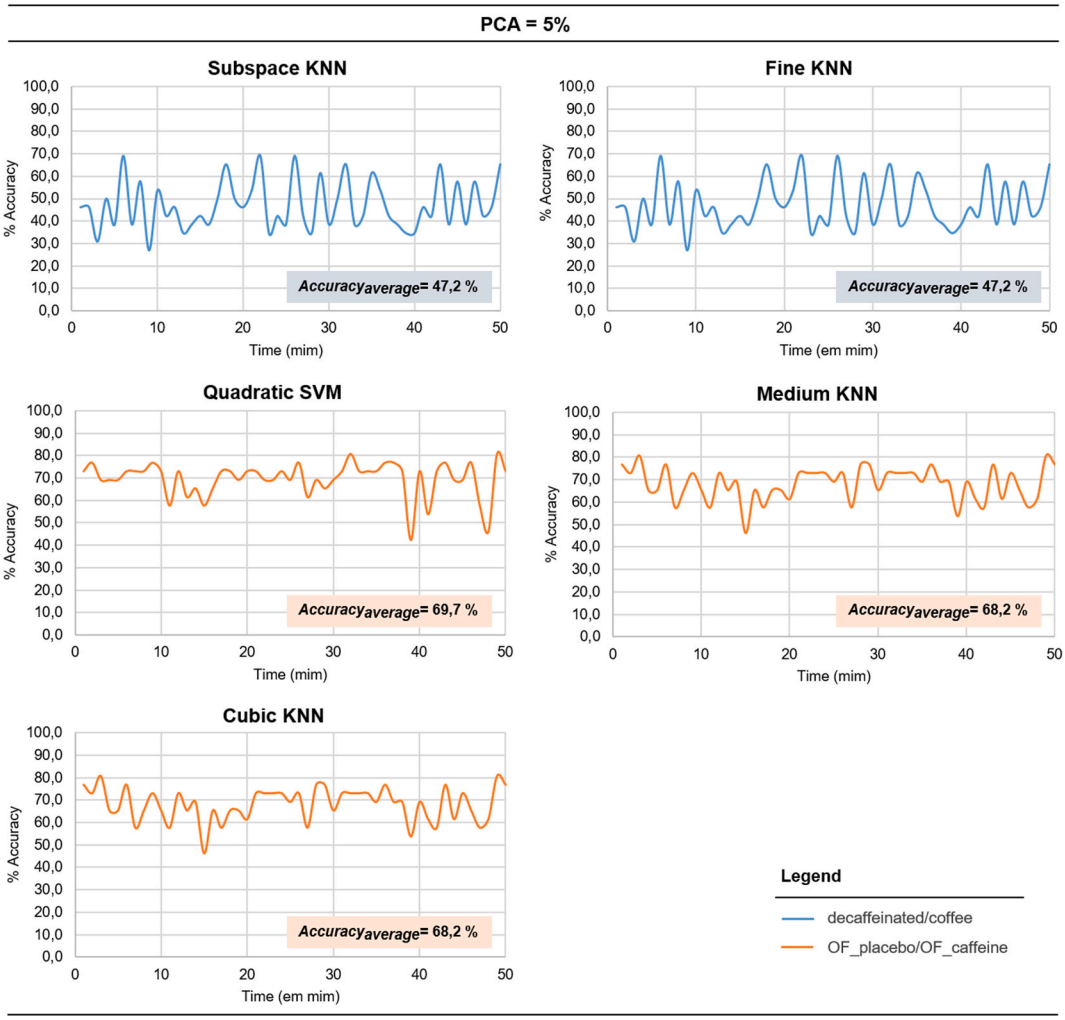

Fig. 10. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 5 %.

decision tree as classifier plus PCA 100 % and 72 % for OF_placebo/OF_caffeine with Fine Gaussian plus PCA 95 % as feature selector. Nevertheless, higher amplitude discriminatory peaks are camouflaged by the average accuracy over time. For the Decaffeinated Coffee/Coffee binary group the accuracy ranged between 30 and 70 % and for the OF_placebo/OF_caffeine it ranged between 62 and 84 %. Analyzing the respective accuracy curves, we conclude that, contrary to decaffeinated and coffee, there were significant differences between OF_placebo and OF_caffeine, namely in the rapid dissolution efficiency and in the controlled release rate over time. This finding evidence shows that caffeine delivery through OFs is indeed a promising technology.

