Wet-coffee processing production wastes: Quality, Potentials, and Valorization Opportunities

---

vorgelegt von
Gustavo Adolfo Figueroa Campos

Dissertation
zur Erlangung des akademischen Grades
"doctor rerum naturalium"
(Dr. rer. nat.)
in der Wissenschaftsdisziplin „Lebensmittelchemie”

eingereicht an der Mathematisch-Naturwissenschaftlichen Fakultät
Institut für Ernährungswissenschaft
Universität Potsdam

Potsdam, 14.07.2022

Hauptbetreuer: apl. Prof. Dr. Harshadrai M. Rawel
Zweitbetreuer: Prof. Dr. Andreas Taubert
Gutachter: Prof. Dr. Dennis Salvador Argueta Mayorga

Published online on the
Publication Server of the University of Potsdam:
https://doi.org/10.25932/publishup-55882
https://nbn-resolving.org/urn:nbn:de:kobv:517-opus4-558828

Content

Abbreviations

11S	Storage protein from C. arabica
AUC	Area under the curve
BOD	Biological Oxygen Demand
BSA	Bovine Serum Albumin
COD	Chemical Oxygen Demand
CQA	Caffeoylquinic Acid
CB	Coffee Beans
GCB	Green Coffee Beans
CP	Coffee Pulp
CW	Coffee Wastewater
DAD	Diode array detector
DCB	Depulped Coffee Beans
DMSO	Dimethyl Sulfoxide
DNS	3,5-Dinitrosalicylic Acid
DO	Dissolved Oxygen
DTE	Dithioerythritol
DW	Dry Weight
DCW	Depulping Coffee Wastewater
ESI	Electrospray ionization
ESI-MS	Electrospray Mass Spectrometry
FAN	Free Amino Nitrogen
FCW	Fermentation Coffee Wastewater
FRAP	Ferric Reducing Ability of Plasma
FE	FRAP Equivalent
FQA	Feruloylquinic Acid
FFCB	Final Fermentation Coffee Beans
GAE	Gallic Acid Equivalent
GCB	Green Coffee Beans
GE	Glucose Equivalent
HPLC	High Performance Liquid Chromatography
HPLC-MS/MS	HPLC coupled tandem Mass Spectrometer
IFCB	Initial Fermentation Coffee Beans
LE	Leucin Equivalent

MALDI-TOF-MS	Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
MRM	Multiple Reaction Monitoring
PAGE	Polyacrylamide Gel Electrophoresis
PVPP	Polyinylpolypyrrolidon
P	Parchment
PCB	Parchment Coffee Beans
RCW	Reception Coffee Wastewater
SK	Silverskin
SDS	Sodium Dodecyl Sulfate
SC	Spent Coffee
TCEP	Tris-(2-carboxyethyl)-phosphine
TFA	Trifluoro Acetic Acid
TIC	Total Ion Chromatogram
TPTZ	2,4,6-Tripyridyl-S-Triazine
Tris	Tris-(hydroxymethyl)-phosphine hydrochloride
WCW	Washing Coffee Wastewater
WCB	Washed Coffee Beans

List of figures

List of tables

1. Introduction

Coffee is one of the most widely produced and traded agricultural products. World production for 2019 was estimated at around 167 million 60-kg bags, with 95 million bags of Arabica (*C. Arabica*) and 72 million bags of Robusta coffee (*C. canephora*) [1]. Robusta coffee has a stronger and harsher taste with a high concentration of caffeine, while Arabica coffee, considered to be of higher quality, has a sweeter taste, with a higher amount of sugars and an excellent acidity evaluation [2]. Besides Arabica and Robusta coffees, there are species such as Liberica (*C. liberica*) and Excelsa (*C. excelsa*) representing around 1% of world production, mainly grown in Africa and of low commercial importance [3].

Two methods are generally used to process coffee cherries: the dry method, also known as the natural method, and the wet also called the washing method. Processing of coffee cherries by dry or wet method is principally similar for all varieties and generally involves elimination of the five layers (skin, pulp, parchment, mucilage and silverskin) in order to release the coffee beans, also defined as green coffee beans. The dry method consists of drying the coffee cherry just after harvest and is followed by mechanically de-husking. Cherries are later cured and the product obtained is termed in trading society as cherry coffee [4]. It is preferably the simplest way of processing coffee cherries, but also represents a laborious method for obtaining high quality coffee beans [5].

The wet-processing method has become more common and is used by many coffee producers to meet market demands for green coffee beans with higher quality. The wet process also ensures optimal protection against oxidation and top quality. However, large amounts of clean water are required during the production: 40 L/kg of dry parchment coffee [6]. In the wet method, the layers are successively removed in a succession of steps including pulping, fermentation, washing and drying [7]. Pulping consists of removing the red outer skin and the white fleshy pulp. Fermentation aims to degrade the mucilage, which consists of hemicelluloses, pectin substances and sugars [8]. During fermentation, natural enzymes and bacteria operate together to break down the mucilage (the amorphous and water-insoluble gel). Beans are washed, dried to a moisture content of about 10-12%, and the thin layer of parchment around the coffee beans are then removed using a de-husker [9]. This method allows during the fermentation step the release into wastewater of reducing sugars and free amino acids. Green coffee beans are later roasted, constituting the initial step in the preparation of beverages from coffee. Numerous and complex chemical reactions take place during roasting and one of the most important categories of these interactions is the Maillard reaction. This involves the participation of free amino acids and reducing sugars, finally contributing to the development of the color, aroma and taste of the roasted coffee beans [10]. Green coffee beans are rich in bioactive compounds such as caffeine, free amino groups, polyphenols and chlorogenic acids. They contain a number of functional biological properties

such as stimulation of the central nervous system, myocardial stimulation and peripheral vasoconstriction [11]. These characteristics of green coffee beans have been subject of numerous works and interest from the nutraceutical and pharmaceutical industries [12]. Although taking into account these different classifications, the composition and concentration of coffee constituents differ according to the type of coffee beans, the environmental conditions, soil type and geographical areas of cultivation [13,14].

It has often discussed that processing factors were responsible for the specific flavor expression of differently processed coffees [5]. In other words, the type of processing influences the differences in the constituents of green coffee which, during roasting, give rise to aroma compounds and express the characteristics of roasted coffee beans [15]. In addition, large amounts of co-products such as coffee pulp, mucilage, parchment, and wastewater are generated during the wet processing [16]. Skin and pulp represent about 43% w/w, mucilage and soluble sugars 11.8% w/w, and finally parchment about 6.1% w/w [17]. These characteristics also differ according to the mode of production and can be valorized in selected sub-sectors to a limited extent. If wet processing of coffee beans is being performed on a large scale, the untreated effluents produced will greatly exceed the self-purification capacity of the natural waterways. In order to overcome the pollution potential of processing such wastewaters, a clear understanding of its constitution is essential to design a feasible treatment system [18]. Further, studies on the effects of wet processing methods on the characteristics and quality of green coffee beans will also provide a broad range of improvement possibilities. The recovery of coffee processing by-products for potential uses in the food sector has been investigated [19]. The potential uses in the food sector for the solid coffee wastes are the production of enzymes and secondary metabolites. The utilization of these in the non-food sector has been directed amongst others towards reinforcement of polymer composites [20], in bio-sorbents [21-24], for cosmetic products [25], cellulose production [26] and application in water treatment [27].The valorization of coffee wastes through modification to activated carbon has been considered as a low-cost adsorbent. The coffee by-products more evaluated as adsorbents are spent coffee grounds, coffee husk, and coffee pulp. These have been investigated for the extraction of secondary plant metabolites [22], removal of organic compounds [21,23] and heavy metals [28].

Activated carbons are versatile adsorbents, their adsorbent properties lie in the large surface area, area activity, and favorable pore size. Studies have examined the application of coffee waste activated carbons in the removal of organic molecules (dyes, acids, pesticides), and cationic and anionic components (lead, cadmium, copper, chromium). These have shown that adsorption capacity of the molecules is correlated with the activation protocol, textural properties of the raw material, size of the organic molecules, and charge of the ions of the adsorbate [24,29,30]. Adsorption of gases and vapor phase has been evaluated. Authors have

determined that adsorption capacities for methane (CH_4), hydrogen sulfide (H_2S), and carbon dioxide (CO_2) are due to the high surface area, volume, narrow size of micropores, and chemistry of the adsorbent [31-33].

No studies of the effect of batch or continuous wet production on the characteristics of green coffee beans and by-products are currently available. Moreover, very few studies have referred to the valorization of coffee by products into activated carbon using low cost and eco-friendly activation protocols. The aim of this work was contributing to develop alternative methods of improving the waste by-product quality and thus making the process economically more attractive with valorization option that can be brought to the coffee producers.

2. Theoretical background

2.1. Coffee cherry

After successful pollination of the coffee flower, a 10-15 mm cherry develops [34]. It contains two seeds: the coffee beans. Before roasting, they are called green coffee beans. On the outside, the coffee cherry is covered by a skin. This exocarp is a monocellular layer [35]. In the course of maturation, it changes its color from green to yellow or from green to pink to red [36,37]. It is covered by a waxy layer. Below this follows the mesocarp. This consists of approximately 80% water and can be divided, from the outside inwards, into pulp and mucilage layer. The dry mass of the pulp consists of more than 60% cellulose and is rich in lignin (>17%) [38]. The mucilage layer is internally adjacent to the endocarp, which is called the parchment [35]. The parchment is a thin, paper-like polysaccharide layer. Together with the silverskin (perisperm), it encloses the seeds. Botanically, the seeds are drupes. Thus, the term bean is not entirely correct botanically, as it is actually reserved for legumes [39] Figures 1 shows the composition of the coffee cherry.

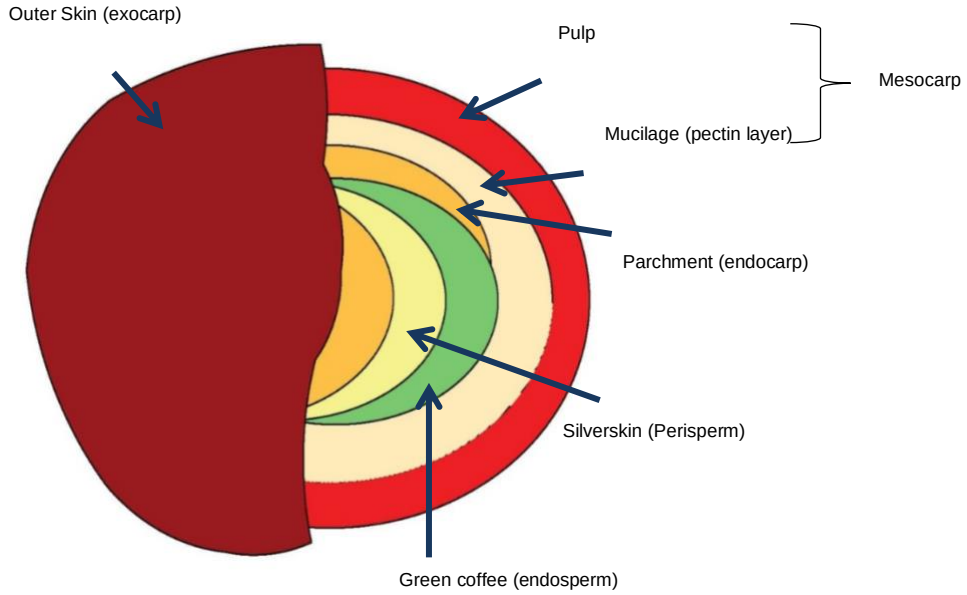


Figure 1 Coffee cherry composition

The green coffee beans consist mainly of water, carbohydrates and dietary fiber, lipids, proteins and peptides, and phenolic compounds mainly chlorogenic acid [40]. Significant amounts of minerals, caffeine, and trigonelline can be also detected. Table 1 summarizes the main constituents of the green coffee beans.

Table 1 Main constituents of green *C. arabica* beans

Constituent	**Concentration (%)**	**Components**
Soluble carbohydrates	9.0 - 12.5	
Monosaccharides	0.2 - 0.5	Fructose, glucose, galactose, arabinose (traces)
Oligosaccharides	6.0 - 9.0	Sucrose (>90%), raffinose (0.0 - 0.9%), stachyose (0.0 - 0.13%)
Polysaccharides	3.0 - 4.0	Polymers of galactose (55 - 65%), mannose (10 - 20%), arabinose (20 - 35%) and glucose (0 - 2%)
Insoluble polysaccharides	46.0 - 53.0	
Hemicellulose	5.0 - 10	Polymers of galactose (65 - 70%), arabinose (25 - 30%) and mannose (0 - 10%)
Pectin	2.0	
Cellulose, β(1 - 4)mannan	41 - 43	
Acids and phenols		
Volatile acids	0.1	
Non-volatile aliphatic acids	2.0 - 2.9	Citric acid, malic acid, quinic acid
Chlorogenic acid	6.7 - 9.2	Mono, di-caffeic and ferulochinic acids
5-Caffeic quinic acid	3.0 - 5.6	
Lignin	1.0 - 3.0	
Lipids	15.0 -18.0	
Wax	0.2 - 0.3	
Oil	7.7 - 17.7	Main fatty acids: 16:0 and 18:2 [41,42]
N-containing components	11.0 - 15.0	
Free amino acids	0.2 - 0.8	Mainly: Glu, Asp, Asp-NH^2
Proteins	8.5 - 12.0	
Caffeine	0.8 - 1.4	Traces of theobromine and theophylline
Trigonelline	0.6 - 1.2	
Minerals	3.0 - 5.4	

Taken from Belitz et al. [43]. The composition varies depending on the cultivation method, climate soil conditions and analysis method. Percentages are given in relation to dry matter.

2.2. Coffee processing

Coffee is the most important food commodity worldwide and ranks second, after crude oil, among all commodities [44]. Coffee is one of the most widely produced and traded agricultural products. World production in 2019 was estimated at around 167 million 60-kg bags, with 95 million bags of Arabica (*Coffea arabica*) and 72 million bags of Robusta (*Coffea canephora*) [45]. Environmental conditions are vital to grow the coffee plant properly. The most important environmental aspects are a temperature between 20 and 26° C, absence of frost, and sufficient sunshine. Moreover, coffee cultivation requires a precipitation of 1,500 to 2,000 mm per year.

Coffee preparation proceeds in the first step by the elimination of the layers adhering to the beans and can be performed by a dry or a wet process. Coffee cherry husks thus obtained

represent about 12% of the berry on dry-weight basis [46]. The dry process, also termed as “unwashed”, is the oldest practiced method, where the entire cherry is first cleaned after harvest and then placed in the sun to dry in thin layers or on patios. Finally, the husk layer, which is composed of pulp, skin, mucilage, and parchment, is mechanically removed [44]. The wet method is called wet process or “washed” coffee and in this case the coffee cherries are immersed in water. Unripe fruits will float, and the good ripe ones will sink. This allows a first separation of unripe and ripe cherries. The skin of the cherry and some of the pulp is removed by pressing the fruit in water through a screen by a machine. The coffee pulp represents 29% dry-weight of the whole berry [46]. The beans are then put in a fermentation tank with a water stream and allowed to ferment to degrade the hygroscopic mucilaginous layer which is an obstacle to the drying. In this process wastewater is generated which contains organic matter and involves mucilage removal with high levels of environmental pollutants. Figure 2 shows exemplary a typical small-scale factory where such wastes are produced. Both these approaches of coffee preparation are well described [46]. Coffee parchment is obtained after the drying step. Colombia wet-coffee processing shows that 100 kg of mature fruits generate 39 kg of coffee pulp, 22 kg of mucilage and 39 kg of parchment [47]. Coffee silverskin is an integument of the coffee bean obtained as a by-product of the roasting process [46]. Finally, spent coffee is the by-product produced after coffee brewing, this is obtained by industrial preparation of instant coffee as well as at restaurants and homes [48]. Spent coffee is generated in large quantities worldwide, approximately 6 million tons yearly [27,49].

In addition to the dry and wet processing, there are some hybrid forms of processing. What they have in common is the mechanical removal of skin and pulp. However, the mucilage layer is only partially removed [5]. In the semi-washed processing, which is common in Indonesia, the mucilage layer is removed after one day of sun drying before the beans are dried. In the semi-dry processing, after the skin and pulp have been removed, with 20 to 80% of the slime layer the beans are placed directly outside to dry [5]. Depending on the water and slime layer content of the beans, the drying time varies from about 8 to 30 days [5]. A common processing method in Brazil involves stacking the beans in the open air after the skin and pulp have been removed. The beans are rinsed repeatedly with water, whereupon the mucilage layer is loosened both mechanically and by fermentation [5]. Monsooned Malaba coffee is a variety from India. After processing, the green beans, which are filled into coffee bags, are stored in open warehouses for six to seven weeks, exposed to the warm, humid winds of the monsoon [50,51].

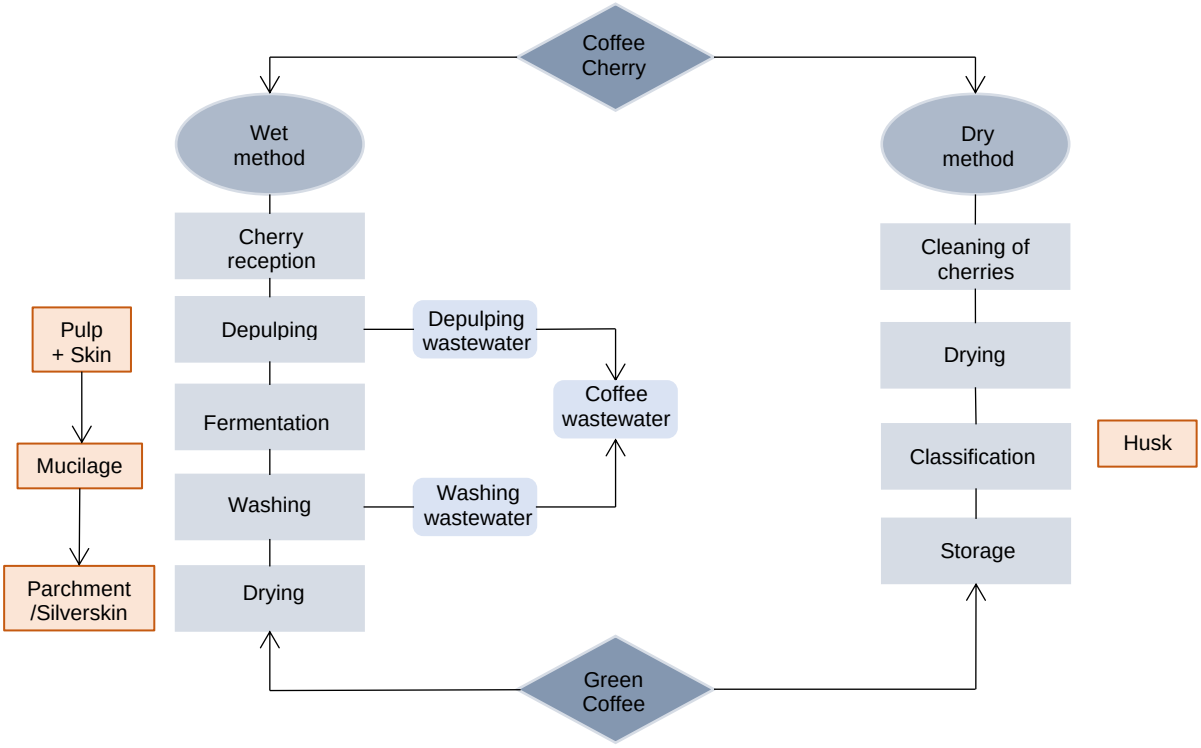


Figure 2 Methods for the processing of coffee cherries and by-products generated on the processing steps

2.3. Coffee proteins

The protein content of *C. arabica* green coffee beans ranges from 8.5 to 12.7% [43,52-55] . The variation in the data found in the literature is due to different measurement methods, differences in the genetics, the degree of ripeness of the coffee cherries, the environmental factors affecting them, as well as processing and storage methods [55-57]. In order to include only enzymes and proteins, as well as peptides and free amino acids, the content of caffeine and trigonelline must be subtracted in the usual determination by nitrogen content.

Proteins are considered the most important fraction for the formation of coffee aroma [10,56,58]. During roasting, the Maillard reaction takes place [59]. Here, free amino acids of proteins and peptides combine with carbonyl groups of reducing sugars. The final product consumed consists of 25% of the resulting melanoidins [52]. These account for the brown color and aroma of coffee. The fact that melanoidins are formed before roasting from a reaction of amino acids residues with phenolic compounds is one of the subjects of the present study.

Among the nitrogen compounds, free amino acids are particularly important for flavor development [55]. However, their proportion in green coffee beans is only 0.15 - 2.5% [55,58]. When coffee beans germinate and differentiate into coffee plants, proteins and polypeptides serve as reservoirs of free amino acids. This can be observed in the example of the 11S storage protein of *C. arabica* beans (11S).

The legume-like storage protein with sedimentation constant 11S occupies the largest of the coffee bean protein fraction [60,61]. A detailed characterization can be found in Rogers et al.

(1999). The 11S accounts for approximately 45% of the soluble protein fraction in the endosperm [60]. However, in a 2D electrophoresis, Rogers et al. also found 11S in the embryo. The 11S contains low content of sulfur. Thaler et al. showed the content of methionine at 2.38% of amino acids in the 11S of green Brazilian *C. arabica* beans [62]. According to Rogers et al. only 1.1 and 0.2% of the amino acids are cysteine and methionine, respectively [60]. In the soluble protein fraction of the 11S, glutamate and glutamine are the most abundant amino acids in coffee beans [60]. The amino acid sequence for the 11S of *C. arabica* beans, used in the present work, comprises 487 amino acids. The sequence was obtained using the sequence P93079 of the UniProt database [63].

