Aflatoxin detection on direction of the 4.0 age at 3.0 costs

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Abstract

Articulate the most diverse and sophisticated technologies, such as Remote Sensing, Big Data, Cloud Computing, Internet of Things, 3D Printing, among others, is part of universe 4.0, whether industrial or agricultural. Focusing on agricultural context, this paper proposes a low-cost 4.0 device to perform the monitoring and control of certain environmental variables for the detection of aflatoxins in peanut crops. Aflatoxins are toxic metabolite of fungi genus Aspergillus that can cause toxic and carcinogenic effects in humans and animals. The device developed was able to monitor temperature and humidity variations helping the aflatoxins identification. The equipment portability allows its use in silos with encapsulation via Additive Manufacturing, besides the aflatoxin prediction from Machine Learning algorithms.

Keywords: Agriculture 4.0; Remote Sensing; Peanut Culture; Aflatoxin.

1. Introduction

The industry has been going through a series of revolutions since the Industrial Revolution of the eighteenth-century, which is recognized as Industry 1.0. Then, electricity has been the guiding element with the emergence of steam engines (in Industry 2.0) leading to development of the electronics area using programming languages to machinery automation (Industry 3.0), which has evolved into Industry 4.0.

Industry 4.0 has gaining attention in Brazilian research (government) programs composing advances to interrelate physical, digital and biological elements [1]. Artificial Intelligence, Additive Manufacturing (or 3D Printing), Big Data, IoT (Internet of Things), Cloud Computing, Synthetic Biology and Cyber Physical Systems (CPS) are the main technologies helping such a fusion [1], [2].

The principle of Industry 4.0 has naturally reached sectors of agriculture and livestock. The use of sensors and devices is established in agricultural production due to the interrelationship of (wireless) network structures that are controlled and monitored via specialized algorithms aiding in decision-making. These mechanisms (in the context of the so called Agriculture 4.0) are becoming an increasingly need for agricultural producers [1].

Peanut production is a highlight in Agriculture 4.0, particularly in the city of Tupã, in the São Paulo state, Brazil, which accounts for 23% from total of exports of this product. Such a region of Alta Paulista presents 43% of national exports among the U$ 10 million in regional agriculture [3]. However, peanuts are food subject to contamination by fungi which produce toxins and aflatoxins from the catabolism of carbohydrates and proteins [4]. National legislation allows up to 20 $\mu g\ Kg^{-1}$ and 50 $\mu g\ Kg^{-1}$ aflatoxin for human and animal consumption, respectively. However, food for export, mainly to Europe, cannot present a limit higher than 4,0 $\mu g\ Kg^{-1}$.

Aflatoxin is a toxic metabolite of fungi genus *Aspergillus* that can cause toxic and carcinogenic effects in humans and animals. Aflatoxins B1, G1, B2 and G2 are the main types of these toxins among more than 18 of these compounds. Aflatoxin B1 is considered the most carcinogenic natural agent [5].

In the face of demand for quality in peanut production, specifically from farmers inserted in the region of Alta Paulista, this work makes room for the teacher-student-society relationship regarding the exchange of ideas and learning through simultaneous and transformative actions among the constituents university and society, focusing on solutions for small farmers.

This paper describes the development of a low-cost educational device for the detection of peanuts' contamination by aflatoxin, which is composed by temperature and humidity sensors aiming to help low-income farmers.

2. Background

2.1 Industry 4.0

The term Industry 4.0 refers to a handful data-based technologies that allow the combination of physical and digital worlds, naturally combining sustainability and corporate social responsibility with advances based on Big Data, Machine Learning, Internet of Things (IoT), and Cyber-Physical Systems [6]. In this context, the development of parts and objects from the successive layered sequence of various materials such as polymers is defined as Additive or 3D Printed Manufacturing [7].