Although the results were generally satisfactory, and the main objective of the OF study was achieved, some limitations are recognized in this methodology. Thus, the suggestions for future perspectives presented should be taken into consideration: (1) Test the algorithm on a larger and more robust database, so that the results can have statistical significance. Namely, the number of women and men should be balanced to address the lack of gender heterogeneity and consider a sample with pre-existing health conditions in terms of physiological and neuropsychological pathologies, as according with state-of-the-art caffeine promotes i.e. the risk for anxiety, hypertension, ischemic stroke, substance misuse among others [12,14,17,75]. Also, this study did not account for individual variations in caffeine metabolism and bioavailability, influenced by genetic predispositions. These genetic factors can significantly impact the cardiovascular responses to caffeine and its effectiveness. The absence of controls for genetic variability in caffeine metabolism may limit the generalizability of our findings, as the metabolic rate can affect both the magnitude and duration of caffeine's physiological effects. Future research could enhance understanding by incorporating genetic profiling to assess individual differences in caffeine metabolism; (2) Perform an individual analysis of the discriminative power of each metric, to understand which ones best describe the signal. It would be interesting to test other metrics; (3) Tune-fine the hyperparameters of each classifier used by grid search processes; (4) Only short-term measurements of cardiovascular parameters were studied, establishing a compromise between the peak plasma concentration of caffeine in the body/start of its decline and the computational time required to process the volume of data resulting

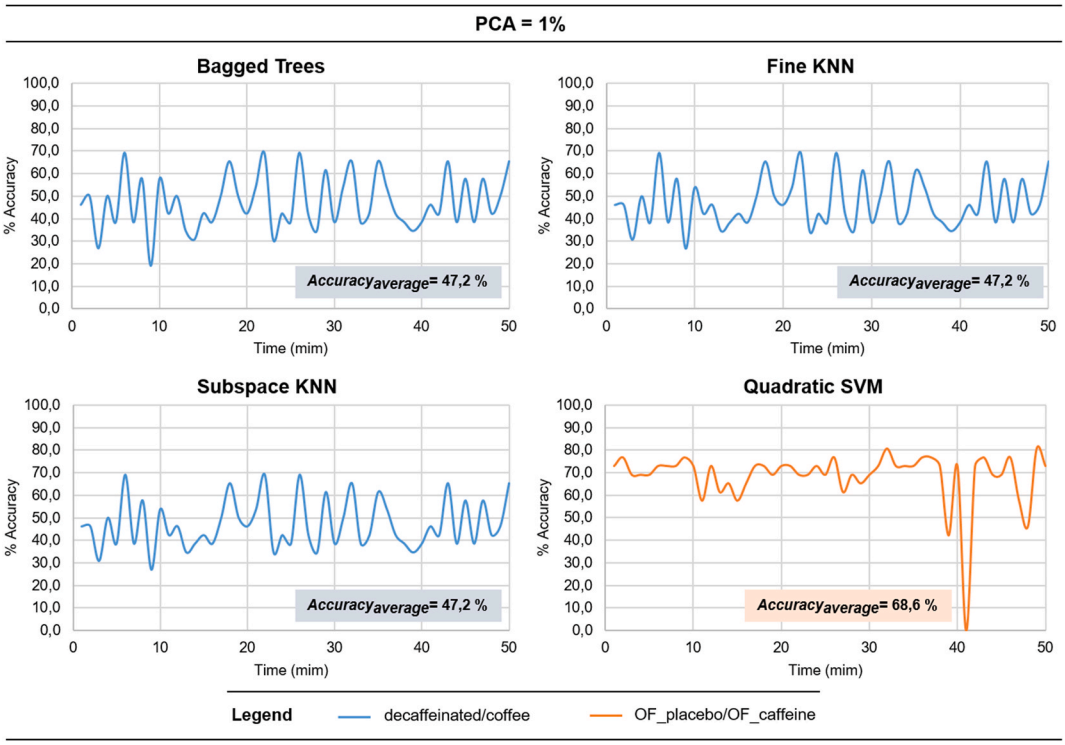

Fig. 11. 50-point accuracy curves of the classifiers with the best results for both binary classifications, when the system was trained with PCA = 1 %.