Reducing SDS electrophoresis of the soluble protein fraction shows mainly three bands at 55, 33 and 24 kDa [52,60,61,64]. The 55 kDa belongs to the intact 11S globulin monomer. This consists of α- and β-subunits. The two subunits are connected by a disulfide bridge. The involved amino acids can be found at position 113 (subunit) and 308 (subunit) of the amino acid sequences in Figure 3. In an alkaline environment, the linked cysteine residues are reduced, and a separate band appears for each of the 33 kDa and subunit 24 kDa [53,65]. Baú et al. (2001) were able to identify a cysteine subunit by means of one-dimensional SDS polyacrylamide gel electrophoresis (SDS-PAGE) of different variants of *C. arabica*. Comparable ratios of α- and β-subunits for the different variants were shown [66]. In contrast, when different species of the subgenus *Coffea* were analyzed, significant differences in mass, amount and ratio of the subunits appeared. While the α-subunit is hydrophilic, polar and hydrophobic amino acids of the β- subunit balance each other [60]. A hydrophobic signal peptide is connected in front of the α-subunit [60,61]. In the sequence used here, this comprises 22 amino acids [63].

```
        10         20 ▼       30         40         50
MAHSHMISLS SYVLLLFLGC LAQLGRPEPR LGGKTQCNIQ KLNAQEPSFR
        60         70         80         90        100
FPSEAGLTEF WDSNNPEFGC AGVEFERNTV QPKGLRLPHY SNVPKFVYVV
       110        120        130        140        150
EGTGVQGTVI PGCAETFESQ GESFSGGQEQ PGKGQEGSKG GQEGQRQRFP
       160        170        180        190        200
DRHQKLRRFQ KGDVLILLPG FTQWTYNDGD VPLVTVALLD VANEANQLDL
       210        220        230        240        250
QSRKFFLAGN PQQGGGKEGH QGQQQQHRNI FSGFDDQLLA EAFNVDLKII
       260        270        280        290        300
QKLKGPKDKR GSTVRAEKLQ LFLPEYSEQE QQPQQQQGQQ QQGVGRGWRS
▼      310        320        330        340        350
NGLEETLCTV KLSENIGLPQ EADVFNPRAG RITTVNSQKI PILSSLQLSA
       360        370        380        390        400
ERGFLYSNAI FAPHWNINAH SALYVIRGNA RIQVVDHKGN KVFDDEVKQG
       410        420        430        440        450
QLIIVPQYFA VIKKAGNEGF EYVAFKTNDN AMINPLVGRL SALRAIPEEV
       460        470        480
LRSSFQISSE EAEELKYGRQ EALLLSEQSQ QGKREVA
```

Figure 3 Amino acids sequence of the storage 11S protein.

Sequence of the 11S storage protein of C. arabica P93079 of the UniProt database. The first arrow marks the end of the upstream signal chain and the beginning of the α-chain. The second arrow shows the end of the α-chain and the beginning of the β-chain.

The secondary structure is dominated by β-sheets [52,64]. Three 11S monomers each combine for transport into storage nodes (protein bodies) [53,60]. They combine by non-specific hydrophobic interaction to form trimers and hexamers with molecular weights between 150 and 400 kDa [52,53,67].

The coffee storage protein 1 gene encodes the 11S [60]. Marraccini et al. characterized a promoter for the expression of the protein [60]. The α- and β-subunits have different isoforms [60,61,64,66].

Bandil et al. compared protein extracts of green *C. arabica* beans from different development stages by 2D electrophoresis. They found that only very specific proteins changed their expression. Among them, the expression of 11S increased during germination [68]. Rogers et al. already described an accumulation of 11S during bean development [60]. These findings are consistent with the function of 11S as a storage and ultimately also as a source of amino acids.

When green coffee beans were stored, Ludwing et al. could not detect any significant change in the amino acid profile, apart from a decrease in the glutamine content [69]. This was due to the fact that proteolytic activity in beans of the *C. arabica* species remained low and constant for several months. Montavon et al. [56] observed the degradation of 11S in ground green coffee beans under aerobic conditions. The band of the α-subunit disappeared from SDS gel electrophoresis, while that of the β-subunit remained [56]. This led the authors to suggest that enzymatic cleavage occurs in combination with oxidative fragmentation which is the trigger for proteolysis of 11S [70].

2.4. Polyphenols in Coffee

Polyphenols are the largest group of secondary plant compounds [71,72]. They are synthesized by numerous plants for their protection against environmental factors, such as UV radiation of pathogens [73]. Health-promoting properties, especially regarding the prevention of cancer, cardiovascular and neurodegenerative diseases, are attributed to them [72,74-76] because polyphenols are potent antioxidants [72]. They complement the antioxidant activity of enzymes and vitamins in the fight against the ever-present threat of reactive oxygen species. More than 10,000 known phenolic compounds are consumed in the diet in the form of staple food such as fruits, vegetables, and cereals, but also stimulants such as tea, chocolate, wine, and coffee [72,77].

From a chemical point of view, polyphenols are naturally occurring compounds with phenolic groups and associated properties [72]. Based on their structural elements and number of aromatic rings, they can be divided into five groups: Flavonoids, phenolic acids, phenolic alcohols, stilbenes, and lignans [73,78,79]. In coffee, derivatives of cinnamic acid (trans-phenylpropionic acid) are the most abundant [41,72]. Along with benzonic acids, these belong

to the group of phenolic acids, namely caffeic acid (3,4 di-hydroxycinnamic acid), ferulic acid (3-methyl-4 hydroxycinnamic acid), sinapinic acid (3,5-dimethoxy-4 hydroxycinnamic acid) and *p*-coumaric acid (4-hydroxycinnamic acid) [72]. Figure 4 shows the structural formulas of cinnamic acid and its most common derivatives.

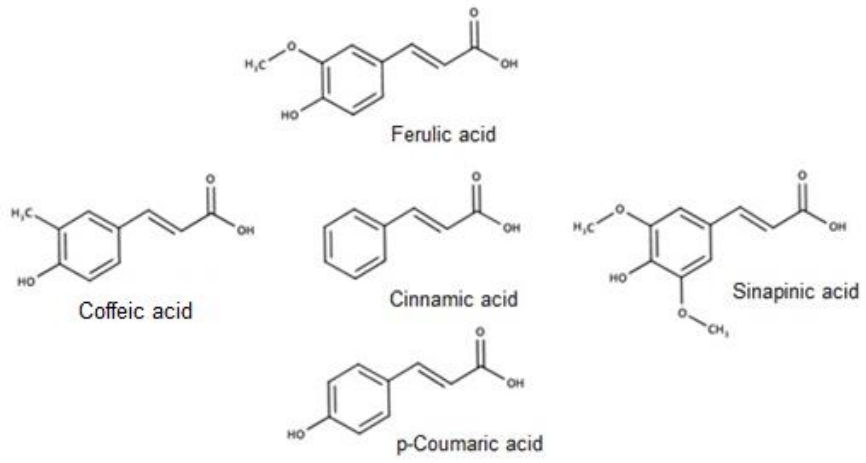


Figure 4 Structural formulas of cinnamic acid and its most common derivatives

However, in unprocessed plant material, hydroxycinnamic acid derivatives are hardly present as free acids [41]. In coffee beans, they are usually esterified with quinic acid (3-R,5R)-1,3,4,5 tetrahydroxycyclohexane-1-carboxyl acid) to form chlorogenic acid (CA) [41,43,80]. Various chlorogenic acids can be distinguished according to the type, number, and position of the acyl residues. These include monoesters, such as caffeoyl quinic acid (CQA) or feruloyl quinic acid (FQA). In addition, *C. arabica* contains characteristic diesters, triesters and tetraesters of caffeic acid (DiCQA, TriCQA, TetraCQA) or mixed diesters of caffeic and ferulic acid, such as caffeoylferuloylquinic acid (CFQA) [41]. The most common chlorogenic acid is 5-O-caffeoylquinic (5-CQA) [41]. Ali et al. found that 4,5-dichlorogenic acid (4,5-DiCQA) has the highest antioxidant capacity of all chlorogenic acids present in coffee [53].

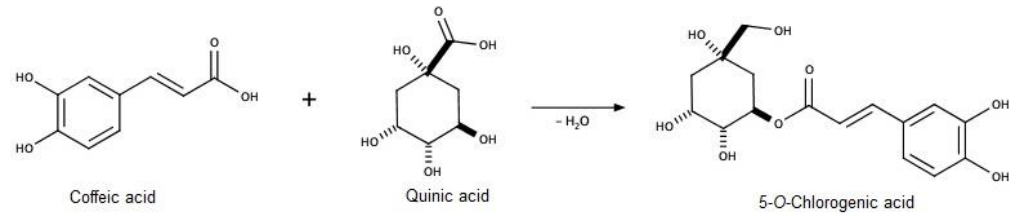


Figure 5 Esterification of caffeic acid and quinic acid to 5-O-Chlorogenic acid

Coffee is one of the most important productive food sources of CA [41]. Depending on the species, green coffee contains 6 - 11% CA in dry weight [55]. However, the parts of the coffee cherry that are separated during processing also contain 2.3 - 3.0% CA dry weight [38]. As shown in Table 1, green *C. arabica* beans consist of 6.7 - 9.2% CA [43]. One cup of coffee (200 mL) of *C. arabica* contains between 70 and 200 mg of CA [41].

2.5. Protein modifications in connection with polyphenols

Proteins can be modified by reaction with phenolic compounds. The bulk of interactions between these two fractions initially result in non-covalent interactions [70,81]. These can be based on hydrophobicity, hydrogen bonds, or ionic bonds [80]. Suryaprakash et al. [82] demonstrated that proteins from sunflower seeds can act as non-covalent ligands for caffeic and quinic acid. In this context, these interactions frequently occurred between caffeic acid and tryptophan, tyrosine, or lysine side chains [82]. Protein modification takes place when the interactions of proteins and phenolic compounds lead to a covalent bond. Such modification can also occur in parallel with non-covalent reactions. This was shown in a study by Prigent et al. [83] using the example of the reaction of 5-CQA with bovine serum albumin (BSA), lysozyme, and lactalbumin.

Covalent bonds are catalyzed both enzymatically and non-enzymatically. Both reactions require the presence of oxygen and can be divided in two steps [80,84,85]. Since dark colored pigments are also formed here, as in the Maillard reaction between proteins and polysaccharides, the reactions are also referred to as browning [43,84].

In the first step, the enzyme-catalyzed reaction begins with the hydroxylation of a monophenol to a diphenol (catechol) [80,85]. This reaction is catalyzed by the enzyme monophenolase [80]. In the second step, the diphenol is oxidized to a quinone [85]. In turn, *o*-diphenolase or laccase are biocatalysts for this oxidation reaction [80]. The responsible enzymes belong to the polyphenol oxidases. Ali et al. [53] showed that the presence of polyphenol oxidases promotes the formation of protein chlorogenic acid adducts more strongly than a mere increase in chlorogenic acid concentration.

Non-enzymatic autoxidation is mostly induced by high temperatures and favorable pH conditions during food processing [84]. The role as antioxidants, makes phenolic compounds and hydroxycinnamic acids, susceptible to oxidation [80]. Hydroxycinnamic acids are oxidized in a first step to semiquinones or quinone radicals [84]. These are reactive intermediate radicals. In combination with oxygen, they are oxidized again and quinones are formed as well [80].

Thus, both the enzymatically and non-enzymatically catalyzed reactions consume oxygen by oxidizing the phenolic compound to a quinone ion. Quinones are diketones that act as oxidants. Their double bound leads to a positive partial charge inside the ring. In conjunction with

nucleophilic groups of amino acids side chains, nucleophilic addition can occur [80,84]. In particular, the amine of lysine and the thiol group of cysteine are preferred reaction partners [86]. However, the indole group of tryptophan, the imidazole group of histidine, the thioether group of methionine or the phenol group of tyrosine may also be involved in the reaction [52,87]. The presence of oxygen is only a prerequisite for the reaction to take place [87]. The reaction of a protein with a phenolic compound is mainly influenced by the nature of the two reaction partners [81]. Prigent et al. [83] showed that differences in hydrophobicity, isoelectric point and amino acid sequence have a significant influence on the interaction of the protein. Regarding the phenolic compound, position and number of hydroxyl groups, molecular weight, and structural flexibility play an important role [81,87,88]. Reaction parameters such as exposure time, temperature, pH, and concentration of phenolic compounds also have an influence [87,89,90]. In addition, enzymes such as polyphenol oxidases, which catalyzes the oxidation of phenolic compounds, drive the modification of proteins.

The 11S has a strong hydrophilic C-terminal on its surface region of the α-subunit [52]. Here, it protects the inner domains by itself becoming a preferred target of chlorogenic acid. This was demonstrated by Schwenke et al. [64] and by Rawel et al. [52]. Trypsin digestion is also most likely to occur at the more exposed α-subunit. Rawel et al. also demonstrated the stability of the conformation of 11S in the alkaline milieu upon addition of chlorogenic acid. The proportions of random turns decreased in favor of stabilizing β-sheets [52].

Montavon et al. [56] noticed a smearing of the SDS-PAGE bands especially in samples of immature coffee cherries. Besides a stronger activity of oxidizing enzymes, it was concluded that the increased multiprotein complex was due to a higher concentration of phenolic compounds. As mentioned above, aerobic incubation of ground green coffee beans result in degradation of storage proteins [52,56,58]. The released amino acids may react with autoxidizing chlorogenic acid. This process can also occur during roasting as well as during the cultivation, ripening and processing of coffee which presumably contributes to the development of the coffee aroma [53,58,80].

2.6. Protein-protein crosslinks

Protein-protein crosslinks (PPCs) are covalent bonds between or within proteins [91,92]. When they occur within a protein, they are referred to as intramolecular PPCs. If, however they covalently link at least two separate proteins, it is called intermolecular PPCs. The nature of the connection depends primarily on the amino acid residues involved. PPCs can be formed in a variety of ways. In food matrices Mckerchar et al. [92] distinguish between naturally occurring, processing derived and targeted PPCs.

The most naturally occurring form is the disulfide bridge [91]. In an oxidation reaction, the enzyme called protein disulfide isomerase links two cysteine residues [92]. Disulfide bridges

are a crucial building block of the tertiary protein structure. In medical diagnostics, they are regarded as indicators of oxidative nutritive stress [92,93]. Breads baked with wheat flours in particular showed higher dityrosine concentrations after baking [93].

Dehydroproteins can be formed during the preservation of foods with bases, or simply during their processing at high temperatures [92]. These can then combine with other amino acid residues by nucleophilic addition [94]. High temperatures are also responsible for PPCs through the above-mentioned Maillard reaction [95]. In addition, isopeptide bonds can be thermally induced. They are formed by condensation of the lysine amino group with asparagine or glutamine residues [96,97].

In addition to the examples mentioned, proteins can also form crosslinks by reacting with phenolic compounds [80,98]. After a quinone has linked to a primary amine of an amino acid side chain by nucleophilic addition, the adduct can be oxidized again [80]. This diketone can now perform a nucleophilic attack on a primary amine [80]. A PPC is formed via a phenolic linkage.

2.7. Consequences of protein modification with polyphenols

The consequences of protein modifications in connection with polyphenols are numerous [80,88]. Many of them indicate reduced nutritive quality [80,81,88]. In a study with whey protein, Rawel et al. [99] showed that protein modification by reaction with selected polyphenols decreased protein solubility over a wide pH gradient [99]. In addition, hydrophobicity increased, and the isoelectric point decreased. *In-vitro* cleavage of modified whey proteins by gastrointestinal tract enzymes (trypsin, chymotrypsin, pepsin, and pancreatin) was decreased. When studying the interaction of myoglobin and selected phenolic compounds, Kroll et al. [100] found also that the solubility of the resulting adducts was lower than that of the native protein. The isoelectric point shifted toward a more acidic pH. Similarly, the endopeptidases trypsin, chymotrypsin, and pepsin had difficulty digesting the modified proteins in vitro [100].

In experiments with soy protein, Rawel et al. [101] also noticed lower lysine, cysteine and tryptophan concentrations after interaction with various phenolic compounds, which is a further indication of lower usability by the human organism. O'Connell et al. [102] found a decrease in accessible lysine and thiol groups when milk proteins were modified with caffeic acid to increase heat stability. Petzke et al. [103] were able to show in rats that modification of lactoglobulin with chlorogenic acid resulted in decreased nitrogen recovery.

Modified proteins are crucial flavoring agents [66]. This has been adequately documented for the products of the Maillard reaction [43,104]. For a beverage rich in protein and hydroxycinnamic acid as coffee, it is important to seek the origin of its complex aroma in the compounds of these components [52,53,56,105].

2.8. Analysis of covalent protein-polyphenol interactions

The described chemical and physical consequences associated with a covalent interaction of proteins and polyphenols can in turn be the starting points for analysis. Since most of these interactions are non-covalent in nature.

Among the methods to explore these interactions, there are several spectro and fluoro spectroscopic methods. Excitation of proteins with a wavelength between 240 and 280 nm leads to the emission of fluorescence in the range of 340 - 350 nm. Covalent bound polyphenols can quench this emission. Liu et al. [106] were able to demonstrate the formation of flavonoid-BSA complexes. Czubinski et al. [81] also investigated flavonoid dependent protein modification using fluorescence spectroscopy and found that exactly one molecule of vitexin binds to one molecule of γ-conglutin at a time. Fluorescence spectroscopy was also used in the study of soy protein by Rawel et al. [89]. They found that the tertiary structure of the protein changes by modifications after interaction with various polyphenols. Rawel et al. [101] obtained comparable results regarding the interaction of BSA and CA using fluorescence spectroscopy. The interaction of proteins and polyphenols results in molecular weight change and net charge. Kluger et al. [107] suggest the use of polyacrylamide gel electrophoresis (PAGE) or size exclusion chromatography (SEC), mainly for the analysis of PPC. Lui et al. [108] applied SEC to determine the arrangement of covalent zein-polyphenol conjugates at pH 9.0 and an expected increase in molecular weight was observed. PAGE is also used in the present work in the analysis of protein fraction of green *C. arabica* beans. Following Ali et al. [53], polyphenols and proteins polyphenol aggregates are analyzed by reversed phase high performance liquid chromatography (RP-HPLC) using a UV-vis detector. While peptide bonds have their maximum absorption at 214 nm and aromatic amino acids at 280 nm wavelength, the maximum absorption of phenolic acids is between 270 - 280 nm and 305 - 330 nm [81]. Ali et al. [53] separated extracted coffee bean proteins by RP-HPLC and determined the maximum absorbance of 5-CQA at 325 nm. Using this relative quantification, the authors found significant differences in the proportion of covalent protein-polyphenol aggregates between green beans of the species *C. arabica* and *C. canephora*.

Gallo et al. [109] used electrospray mass spectrometry (ESI-MS) to analyze the covalent interaction of casein and whey protein with polyphenols from cocoa. They were able to identify a specific cysteine molecule of β-lactoglobulin as a binding site for catechin and epicatechin. Thus, with ESI-MS, it is possible to identify specific amino acids in the primary structure of a protein as binding sites for polyphenols [81].

2.9. Coffee cup quality

Coffee producing countries normally have their own coffee quality standards. These standards should be accepted for the local as well as for the international market. Erna Knutsen of

Knutsen Coffee Ltd, in a speech held in France in 1978, brought for the first time the term “specialty coffee”. This term refers to special geographic microclimates which produce coffee beans with unique flavor profiles. Specialty coffee beans require adequate preparation, roasting and brewing methods. Specialty coffee industry has evolved during the last 10 years, however this term is confined as a potentially wonderful gustatory experience. In order to preserve high quality of coffee cherries, several processing steps need to be considered. First, plant farming factors like right soil, appropriate climate conditions, proper maintenance, right ripeness, and harvesting are essential to develop high quality coffee fruits. Specialty coffee cherries need to be processed to a wet mill right after harvesting. Time between harvesting and processing plays an important role for the final coffee-cup quality. Processing steps must be carefully undergone so that coffee beans are not damaged. The drying step is another key activity. If coffee beans are dried too quickly or too slowly, unevenly, dried and then rewetted, or not dried enough, it can be averse to the final coffee quality. Parchment green coffee must be adequately stored, guaranteeing proper temperature, and humidity. Finally, parchment coffee beans must be dehulled and subsequently separated by size and packaged for shipping [110].

The Specialty Coffee Association (SCAA) is a non-profit organization founded in 1982 for the specialty coffee industry. SCAA defines specialty coffee in its green stage (green coffee) as coffee free of primary defects, properly sized and dried, resulting in no faults or taints in the cup. This means that coffee must be able to pass cupping tests. After roasting, coffee must be milled. Since aromatic compounds are released from grinding, milling and brewing need to be close in time. Size of the milled coffee is also important depending on the brewing preparation. Finally, water quality, brewing temperature, coffee to water ratio and extraction need to be taken into account to produce a specially coffee beverage [111].

The Technical Standards Committee (TSC) of the SCAA proposed standards for cup quality such as sample preparation to evaluate the quality. For the evaluation of the coffee cup quality a sensory terminology has been established. Figure 6 shows the Coffee Taster’s Flavor Wheel by SCAA and World Coffee Research (WRC). The Flavor Wheel provides means of recording important flavor attributes for coffee such as fragrance/aroma, flavor, aftertaste, acidity, body, balance, uniformity, clean cup, sweetness, defects, and “overall” attributes. The overall cupping quality is based on the flavor experience of the individual as a personal assessment.

Figure 6 Coffee taster´s flavor wheel.

Taken from the Specialty Coffee Association of America (SCAA) and World Coffee Research (WCR) (2006) [111]

2.10. Coffee by-product utilization

The treatment of coffee by-products is generally realized by oxygen-driven biological methods, such as composting, which serves a dual purpose, i.e. valorization via manurial value and as well as decreasing the pollution potential [46,112]. Many studies have been directed towards this goal of composting coffee wastes [112-119], while implementing among them earthworms [120-122] or with the intention of modulating the mineral recycling [123-126]. Other approaches concern the realization of sustainability by application in fuel production including biogas [127-139], ethanol [140-142], biodiesel [143] or in briquetting of wastes from coffee plants with efforts also directed towards torrefied coffee residues [144-155]. Table 2 shows a compilation of recent advancements (2012-2016) and available studies featuring the utilization of coffee wastes.

Table 2 Recent advances on coffee waste utilization (2012-2016)

Nr.	Type of coffee waste used	Method of utilization	Reference
1	Coffee by- and waste products (husks, skin, pulp, mucilage, parchment, silverskin, spent coffee, wastewater)	Reviews: Different methods of utilization	[46,132,152,156-161]
2	Spent coffee grounds from different coffee making art	Source of bioactive compounds (e.g. phenolic compounds - caffeoylquinic acids, antioxidant dietary fibers, triglycerides)	[157,162-166]
3	Spent coffee	Natural biosorbents for removal of heavy metals; Activated carbons	[167-169] [170-174]
4	Spent coffee	Decolorization e.g. of real textile wastewaters	[175-180]
5	Coffee by- and waste products	Enzymes – e.g. xylanase in solid-state fermentation, beta-glucosidase	[158,181,182]
6	Coffee silverskin, pulp / spent coffee	Bioethanol production via yeast strains	[140-142]
7	Coffee by- and waste products	Bioenergy – incl. pyrolysis and gasification of biomass residues – char tar and gas production, biogas, bio-oil, torrefied biomass, briquetting etc.	[127,129,132,135-139,144,145,147,149-153,163,183-186]
8	Coffee husks / spent coffee	Composting / fertilizer	[112,117,119,120,126]
9	Coffee husks / spent coffee	Polymer composites	[159,187]
10	Coffee husk ash reject material	Ceramic production	[188-190]
11	Coffee husks	Bacterial cellulose	[191]
12	Coffee husks	Potential utilization in food production	[192]
13	Spent coffee	Cosmetic formulations	[193,194]
14	Spent coffee	Building materials	[195-198]
15	Spent coffee	Biosynthesis of polyhydroxyalkanoates	[164]

The basic/proximate chemical composition of these four main coffee by-products (coffee pulp, coffee husks, coffee silverskin, and spent coffee) and their utilization are well documented [46]. The potential uses in the food sector for the solid coffee wastes amongst others are the production of enzymes and secondary metabolites. The use of waste streams with novel biotechnological methods has been proposed for the production of bulk chemicals and value

added products such as single cell protein, ethanol, organic acids, amino acids, secondary metabolites, mushrooms and enzymes [199]. Spent coffee grounds, coffee pulp and husks for examples have been utilized for polyphenol [157,162-166,200] or auxin recovery [201] among other bioactive compounds [46,157].

