Alfalfa Leaf Powder and its Potential Utilisation in Raw Vegan Chocolate

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Abstract

Utilization of new or unusual sources of nutrients has led to a wider market regarding food product diversity. The utilization of alfalfa in food products leads to an increase in nutrients as proteins, from macro elementary point of view and in polyphenols, chlorophylls, and carotenoids if is to look in the micro elementary system. The present paper proposes the increase of the bioavailability of alfalfa powder by adding in a raw vegan chocolate. Characteristics as total phenolic content, antioxidant activity, FTIR fingerprint, and antimicrobial activity were investigated.

Keywords: alfalfa, antioxidant, phenol content, raw chocolate.

Introduction

Chocolate can be defined as a product composed of cocoa butter, cocoa mass and sugar (Andarea-Nightingale et al, 2009). It may contain other various and ingenious ingredients as from hazelnuts to chilly pepper and probiotics. Chocolate categories are known as dark, milk and white that is different regarding their content of cocoa solids, milk fat, and cocoa butter. From the colloidal point of view, chocolates can be characterized as semisolid suspensions of fine solid particles of sugar and cocoa; representing about 70% in total, in a continuous fat phase (Afaokwa, 2010). Chocolate is a food product consumed all over the world, by all social classes and by people of all ages. This food product is and have been so in vogue and popular due to its potential to awake sensory pleasure and beneficial emotions (El-Kalyoubi et al., 2011).

Scientists, food producers, and health authorities are constantly searching for ways to innovate and tailor novel and functional products in order to stay competitive, promote a healthy lifestyle and meet consumer demands (De Pelsmaeker et al., 2015). Functional food products have acquired fame in the market, with a significant number of food products being developed and studied every day (Morato et al., 2015). In recent years, influenced by increased demands for healthy products the chocolate industry has broth to the market many functional chocolates. Due to the fact that chocolates are food with broad acceptance and wide consumption their enrichment with ingredients that potentially improve the consumers' health is a good strategy. A relevant number of recent studies point towards this trend.

Enrichment of chocolate with an innovative and healthy compound may increase consumer acceptance and promote health.

As an ordinary perennial vegetable, alfalfa is widely cultivated as a forage crop. Because of the important protein content (about 15–20%), this plant is used by the green crop drying business

as material for obtaining fodder pellets for cattle (Hadidi et al., 2019). Nevertheless, alfalfa is also accepted as an important raw material for human protein intake and other various utilizations (Pop et al., 2016). The continuous growth of the world population is directing to raises in the need for sources of cheap and appropriate plant proteins to supplement or even replace expensive and environmental unfriendly sources of animal protein (Woldesellasse et al., 2018). Several characteristics of the alfalfa plant make it less suitable for human food utilization. Among these, consumer perception and presentation form are the most important ones. Thus, the authors have decided to use a worldwide loved desert in order to make the alfalfa leaf powder more bioavailable for the human body.

The present research describes the characterization of functional chocolate containing Alfalfa leaves powder. Characteristics as total phenolic content were investigated among with its, antimicrobial and antioxidant activities.

Materials and methods

The alfalfa powder was purchase from a local market from Cluj-Napoca and kept it its original pack at room temperature in a dry environment prior to use. DPPH, Folin-Ciocalteu's phenol reagents were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used in this experiment were of analytical grade and purchased from the same provider. All the stock solutions were prepared by using distilled water.

Alfalfa raw vegan chocolate production

In order to obtain the proposed food ingredients like cacao butter, cacao, stevia, and alfalfa powder were utilized. All the ingredients were heat treated under 40°C.

Fourier transform infrared spectroscopy (FTIR)

This technique was utilized to characterize alfalfa powder. Was used an IRPrestige-21 FTIR Spectrometer from Shimadzu (Berlin, Germany), equipped with the MIRacle single reflection horizontal attenuated total reflectance accessory, with a single reflection crystal plate and a high-pressure clamp. Each spectrum was from 3,600 to 600 cm^{-1}.