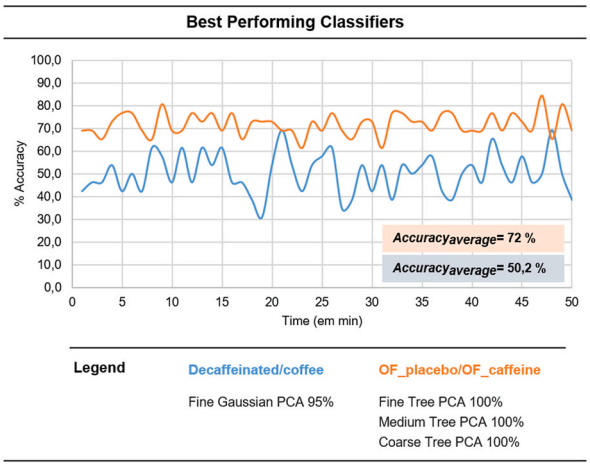

Fig. 12. Accuracy curves of the classifiers with the best research results.

from this monitoring. It would be essential to investigate the long-term physiological effects of caffeine by increasing the monitoring time to track caffeine activity in the body; (5) We will intend to refine our ML discrimination analysis by evaluating shorter periods of time, such as intervals of 10 s or even shorter, to capture more nuanced temporal dynamics and enhance the accuracy of our model's predictions reducing in this way the ML results variation registered in present study; (6) Monitoring cardiac activity using electrocardiographic signals reveals caffeine-specific patterns and it is simple to acquire. Even so, the impact of caffeine on the human organism is vast, and it would be relevant to study it at other physiological and psychological levels by combining stratified ECG data with other complementary technologies such as Electrodermal Activity (EDA), respiratory monitoring, Functional Near-Infrared Spectroscopy (fNIR), among others, enhances the depth and breadth of caffeine insights behavior over time; (7) To increase the robustness of the results, other measures of checking model's performance should be used in addition to accuracy, such as sensitivity, precision, recall, f1-score and the area under the ROC curve (AUC); To summarize, the study's results explored the effects of caffeine on

physiological signals using various machine learning classifiers to analyze the data. The study's results have important implications for developing more accurate and effective methods of monitoring the effects of caffeine on the human body. This approach also contributes to understanding the mechanisms underlying caffeine's effects on physiological signals. It is helpful for researchers studying the effects of caffeine on human physiology, particularly concerning cardiovascular and autonomic responses. Those working in biomedical engineering, signal processing, and machine learning interested in developing and evaluating algorithms for analyzing physiological signals will also contribute to this study. The food and beverage industry, particularly coffee producers, as it sheds light on the differences between decaffeinated and caffeinated coffee and the efficiency of as a vehicle for caffeine delivery, will also benefit from the evidence in this paper. Finally, consumers interested in understanding caffeine's effects on their bodies may find this valuable approach as it provides a way to analyze the physiological signals related to caffeine consumption. As a result, consumers may better understand how their bodies respond to caffeine and how long the effects of caffeine last. This information could help consumers make more informed decisions about their caffeine consumption, such as when to consume caffeine and the most appropriate amount for their bodies. Additionally, by understanding the differences between caffeinated and decaffeinated coffee, consumers may be able to choose the option that best suits their needs and preferences. Overall, this approach allows researchers, producers, and consumers to gain insights into caffeine consumption and make more informed decisions about caffeine-related issues.

Data availability

Data will be made available on request.

Statements of ethical approval

The study complied with all ethical procedures required by the institutions involved, also the principles expressed in the Declaration of Helsinki. Furthermore, the project also relies on a favorable ethical opinion carried out by the "Ethics Lab" of the "Institute of Bioethics" of the Universidade Católica Portuguesa (reference number 05/2018). Additionally, the project received a favorable opinion from the "National Data Protection Commission" (Proc. No. 6129/2018 of May 14, 2018).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Rita Domingues: Writing – original draft, Investigation, Conceptualization. **Patrícia Batista:** Writing – review & editing, Validation. **Manuela Pintado:** Writing – review & editing, Validation. **Patrícia Oliveira-Silva:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Conceptualization. **Pedro Miguel Rodrigues:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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