The valorization of the solid coffee by-products in the non-food sector has been directed amongst others towards reinforcement in polymer composites [159,164,202], in activation [46,203] / de-colorization of organic dye [46,177,178], use as electrode material [167,170,171,175,176,204], application in wastewater treatment [167,170,171,178,179], for cosmetic products [193], cellulose production [191], enzyme production [118,158,181,182,205,206], building materials [188,189,195,196,207,208] including crystalline nanoparticles [209,210], utilization in storage of gases [211] as well as in control of plant diseases [212].

Coffee pulp residues have been researched with various treatments [213,214] as already mentioned above, but the characteristics and approaches to the treatment [215] and utilization [160] of coffee wastewater generated during the wet processing require more concentrated efforts and attention. Furthermore, the process of fermentation generates also wastewater with serious consequences for the environment because of their high Biochemical Oxygen Demand, Chemical Oxygen Demand values and acidic nature such. Therefore, it is necessary to develop better methods of treatment in order to decrease their content, the envisaged result being to produce higher wastewater quality and gaining new valuable products.

If wet processing of coffee beans is being performed in big style, the untreated effluents produced will greatly exceed the self-purification capacity of the natural waterways. In order to overcome the pollution potential of processing such wastewaters, a clear understanding of its constitution is essential to design a feasible treatment system [18].

The wastewater generated in the cleaning and pulping steps contains many individual compounds including proteins / enzymes, peptides and free amino acids. The extensive literature research revealed that there is no detailed data on exact composition available for wastewater. The secondary plant metabolites present will certainly include nitrogen containing compounds such as caffeine and trigonelline [216,217]. Coffee beans are also a major source of antioxidants which include phenolic compounds such as chlorogenic acid, an ester formed between caffeic - and quinic acid. The results, as mentioned formerly, are caffeoylquinic acids (CQA), their corresponding three isomers (3-CQA, 4-CQA and 5-CQA), dicaffeoylquinic acids (diCQA) with respective three isomers (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) and feruloylquinic acids with the known three isomers (3-FQA, 4-FQA and 5-FQA), representing further derivatives of hydroxycinnamic acids found in coffee beans [218]. Recent studies reporting on the composition of coffee phenols have documented more than 50 hydroxycinnamic acid derivatives being present [219,220].

The interest in these two compound classes (proteins and phenolics) from the chemical point of view is related directly or indirectly to their dual role as substrates for oxidative monitored reactions. The phenolic compounds are susceptible to both enzymatic and non-enzymatic oxidation in the presence of oxygen [221,222]. The resulting reactive *o*-quinones are capable of undergoing a nucleophilic addition to proteins as already mentioned, thereby covalently modifying the proteins [222]. The development of mass spectrometric methods has recently provided a more detailed biochemical characterization of the interactions of CQA and amino acids [222-224].

2.11. Coffee wastewater

The term virtual water is defined as the volume of water required to produce a commodity or service (Allan, 1998, 1999; Hoeskstra, 1998). When there is a transfer of products or services from one place to another, there is little direct physical transfer of water. The virtual water in terms of the production point of view can be defined as the real water used in the production of the commodity. It is production site specific, as it depends on the production conditions, including place and time of production and local water use efficiency [225]. Virtual water content of coffee therefore can be defined as the total volume of water required for producing the coffee. The virtual water of roasted coffee is the sum of the water requirements in each production stage. Meaning, the crop water requirements of coffee plant, and water of the post-harvesting production. In the Netherlands when drinking one standard cup of coffee (125 mL) about 140 liters of water are needed and in terms of world population about 110 billion cubic meters of water per year are required in order to be able to drink coffee [226]. Table 3 shows the steps for the calculation of the virtual water content of coffee produced in Guatemala by the wet processing.

Table 3 Calculation of the virtual water content of coffee produced in Guatemala by wet processing

Variable	Value	Unit	Source
Crop water requirement	1.338	mm	CROPWAT
Yield of fresh cherries	5,6	ton/ha	Calculated from yield of green coffee given by FAO (2003c)
Virtual water content of fresh cherries	**2.389**	**m^3/ton**	**C = 10 A/B**
Water use for pulping	7,5	m^3/ton of fresh cherries	Assumption, based on GTZ (2002b)
Remaining fraction after pulping	0,44	ton/ton	Bressani (2003), GTZ (2002a)
Virtual water content of pulped cherries	5.447	m^3/ton	E = (C+C1)/D
Water used for soaking and washing	22	m^3/ton of pulped cherries	The roast and post coffee company (2003), GTZ (2002b)
Remaining fraction after fermentation and washing	0,9	ton/ton	Bressani (2003)
Virtual water content of wet parchment coffee	6.077	m^3/ton	G = (E + E1)/F
Remaining fraction after drying	0,506	ton/ton	GTZ (2002c)
Virtual water content of dry parchment coffee	**12.010**	**m^3/ton**	**I = G/H**
Remaining fraction after hulling (removing the parchment layer)	0,9	ton/ton	GTZ (2002a)
Virtual water content of hulled beans	13.344	m^3/ton	K = I/J
Remaining fraction after polishing, grading and sorting	0,89	ton/ton	GTZ (2002a)
Virtual water content of green coffee	**14.993**	**m^3/ton**	**N = K/L**
Remaining fraction after roasting	0,84	ton/ton	GTZ (2002a), Hicks (2001), ICO (2003), Sovrana (2003)
Virtual water content of roasted coffee	**17.849**	**m^3/ton**	**P = N/O**
Total Production	**240.222**	**ton/yr**	

Note: Virtual water content was determined as described by Chapagain et al. [226]

Coffee wastewater is acidic with a high content of suspended and dissolved organic matter. The effluent is rich in sugars, tannins, and alkaloids [227]. The major problems and consequences for the environment are due to their high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), acidic nature and unacceptable color. Using oxygen from water microorganisms will slowly break down the organic matter. Whenever oxygen demand starts to exceed, oxygen content decreases resulting in anaerobic conditions [2]. This results in high amounts of oxygen needed to break down organic matter (BOD) and oxidation of inorganic chemicals (COD). Anaerobic conditions can trigger health threatening bacteria with unpleasant smells and dark color appearance that can be harmful for aquatic inhabitants [2,228]. Besides bacteria that leak out into potable water can impact human health [229]. It is

important to mention that color plays also an important role in aquatic life from sun rays. When water exhibits brown or dark color, photosynthesis activity decreases [228], thus affecting other parameters like temperature, Dissolved Oxygen (DO), BOD and COD [230]. Organic acid from the fermentation of sugars makes the wastewater acidic. The acidity of the effluent might exhaust life supporting of water bodies [231], besides under such conditions plants and animals can hardly survive [232]. Therefore pH adjustment is required to increase microbial growth and activity for decreasing organic matter [233].

2.11.1. Coffee wastewater treatment

In the developing countries, where the coffee processing takes place, wet-coffee processing plants are still discharging coffee wastewater without any treatment. The main problem is that the conventional wastewater treatments are too expensive for most of the coffee producers [234]. Therefore, besides effective, coffee wastewater treatment, operational and maintenance costs as well as environmentally friendly technology must be considered. Apart from that, when wastewater is discharged it must be based on discharge limits available in the respective country.

Wastewater treatment can be divided into biological and physicochemical methods, the latter have been more chosen to treat industrial wastewater. This is because the ability of such methods to break down complex compounds in wastewater in short time. Some studies have addressed the coffee wastewater treatment using different treatment technologies which vary from low to high technologies. Coffee wastewater has been tested to be a potassium source for coffee plant production. The authors showed the viability in the use of coffee wastewater in the production of coffee plants, though dilution should be considered [235]. However, application of higher volumes of coffee wastewater might affect the soil respiratory rate, soil fertility, pH, available phosphorous, and physicochemical properties of the soil. Moreover, population of soil macro fauna and microbial population are also affected [236]. Oliveira et al. [237] investigated the performance of drip irrigation subunits using wastewater from coffee processing, even though the wastewater must undergo a physical treatment (filtration) to avoid the risk of clogging dippers [238]. Another well-known and attractive food industry waste treatment technology in developing countries is the anaerobic technology. This requires low maintenance and operation and has the capacity to treat seasonally produced wastes. An anaerobic hybrid reactor using an Up-flow Anaerobic Sludge Blanket (UASB) and filter was tested for treating coffee wastewater, finding that variations in volume, wastewater concentration and pH prevent a stable reactor operation [239-241]. Furthermore, some of the constituents present in coffee wastewater like caffeine, chlorogenic acid, and tannins could be potential toxic compounds for anaerobic bacteria [242]. Another approach is combining anaerobic and aerobic technologies. This proposal was made using bio-methanation followed

by aeration and wetland technology [243]. The anaerobic-aerobic process is suited for coffee processing water treatment. However, sedimentation and pH stabilization must firstly be done to guarantee adequate treatment efficiency.

Physicochemical and biological treatment methods commonly used in domestic wastewater treatment have also been investigated for the treatment of coffee wastewater. Photo-Fenton, oxidation, zero-valent iron (ZVI), fungal species, coagulation, membrane filtration, electro-chemical coagulation, Gamma radiation and activated carbon. These methods have successfully treated contaminants in coffee wastewater, nevertheless some disadvantages must be taken into consideration like material replacement, additional chemical supplies, method lifespan and time consuming, amount of energy, high level of expertise and high costs.

2.12. Lactic acid in coffee fermentation

Origin of lactic acid bacteria is believed to be plant material [244]. In coffee fermentation lactic acid bacteria find a rich environment for its development in coffee pulp. However, factors affecting the initial load of microorganisms include the quality and integrity of coffee cherries, plant variety, hygiene of fermentation tanks and water used in the process. The microbiota of wet-coffee processing is a complex ecological community belonging mainly to the groups of bacteria, fungi, and yeast. These microorganisms are associated with the degradation of the pulp and mucilage of the coffee fruit (Figure 7), the formation of alcohols and long-chained carboxylic acids [245]. Sugar content in coffee pulp is the main carbon source for the lactic acid bacteria growth. These sugars include glucose, fructose, galactose, and mannose and polysaccharides like pectin and cellulose [246].

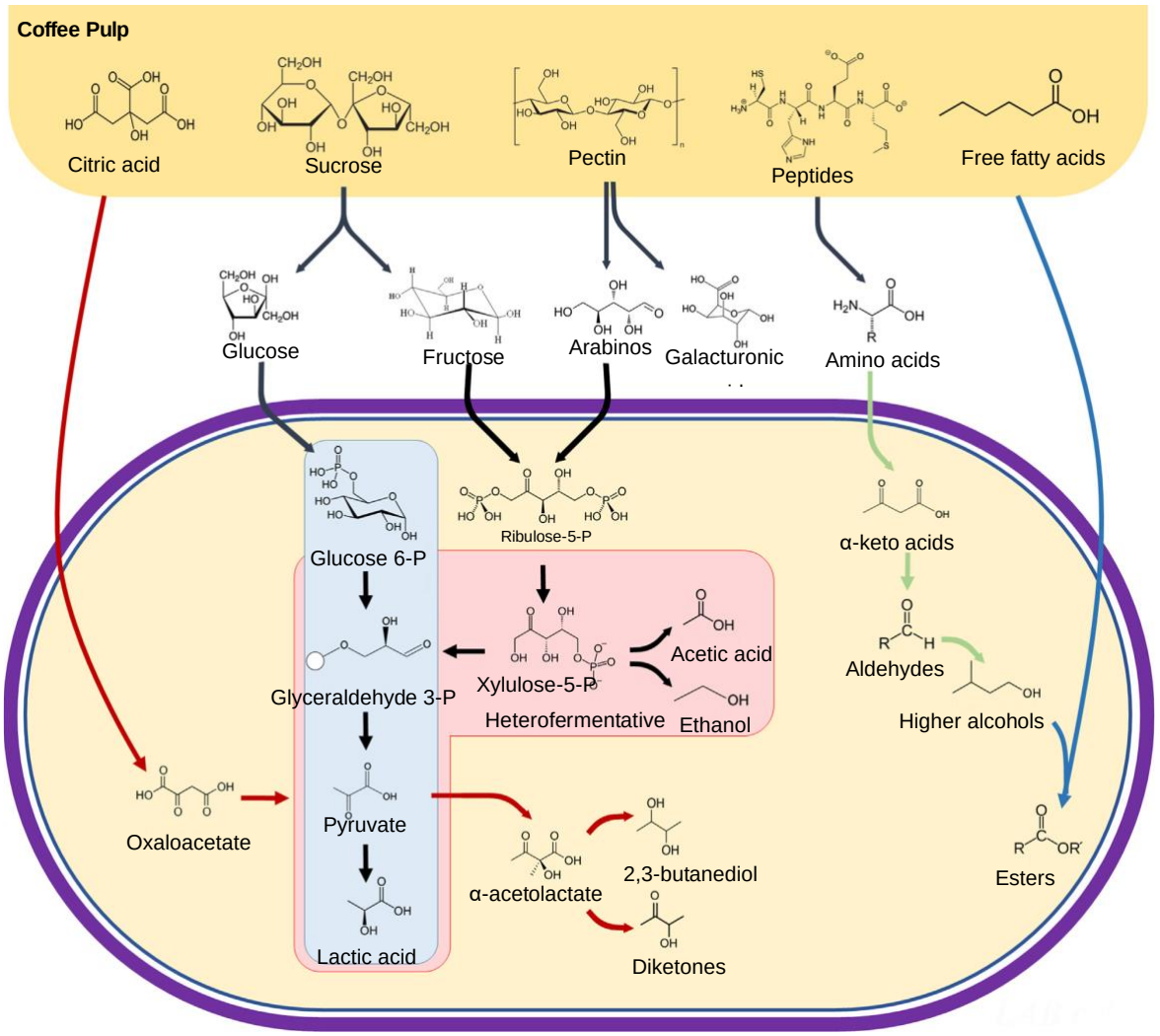


Figure 7 Lactic acid bacteria pathway in wet-coffee processing fermentation.

Taken from de Melo Pereira, et al. [246]

Organic acids are constituents of interest due to their different functionalities. To guarantee the proper drying of green coffee, mucilage layer needs to be degraded and this process takes place during the fermentation stage. It was shown that mucilage layer degradation was correlated to acidification by lactic acid bacteria [247-250]. The reduction in pH below 4.6 is a method used empirically by coffee producers to determine the end of fermentation [251]. Lactic acid bacteria have been related also to improve the organoleptic characteristics like acidity of coffee beverage [250]. Finally, lactic acid bacteria in fermentation have been reported with bacteria with antifungal activity preventing growth of fungi related with poor cup quality as well as mycotoxins potentially harmful to consumers [252].

2.13. Side effects of lactic acid in coffee wastewater

The process of fermentation also generates wastewater with serious consequences for the environment. Lactic acid fermentation contributes to the acidification of the wastewater. The

acidity of the effluent might exhaust life supporting of water bodies [231], besides under such conditions plants and animals can hardly survive [232]. Depending of effluent handling, wastewater can leach into the groundwater sources, resulting in pollution of the drinking water for the surrounding communities. Polluted drinking water might cause health problems like skin irritation, stomach pain, and nausea [231]. Because of their high Chemical Oxygen Demand and dissolved solid content, coffee wastewater is normally treated by a combination of anaerobic and aerobic biological methods. The pH is an essential factor of control during anaerobic digestion, since microorganisms have optimum growth in the pH range between 6.8 and 7.6 [253]. In a pilot scale experimental set-up Zhang et al. [254] have examined the influence of lactic acid on the anaerobic digestion of kitchen wastes where lactic acid is the main fermentation product. The study demonstrated that high concentration of lactic acid in the effluent could decrease the load of Chemical Oxygen Demand and deteriorate the effluent of the subsequent methanogenic process due to the propoionic acid accumulation. Therefore, preventing the presence of lactic acid for the improvement of biological treatment, special anaerobic digestion is advisable.

2.14. Adsorption

Adsorption can be defined as the ability of some solids to retain substances from solution onto their surfaces. Adsorption is a commonly used treatment technique for industrial and domestic wastewater. Besides, separation of selected compounds by adsorption in the food industry is widely performed. The substance gathered on the surface is named adsorbate and the solid or surface is called adsorbent. Mistakenly adsorption and absorption are used interchangeably, however absorption is where a liquid is soaked up into the adsorbent, whereas adsorption refers to individual molecules gathering on the surface of the adsorbent. Desorption is another term equally important, and it is defined as the removal of the molecules from the surface. Desorption results when the adsorbent on the surface becomes saturated with the adsorbate. Figure 8 shows the adsorption-desorption mechanism.

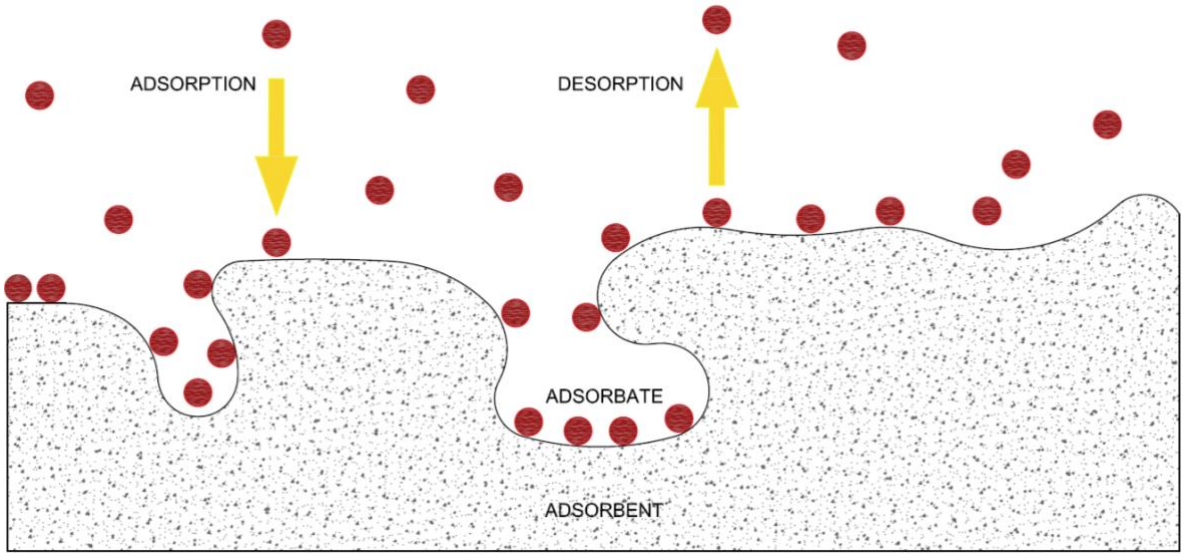


Figure 8 Schematic representation of adsorption and desorption mechanism.

Depending on the nature of the forces involved between adsorbent and adsorbate, the adsorption can be divided in two types: physical and chemical adsorption. In physical adsorption, the adsorbate binds the adsorbate by relatively weak van der Walls forces which are independent of the electronic properties of the adsorbate and the adsorbent, therefore electron exchange does not happen. Chemisorption, however, involves exchange of electrons between adsorbate molecules and surface of the adsorbent forming a chemical bond. The bond formed between adsorbent and adsorbate is stronger in chemical adsorption compared to the physical adsorption. The two types of adsorption work together, where lower energies are associated with physisorption, while higher energies are associated with chemisorption. Nevertheless, in practice it is difficult to differentiate between the two of them.

2.14.1. Adsorption isotherms

The process of adsorption is studied through mathematical models known as adsorption isotherms. Adsorption isotherm is a representation of the amount of adsorbate adsorbed on the adsorbent as a function of the equilibrium concentration remaining after adsorption, at constant temperature. Two isotherm models are mainly used to explain the behavior of adsorption on activated carbon, the Langmuir and Freundlich isotherms.

The Langmuir adsorption isotherm model assumes homogeneous and monolayer adsorption with no lateral interaction between adjacent adsorbed molecules when a single molecule occupies a single surface site [255]. The non-linearized form of the Langmuir isotherm is expressed as:

$$q_e = q_m * K_L * C_e$$

where q_e is the amount of adsorbate in the adsorbent at equilibrium (mg/g), K_L is the Langmuir isotherm constant (L/mg), C_e is the equilibrium concentration of lactic acid in the solution (mg/L).

The Freundlich isotherm model describes a non-ideal and reversible adsorption process, which is applied to explain the multilayer adsorption onto a heterogeneous adsorbent surface. The non-linear form of the Freundlich isotherm model is represented as:

$$q_e = K_F * C_e^{1/n}$$

where K_f is the Freundlich isotherm constant (mg/g) $(L/g)^n$ and n is the adsorption intensity. Adsorption isotherm models are important because they give essential information to understand how a specific adsorbent will interact with the adsorbent surface. Furthermore, the affinity between absorbent and adsorbate, and maximum adsorption capacity are elucidated from the isotherm models.