Considering embedded systems in technology 4.0, essentially composed of electronic devices, a point to be approached is the type of encapsulation to be employed, especially if the purpose is to monitor an agricultural environment. In this context, additive manufacture is capable of designing and printing parts and objects of the most varied and complex forms.

In the digital era, in which plenty devices capture users' data - even from everyday activities like (virtual) games, household chores, or sports - the increase of computers' power in terms of data storage (and processing) has become not only essential, but also a viable reality. Especially, with hardware advances, companies can capture a huge quantity of (more and more detailed) information about their customers, suppliers, and operations, whereas mobile phones, multimedia devices, radio-frequency identification (RFID) readers, and wireless networks are creating and communicating data to computers and/or each other [8].

As a result, the nowadays dynamics of collecting and generating data far exceeds the human ability to analyze and extract knowledge from them [9], [10]. Also, generated data are typically complex, have no apparent patterns and change at enormous speed because of the dynamics of the network itself. From this scenario, the term "Big Data" emerged, emphasizing ways to computationally overcome the problem of deal with these data [11]. While Internet of Things (IoT) is based on the network communication of any physical object [12]; basically, a system consisting of everyday objects that interact through network connections only makes sense when spreading its links connecting the above mentioned technologies to a more sustainable World perspective, with efficient use of our natural resources.

2.2 Aflatoxin in peanut culture

In Brazil, the most frequently encountered aflatoxins are those of type B1 and B2, according to H. Fonseca, et. al.[13], the tolerable limit of aflatoxin type B1 + G1 in kg of peanut was 30 μg, ie 30 μg/kg. ANVISA [14] published a resolution with the tolerable limit of aflatoxin types B1, B2, G1 and G2 at 20 μg/kg.

According to K. B. Imamura, et. al. [15] observed that, between 2010 and 2012, Arachis hypogaea L raw peanut crop from the region of Alta Paulista/SP, showed aflatoxin contamination rates from 2010 to 2011 of 21-52 mg/kg, which in 2011-2012 increased by five times, ie 28-260 mg/kg.

Aspergillus sp. shows cell growth and active metabolism at low hygroscopic grain equilibrium between 13.5 and 18 (% b.u.) [16]. Therefore, the final moisture contents after the drying stage of the pods should be less than 10%, to allow a longer storage time. Thus, upon reaching the average of three moistures below 9%, provided that none of the measures to be greater than 11.5% moisture drying step is considered complete and is then terminated.

Cultures in tropical and subtropical areas are more prone to contamination. The best conditions for the development of fungi and thus for the production of aflatoxin are found in areas with high temperatures (25 to 30 ° C) and high humidity (80-90%). The storage in humid and airless, as well as inadequate transport, favoring not only contamination with spores but also fungal growth in the already contaminated products. Thus, the balance between temperature and humidity in the storage environment should be monitored taking into account the areas of silos and sheds that can reach higher temperatures and humidities and favor the growth of microorganisms and their mycotoxins.

The values of equilibrium moisture content of biological products depend on several factors such as temperature and relative humidity, the species and/or variety of the product and its physiological maturity, the product's history and the manner in which equilibrium was obtained. The importance of obtaining experimental data on the hygroscopic balance of agricultural products is well known. Considering the importance of this concept, in the numerous applications in the field of drying, storage and handling of agricultural products, efforts have been made to obtain equations expressing the equilibrium moisture content for each product, in environmental factors.

Among the various equations used to express the of grain moisture, as a function of temperature and relative humidity, for wider ranges of temperature and relative humidity, we find the Henderson equation:

$$U_e = \frac{1}{100}\left[\frac{ln(1-0{,}01UR_e)}{-K(T+C)}\right]^{\frac{1}{N}}, \qquad (1)$$

where T is temperature in °C, UR is a relative humidity in %, K, C and N are constants defined for each type of grain [17].