Ultrasound-Assisted Extraction of Flavonoids and Antioxidants from Alfalfa

Ultrasonic-assisted extraction was performed in a digitally-controlled ultrasonic bath (KQ3200E, Kunshan Ultrasonic Instrument Co. Jiangsu, China). Sample of 3 g was placed into a flask (250 mL), soaked with ethanol solvent at the given concentration in a scheduled ratio of liquid to solid and then placed in an ultrasonic cleaning bath at 40 kHz for a certain time at a constant temperature. Extracts were filtered through a filter paper under vacuum and the residue was extracted again (three times) with the same volume of fresh solvent. Then, filtrates were combined and concentrated using a rotary evaporator at 50 °C under vacuum. Finally, the filtrate was prepared to a constant volume (150 mL) using 60% ethanol for estimation of flavonoids and antioxidant measurements through various chemical assays.

Determination of Total Phenolic Content

Total phenolic content from extracts was measured according to the Folin-Ciocalteu procedure, as described by (Ainsworth, 2007). The extract was measured at absorbance of 756 nm. Measurements were calibrated to a standard curve of prepared gallic acid solution ranging from 0–0.2 mg/mL with y = 4.214x – 0.0118,

(R2 = 0.999) and the results was then expressed as mg of gallic acid equivalents (GAE) per g of dry crude extracts.

TPC =the phenolic content of extracts (mg GAE) / weight of dried extracts (g)

Determination of Antioxidant Activity

DPPH Radical Scavenging Capacity Measurement

The radical-scavenging ability of DPPH was measured according to the method of (Sharma, 2009). Thus, the DPPH radical solution was produced by gently mixing 0.1 mM DPPH solution (0.75 mL) and the sample solution (1.5 mL) with various concentrations. This was allowed to stand in the dark for 30 min, and absorbance was measured at 517 nm. The free radical scavenging activity was calculated as following:

Scavenging effect % = 1 −A1 − A2 (1)

A0 × 100% (2)

where A1 was the absorbance of the sample, A0 was the absorbance of the solvent control, and A2 was the absorbance of the reagent blank without DPPH.

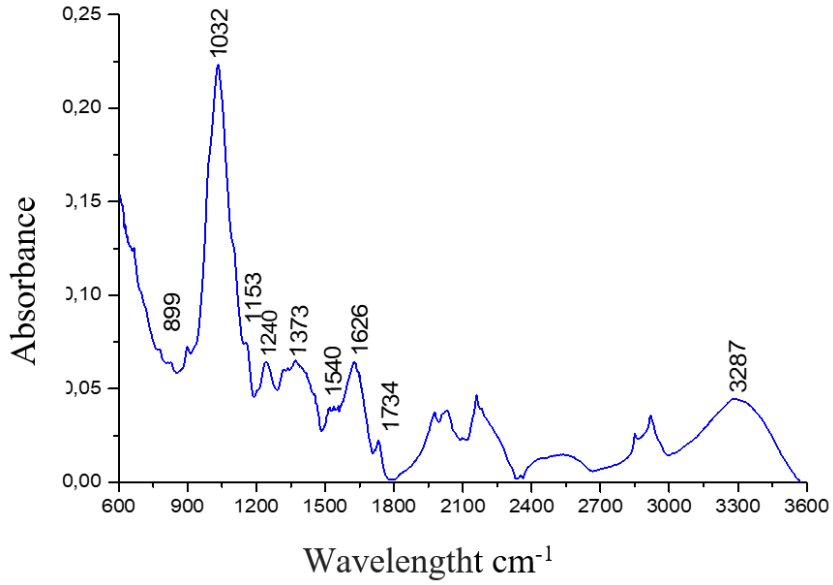

Figure 1. FTIR absorbance spectrum of alfalfa powder (600-3600 cm^{-1})

Antimicrobial activity of alfalfa powder extract

Antibacterial activity of the tested solution was performed by using the resazurin assay. The four pathogenic strains used in the study were *Escherichia coli* ATC 25922 (Microbiologics), *Salmonella typhimurium* ATCC 14028 (Microbiologics), *Listeria monocytogenes* ATCC 35152 (Liofilclem), and *Staphylococcus aureus* ATCC 65389 (MediTech).