2.15. Valorization of coffee wastes through modification to activated carbon

Activated carbons are versatile adsorbents, their adsorbent properties lie in the large surface area, area activity, and favorable pore size. Besides physical characteristics, adsorption capacity is greatly influenced by the chemical structure of the surface, which affects the characteristics of the surface such as polarity, acidity, and hydrophobicity [256]. The surface of the activated carbon is normally modified to change its adsorption properties. The modification is carried out mainly by two manufacturing processes, implicating physical or chemical activation. Physical activation involves heating in the absence of air with steam or other gases like carbon dioxide between 800 and 1000° C, while chemical activation is commonly performed using several activation agents like potassium hydroxide, zinc chloride, and phosphoric acid [257]. In the chemical activation, removal of the activation agent is required, causing environmental burden and corrosion risks [258] while for the physical activation steam or carbon dioxide are necessary to oxidize the carbon surface elevating the production costs. Recently, Kong et al. [259] have proposed an activation step via co-calcination with limestone. This method relies on the fact that limestone is decomposed above 800° C producing quicklime and carbon dioxide effluent. Coffee processing by-products have been transformed into activated carbon using different carbonization and activation protocols. Chemical activation is mainly used to produce activated carbon from coffee wastes, nevertheless few investigations have addressed physical activation method using carbon dioxide (CO_2) and steam mixed with a chemical agent [260]. Characteristics such as contact surface, pore distribution and surface chemistry have been enhanced through activation methods. Adsorbents analyzed by scanning electronic microscopy (SEM) have shown that after physical activation a well-developed porosity was obtained. Whereas, after chemical activation disrupted structure was observed [261]. Studies have examined the application of coffee waste activated carbons in the removal

of organic molecules (dyes, acids, pesticides), and cationic and anionic components (lead, cadmium, copper, chromium). These have shown that adsorption capacity of the molecules is correlated with the activation protocol, textural properties of the raw material, size of the organic molecule, and charge of the ions of the adsorbate [24,29,30]. Adsorption of gases and vapor phase has been evaluated. Authors have determined that adsorption capacities for methane (CH_4), hydrogen sulfide (H_2S), and carbon dioxide (CO_2) are due to the high surface area, volume, narrow size of micropores, and chemistry of the adsorbent [31-33].

3. Aim

Coffee consumption is a global issue and many countries with rich economies import coffee beans to primarily roast these according to the individual consumer demand. But those countries producing and primarily processing the raw beans are burdened with low economical incomes to fight the severe contamination. This poses serious environmental problems caused by the by-products and wastewater that is being generated during wet processing. It is in this context that the present research has its importance, contributing to develop alternative methods of controlling the waste by-product quality and solutions to increase not only the pollution awareness but to make re-processing economically more attractive. This resulted in the following aims:

1. Determination of the main constituents present in wet-coffee processing by-products

The high acidity of coffee wastewater and other components that contribute to the pollution of the sewage and environment will be analyzed to have an overview of the individual substances present. The specific aims include:

- Characterization of coffee by-products to determine the acidity compounds such as phenolic compounds, and other organic acids present
- Characterization of the other constituents present and their contribution to pollution
- Characterization of the macromolecules present
- Determination of the bioactive properties of selected main constituents of substances present
- Separation and concentration of selected target substances from coffee by-products
- Determination of alternative uses of the selected target substances from coffee by-products
- Formulation of suggestion for an adequate low-cost technological strategy for acquiring economical add-on value to coffee by-products

2. Characterization of the protein modification present

The proteins present are liable to modification due to the reactive components present. This part will deal with the establishment of a new strategy to encompass such modifications, and to give recommendations for improving the individual steps of wet processing. The specific objectives include:

- Establishment of new protocol for the fragmentation of the proteins using specific enzymes, chemical cleavage or a combination of these two options
- Characterization of the protein modifications via analysis of the fragments produced with MS/MS methods
- Purification/separation/characterization of protein/peptide adducts
- Preliminary tests to the applicability of the established protocol for green coffee beans

3. Treatment of coffee wastewater with activated carbon and analyzing the effect and changes in the constituent composition

This part will address the changes in the wastewater quality by the appropriate treatment while using activated carbon made from other waste products of the coffee processing industry. The specific objectives include:

- Evaluation of the analytical data of coffee wastewater from aim 1 to establish the strategy needed for treatment of selected compounds
- Observation of the treatment for the corresponding changes in the composition of the relevant substances
- Design of an activated carbon species considering the operating parameters and following design: material particle size, mixture ratio, pyrolysis temperature; adsorbate to adsorbent ratio, effect of pH, effect of initial concentration, effect of contact time, and adsorption isotherm

4. Experimental part

4.1. Samples

Characterization part: Samples from *arabica* species were used in this work. Depulped coffee beans, initial fermentation coffee beans, during fermentation coffee beans, final fermentation coffee beans, green coffee beans, coffee pulp, parchment, depulping coffee wastewater, fermentation coffee wastewater, and washing coffee wastewater were obtained from a pilot continuous processing (Rio Colorado Company, located in Palencia, Guatemala) and from a pilot batch processing (Santa Sofia, located in Santa Rosa, Guatemala). Samples were taken in 2018- and 2019-production year for both pilot coffee processes.

Modification of proteins part: Arabica species processed by different type of process were selected for this part. Samples were purchased as green coffee from Coffeewell GmbH, Mettmann, Germany. Table 4 shows the information of the green coffee samples from Africa, Asia, Middle and South America.

Table 4 Samples of *C. arabica*

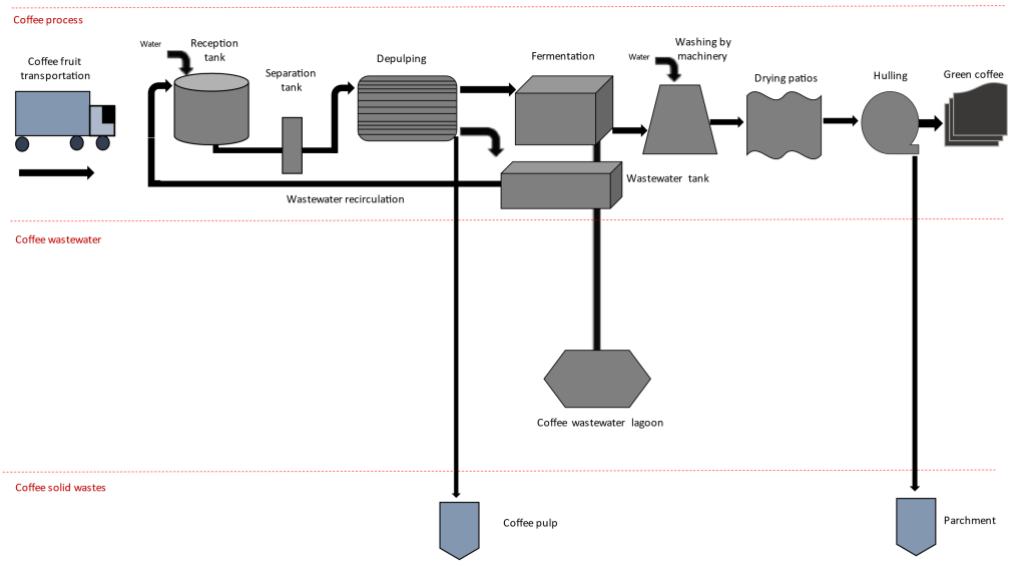
Abbreviation	**Country**	**Name**	**Type of process**	**Type of sample**
AM	Ethiopia	Maji	Half-wet	Green coffee
KJ	Kenia	Josra	Wet	Green coffee
TM	Tanzania	Mbeye Peak	Wet	Green coffee
CT	Costa Rica	Terrazu	Wet	Green coffee
MF	Mexico	Flamingo	Wet	Green coffee
BL	Brazil	Logoa	Dry	Green coffee
BS	Brazil	Santos	Dry	Green coffee
KS	Colombia	Supremo	Wet	Green coffee
PU	Peru	Urubamba	Wet	Green coffee
IM	India	Malabar	Monsooned	Green coffee
JL	Indonesia	Kayumas	Half-wet	Green coffee
SG	Indonesia	Gayo	Half-wet	Green coffee

Adsorption part: Spent Coffee was obtained from Deutsche Extrakt Kaffee GmbH (Berlin, Germany). Coffee parchment was collected from Rio Colorado Coffee Company (Palencia, Guatemala). Commercial carbon Norit® GAC 1240EN was purchased from CABOTCORP (Boston, Massachusetts, United States)

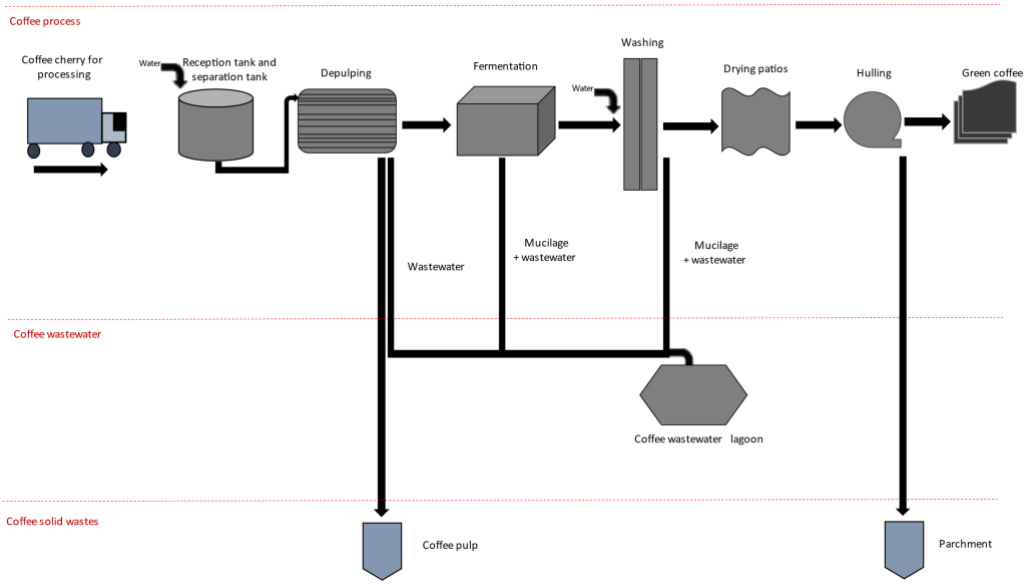
4.2. Methods

4.2.1. Batch and continuous pilot configuration for coffee cherries processing

Two major processing options (pilot batch and continuous wet processing, Figure 9) are commonly practiced in many wet coffee producing countries of Latin America and especially in Guatemala. They differ in the composition of the wash water used to produce the final dried coffee beans. While visiting different small-scale companies in Guatemala, also indicated that slight individual changes to these two principle processing outlines may be employed. An overview of the different technical possibilities is given in a textbook on coffee production [262]. Shortly, in the batch processing, the coffee cherries are immersed in water to select cherries between unripe and the good ripe ones. Cherry skin and pulp were removed by pressing the fruit through a sieving screen, and beans were put in a fermentation tank with a water stream for 26 hours. Fermented coffee beans were washed out to remove mucilage and then finally sundried. Water applied for washing the fermented beans consisted of 50% fresh water and 50% wastewater coming from preceding de-pulping and fermentation steps. This water was gathered and pumped back to the reception tank to be included in processing the next coffee batch. To process 60,000 kg of coffee cherries, a flow rate of 6.9 L/s for 1.5 hours was applied. Therefore, 1 kg of processed coffee required ca. 0.63 liters of water. Fresh water was used during the whole continuous processing and fermentation required 48 hours. Afterwards fermented coffee beans were allowed to rest in a shallow tank for another 40 hours, followed by a washing step. To process 12,000 Kg of coffee, a flow rate of 4.9 L/s for 2 hours was generally applied, thus consuming at least 3 liters of water to produce 1 kg of processed coffee. In comparison, a mechanical system was reported in Central America to remove the mucilage from the beans. This ecological process required only ca.1 L/kg of dry parchment coffee and largely preserves the original quality of the coffee beans [6].



(a)



(b)

Figure 9 Batch (a) and Continuous (b) wet-coffee processing

4.2.2. Sample preparation

Sample preparation for analysis was conducted according to the type of samples (bean and solid by-products or wastewater samples). Coffee beans, pulp and parchment samples were freeze-dried, grinded with a laboratory blender and then fractionated to obtain flours with particle size < 0.2µm.

To determine the total phenolic content, antioxidant capacity, reducing sugars and free amino groups, 10 mg of flour were mixed with 1 mL of 80% methanol in water (80:20, v/v). Extraction was performed at room temperature under shaking conditions for 30 min. After centrifugation at 9300 g for 10 min, supernatants were collected and stored at -20° C. Likewise, wastewater samples were directly centrifuged, supernatants collected and stored at -20° C.

To analyze the organic acids, extraction was performed under the same conditions as described above, using distilled water as solvent. Wastewater samples were directly centrifuged. The supernatants were collected and applied for solid phase extraction (SPE) using a column containing 300 mg of MN SC 6 Polyamide. After activating the column with 3 mL of 0.01 N sulfuric acid, 1 mL of sample was loaded, washed/eluted with 0.01 N sulfuric acid, and stored at -20° C. Afterwards, the organic acids remained in the washing out phase. This treatment also allows the removal of different compounds (e.g., colored fractions) which may interfere with the analysis of the organic acids.

To determine the protein content, 10 mg of flour was initially mixed with 1 mL of 80% methanol for 30 min and centrifuged (9300 g, 10 min at 4° C). Supernatants were removed, the precipitates washed with 1 mL of acetone for 5 min and dried under a hood. Extraction was done overnight using 1 mL of 1% sodium dodecyl sulfate containing 1 mM dithioerythritol.

Mixtures were then centrifuged (9300 g, 5 min at 4° C), the supernatants collected and stored at -20° C.

4.2.3. Folin-Ciocalteu method for the analysis of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method as described by Singleton et al. [263] with some modifications. Gallic acid was used as standard. Briefly, 20 mL of sample or standard solutions was mixed with 180 mL of the Folin reagent (mixture of Folin-Ciocalteu and 0.01 M NaOH, 1:1, v/v) on the iMark microplates while using the corresponding reader (Bio-Rad Laboratories, Hercules, CA, USA). The mixtures were incubated at room temperature for 30 min and absorbance was measured at 750 nm. The results were expressed as g/100 g gallic acid equivalent (GAE) of dry weight (DW) for coffee beans and by-product samples; and as mg/L GAE for wastewater samples.

4.2.4. Determination of caffeine content and composition of major phenolic compounds by HPLC-MS

Caffeine content and composition of phenolic compounds were determined using a HPLC system (Shimadzu HPLC system GmbH, Leonberg, Germany). Analyzes were performed with a C18 column (150 mm × 4.6 mm, Ø 3 µm, at 40° C; BISCHOFF GmbH, Leonberg, Germany) with a pore size of 120 Å. Flour samples were mixed in a ratio of 10:1 (w/v) with a solution of 50% methanol containing 1% acetic acid. Wastewater samples were mixed in a ratio of 1:1 (v/v) with a solution of 98% methanol containing 2% of acetic acid. 10 µL of each solution was injected onto the column. The separation was done at a flow rate of 1 mL/min for 45 min. Mobile phase consisted of 0.1% trifluoroacetic acid in distilled water (eluent A) and methanol (eluent B). The gradient applied was 20% eluent B from 0.01 to 3 min; 35% eluent B, from 3 to 20 min; 68% eluent B from 20 to 37 min; 68% eluent B from 37 to 40 min; 20% eluent B from 40 to 45 min. The detection was performed with dual wavelength at 280 nm for caffeine and at 325 nm for phenolic compounds (with an UV-Vis SPD-10 AVP detector, Shimadzu, Kyoto, Japan). The results were expressed in g/100g chlorogenic acid equivalent (CAE) DW for coffee bean and by-product samples; and as mg/L CAE for wastewater samples.

4.2.5. Determination of reducing sugars

Reducing sugars were determined under isocratic conditions with a Shimadzu HPLC system equipped with an evaporative light-scattering detection (Shimadzu ELSD-LT II, Gain=8, Shimadzu, Kyoto, Japan). Samples were analyzed on an X-bridge Amide column (100 mm × 4.6 mm with particle size of 3.5 µm; Waters GmbH, Eschborn, Germany). Column and ELSD temperatures were set at 65° C and 40° C, respectively. The mobile phase consisted of solutions of 84% acetonitrile and distilled water (eluent A) and 0.1% ammonia (eluent B). Flow

rate was fixed at 0.8 mL/min for an injection volume of 5 µL. Fructose, glucose, sucrose, and maltose solutions prepared with 60% methanol were used as external standards for a calibration range from 0.00 to 0.4 mg/mL. The results were expressed as g/100g DW for coffee bean and by-product samples and as mg/L for wastewater samples.

4.2.6. Determination of organic acids

A HPLC system (Shimadzu Europa GmbH, Duisburg, Germany) equipped with an UV-Vis Detector SPD-10 AVP (Shimadzu, Kyoto, Japan) was used to analyze the organic acids. 50 µL of samples were injected into an Aminex HPX-87H column (300 mm x 7.8 mm, Ø 9 µm, 65° C). The separation was done under isocratic conditions with solution of 0.01 N sulfuric acid. Flow rate of 1.0 mL/min was applied for a separation time of 20 min. Citric acid, malic acid, lactic acid, acetic acid, propionic acid and butyric acid were used as standards for a calibration range from 0.00 to 0.2 mg/mL. The detection was done at 210 nm and the final results were expressed as g/100g DW for coffee bean and by-product samples and as mg/L for wastewater samples.

4.2.7. Antioxidant capacity

Antioxidant capacity was measured with the Ferric Reducing Ability of Plasma (FRAP) method using ascorbic acid as standard. FRAP-reagent was prepared by mixing in a ratio of 1:1 20 mM of iron (III) chloride hexahydrate with 10 mM of 2,4,6-Tripyridyl-S-Triazine (TPTZ). 10 µl of samples or standard solutions were then mixed with 150 µL of FRAP-reagent and incubated at room temperature for 6 min. The absorbance was taken at 595 nm using the microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA). The results were expressed as g/100g FRAP equivalent (FE) of dry weight (DW) for coffee bean and by-product samples and as mg/L FE for wastewater samples.

4.2.8. Free amino groups

Free amino groups were analyzed using the fluorescamine free amino groups assay. Fluorescamine reagent was prepared by mixing 3 mg of fluorescamine (Thermo Fischer Scientific, Waltham, MA, United States) with 1 mL of dimethyl sulfoxide (DMSO). 150 µL of sample or standard were mixed with 50 µL of fluorescamine reagent and incubated at room temperature for 15 min. Excitation was set at 365 nm and emission at 470 nm while using a microplate reader (TECAN infinite M200 PRO, Männedorf, Switzerland). L-Leucin with concentrations ranging from 0.00 to 6.00 mM was used as standard and the results were expressed as g/100g DW for coffee bean and by-product samples and as mg/L for wastewater samples.

4.2.9. Extraction of protein

Samples from two coffee plantations located in Guatemala were extracted by three different methods for protein determination. Extraction with polyvinyl polypyrrolidone(PVPP)(option I) was based on Laing et al. [264] and Ali et al. [53]. The extraction protocol with sodium dodecyl sulfate (SDS) (option II) was developed as described by Want et al. [265] and Figueroa et al. [266]. Extraction protocol option II was further improved and used for further analysis. A schematic flowchart of the three extraction protocols is provided in Figure 10 presents the schematic flowchart of the extraction protocols.

Briefly, for method I: 100 mg of powder sample and 50 mg of polyvinylpolypyrrolidone (PVPP) were mixed with 1 mL of 0.04% ascorbic acid in water (*v/v*). Extraction was performed at room temperature under shaking conditions for 2 hours. After centrifugation at 4000x *g* for 20 min, supernatants were collected and stored at -20 ° C. For method II: 100 mg of powder sample were mixed with 1 mL of hexane under shaking conditions for 10 min. After centrifugation at 7000x *g* for 10 min, supernatants were discharged. Subsequently, 1 mL of hexane was added, and samples were let dry in opened microtubes for 10 min at room temperature. Finally, 750 µL of SDS buffer were added to each sample and heated for 20 min at 50 ° C. After centrifugation at 7000x *g* at 4° C for 5 min, supernatants were collected. Method III consisted of mixing 20 mg of powder sample with 1 mL of hexane under shaking conditions for 10 min. After centrifugation at 7000x *g* at 4° C for 10 min, supernatants were discharged. Thereafter, the precipitates were washed with 1 mL of hexane and let them dry in opened microtubes for 1 hour at room temperature. Subsequently, 750 µL of SDS buffer were added to each precipitate. Then 20 µL of 0.25 M of tris-(2-carboxyethyl) phosphine (TCEP) solution were briefly mixed with the precipitates and next heated at 50 ° C for 20 min in a dark room. Subsequently, 20 µL of 0.25 M of iodoacetamide (IAA) solution were added to the precipitates and incubated at 50 ° C for 20 min in a dark room. Mixtures were afterwards centrifuged for 5 min. Finally, the supernatant was transferred to a new microtube, and precipitates were discharged.

Protein fractions extracted with the three methods were finally mixed with 1 mL of acetone at 4 ° C and incubated at-20 ° C for 20 min. After centrifugation at 7000 ×g for 5 min, supernatants were discharged. Right after, 1.5 mL of methanol at 4 ° C was added and mixed for 20 seconds and incubated for 20 min at -20 ° C. After centrifugation conditions for 5 min, supernatants were transferred to new microtubes.

After extraction with the methods I and II, samples were mixed with 500 µL of urea buffer under shaking for 1 min flowed by 10 min of ultrasonic bath. Mixtures were centrifugated at 10000x g for 5 min and supernatants were stored at -20 ° C.