Tables 1 and 2 show the equilibrium moisture values for the peanut according to the temperature (°C) and relative humidity (%) of the air in two different conditions: peanut in the pod and benefited, respectively. As already mentioned, above 9% moisture, the grains already present risks of manifesting the fungus and consequently its mycotoxin. At temperatures of 15 °C and relative humidity 50% (environment) the grains are already in conditions for fungal growth, as seen in Tables 1 and 2.

Table 1. Equilibrium moisture (Ue) to peanut pod depending on the temperature and relative humidity.

	Relative humidity (%)			
Temperature (°C)	50	60	70	80
10	8.8	9.7	10.7	11.8
15	**8.4**	9.2	10.2	11.3
20	**8**	8.8	9.7	10.8
25	**7.7**	**8.5**	9.4	10.4
30	7.4	**8.2**	9	10
35	7.2	**7.9**	8.8	9.7
40	7	**7.7**	8.5	9.4
45	6.8	7.5	**8.3**	9.2

Conditions highlighted in bold correspond to the range of 0.5% above and below the ideal moisture content for commercialization (8.0%).

Table 2: Equilibrium humidity for the peanut benefited in the function of the temperature and relative humidity of the air.

	Relative humidity (%)			
Temperature (°C)	50	60	70	80
10	6.4	**7.6**	8.9	10.6
15	6	7.2	8.5	10.1
20	5.8	6.9	**8.1**	9.7
25	5.5	6.6	**7.8**	9.3
30	5.3	6.3	7.5	9
35	5.1	6.1	7.2	8.6
40	4.9	5.9	7	**8.3**
45	4.8	5.7	6.7	**8.1**

Conditions highlighted in bold correspond to the range of 0.5% above and below the ideal moisture content for commercialization (8.0%).

3. Material and methods

In order to detect the variables temperature and humidity, the device is divided into a central module comprises a microcontroller and four others peripheral, power supply, sensors and a man-machine interface, illustrated in Figure 1a.