Minimum Inhibitory Concentration (MIC)

Broth dilution tests were conducted in standard trays containing 96 wells in order to evaluate the MIC of the solution. In order to obtain sequential dilutions, 100 µl of the alfalfa powder extract (in acetone and ethanol) was mixed with 100 µl of nutrient broth, mixed well and then 100 µl of the mixture was transfused in the next well containing 100 µl nutrient broth. The final volume was 110 µl in each well after we added 10 µl of 24 h old bacteria inoculum (~105), and allow to grow overnight at 37°C for 24h. Afterwards, 30 µl of resazurin solution was added to each well, and the plate was re-incubated for 2 more hours. The MIC value was the lowest concentration of the solution that did not permit any visible growth of the pathogenic. A change from blue to pink indicates a reduction of resazurin and therefore bacterial growth. A change in color from blue to pink indicated the growth of bacteria, and the minimal inhibitory concentration (MIC) was defined as the lowest concentration of the alfalfa leaf powder that prevented this change in color.

Results and discussions

Fourier transform infrared spectroscopy

The FTIR spectrum of Alfalfa powder (Figure 1) reveals a specific band at approximately 3300 cm^{-1} that corresponds to O-H stretching and N-H stretching vibrations. The absorption band that stands out in the infrared region with an average wavelength between 1800 and 600 cm^{-1} has been shown to be useful for identifying polysaccharides with different structure and configurations (Kacøuráková 2008, Wilson 2000). The absorption band around 1730 cm^{-1} corresponds to the carbonyl (C=O) and the carboxylic (COOH) group, giving the presence of ester compounds generally present in the pectin shell.

Two protein uptake bands at about 1626 (C = O); 1540 (N-H); and 1240 (C-N) cm^{-1} were assigned as amide (I), (II), and (III), respectively.

Carbohydrates have been shown to be the majority constituents according to the absorption bands obtained. The strips lying between 1200 and 900 cm^{-1} indicate stretches of C-O, C-C or C-O-C linkages that are frequently found in polysaccharides (celluloses, hemicelluloses and pectins)

Total Phenolic Content and Antioxidant Activity

The total phenolic content and the antioxidant activity was determined for the alfalfa powder and

Table. 1. The content of total polyphenols (mean ± S.E. in g) and antioxidant activity of alfalfa leaf powder and of the raw vegan alfalfa chocolate

Sample	Mg Gallic Acid Eq/100g sample	F (mM) Trolox/1g sample
Alfalfa leaf powder	142±92,5	24.690±0,6
Raw vegan alfalfa chocolate	571±35,1	102.437±24,1

also for the final product, the alfalfa chocolate. The phenolic content from the chocolate was influenced by the presence of these compounds in other ingredients than the alfalfa powder. The DPPH assay was utilized in order to evaluate the antioxidant activity of the alfalfa powder extract and the extract from the chocolate containing the valuable ingredient. Table 1 shows the total phenolic content and antioxidant activity of the alfalfa powder and of the final product.

Minimum Inhibitory Concentration (MIC)

The alfalfa powder exhibited some antibacterial action, which was more profound against L. monocytogenes and ordinary against E. coli and S. aureus. A better antimicrobial activity was evidenced for the ethanol extract. The methanol extract showed almost no antimicrobial activity, with modest results against B. subtilis. Our results are consistent with previously reported MIC values concerned with E. coli and ranged between 100 and 250 mg/mL (Kobbi et al. 2015), although in other studies MIC values as low as 75 mg/mL and 40 mg/mL (Joy, 2014) have been observed.

Conclusion

The present paper revealed results that sustain more and more the utilization of alfalfa powder in proved the presence of valuable compounds like polyphenols, chlorophyll, carotenoids, and compounds with antimicrobial effect.

Further investigations need to be conducted regarding the nutritional improvement of the food products by alfalfa powder addition and regarding the customer acceptance of such products. Overall, the utilization of cheap and convenient ingredients, that bring an added value to food products is an excellent alternative for undeveloped countries and for the environment.

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