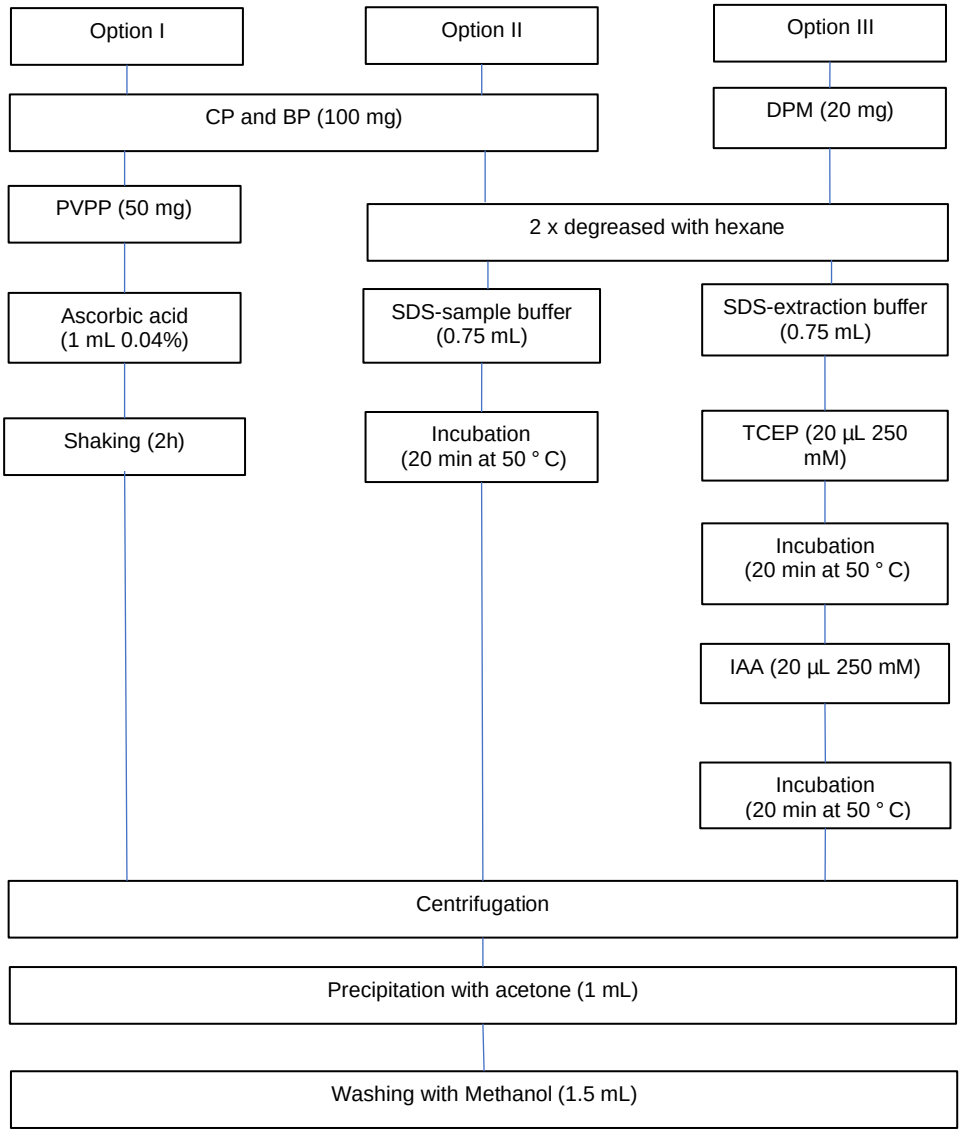


Figure 10 Schematic flowchart of the three protein extraction protocols used

CP are samples from continuous process; BP are samples from the batch process; DPM are samples from different processing methods; TCEP the reducing agent tris(2-carboxyethyl)phosphine and IAA the alkylation agent iodoacetamide.

4.2.9.1. Free amino nitrogen

Free amino nitrogen was performed to measure the concentration of the protein bound free amino groups. Glycine stock solution (2mg/ L) was used as standard. 40 µL of the mixed solution or standard was diluted in distilled water. Briefly, 400 µL of the diluted solution was mixed with 200 µL of ninhydrin staining reagent. The resulting ninhydrin mixtures were heated at 100 ° C for 16 min, solutions were cooled at 20 ° C for 20 min and 1 mL or potassium iodide solution was added. The absorbance against distilled water was measured using the corresponding spectrophotometer (Jenway Genova, Staffordshire, UK). Absorbance was measured at 570 nm. The content of free amino nitrogen was calculated using the Equation 1 and the results expressed as µg/mg protein:

$$FAN = (A_S - A_B - A_C) / (A_G - A_B) \times 2 \times F \quad (1)$$

where, A_S is the absorbance of the sample, A_G is the absorbance of Glycine standard solution, A_B is the absorbance of the blank, A_C is the absorbance of the corrected blank, F is the dilution factor and 2 is the concentration of Glycine standard solution (mg/L).

4.2.9.2. Free thiol groups

Free thiol groups were measured to examine the modification of certain amino acids side chains that is induced by oxidation reaction of phenolic compounds. Reduced glutathione and N-acetylcysteine was used for the calibration curves. The extracted solutions were first dissolved in 0.2 M Tris-SDS buffer. Briefly, 450 µL of the dissolved samples or standard were mixed with 30 µL of (5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in the cuvettes while using the spectrophotometer (Jenway Genova, Staffordshire, UK). The mixtures were incubated at room temperature for 10 min and absorbance was measured at 421 nm. The results are expressed as nmol SH groups/mg protein.

4.2.9.3. Phenolic compounds bound to proteins by HPLC

Composition of the phenolic compounds were determined using a HPLC system (Shimadzu HPLC system GmbH, Leonberg, Germany). Analyzes were performed with a C8 column (250 × 3.0 mm, particle size 5 µm, at 37 ° C; MZ-Analysetechnik GmbH, Mainz, Germany) with a pore size of 300 Å. Undigested samples extracted by the improved SDS extraction method (Option III) were used. After extraction, the protein pellets were dissolved in 0.5 mL urea extraction buffer and injected onto the column. The separation was done at a flow rate of 0.6 mL/min for 20 min. Mobile phases consisted of 0.1% trifluoroacetic acid in distilled water (eluent A) and acetonitrile (eluent B). The gradient applied was: 0% eluent B from 0.01 to 3 min; 40% eluent B, from 3 to 7 min; 40% eluent B, from 7 to 10 min; 80% eluent B from 10 to 11 min; 80% eluent B, from 11 to 13 min; 0% eluent B from 14 to 20 min. The detection was performed with a dual wavelength; at 280/325 nm for the determination of phenolic compounds (with an UV-Vis SPD-10 AVP detector, Shimadzu, Kyoto, Japan). The results were expressed as µg phenolic compound/mg protein.

4.2.9.4. Determination of protein content

Protein content was determined using Lowry method. In an alkaline environment, complex peptides bonds Cu2+ ions are reduced to Cu+. Folin-Ciocalteu reagent is used to strengthen the color of the reaction. The amino side chains of asparagine, cysteine, histidine, tryptophan, tyrosine, and the monovalent copper catalysis react with the Folin-Ciocalteu reagent. Consequently, phosphomolybdic acid and phosphotungstic acid are reduced to molybdenum

and tungsten blue. The resulting chromophores absorb light at 750 nm. The absorption is measured photometrically. BSA was used as external calibration [267,268].

Protein content was measured by using the method of Lowry et al. [268] with BSA as standard. Lowry reagent was prepared by mixing two reagents. Reagent A consists of 1 part copper tartrate complex, 2 parts of distilled water, and 1 part of 0.8 mol/L NaOH. Reagent B contains 1 part of Folin-Ciocalteu solution, and 5 parts of distilled water. Briefly, 75 µL of standard or sample was mixed with 75 µL of reagent A and incubated for 10 min at room temperature. Thereafter, 40 µL of reagent B was added, incubated for 30 min and the extinction was measured at 660 nm. The results were expressed as mg/L.

4.2.9.5. Fluorescence spectroscopy

Fluorescence spectroscopy was performed to describe covalent protein-polyphenol interactions. The visible fluorescence spectrum of the protein extracted with the three different extraction protocols were investigated. Samples were dissolved in 0.5 mL urea extraction buffer. Samples were diluted 1:1280 with the same extraction buffer. The light emitted in the range of 300 – 500 nm light wavelength was recorded with a Spectro fluorophotometer (Shimadzu, Duisburg, Germany) using an excitation wavelength of 280 nm. The signal intensity was determined by plotted the light wavelength against the area under the curve (AUC). Pure urea buffer extraction was used for the blanks and subtracted from the AUC of the samples.

4.2.9.6. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed to determine the resulting molecular weight change and net charge of the interaction of protein and polyphenols. SDS-PAGE was performed under reducing conditions using Novex™ NuPAGE™ 4-12% Bis-Tris gels according to the manufacturer's instructions (Thermo Scientific™, Carlsbad, CA, USA). Samples were mixed with Novex™ NuPAGE™ LDS sample buffer to a ratio of 1:1 and then heated a 95 °C for 10 minutes. 10 µL of the mixture was loaded into the gels. After separation, gels were stained overnight with a solution of Coomassie Blue G250, then destined for ca. 2 hours with 10% acetic acid and finally scanned (Bio-5000 Professional VIS Gel Scanner, SERVA Electrophoresis GmbH, Heidelberg, Germany). Spectra Multicolor Broad Range Protein-Marker (Thermo Fisher Scientific, Vilnius, Lithuania) was used as standard.

4.2.9.7. In-gel digestion

Gel bands obtained from the SDS-PAGE were cut with a scalpel and placed in a 0.5 mL microtubes. Thereafter, 200 µL of Coomassie blue G250distaining solution was added and incubated at 37 ° C for 30 min. Subsequently, 100 µL oftris-(2-carboxyethyl)-phosphine(TCEP) reduction buffer (25 mM) was added and incubated at 60 ° C for 10 min. Next, 100 µL of

iodoacetamide (IAA) buffer (25 mM) was mixed and incubated at room temperature for 1 hour in a dark room. IAA buffer was removed and 200 µL of distaining solution was added and incubated at 37 °C for 15 min. 100 µL of acetonitrile were added and incubated at room temperature for 15 min. Acetonitrile was removed and gel pieces were allowed to dry for 15 min. The digestion was performed by using trypsin and pepsin enzymes. Digestion using trypsin: the treated gel pieces were mixed with 15 µL of trypsin. After incubation at room temperature for 15 min, 25 µL of 25 mM ammonium bicarbonate buffer was added; digestion using pepsin: the treated gel pieces were mixed 15 µL of pepsin. After incubation at room temperature for 15 min, 25 µL of 0.09 M HCl was added. Finally, gel pieces were macerate manually and incubated in a dark room for 20 hours.

4.2.9.8. Solid-phase extraction (SPE)

SPE was performed to remove any impurities of the extracted samples. Briefly, 300 mg of C18 material (Chromabond C18 ec, Marchery-Nagel, Düren, Germany) was placed into a glass chamber.After activating the column using 6 mL of a buffer containing 50% acetonitrile, 50% double-distilled (bidest) water and 0.1% formic acid (SPE buffer); conditioned with 6 mL of bidest water, the digested samples were loaded onto the columns, washed with 6 mL of bidest water and the peptides were collected using 1 mL of SPE buffer. The eluates were finally diluted in a ratio 1:5 (v/v) using bidest water, and the samples were filled into the vials for the LC-MS/MS analysis.

4.2.9.9. MALDI-TOF-MS

Samples separated by SDS-PAGE were analyzed by mass spectrometry using matrix-assisted laser desorption ionization time of flight (MALDI-TOF) to analyze covalent bonds between 5-CQA and 11S. Samples digested for 20 hours were centrifugated for 10 min and subsequently placed in the ultrasonic bath (BANDELIN electronic GmbH, Berlin, Germany). After centrifugation conditions for 10 min, the supernatant was removed and transferred to a fresh reaction vessel. The matrix solution was freshly prepared by mixing 20 mg of α-Cyano-4-hydroxycinnamic acid with 300 µL of acetonitrile and 700 µL of 0.1 % TFA. 2 µL of samples was mixed with 2 µL of matrix solution and 3 µL of the matrix-sample mixture was placed on a MALDI plate for each sample and standard. The matrix-sample mixtures were crystallized at room temperature after 20 min. Peptide calibration standard II (Bruker, Massachusetts, USA) was used as standard. The time-of-flight analyzer (Autoflex Speed, Bruker, Massachusetts, USA) was run with the software FlexControl 3.4 (Bruker). After calibration, 30 – 40 % of laser intensity was applied to the crystallized matrix samples until clear peaks were recognized. The mass spectra obtained were analyzed using the software FlexAnalysis 3.3 (Bruker). The background noise was minimized by using the baseline subtraction function. Calibration was

performed by using the spectra of the closest standard. For each spectrum, a mass list of the most important peaks was imported to the software BioTool 3.2 (Bruker).

4.2.9.10. In-Solution digestion

For the trypsin digestion, the extracts after acetone precipitation were re-dissolved in 400 µL of digestion buffer containing 0.1 M ammonium bicarbonate. After addition of 20 µL of the trypsin solution (4mg/mL), incubation at 37 ° C under shaking conditions for 20 hours was performed. The digestion was stopped by adding 15 µL of 40 % formic acid. For pronase digestion, samples digested from the tryptic digestion were used. First, 1 mL of the eluate was freeze-dried, and the resulting pellets were dissolved in 0.4 mL of digestion buffer containing 50 mM of ammonium bicarbonate using an ultrasonic bath. Afterwards, 16 µL of a pronase solution (1 mg/mL) (Sigma-Aldrich, Steinheim, Germany) were added and incubated at 37 ° C under shaking condition for 20 hours. The reaction was stopped by adding 15 µL of 40 % formic acid. Prior to LC-MS/MS analysis, digested extracts were applied for SPE.

4.2.9.11. Development of a Multiple Reaction Monitoring (MRM) assay

For the analysis of protein modification in connection with phenolic compounds, an MRM method was developed using HPLC-MS/MS. The method focuses on the detection of unmodified and lysine-modified peptides of the α- and β-chain of the trypsin digested protein 11S present in protein extracts of *C. arabica*. For the method development, a define workflow was followed and corresponding steps are defined in Figure 11.

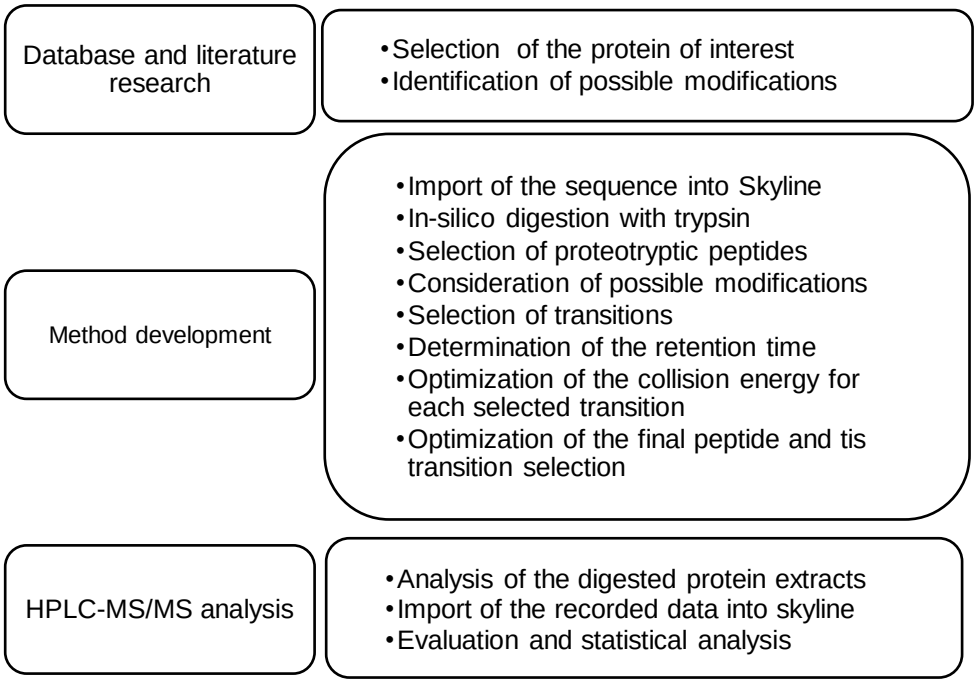


Figure 11 Workflow for developing a Multiple Reaction Monitoring (MRM) assay using a high performance liquid chromatography mass spectrometry HPLC-MS/MS.

The information of the 11S sequence was obtained from the UniProt online database (https://www.uniprot.org) and while considering the published data [269,270]. A total of four unreviewed records with information obtained from literatures and curator-evaluated computational analysis was compiled from UniProt database. The compilation is given in section 7.12

The sequence of the four selected sequences of 11S were imported into the MacCoss Lab Skyline Software via FASTA (fast-all) data file. FASTA is a program developed by Pearson et al. for database search and sequence comparison of proteins and DNA [271]. The Skyline program performs an in-silico digestion with the imported sequences of the target proteins. Skyline generates a list of all possible peptides, considering the probability of their formation. Which peptides are best suited for the analysis is determined by the ionization capability, signal strength, and specificity of the peptides for a certain protein [269].Following settings were applied: trypsin was selected as the proteolytic enzyme. The maximum number of missed interfaces (partials) was set at zero. No background proteome was set. The length of the peptides was set at 5-25 amino acids and the modification considered was the carbamidomethyl referring to the alkylation of the cysteine residues by iodoacetamide. The transitions are filtered for double charged precursors and single charged ions. In addition, it was set that only y-ions are allowed through. Each transition should search for six fragment ions. Skyline calculated the retention time to be 8.6 min and a preliminary collision energy of the individual transitions. The collision energy was optimized. For this purpose, different collision energies were tested for each intact mass and the one which achieved the largest peak area after the MS/MS run was selected. The selected transitions of the α and β-chain are listed in section 7.10.1 and 7.10.2. To measure lysine-modified masses, a structural modification of an additional 176 m/z for the CQA monomer and 341 m/z for DiCQA was chosen in Skyline.

Tryptic digested protein in coffee beans were measured through an HPLC-MS/MS Agilent 1260 system (Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn, Germany) with a binary pump, multicolumn thermostat provided with an Agilent G6470A series triple Quad LC/MS (Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn, Germany), integrated with an electrospray (ESI) source operating in positive and negative ionization mode. One microlitre of sample was injected into the system through a Kinetex C8 analytical column (150 × 4.60 mm, 2.6 µm, 100 A; Phenomenex, Torrance, CA, USA) set at a temperature of 30° C with a flow rate of 0.5 mL/min. The mobile phase was composed of HPLC grade water containing 0.1% formic acid (eluent A) and 100% LC-MS grade Acetonitrile (eluent B). Mobile phase gradient conditions were set as follows: 100% solvent A from 0 to 5 min, 50–5% solvent A from 20 to 24 min, and 100% solvent A from 25 to 28 min. The desolvation gas temperature in the ionization source was set at 275°C, gas flow rate of 11 L/min, nebulizer

pressure of 35 PSI, fragmentor voltage of 130 V, and dwell time of 20 min. The collision gas used was nitrogen. Detection was performed in the multiple reaction monitoring (MRM) mode, in which a specific transition was monitored at a specific time according to the retention time of the peptides. The MS-Data collection was obtained between 3 – 20 min. The relative abundance of each peptide was measured considering the total area of all the transitions analyzed.

4.2.10. Modification of coffee by-products to activated carbon

4.2.10.1. Sample preparation

Four different types of adsorbents were studied for the adsorption experiments: one from spent coffee grounds, one from coffee parchment, and two commercial activated carbons. Spent Coffee (SC) was collected from the Deutsche Extrakt Kaffee GmbH (Berlin, Germany). Parchment (P) was collected from Rio Colorado coffee company (Palencia, Guatemala). Spent Coffee was stirred to obtain a homogeneous sample, and oven-dried at 110° C for 24 h. Coffee Parchment was first grinded in a ball mill until a particle size of < 64 µm was achieved. Four separate mixtures of calcium carbonate with spent coffee or parchment were prepared with mass ratios 0:1 (spent coffee or parchment directly carbonized without the addition of calcium carbonate), 1:1, 1:2, and 2:1. The mixtures were put in an ark, which was placed in a programmable quartz tube electric oven to be carbonized at 800° C and 850° C for 1 h ata heating rate of 5° C/min under an argon atmosphere. The carbonized samples were washed with 2 M HCl, and later washed with deionized water on filter paper, until neutral pH was reached. Finally, samples were dried at 110° C for 24 hours and cooled down to room temperature for further separation. The activated carbon varieties of spent coffee (SC) and parchment (P) resulting from ratios 0:1, 1:1, 1:2, and 2:1 (w/w), and temperatures 800° C and 850° C were labelled SC1-80 and P1-80 (0:1, 800° C); SC1-85 and P1-85 (0:1, 850° C); SC2-80 and P2-80 (1:1, 800° C); SC2-85 and P2-85 (1:1, 850° C); SC3-80 and P3-80 (1:2, 800° C); SC3-85 and P3-85 (1:2, 850° C). The commercial carbons were purchased from Merck (Darmstadt, Germany) and CABOTCORP (Boston, Massachusetts, United Sates), in this work labeled PAC and GAC, respectively and used without further treatment.

4.2.10.2. Infrared spectroscopy (IR)

Functional groups in activated carbon samples were analyzed by IR spectroscopy. Pulverized samples were placed on the spectrometer Nicolet iS5 (Thermo Scientific, Massachusetts, USA) coupled with a diamond crystal unit iD7 ATR (Thermo Scientific). 32 scans were performed from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

4.2.10.3. Thermogravimetric analysis (TGA)

Changes in the mass as a function of the temperature of the materials were measured as samples were heated and held at a constant temperature in a nitrogen atmosphere. The mass of the samples was analyzed using a Linseis TGA/DTA L81 (Linseis Messgeräte GmbH Selb, Germany). Temperature was set from 25 to 1,000° C with a heating rate of 10° / minute.

4.2.10.4. Organic elemental analysis

The carbon, hydrogen, nitrogen, and sulfur amount of the materials were determined using an Elementar Vario EL III (Elementar Analysensysteme GmbH, Langensbold, Germany).

4.2.10.5. X-ray powder diffraction (XRD)

The structure characteristics and composition of the samples were analyzed by using an empyrean powder X-ray diffractometer (Malvern Panalytical, Malvern, United Kingdom) in a Bragg-Brentano geometry coupled with a PIXce11D detector using Cu Kα radiation (λ = 1.5416 Å) operating at 40 kV and 40 mA. The θ/θ scans were performed from 4-70° 2θ with a step size of 0.0131° and a sample rotation time of 1second. The diffractometer was configured with a programable divergence, anti-scatter slit and a large Ni-beta filter. The detector operated on a continuous mode with an active length of 3.0061° .Data of IR, TGA, and XRD was processed using OriginPro, version 2019b (OriginLab Corporation, Northampton, MA, USA).

4.2.10.6. Scanning electron microscopy (SEM)

SEM was used to analyze the surface properties and morphology of the prepared activated carbons. Samples were loaded onto a double-sided carbon tape attached to SEM tubes and then coated with gold/palladium using a sputter coater (SC7620 coater, Quorum Technologies, Lewes, United Kingdom) for 75 seconds at 18 mA to avoid charging effects. SEM images were acquired using a JEOL JSM-6510 mode Field Emission Scanning Electron Microscope (JEOL Ltd. Manufacturing company, Akishima, Tokyo, Japan). The acceleration voltage was set at 15 kV and the images were magnified 4,000 times.