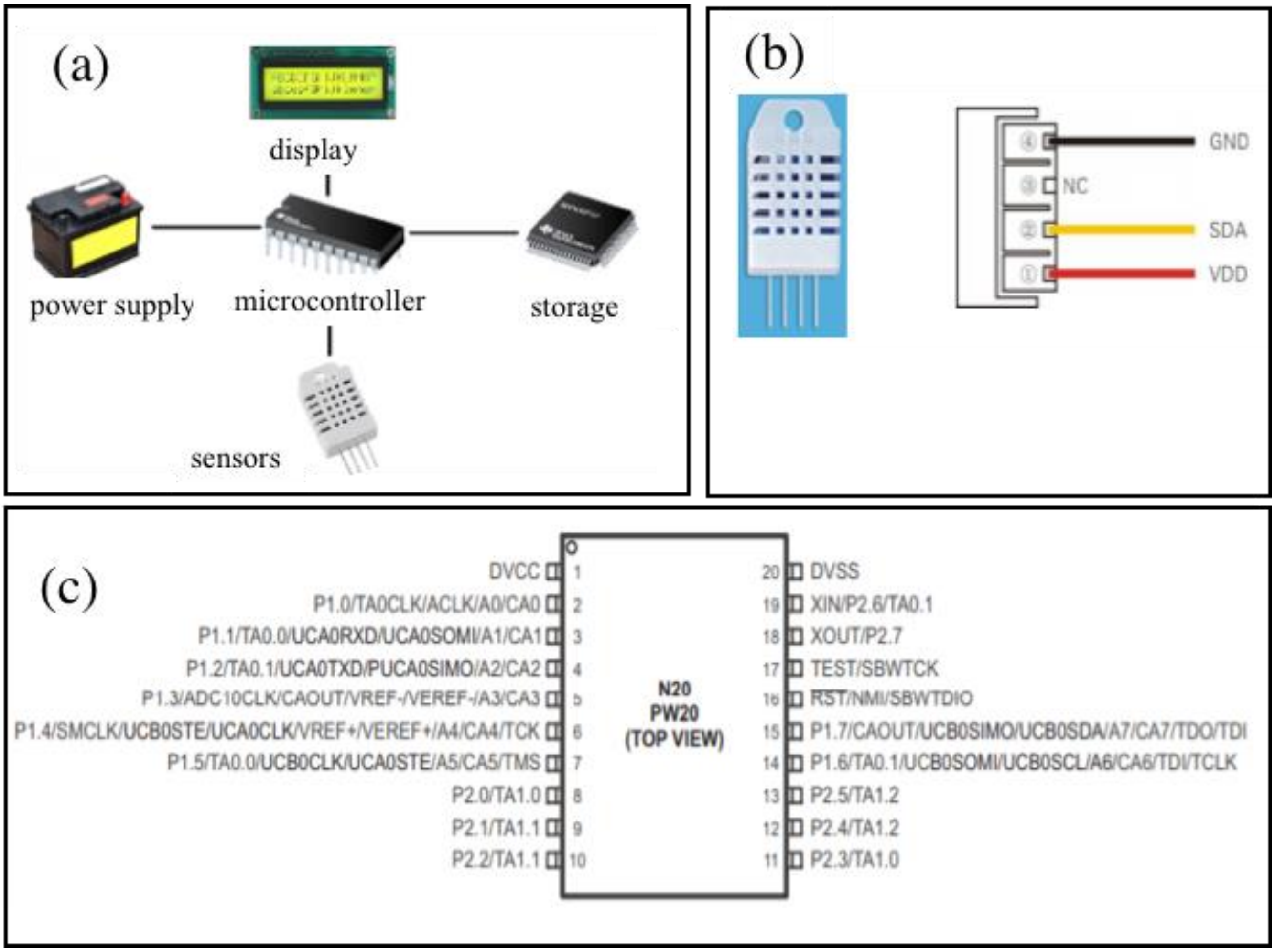


Figure 1 – (a) device architecture, DHT22 sensor and (c) microcontroller Pinout MSP430G2553IN20, developed by the authors.

The central module consists of a mixed signal microcontroller MSP430G2553IN20, including analog/digital circuits and microprocessor on a single chip. Besides, the analog-digital converter, timer, and direct memory access are built into the module. Regarding integrated communication capability, this microcontroller uses a universal serial communication interface supporting UART, I2C and SPI protocol standards, as shown in Figure 1b.

The sensors used in the device are of the type DHT22 - AM2302, of high precision to measure relative humidity and temperature. It operates in the 3.3 to 5.5 V voltage range; the temperature is from -10 °C to +80 °C and relative humidity from 0 to 100%. The error range is ± 0.5 °C and ± 0.2% for temperature and humidity, respectively.

The physical encapsulation and the pin assignments of the sensor are shown in Figure 1c, SDA being the serial acquisition of data; GND grounding; NC pin floating or GND and VDD supply. The communication between the microcontroller and sensors was performed using the I^2C protocol. The man-machine interface was made by an LCD display for integrating standardized electrical interfaces, allowing a greater permutation and variability of equipment, making it more accessible. The display is supplied with a voltage of 5V and is integrated with a backlight system that allows reading in low light environments. An

MMBT2222 transistor was used to turn off the backlight of the device, thus increasing battery life. Energy saving is the most important factor in this work, as it will be used in peanut plantations and in remote grain storage sites.

Two voltage regulators, TPS70950 connected to 10 and 0.1 μf capacitors were connected, regulating the output at 5V and MCP1700, to a 3.3 V output, with capacitors of 10 and 0.1 μf connected at the input and output of the component. A Bluetooth device was attached to the data transmission remotely.