4.2.10.7. Zeta potential measurements

Zeta potential measurements were performed to determine electrostatic magnitude between particles of activated carbon samples. Materials were first mixed with 30 mL distilled water and thereafter treated in ultrasonic bath for 30 min. Samples were then analyzed by using the zeta meter Zetasizer Ultra (Malvern Panalytical, Malvern, United Kingdom). Data was calculated using the software ZS XPLORER (Malvern Panalytical)

4.2.10.8. Specific surface area and porosity by nitrogen sorption analysis

Nitrogen (N_2) sorption isotherms at 77 K were measured on a Micromeritics Gemini VII Surface Area and Porosity Analyzer (Micromeritics, Norcross, GA, USA). The specific surface area was calculated using the Brunauer-Emmett-Teller (BET) equation [272] and applying the Rouquerol criterion [273]. The pore size distribution and pore volume were calculated using the heterogenous surface non-local density functional theory (HS-NLDFT) with a minimum pore width of 1.47 nm in the SAEIUS software Version 3.0 (Micromeritics, Norcross, GA, USA), as previously described by Maziarka et al. [274]. Prior to N_2 sorption measurements, samples were vacuum dried at room temperature for at least 16 h, followed by degassing under heating and vacuum, using a VacPrep™ 061 Sample Degas System (Micromeritics, Norcross, GA, USA). The spent coffee grounds and parchment (~1 g) were degassed at 80° C for 16 h, while the activated carbon materials (~250 mg) were degassed at 200° C for 4 h.

4.2.10.9. Adsorption experiments

The batch equilibrium experiments were conducted in a vertical shaker at a speed of 30 rpm. The model of anionic color dye was prepared from an analytical grade 90% Bromophenol Blue stock solution and demineralized water. Whereas the model of fermentation broth was prepared from an analytical grade of 99.5% citric acid, 99.0% L-(-)malic acid, 98.0% L-lactic acid, 100% acetic acid, 99.0% propionic acid, 98.0% sodium butyrate stock solution and demineralized water. The initial pH of the bulk solution was adjusted by either NaOH or HCl. Batch adsorption studies were performed in 5 mL microtubes containing 2.0 mL of model anionic color dye (50 mg/L) and 4 mg of adsorbent for all Bromophenol Blue studies. Lactic acid batch experiments 10% w/v were used as performed by Pradhan et al. [275] unless stated otherwise, which then were place in a vertical shaker for 4 hours to obtain equilibrium. Samples were taken at predetermined intervals and further filtered with a 0.22 µm syringe filter. The adsorbents named SC1-80, SC1-85, SC2-80, SC2-85, SC3-80, SC3-85, P1-80, P1-85, P2-80, P2-85, P3-80, and P3-85 were studied through Bromophenol Blue at different calcination temperatures (800 and 850° C), various pH (1-11) and different initial concentrations in the range of 10-100mg/L. Adsorbents named SC1-85, P1-85, GAC, and PAC were studied using Lactic acid adsorbate at different pH (2-6), and initial concentrations (0.5-25 g/L) and a mixture solution containing citric acid, malic acid, lactic acid, acetic acid, propionic acid, and sodium butyrate at the initial concentration of 20 g/L, based on pre-screening experiments, and literature survey. All the batch adsorption experiments were performed in duplicate by employing individual microtubes for each data point.

4.2.10.10. Batch Adsorption experiments of color dye and organic compounds

The Bromophenol Blue study comprised three different series of experiments, investigating the effect of (i) calcination temperature; (ii) pH; (iii) initial Bromophenol Blue concentration. The lactic acid adsorption study encompassed two series of experiments, analyzing the effect of (i) pH and (ii) initial concentration. The effect of pH was tested for different pH (2, 2.85, 3.86, 4 and 6), both above and below the pKa value of lactic acid by using a model fermentation broth of 2.5 g/L lactic acid. The effect of lactic acid initial concentration was tested by using a model fermentation broth for different concentrations (0.5, 1.5, 2.5, 3.0, 3.5, 4.5, 5.5, 6.0, 7.5, 10.0, 15.0, 20.0 and 25 g/L) without pH modification. The pH and initial concentration range were particularly selected to represent the lactic acid production from wet-coffee processing [266] and various heterolactic fermentation processes [276,277]. The effect of main organic acids in wet-coffee processing was tested by using a model fermentation broth mixture of 20g/L containing citric acid, malic acid, lactic acid, acetic acid, propionic acid, and butyric acid. The effect of fermentation coffee wastewater adsorption was tested by using samples collected in Santa Sofia coffee company located in Palencia, Guatemala.

The adsorption capacity and efficiency (i.e., % organic acids adsorbed onto the adsorbent) under equilibrium conditions were calculated using equations (1) and (2), respectively:

$$qe = (Co - Ce)\ Vs / 1000 * m \quad (1)$$

$$E = (Co - Ce) / Co * 1000 \quad (2)$$

where qe is the amount of adsorbate in the adsorbent at equilibrium (mg/g), E is Bromophenol Blue or lactic acid recovery efficiency (%), Co is the initial concentration of Bromophenol Blue or lactic acid (mg/L), Ce is the equilibrium concentration of Bromophenol Blue or lactic acid in the solution (mg/L), V is the volume of the solution (L) and m is the mass of the adsorbent (g).

4.2.10.11. Adsorption Isotherm

The batch equilibrium results of different initial lactic acid concentration experiments were used for the isotherm study. Two commonly used isotherm models, Langmuir (eq. I), and Freundlich (eq. II) were selected to explain the lactic adsorption process.

The Langmuir adsorption isotherm model assumes homogeneous and monolayer adsorption with no lateral interaction between adjacent adsorbed molecules when a single molecule occupies a single surface site. The form of nonlinearization equation of Langmuir (Table 5, equation I) is represented by Ce which is equilibrium aqueous-phase concentration of adsorbate (mg/L), and KL is the constant related to the free adsorption energy and the reciprocal of the concentration at which half saturation is reached.

On the other hand, the Freundlich isotherm model describes a non-ideal and reversible adsorption process, which is applied to explain the multilayer adsorption onto a heterogenous adsorbent surface. In Freundlich equation (Table 5, II), KF is the Freundlich constant of maximum absorption capacity, Ce is the solution concentration equilibrium (mg/L), and 1/n is the sorption constant having a value between 0 and 1.

Table 5 Non-linear form of adsorption isotherm model equations

Isotherms	Non-linear form	Equation
Langmuir	$qe = qm * KL * Ce$	(I)
Freundlich	$qe = KF * Ce^{1/n}$	(II)

Notes: qe = amount of adsorbate in the adsorbent at equilibrium (mg/g), Ce = equilibrium concentration of lactic acid in the solution (mg/L), qm = maximum monolayer coverage capacities (mg/g), KL = Langmuir isotherm constant (L/mg), n = adsorption intensity, KF = Freundlich isotherm constant (mg/g) (L/g)n

4.2.10.12. Organic acid quantification

An HPLC system (Shimadzu Europa GmbH, Duisburg, Germany) equipped with a UV-Vis Detector SPD-10 AVP (Shimadzu, Kyoto, Japan) was used to analyze the organic acids. A volume of 50 µL of samples untreated and treated with different activated carbons was injected into a SUPELCOGEL^{TM} H, 6% Crosslinked column (250 × 4.6 mm, 9 µm; Sigma Aldrich, Steinheim, Germany). The separation was performed at 70° C under isocratic conditions, using a solution of 0.01 N sulfuric acid as eluent. Flow rate of 0.5 mL/min was applied for a separation time of 15 min.

4.2.10.13. Total phenolic compounds

Folin-Ciocalteu method was used to determine the content of total phenolic compounds as described by Singleton et al. [263] with some modifications. Gallic acid was used as standard. Shortly, 20 µL of standard or sample solution was mixed with 180 µL of the Folin reagent (mixture of Folin-Ciocalteu and 0.01 M NaOH, 1:1, v/v) on the iMark microplates while using the reader (Bio-Rad Laboratories, Hercules, CA, USA). The mixtures were incubated for 30 min at room temperature and absorbance was measured at 750 nm. The results were expressed as mg/L GAE.

4.2.10.14. Protein content

Protein content was measured by using the method of Lowry et al. [268] with BSA as standard. Lowry reagent was prepared by mixing two reagents. Reagent A contains 1 part of copper tartrate complex, 2 parts of distilled water, and 1 part of 0.8 mol/L NaOH, while reagent B consists of 1 part of Folin-Ciocalteu solution, and 5 parts of distilled water. Briefly, 75 µL of

standard or sample was mixed with 75 µL of reagent A and incubated for 10 min at room temperature. Thereafter, 40 µL of reagent B was added, incubated for 30 min and the extinction was measured at 750 nm. The results were expressed as mg/L.

4.2.10.15. Scan spectra

Coffee wastewater compounds were measured through HPLC-MS/MS Agilent 1260 system (Agilent Technologies Sales & Services GmbH & Co.KG, Waldbronn, Germany) with a binary pump, multicolumn thermostat provided with an Agilent G6470A series triple Quad LC/MS (Agilent Technologies Sales & Services GmbH & Co.KG, Waldbronn, Germany) integrated with an electrospray (ESI) source operating in positive and negative ionization mode. Compounds were measured using a Kinetex C8 analytical column 2.6 µm, 100 A, 150 x 4.60 mm (Phenomenex, Torrance, CA, USA) set at temperature of 30° C. The mobile phase was composed of eluent A (0.1% formic acid) and eluent B (100% acetonitrile), at flow rate of 0.5 mL/min. The following mobile phase gradient conditions were applied: 100% A from 0 to 5 min, 50-5% A from 20 to 24 min, and 100% A from 25 to 28 min. A total of 1 µL of sample was injected. The dissolvation gas temperature in the ionization source was set at 275° C, gas flow rate of 11 L/min, nebulizer pressure of 35 PSI, fragmentor voltage of 130 V, and dwell time of 20 min. The collision gas used was nitrogen. Detection was performed in the MS2 mode. The MS-Data collection was performed between 2- and 20-min. Mass spectrometric scans were made for the range of 50-1500 m/z. Compounds sorption onto AC was measured considering the total area of all the transitions analyzed.

4.3. Data analysis

All experiments were carried out at least in triplicate and data are expressed as mean ± standard deviation. Data were analyzed with GraphPad Prism 8® (GraphPad Software, Inc., San Diego, CA, USA) using two-way ANOVA and Tukey's test, and the results were considered statistically significant for $p < 0.05$. Isotherm models were analyzed with OriginPro, version 2019b. OriginLab Corporation, Northampton, MA, USA.

5. Results and discussion

The results and discussion presented are divided into three studies reflecting the content of the three manuscripts published.

5.1. Study 1: Comparison of Batch and Continuous Wet-Processing of Coffee: Changes in the Main Compounds in Beans, By-Products and Wastewater

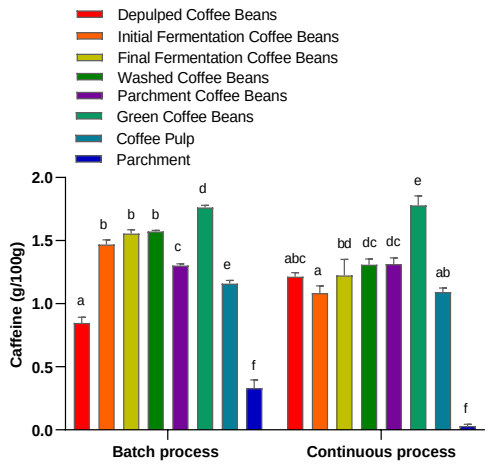
Many technical challenges still need to be overcome to improve the quality of the green coffee beans. The wet Arabica coffee processing in batch and continuous modus were investigated. Coffee beans samples as well as by-products and wastewaters collected at different production steps were analyzed in term of their content in total phenols, antioxidant capacity, caffeine content, organic acids, reducing sugars, free amino group and protein content. The results showed that 40% of caffeine was removed with pulp. Green coffee beans showed highest concentration in organic acids and sucrose (4.96 ± 0.25 and 5.07 ± 0.39 g/100g DW for the batch and continuous processing). Batch green coffee beans contained higher amount of phenols. 5-caffeoylquinic Acid (5-CQA) was the main constituent (67.1 and 66.0% for the batch and continuous processing). Protein content was 15 and 13% in the green coffee bean in batch and continuous processing, respectively. A decrease of 50 to 64% for free amino groups during processing was observed resulting in final amounts of 0.8 to 1.4% in the processed beans. Finally, the batch processing still revealed by-products and wastewater with high nutrient content encouraging a better concept for valorization.

5.1.1. Caffeine, total phenols, and antioxidant analysis

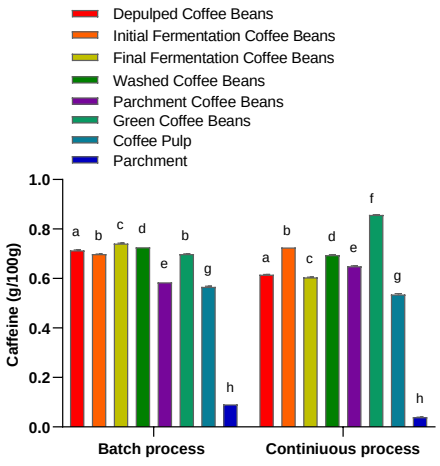
Coffee beans, coffee pulp and parchment were collected at different steps of the wet batch and continuous pilot scale processing, and samples were analyzed. Figure 12 presents the concentrations of caffeine, phenolic compounds and antioxidant capacity of de-pulped coffee beans, initial and final fermented coffee beans, washed coffee beans, green dried coffee beans, coffee pulp and parchment for 2018 and 2019 production year. An increase of caffeine, total phenols and antioxidant capacity from the de-pulped coffee beans to the green coffee beans can be observed, depending on whether the cherries were processed in batch or in continuous workflow. Caffeine content for 2018 production year in de-pulped coffee beans and green coffee beans was 0.85 ± 0.04 and 1.21 ± 0.03; and 1.76 ± 0.02 and 1.78 ± 0.08 g/100g DW for the batch and the continuous process, respectively. Caffeine content for 2019 production year shows lower content compared to 2018; de-pulped coffee beans and green coffee beans were 0.75 ± 0.00 and 0.70 ± 0.00; and 0.62 ± 0.00 and 0.86 ± 0.04 DW for the batch and continuous process, respectively. Statistical analysis shows that there were no

significant differences while comparing the values in green coffee beans for both production years. Contents of caffeine in Arabica green coffee beans were reported to range between 0.7 and 1.7 g/100 g [278,279]. Caffeine values of coffee beans at different processing steps were all in that range. High values of caffeine were obtained in coffee pulp (1.16 ± 0.02 and 1.09 ± 0.03 g/100g DW for the batch and continuous processing 2018, respectively), caffeine was detected in the parchment of the batch process (Figure 12a, b). This result indicates that up to 40% of caffeine is removed with pulp during the wet processing, regardless of whether the processing is performed in batch or in a continuous way. Caffeine (1,3,7-trimethyl-xanthine), was reported to be basically related to the protective function of coffee beans and viewing the inner part of the cherry, caffeine is found as much in the beans as in the pulp and skin [280]; justifying the higher amount of caffeine in the pulp documented here.

Phenolic compounds and antioxidant capacity were analyzed, and data processing did not show a significant difference in green coffee bean samples ($p > 0.05$). The values of 1.43 ± 0.18 and 1.16 ± 0.28 g/100g DW; 2.04 ± 0.09 and 2.08 ± 0.57 g/100g DW were obtained for the batch and the continuous processing 2018 production year, respectively (Figure 12c). The values of 0.98 ± 0.13 g/100g DW; 1.43 ± 0.33 g/100g DW were obtained for the batch and the continuous processing 2019 production year, respectively (Figure 12d). Significant differences in term of antioxidant capacity were observed between the de-pulped coffee beans for the two production years (0.81 ± 0.22 and 1.41 ± 0.14 g FE/100g for the batch and the continuous processing 2018, respectively; 2.04 ± 0.33 and 1.28 ± 0.07 for the batch and the continuous processing 2019, respectively).



(a)



(b)

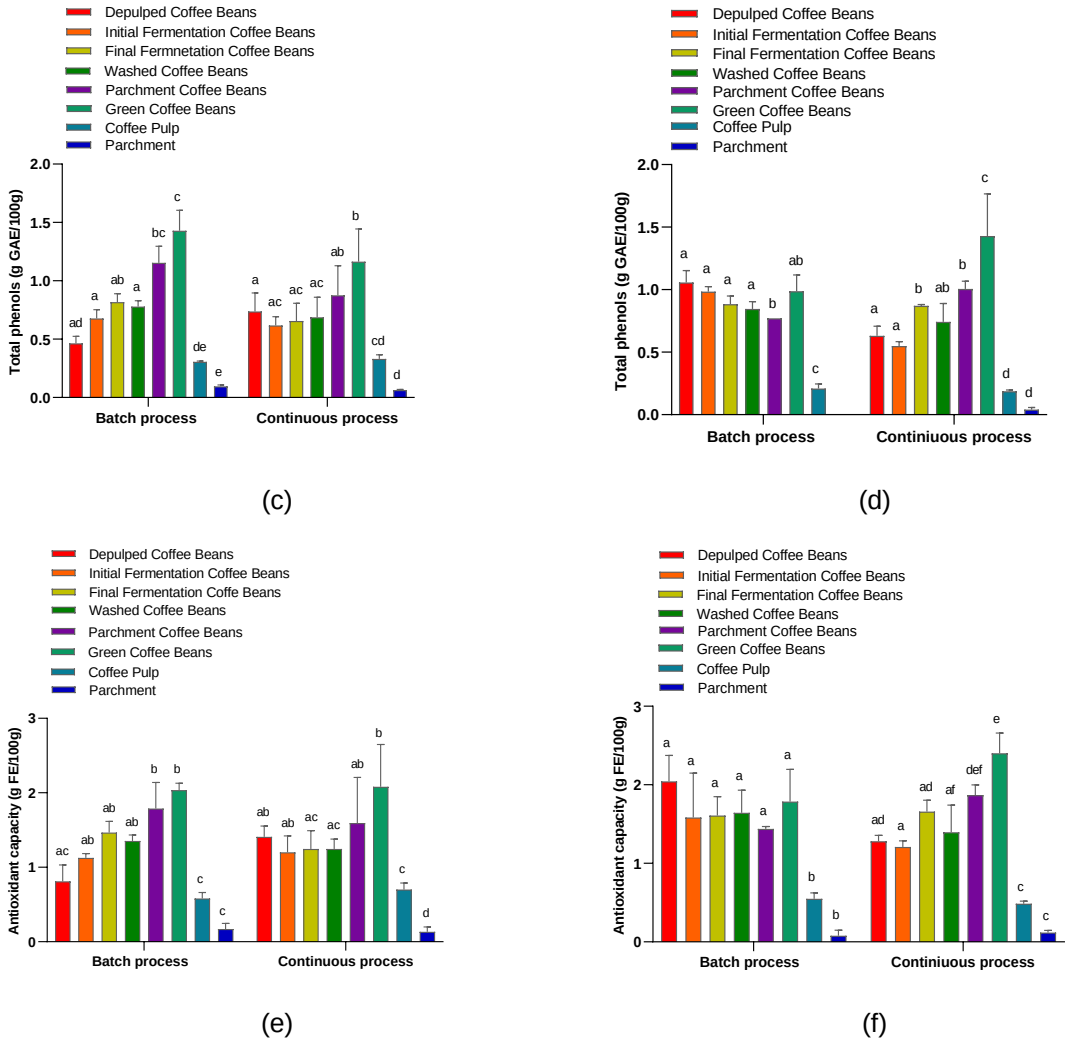


Figure 12 Content of caffeine, total phenols and antioxidant capacity of coffee beans and by-products.

Content of (a) caffeine, (c) total phenols and (e) antioxidant capacity of coffee beans and by-products during the wet batch and continuous processing. (a), (c), and (e) represent data of coffee processing year 2018. (b), (d), and (f) represent data of coffee processing year 2019. The antioxidant capacity is measured in FRAP equivalents (FE). Data is expressed as means ± standard deviation with n = 3. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

Moreover, in terms of total phenol and antioxidant capacity no significant ($p < 0.05$) changes were recorded during the fermentation step in the batch processing while significant ($p > 0.05$) changes were showed in the case of continuous process 2019 for total phenols.

Total phenol values of 0.68 ± 0.08 and 0.82 ± 0.07 g GAE/100g DW; and antioxidant capacity values of 1.13 ± 0.06 and 1.47±0.15 g FE/100g DW were obtained for the initial and the final fermentation coffee beans with the batch processing 2018 production year, respectively. These values were 0.62 ± 0.08; 0.66 ± 0.15; 1.20 ± 0.22 and 1.25 ± 0.25 g/100 g DW for the total phenol and antioxidant capacity for initial and final step of fermentation of coffee beans during the continuous processing 2018 production year, respectively. Alike values of total phenol values of 0.98 ± 0.04 and 0.88 ± 0.07 g GAE/100g DW; and antioxidant capacity values of 1.58

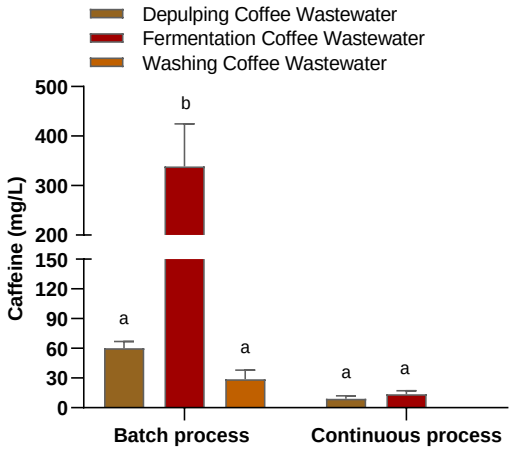
± 0.56 g FE/100g DW and 1.61 ± 0.24 g GAE/100g DW were obtained for the initial and the final fermentation coffee beans with the batch processing 2019 production year, respectively. Similar values of phenolic content and antioxidant capacity were previously reported from Arabica green coffee beans [278,279,281].

Wastewater resulting from the de-pulping, fermentation and washing steps was also investigated and the results are presented in the Figure 13. It can be seen that batch processing exhibited higher content of caffeine, total phenols, and antioxidant than continuous processing. Values of 2018 processing year for caffeine, total phenols and antioxidant capacity of wastewater from the fermentation step in batch processing were 388.6 ± 85.9; 53.2 ± 1.6 and 88.7 ± 4.9 mg/L, respectively. Corresponding values for the continuous processing were significantly different (p > 0.05):13.6 ± 3.6; 6.8 ± 0.2 and 20.5 ± 0.2 mg/L, respectively. Whereas values of 2019 processing year of caffeine, total phenols and antioxidant capacity of wastewater from the fermentation step in batch processing were 103.1 ± 0.01; 67.9 ± 3.5 and 124.4 ± 10.38 mg/L, respectively. Corresponding values for continuous processing were significantly different (p > 0.05):12.0 ± 0.1; 10.21 ± 1.3 and 19.6 ± 1.30 mg/L, respectively.