Initially the device mounted on a protoboard to check the behavior of the components in the circuit: sensors, display, microcontroller MSP430G2553IN20, potentiometer (display blacklight adjustment), a launchpad MSP-EXP430FR5994 to transfer the settings to the microcontroller of the device (see Figure 2a). After the tests, we started the development of circuit board in CircuitMaker software, which offers two types of files to the project: PCB, boards Prototyping Printed Circuit (see Figure 2b) and schematic, file in which designed the wiring diagram of the device (see Figure 2c). After the design of the board, all components of the circuit were placed inside a box, making the device portable allowing us to carry out measurements in different environments (see Figure 2d).

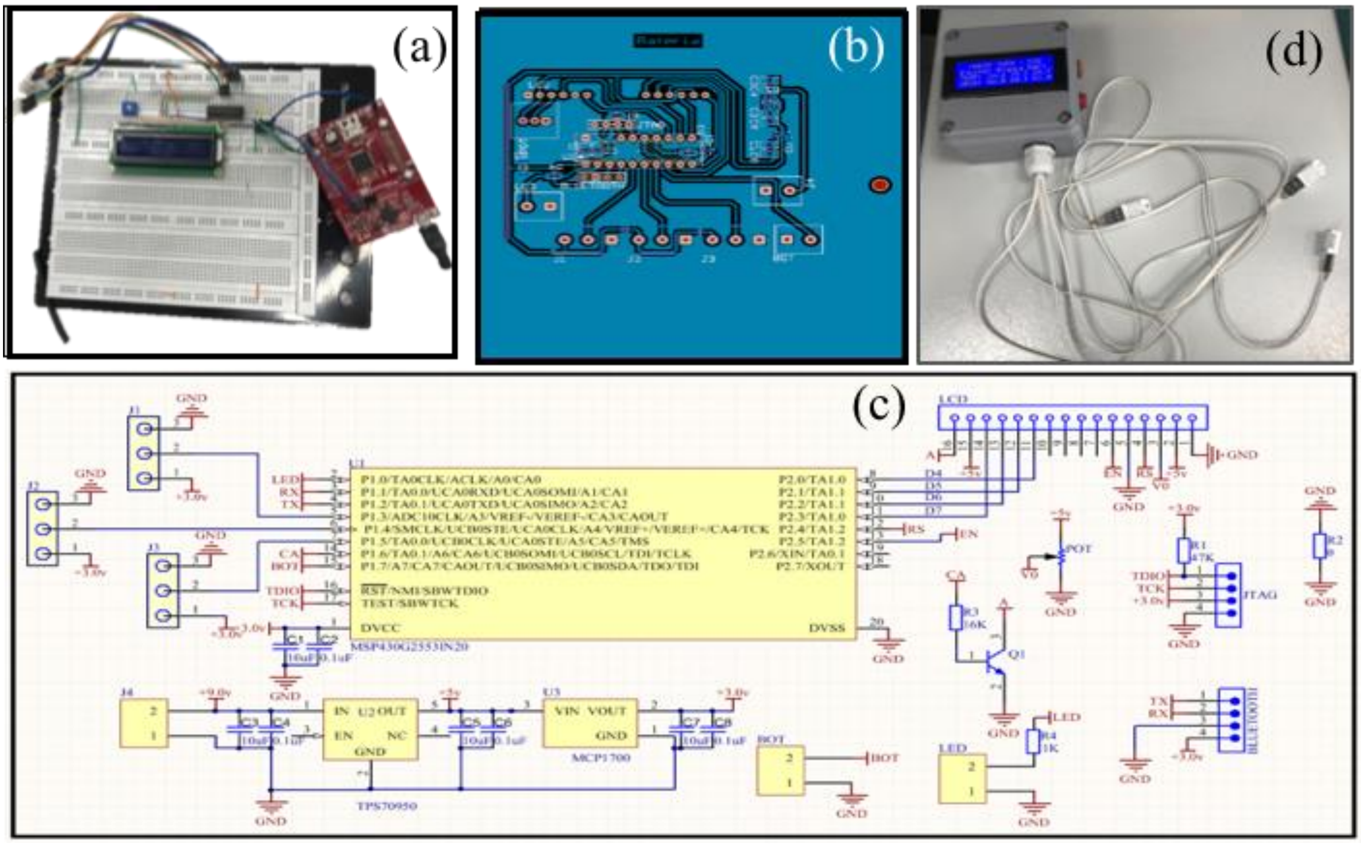
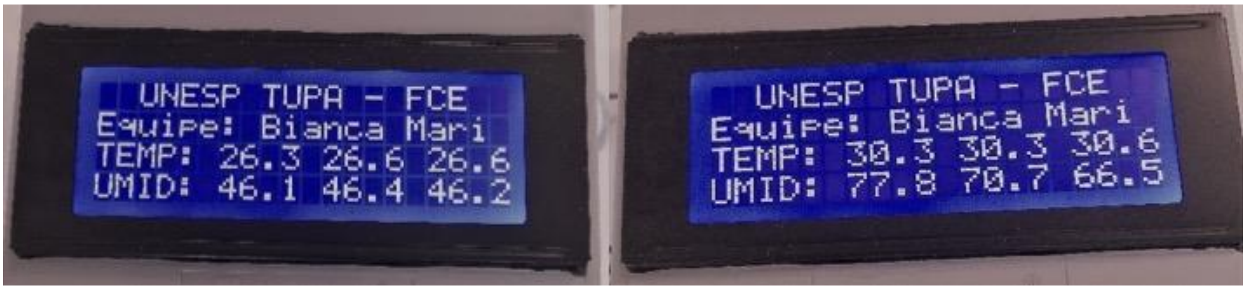