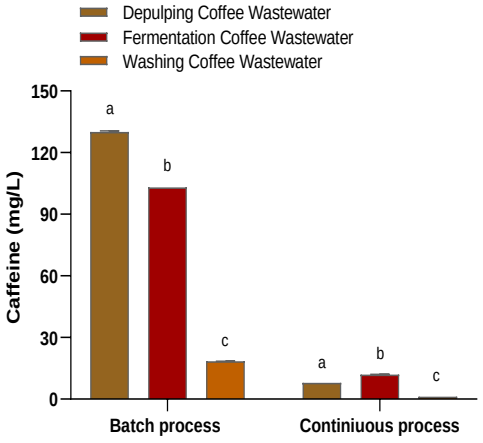
The results of processing year 2018 document an increase in caffeine content up to 5 times in the wastewater resulting from the fermentation step of the batch processing. Contrary to the production year 2019, results exhibit decreases in caffeine content to 1.5 times in the wastewater from fermentation step. During the fermentation, organic acids are also produced and it has been shown that caffeine solubility may increase with acidity of the medium [280]. The main reason for the observed increase in caffeine but also that of phenolic compounds and the corresponding antioxidant capacity most probably lies in the re-using of the wastewater from former/proceeding treatments during the batch processing. Caffeine was not detectable in wastewater generated by the washing step in the continuous processing and the corresponding total phenols, and the antioxidant capacity values were significantly (p > 0.05) different with 3.7 ± 0.5 and 9.6 ± 0.3 mg/L as compared to batch processing, respectively. As for 2019 production year, corresponding values were 2.5 ± 1.6 and 11.3 ± 0.6 mg/L. These results clearly show that the composition of wastewater is dependent on how much and how often the wastewater is recycled in the batch processing. Thereafter, the first indication for the potential of valorization is given in the proper re-utilization for the so-produced wastewater. After being used the wastewater is disposed in lagoons where one part evaporates and the other one infiltrates into the subsoil. The effect of the nutrient density on the recycled wastewater especially of phenolic compounds may therefore not only influence the micro-organism development but eventually also their composition during the fermentation. Studies to this respect have not yet been conducted.

The composition of green coffee beans for the major chlorogenic acids was analyzed using the HPLC and a total of 9 caffeoylquinic acid (CQA) and feruloylquinic acid (FQA) isomers

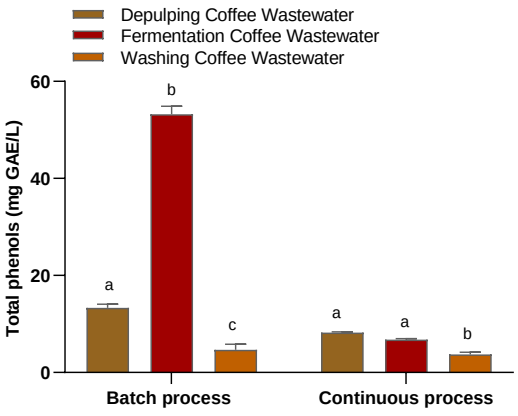
were identified as described in our former work [53]. There were similarities in the composition for the green coffee beans resulting from both processing options and production years. Main constituents were caffeoylquinic and feruloylquinic acid esters 5-CQA (67.1 and 66.0%; 65.1 and 63.2 %), and 5-FQA (5.1 and 5.4%; 5.9 and 4.9%); and the dimer form 3,5-di-CQA (10.8 and 10.0%; 9.2 and 10.2 %) for the batch and the continuous processing of production year 2018 and 2019, respectively (Figure 14b, c). Chlorogenic acids found in coffee beans are valued for their health benefits. They are known to have antimicrobial activity, preventing the degradation of bioactive compounds and strongly influencing the taste and color of coffee beverages. [282]. The presented results clearly show that carrying out the wet-processing in batch or in continuous way does not change the pattern of the major phenolic compounds present – the proportion and especially the composition of these compounds remains roughly constant, but the overall total content is likely to be affected as documented in Figure 12.



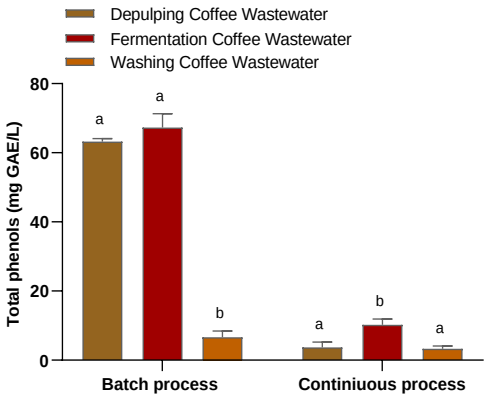
(a)



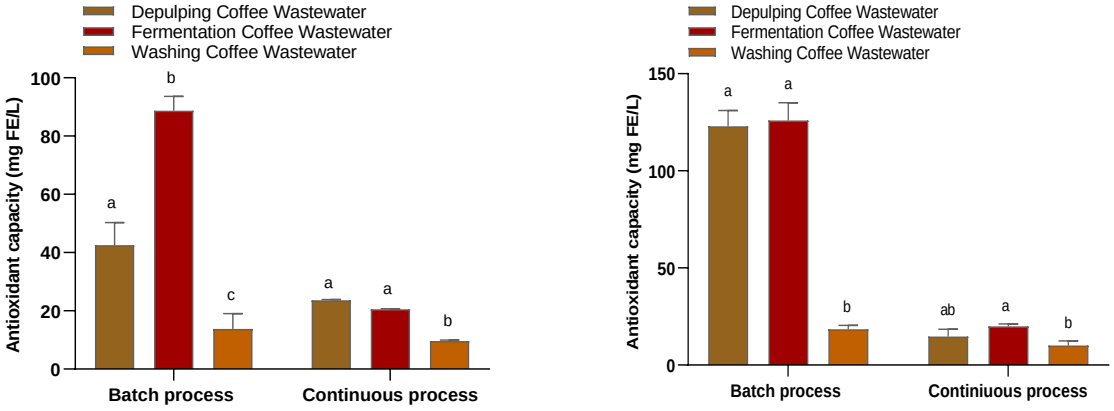
(b)



(c)



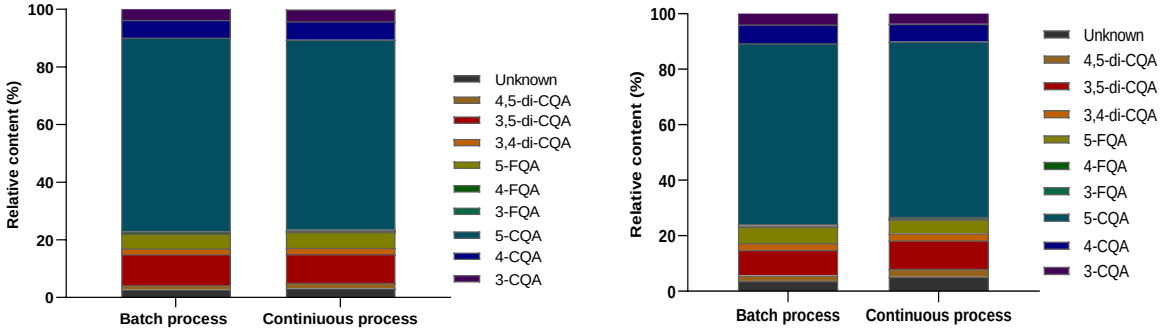
(d)



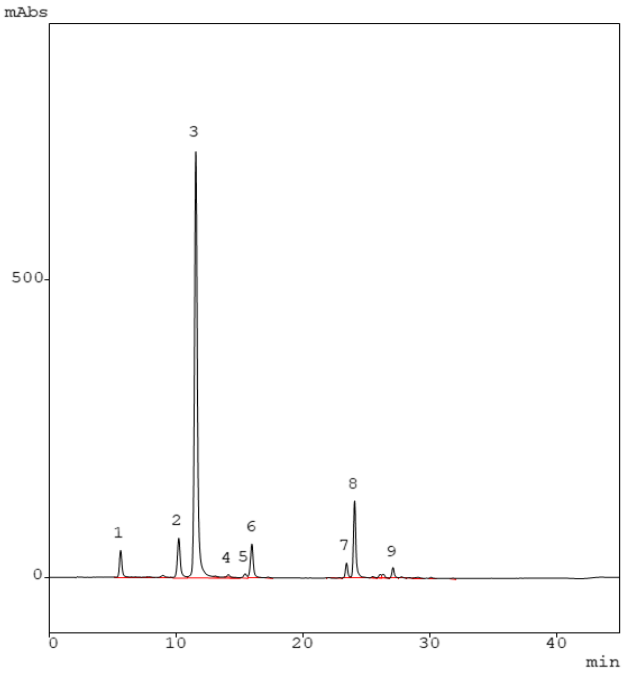
(e) (f)

Figure 13 Content of caffeine, total phenols, and antioxidant capacity of coffee wastewater

Amounts of (a) caffeine content, (c) total phenols and (e) antioxidant capacity of de-pulping coffee wastewater, fermentation coffee wastewater and washing coffee wastewater during the wet batch and continuous processing. (a), (c), and (e) represent data of coffee processing year 2018. (b), (d), and (f) represent data of coffee processing year 2019. Data is expressed as means ± standard deviation with n = 3. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).



(a) (b)



(c)

Figure 14 Major phenolic compounds and Chromatogram of green coffee beans

(a) represents data of coffee processing year 2018 and (b) year 2019; (c) exemplary chromatogram of green coffee beans obtained from batch processing (a) and the composition of the major phenolic compounds (b). (1) 3-O-Caffeoylquinic acid (3-CQA), (2) 4-O-Caffeoylquinic acid (4-CQA), (3) 5-O-Caffeoylquinic acid (5-CQA), (4) 3-O-Feruloyquinic acid (3-FQA), (5) 4-O-Feruloyquinic acid (4-FQA), (6) 4-O-Feruloyquinic acid (5-FQA), (7) 3,4-O-Dicaffeoylquinic acid (3,4-di-CQA), (8) 3,5-O-Dicaffeoylquinic acid (3,5-di-CQA), (9) 4,5-O-Dicaffeoylquinic acid (4,5-di-CQA), allocation as reported in [53].

5.1.2. Organic acids

Organic acids, especially in wet coffee processing, are constituents of interest due to their different functionalities. In order to guarantee the proper drying of green coffee, the mucilage layer needs to be degraded and this process takes place during the fermentation stage. It was showed that mucilage layer degradation was correlated to acidification by lactic acid bacteria [247-250]. Digested mucilage being precipitated out of the solution, making a thick crust on the surface of wastewater [2]. Results of organic acids analysis for 2018 and 2019 production years are compiled in Tables 6 and 7, respectively. Data indicate that citric acid, malic acid and lactic acid were found to be present in the coffee bean samples and by-products during the different steps of treatment, either in the batch or the continuous processing. As it can be seen for 2018 production year samples, among the coffee beans, green coffee beans showed the highest concentration of organic acids. Citric acid was found to be the predominant organic acid. Concentrations ranged from 0.6 to 1.0 g/100g DW and from 0.7 to 1.0 g/100g DW in the batch and the continuous system, respectively. Concentrations were higher in coffee pulp than in coffee beans. For example, malic acid was found to be 6 times higher in pulp than in green coffee beans when operating in continuous system, and about 4 times with batch processing.

Coffee pulp exhibited the highest concentrations of malic acid, 1.6 ± 0.05 g/100g DW for batch processing and 3.4 ± 0.26 g/100g DW for continuous processing. Coffee processing in 2019 shows a different pattern in organic acid composition. Lactic acid was found to be the predominant organic acid for green coffee beans. Concentrations ranged from 0.42 to 1.1 g/100 g DW from 0.27 to 0.9 g/100g DW in the batch and continuous system, respectively. Like samples taken in 2018, concentrations were higher in coffee pulp than in coffee beans. Malic acid was found to be 22 times higher in coffee pulp than in green coffee beans when operating in continuous system, and about 3.5 times with batch processing. Coffee pulp 2019 resembles the organic acids composition of coffee pulp 2018, showing the highest concentration in malic acid, 2.53 ± 0.03 g/100g DW for batch processing and 3.42 ± 0.26 g/100g DW for continuous processing. Fermentation is mainly carried out by lactic acid bacteria, *enterobacteriaceae*, and *bacillus* [8]. They are heterofermentative producing acetic and lactic acids [250]. However, malic and citric acid confer desirable acidity to the coffee beverage [245,283-285]. Moreover, enzymes produced from lactic acid fermentation might lead to the hydrolysis of macromolecules such as proteins, carbohydrates and polyphenols generating aroma precursors assuming that they can penetrate e.g. by diffusion into the coffee beans [105].

It can be clearly seen that for batch processing 2018, lactic acid (2,687.1 ± 23.0 mg/L) and propionic acid (1,679.3 ± 67.9 mg/L) were the main organic acids in coffee wastewater. With the continuous processing, a concentration of 373.5 ± 15.0 mg/L of lactic acid in fermentation wastewater was determined. Different composition was observed in wastewater samples 2019, showing for batch processing, malic acid (1,360.9 ± 3.0 mg/L) and lactic acid (1,185.6 ± 6.6 mg/L). As for the continuous processing, a concentration of 353.7 ± 4.8 mg/L of lactic acid and 204.0 ± 0.7 mg/L of malic acid was exhibited. Propionic acid was not detected. Production of butyric acid and propionic acid during the fermentation indicate an over-fermentation, which can be responsible for the “stinker oniony” coffee aroma profiles [250]. This observation is supported by the fact that, during the fermentation process, microorganisms use part of the nutrients from coffee beans to support their growth while producing secondary metabolites [105]. As the water is only partly renewed in the case of the batch process, it results in an accumulation of secondary metabolites, especially organic acids. There is little information about the effect of water recirculation on coffee bean constituents. Vásquez Morera [286] showed that acidity and aroma of coffee beans increased when recirculating water for two days of fermentation. Contact time between water and coffee beans might also increase acidity and decrease bitterness [287]. The enzymes and organic acids produced from fungal and bacterial fermentation lead to the hydrolysis of macromolecules such as carbohydrates, proteins and polyphenols resulting in simplified products like reducing sugars, amino acids and organic

acids. These are important aroma precursors in roasting process which will impact the coffee beverage final cup quality [288,289].

Temperature and pH of wastewater along both processes were measured using a portable multiparameter HI98194 (HANNA instruments, Woonsocket, Rhode Island, United States). For batch processing, de-pulping water showed 17.8° Cand pH 4.5; fermentation water 18.2° Cand pH 4.2; and washing water 16.8° Cand pH 4.78. With continuous processing, de-pulping water had 16.2° Cand pH 6.3; fermentation water 15.8° Cand pH 6.4; and washing water 13.7° Cand pH 7.4. These results were obtained via field measurements and document that the conditions of fermentation appear to be different. At the same time these slight differences during the treatments may also influence the degradation/biochemical interactions occurring during the processing and eventually also affect the quality of the coffee beans produced.

Table 6 Composition of organic acids in coffee beans, coffee by-products and wastewater during the batch and continuous processing. Processing year 2018

		Coffee and by -product samples (g/100g)								Wastewater samples (mg/L)		
		DCB	F0C	FFC	WCB	PCB	GCB	CP	P	DCW	FCW	WCW
Batch	Citric acid	0.60 ± 0.03^{a}	0.80 ± 0.05^{b}	0.94 ± 0.01^{cd}	0.92 ± 0.02^{cd}	0.90 ± 0.03^{bc}	1.03 ± 0.02^{d}	1.25 ± 0.03^{e}	0.16 ± 0.05^{f}	72.0 ± 4.1^{a}	847.0 ± 74.3^{b}	68.8 ± 17.2^{a}
	Malic acid	1.02 ± 0.00^{a}	0.21 ± 0.00^{b}	0.26 ± 0.06^{b}	0.28 ± 0.07^{b}	0.36 ± 0.01^{b}	0.38 ± 0.02^{b}	1.64 ± 0.05^{c}	0.13 ± 0.02^{b}	111.9 ± 3.0^{a}	483.0 ± 26.8^{b}	35.2 ± 0.7^{c}
	Lactic acid	0.79 ± 0.07^{a}	0.17 ± 0.04^{bc}	0.23 ± 0.01^{b}	0.20 ± 0.00^{bc}	0.09 ± 0.02c	0.48 ± 0.03d	0.96 ± 0.00^{e}	n.d.	1082.1 ± 32.9^{a}	2,687.1 ± 23.0^{b}	182.6 ± 7.2^{c}
	Acetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	241.4 ± 26.6^{a}	839.8 ± 12.0^{b}	63.7 ± 8.8^{c}
	Propionic acid	0.77 ± 0.23^{a}	0.77 ± 0.23^{a}	0.11 ± 0.01^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1679.3 ± 67.9	n.d.
Continuous	Citric acid	0.74 ± 0.01^{a}	0.87 ± 0.02^{bc}	0.69 ± 0.00^{a}	0.71 ± 0.07^{a}	0.80 ± 0.05^{ab}	0.99 ± 0.00^{c}	1.20 ± 0.03^{d}	n.d.	n.d.	8.0 ± 2.4	n.d.
	Malic acid	0.63 ± 0.02^{ab}	0.72 ± 0.00^{a}	0.44 ± 0.01^{b}	0.52 ± 0.09^{ab}	0.49 ± 0.01^{ab}	0.56 ± 0.04^{ab}	3.42 ± 0.26^{c}	n.d.	14.5 ± 0.2^{a}	18.4 ± 0.6^{a}	n.d.
	Lactic acid	0.36 ± 0.01^{a}	0.36 ± 0.02^{a}	0.13 ± 0.02^{b}	0.16 ± 0.01^{b}	0.12 ± 0.00^{b}	0.49 ± 0.05^{c}	0.69 ± 0.08^{d}	n.d.	330.1 ± 8.4^{a}	373.5 ± 15.0^{a}	n.d.
	Acetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	92.4 ± 3.3^{a}	81.7 ± 2.5^{a}	n.d.
	Propionic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10.6 ± 3.3	n.d.	n.d.

Data are expressed as means ± standard deviation. n = 3; n.d.=not detected. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test). DCB: de-pulped coffee beans; F0C: initial fermentation coffee beans; FFC: final fermentation coffee beans; WCB: washed coffee beans; PCB: parchment coffee beans; GCB: green coffee beans; CP: coffee pulp; P: parchment; DCW: de-pulping coffee wastewater; FCW: fermentation coffee wastewater and WCW: washing wastewater.

Table 7 Composition of organic acids in coffee beans, coffee by-products and wastewater during the batch and continuous processing. Processing year 2019

		Coffee and by -product samples (g/100g)								Wastewater samples (mg/L)		
		DCB	F0C	FFC	WCB	PCB	GCB	CP	P	DCW	FCW	WCW
Batch	Citric acid	0.22 ± 0.03^{a}	0.19 ± 0.00^{bd}	0.19 ± 0.00^{bd}	0.18 ± 0.00^{bd}	0.13 ± 0.00^{c}	0.19 ± 0.01^{d}	0.24 ± 0.00^{a}	n.d.	62.0 ± 1.5^{a}	48.7 ± 1.1^{b}	3.2 ± 0.0^{c}
	Malic acid	0.37 ± 0.00^{a}	0.67 ± 0.03^{bc}	0.41 ± 0.02^{a}	0.59 ± 0.03b	0.44 ± 0.02^{a}	0.71 ± 0.04c	2.53 ± 0.03d	n.d.	1095.1 ± 10.3^{a}	1360.9 ± 3.0^{b}	56.5 ± 0.4^{c}
	Lactic acid	0.42 ± 0.00^{a}	0.35 ± 0.01^{bc}	0.24 ± 0.04^{b}	0.13 ± 0.00^{bc}	0.86 ± 0.02^{ce}	1.11 ± 0.11^{d}	0.81 ± 0.00^{e}	n.d.	1631.2 ± 6.2^{a}	1185.6 ± 6.6^{b}	213.4 ± 0.3^{c}
	Acetic acid	n.d.	n.d.	n.d.	0.17 ± 0.03^{a}	0.18 ± 0.02^{a}	0.18 ± 0.01^{a}	n.d.	n.d.	599.1 ± 3.4^{a}	437.0 ± 0.5^{b}	91.9 ± 3.3^{c}
	Propionic acid	n.d.	n.d.	n.d.	n.d.	n.d.	0.17 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.
Continuous	Citric acid	0.17 ± 0.00^{ad}	0.07 ± 0.03^{b}	0.10 ± 0.00^{bc}	0.14 ± 0.01^{ac}	0.10 ± 0.00^{c}	0.17 ± 0.00^{d}	1.90 ± 0.04^{c}	n.d.	8.77 ± 0.3^{a}	6.3 ± 0.1^{b}	17.8 ± 0.1^{c}
	Malic acid	0.28 ± 0.02^{a}	0.22 ± 0.06^{ab}	0.20 ± 0.01^{ab}	0.18 ± 0.07^{ab}	0.10 ± 0.02^{b}	0.15 ± 0.02^{b}	3.42 ± 0.26^{c}	n.d.	228.2 ± 0.6^{a}	204.0 ± 0.7^{a}	25.5 ± 2.7^{a}
	Lactic acid	0.27 ± 0.01^{a}	0.14 ± 0.01^{a}	0.19 ± 0.01^{a}	0.23 ± 0.00^{a}	0.23 ± 0.09^{a}	0.92 ± 0.15^{b}	0.16 ± 0.02^{a}	n.d.	427.0 ± 4.5^{a}	353.7 ± 4.8^{a}	233.8 ± 8.0^{a}
	Acetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	83.3 ± 0.2
	Propionic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12.9 ± 3.8

Data are expressed as means ± standard deviation. n = 3; n.d.=not detected. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test). DCB: de-pulped coffee beans; F0C: initial fermentation coffee beans; FFC: final fermentation coffee beans; WCB: washed coffee beans; PCB: parchment coffee beans; GCB: green coffee beans; CP: coffee pulp; P: parchment; DCW: de-pulping coffee wastewater; FCW: fermentation coffee wastewater and WCW: washing wastewater.