Figure 2 – (a) Circuit mounted on a test board. (b) Project transferred to a printed circuit board, after the tests, (c) schematic figure of the plate constituting the final device and (d) final portable device. Developed by the authors.

4. Laboratory tests and future work.

The optimum temperature for aflatoxin production is influenced by the type of substrate. In peanuts, nutrient solution, the optimum temperature for aflatoxin production by *Aspergillus flavus* was 25 °C in the incubation period of 7 to 9 days. High levels of aflatoxin in peanuts were produced at 25 to 30 °C for 7 and 15 days of incubation, occurring in the same liquid medium. Aflatoxin production by *Aspergillus parasiticus* reached a higher level at temperatures of 20, 25 and 30 °C, in the period from 5 to 21 days. The highest levels were observed at 25 and 30 °C at 7 and 15 days of incubation [18].

Tests were performed using the equipment shown in Figure 2d, with its set of three temperature and humidity sensors to detect differences in these parameters in an experimental way. The tests were carried out in B.O.D. incubator incubators with temperatures in the range of -10 to 60 ºC and were developed for experimental situations that require temperature control, for incubation work, determination of pollution levels, tests for early aging of seeds, storage of materials. The tests verified that the sensor set was able to monitor satisfactorily tested temperature variations, as shown in Figure 3 below.



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UNESP TUPA - FCE
Equipe: Bianca Mari
TEMP: 26.3 26.6 26.6
UMID: 46.1 46.4 46.2
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UNESP TUPA - FCE
Equipe: Bianca Mari
TEMP: 30.3 30.3 30.6
UMID: 77.8 70.7 66.5
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Figure 3 – Instrument display for measuring temperature and humidity at two different times.

From these scans we saw the potential to carry out these measurements in place to monitor temperature and moisture in stored grain. In the future, we intend transmit data using Bluetooth network (for remote locations and without access to the communications network) and wireless; we aim to insert it in peanut silos in order to monitor data that may assist in the proliferation and detection of aflatoxin in peanut production, embedded in a spherical capsule designed and executed in a 3D printer and, finally, to implement the measured data in a set of attributes inherent to the contamination of the peanut by the aflatoxin, in order to elaborate its automatic detection by means of Machine Learning-based methods.

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