5.1.3. Reducing sugars

Low molecular weight carbohydrates are involved in Maillard reactions and color changes during the roasting of green coffee beans. Tables 8 and 9 illustrate the composition of reducing sugars of coffee bean and by-product samples during the wet batch and continuous processing for production years 2018 and 2019, respectively. The following six sugars were detected: Arabinose, fructose, mannose, glucose, sucrose, and mannose. With the batch processing for 2018 production year, arabinose contents in de-pulped coffee beans, initial and final fermentation coffee beans, and coffee pulp, were 0.22 ± 0.01; 0.13 ± 0.04; 0.11 ± 0.01 and 0.18 ± 0.02 g/100 g DW, respectively. Arabinose as well as fructose and mannose were not detected in green coffee beans and values of glucose and sucrose were of 0.66 ± 0.04 and 4.96 ± 0.25 g/100 g DW, respectively. After the de-pulping step, there was no sucrose left in the coffee pulp. However, the concentration of arabinose (0.18 ± 0.02 g/100 g DW), fructose (9.48 ± 0.43 g/100 g), mannose (0.92 ± 0.08) and glucose (8.72 ± 0.48 g/100 g) were significantly different ($p > 0.05$) in coffee pulp. Although sucrose was not detected in parchment and wastewater samples, all the other reducing sugars (fructose, mannose and glucose) were present in higher concentration in wastewater resulting from de-pulping, fermentation and washing.

Arabinose contents in de-pulped coffee beans, coffee beans at the beginning of the fermentation, and coffee beans at the end of the fermentation in 2019 with the batch processing, were 0.13 ± 0.00; 0.14 ± 0.06; 0.14 ± 0.03 g/100g DW, respectively. Arabinose was not detected in coffee pulp as well as parchment with the batch processing 2019. Values for glucose and sucrose in green coffee beans were 0.58 ± 0.06 and 5.02 ± 0.30 g/100g DW, respectively. Alike samples 2018, after the de-pulping step, there was no sucrose left in the coffee pulp. Nevertheless, concentration of fructose (8.63 ± 0.22 g/100g DW), mannose (0.99 ± 0.50 g/100g DW), and glucose (8.31 ± 0.49 g/100g DW) were significantly different ($p > 0.05$) in coffee pulp. Even though sucrose was not detected, reducing sugars fructose, mannose, and glucose were present in higher concentrations in wastewater 2019 resulting from de-pulping, fermentation and washing. These results indicate removal of reducing sugars during the batch processing; sugars in wastewater are most likely liberated from coffee pulp and from mucilage layer which precipitate after fermentation.

In the case of the continuous processing for the samples taken in 2018, although arabinose was not detected in the coffee bean samples, fructose, mannose, glucose and sucrose were present at different concentrations. Fructose (9.74 ± 0.72 g/100 g DW), mannose (0.88 ± 0.08 g/100 g DW) and glucose (8.29 ± 0.59 g/100 g DW) in coffee pulp were similar to those obtained with the batch process. The analysis of the variance showed that differences were not significant. Although, other sugars like fructose and mannose may be present in green coffee beans, glucose and sucrose seem to be the most relevant ones independent of the

processing conditions used. Values of 0.58 ± 0.05 and 5.07±0.39 g/100 g DW were obtained for glucose and sucrose, respectively. Acidri, et al. [290] and Kinyua, et al. [291] showed that sucrose is the main low molecular weight saccharide component of the coffee seeds. Its higher content is correlated with a better cup quality. Depending on the type of coffee species, sucrose ranges from 5.1 to 9.4 % in Arabica coffee have been documented [289]. The data for coffee pulp 2019 shows fructose (8.63 ± 0.22 g/100g DW), glucose (8.31 ± 0.49 g/100g DW), mannose (0.99 ± 0.50 g/100g DW), and maltose (0.21 g/100g DW). These values were similar to those obtained with the batch process.

From Table 8 higher glucose values were obtained in coffee pulp while those of sucrose were recorded in green coffee beans. We can see that with the batch processing 2018 glucose was significantly reduced between the de-pulping (4.91 ± 0.19 g/100 g DW) and final fermentation (1.13 ± 0.07 g/100 g DW) steps while sucrose concentration was increased from 0.49 ± 0.03 to 2.10 ± 0.11 g/100 g DW. The same trend was observed for batch processing 2019. Glucose reduction between de-pulping and final fermentation was 1.47 ± 0.28 g/100g DW and 1.10 ± 0.0 g/100g DW, respectively. However, sucrose content decreased from 4.50 ± 0.01 g/100g DW to 4.08 ± 0.12 g/100g DW. With continuous 2018 production year, glucose decreased from 1.56 ± 0.12 to 0.52 ± 0.04 g/100 g DW from the de-pulping to the fermentation steps, while sucrose fluctuated from 2.66 ± 0.17 to 2.29 ± 0.23 g/100 g DW. Similarly, glucose for continuous processing 2019 decreased from 2.30 ± 0.03 g/100g DW to 0.74 ± 0.01 g/100g DW from the de-pulping to the fermentation step. Contrary, sucrose increased from 2.89 ± 0.16 g/100g DW for de-pulping to 3.89 ± 0.18 g/100g DW for final fermentation. The mucilage acts as a sort of barrier, a protective outer layer to isolate the bean from the rest of the cherry, preventing internal decomposition by enzymatic and kinetic processes during fermentation [5]. In the fermentation process, microorganisms use simple sugars present in the mucilage as a carbon source. Therefore, reducing sugars like glucose and fructose decrease as they are consumed by microorganisms during the fermentation [251]. Compared to the batch process, reducing sugars in the continuous processing were not detected in the parchment and were up to 18 times lower in the fermentation wastewater. The batch processing due to recirculation/reuse of water thus results in by-products and wastewater that contain more reducing sugars – again a potential which should be valorized.

Table 8 Composition of reducing sugars in coffee beans, coffee by-products and wastewater during the batch and continuous processing. Processing year 2018

		Coffee and by -product samples (g/100g DW)								Wastewater samples (mg/L)		
		DCB	**F0C**	**FFC**	**WCB**	**PCB**	**GCB**	**CP**	**P**	**DCW**	**FCW**	**WCW**
Batch	Arabinose	0.22 ± 0.01^{a}	0.13 ± 0.04^{b}	0.11 ± 0.01^{b}	n.d.	n.d.	n.d.	0.18 ± 0.02^{a}	n.d.	25.0 ± 1.0^{a}	610.3 ± 57.2^{b}	24.7 ± 2.1^{a}
	Fructose	5.63 ± 0.15^{a}	0.59 ± 0.04^{b}	0.66 ± 0.04^{b}	0.39 ± 0.03^{b}	n.d.	n.d.	9.48 ± 0.43^{c}	n.d.	43.3 ± 0.6^{a}	327.7 ± 30.4^{b}	47.0 ± 2.6^{a}
	Mannose	0.70 ± 0.02^{a}	0.21 ± 0.02^{b}	0.18 ± 0.02^{b}	0.16 ± 0.01^{b}	n.d.	n.d.	0.92 ± 0.08^{c}	0.17 ± 0.01^{b}	1308.0 ± 46.6^{a}	1720.3 ± 65.5^{b}	151.0 ± 7.9^{c}
	Glucose	4.91 ± 0.19^{a}	0.91 ± 0.06^{b}	1.13 ± 0.07^{b}	0.80 ± 0.05^{bd}	0.56 ± 0.04^{bd}	0.66 ± 0.04^{bd}	8.72 ± 0.48^{c}	0.23 ± 0.01^{d}	253.3 ± 11.1^{a}	1400.3 ± 88.6^{b}	87.0 ± 5.6^{c}
	Sucrose	0.49 ± 0.03^{a}	1.69 ± 0.08^{b}	2.10 ± 0.11^{bc}	2.44 ± 0.17^{c}	4.19 ± 0.22^{d}	4.96 ± 0.25^{e}	n.d.	0.04 ± 0.01^{a}	n.d.	n.d.	n.d.
Continuous	Arabinose	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	34.7 ± 1.2^{a}	34.7 ± 2.1^{a}	n.d.
	Fructose	1.68 ± 0.16^{a}	1.81 ± 0.15^{a}	0.20 ± 0.01^{b}	0.27 ± 0.02^{b}	n.d.	n.d.	9.74 ± 0.72^{c}	n.d.	n.d.	29.0 ± 1.4	n.d.
	Mannose	0.21 ± 0.01^{a}	0.42 ± 0.04^{b}	0.16 ± 0.00^{a}	0.23 ± 0.02^{a}	0.23 ± 0.02^{a}	0.17 ± 0.01^{a}	0.88 ± 0.08^{c}	n.d.	395.0 ± 14.2^{a}	482.7 ± 22.2^{b}	n.d.
	Glucose	1.56 ± 0.12^{a}	1.81 ± 0.15^{a}	0.52 ± 0.04^{b}	0.61 ± 0.04^{b}	0.44 ± 0.03^{b}	0.58 ± 0.05^{b}	8.29 ± 0.59^{c}	n.d.	66.0 ± 3.6^{a}	77.0 ± 2.6^{a}	n.d.
	Sucrose	2.66 ± 0.17^{a}	2.05 ± 0.15^{b}	2.29 ± 0.23^{ab}	2.47 ± 0.18^{ab}	3.74 ± 0.27^{c}	5.07 ± 0.39^{d}	n.d.	n.d.	n.d.	n.d.	n.d.

Data are expressed as means ± standard deviation. $n = 3$; n.d.= not detected. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test). DCB: de-pulped coffee beans; F0C: initial fermentation coffee beans; FFC: final fermentation coffee beans; WCB washed coffee beans; PCB parchment coffee beans; GCB green coffee beans; CP coffee pulp; P parchment; DCW: de-pulping coffee wastewater; FCW fermentation coffee wastewater and WCW: washing wastewater

Table 9 Composition of reducing sugars in coffee beans, coffee by-products and wastewater during the batch and continuous processing. Processing year 2019

		Coffee and by -product samples (g/100g DW)								Wastewater samples (mg/L)		
		DCB	**F0C**	**FFC**	**WCB**	**PCB**	**GCB**	**CP**	**P**	**DCW**	**FCW**	**WCW**
Batch	Arabinose	0.13 ± 0.00^{a}	0.14 ± 0.06^{a}	0.14 ± 0.03^{a}	n.d.	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	n.d.	n.d.	180.0 ± 36.7^{a}	186.0 ± 2.83^{a}	n.d.
	Fructose	1.99 ± 0.01^{a}	1.43 ± 0.01^{b}	0.80 ± 0.05^{c}	0.47 ± 0.05^{d}	0.17 ± 0.01^{e}	0.16 ± 0.02^{e}	8.63 ± 0.22^{f}	0.21 ± 0.01^{e}	12182.5 ± 47.4^{a}	9472 ± 472^{b}	47.5 ± 0.70^{c}
	Mannose	n.d.	n.d.	n.d.	n.d.	0.08 ± 0.03^{a}	n.d.	0.99 ± 0.50^{a}	0.11 ± 0.01^{a}	1813.0 ± 166^{a}	1268.0 ± 103.2^{b}	140.0 ± 2.8^{c}
	Glucose	1.47 ± 0.28^{a}	1.22 ± 0.04^{a}	1.10 ± 0.00^{ab}	1.00 ± 0.14^{ab}	0.54 ± 0.08^{b}	0.58 ± 0.06^{b}	8.31 ± 0.49^{c}	0.15 ± 0.01^{b}	253.3 ± 11.1^{a}	1400.3 ± 88.6^{b}	87.0 ± 5.6^{c}
	Sucrose	4.50 ± 0.01^{ac}	4.35 ± 0.23^{abc}	4.08 ± 0.12^{ab}	4.00 ± 0.13^{b}	4.17 ± 0.06^{cb}	5.02 ± 0.30^{d}	n.d.	n.d.	n.d.	n.d.	n.d.
	Maltose	0.20 ± 0.05^{a}	0.35 ± 0.06^{a}	0.16 ± 0.03^{a}	0.15 ± 0.01^{a}	0.16 ± 0.02^{a}	0.15 ± 0.01^{a}	0.21 ± 0.01^{a}	0.23 ± 0.01^{a}	n.d.	292.5 ± 12.0^{a}	27.5 ± 0.71^{b}
Continuous	Arabinose	0.13 ± 0.04^{a}	0.11 ± 0.01^{a}	n.d.	n.d.	n.d.	n.d.	0.10 ± 0.01^{a}	n.d.	39.5 ± 2.12	n.d.	n.d.
	Fructose	2.76 ± 0.10^{a}	1.51 ± 0.02^{b}	0.35 ± 0.01^{c}	0.25 ± 0.03^{c}	0.14 ± 0.01^{c}	0.16 ± 0.01^{c}	8.52 ± 0.06^{d}	0.15 ± 0.01^{c}	979.5 ± 41.7^{a}	834.5 ± 7.8^{a}	n.d.
	Mannose	1.21 ± 0.05^{ac}	0.41 ± 0.03^{b}	0.24 ± 0.08^{bc}	n.d.	0.10 ± 0.03^{bc}	n.d.	1.80 ± 0.69^{a}	0.07 ± 0.01^{c}	98.5 ± 9.19^{a}	97.5 ± 4.9^{a}	n.d.
	Glucose	2.30 ± 0.03^{a}	1.34 ± 0.02^{b}	0.74 ± 0.01^{c}	0.61 ± 0.10^{c}	0.47 ± 0.00^{c}	0.71 ± 0.01^{c}	9.66 ± 0.29^{d}	n.d.	672.0 ± 5.7^{a}	572.0 ± 22.6^{a}	n.d.
	Sucrose	2.89 ± 0.16^{a}	2.41 ± 0.04^{b}	3.89 ± 0.18^{c}	2.96 ± 0.02^{a}	4.38 ± 0.18^{d}	5.62 ± 0.06^{e}	n.d.	n.d.	n.d.	86.5 ± 12.02	n.d.
	Maltose	0.20 ± 0.02^{a}	0.18 ± 0.02^{a}	0.16 ± 0.02^{a}	0.18 ± 0.00^{a}	0.17 ± 0.01^{a}	0.20 ± 0.07^{a}	0.1 ± 0.01^{a}	0.15 ± 0.01^{a}	70.0 ± 2.8^{a}	n.d.	30.5 ± 2.1^{b}

Data are expressed as means ± standard deviation. $n = 3$; n.d.= not detected. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test). DCB: de-pulped coffee beans; F0C: initial fermentation coffee beans; FFC: final fermentation coffee beans; WCB washed coffee beans; PCB parchment coffee beans; GCB green coffee beans; CP coffee pulp; P parchment; DCW: de-pulping coffee wastewater; FCW fermentation coffee wastewater and WCW: washing wastewater.

5.1.4. Soluble proteins and amino acids

Lowry method was used to quantify the soluble proteins in coffee beans and their by-products. The results for production years 2018 and 2019 are presented in Figure 15. Soluble proteins were present in considerable amounts in coffee samples, ranging from 5 to 15%. Protein content averages of 10-14% in coffee Arabica have been reported [292]. Different trends in the protein contents were exhibited along the processing as well as dependent on the production year. With the batch processing 2018, an insignificant increasing of protein content during the fermentation step was registered, rising from 13.93 ± 2.50 to 14.05 ± 2.91 g/100 g DW from the initial to the final fermented coffee beans. This amount decreased with the washing step to a value of 13.58 ± 1.64 g/100 g DW in the washed coffee beans. Finally, protein content increased up to 17.21 ± 2.71 g/100 g DW in the green coffee bean after removing the parchment. For 2019 batch processing an insignificant increasing of protein content during the fermentation steps was registered, similar to 2018 batch processing, from 8.99 ± 0.76 g/100g DW to 10.64 g/100g DW from the initial to the final fermentation step. Contrary to 2018 washed coffee beans, the amount of protein content significantly increased to a value of 16.6 ± 2.30 g/100g DW with the washing step. Protein content in green coffee insignificantly decreased to 15.5 ± 1.42 g/100g DW.

The situation was similar with the continuous processing. An insignificant increase of protein content was observed between the coffee beans at the beginning of fermentation (11.20 ± 1.77 g/100 g DW) and the final fermented coffee beans (11.63 ± 0.56 g/100 g DW) for 2018 samples. Contrary 2019 samples insignificantly decreased from 11.06 ± 0.67 g/100g DW to 8.26 ± 0.79 g/100g DW from beginning to final fermentation steps. After washing and parchment removal, the protein content in green coffee beans was 10.62 ± 1.94 g/100g DW and 13.37 ± 1.19 g/100g DW for 2018- and 2019-year productions, respectively. The results indicate that the proteins or their structures might be affected during both processing steps. A positive effect of the fermentation on the solubility and accessibility of proteins seems to be probable. By-products were also analyzed and protein values of 9.25 ± 1.08 and 10.13 ± 0.53 g/100 g DW in coffee pulps were recorded for the batch and the continuous systems for 2018 samples, respectively. The same trend but lower values were found for 2019 samples of the batch process (7.98 ± 0.53 g/100g DW) and the continuous process (5.45 ± 0.96 g/100g DW). Furthermore, while a similar protein content of 3.36 ± 0.33 g/100 g DW (2018 production year) and 3.33 ± 0.62 g/100g DW (2019 production year) in the parchment sample of the batch system was registered, no protein was detected in the parchment from the continuous system. Similar behavior was observed with wastewater samples. However, wastewater produced with batch system in 2018 showed higher values compared to those with 2019 production year. Protein values of 801.25 ± 94.6, 9,292.5 ± 941.0 and 203.0 ± 34.0 mg/L were obtained for the wastewater form de-pulping, fermentation and washing steps, respectively. While protein value

of 1,699.0 ± 122.3, 2,384 ± 257.9, and 297.6 ± 51.1 mg/L were recorded for 2019 production year for the wastewater from de-pulping, fermentation and washing step, respectively. Protein content was detected in low concentration only for 2019 production year in the washing step of the continuous system.

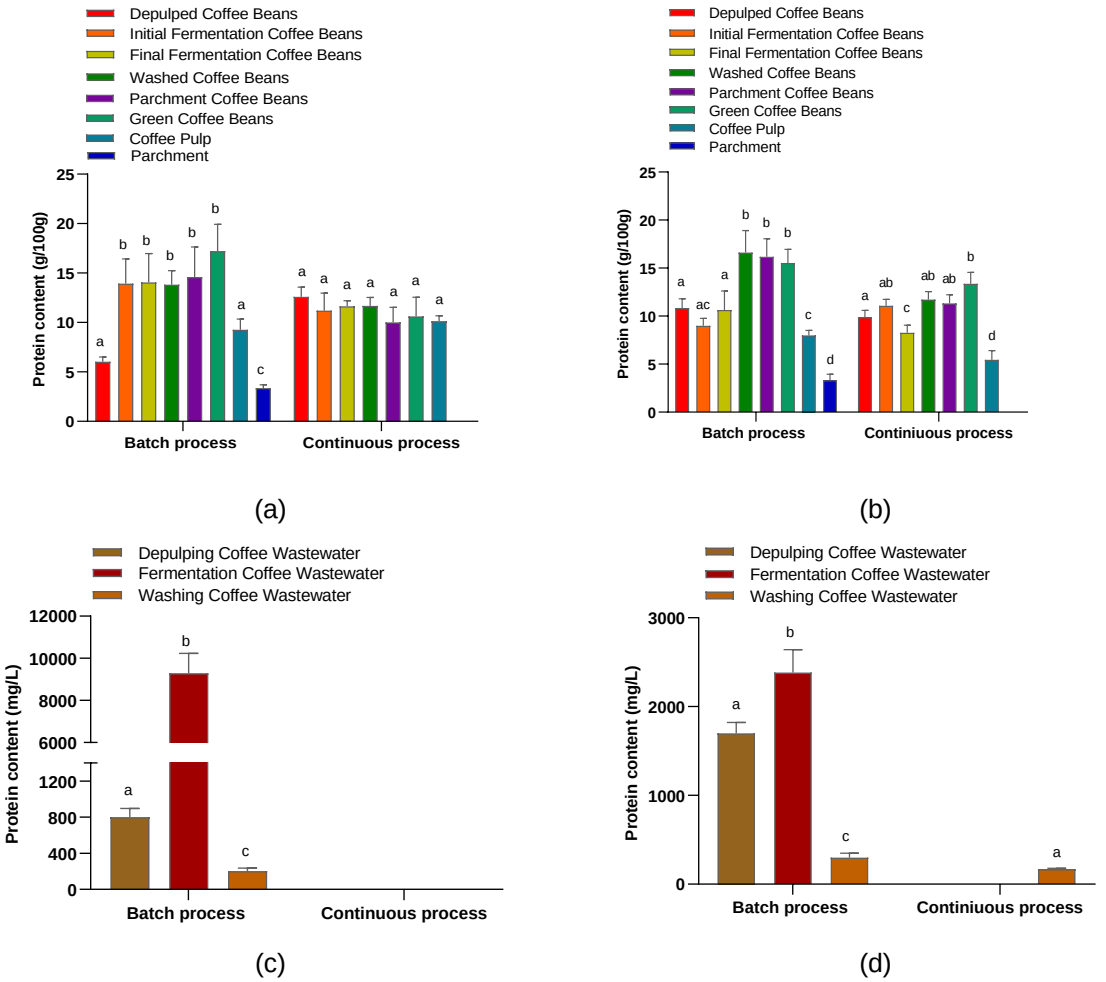
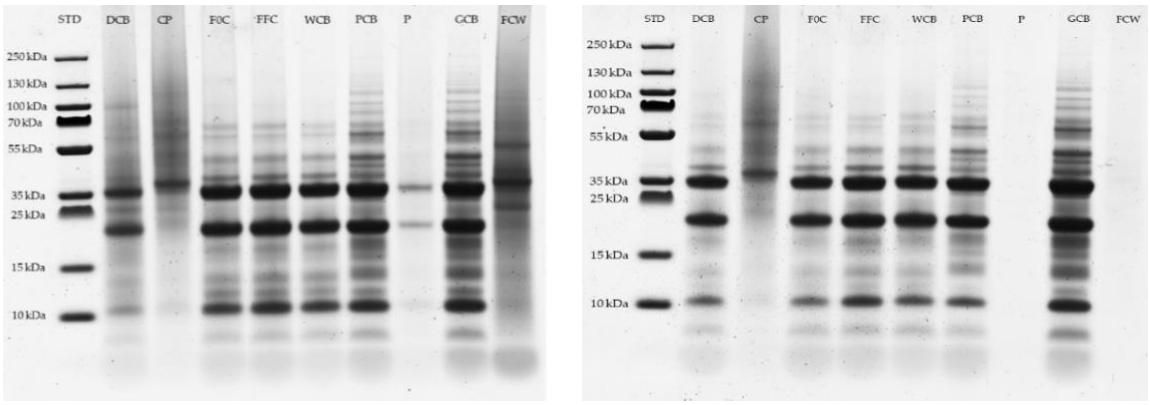


Figure 15 Protein content of coffee beans, by-products and wastewater

Amounts of protein content of coffee beans, by-products and wastewater samples during the wet batch and continuous processing for production year 2018 (a, c) and 219 (b, d). Data are expressed as means ± standard deviation. $n = 3$. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

Storage protein accounts for 45% of total proteins in the green coffee beans. The most abundant of these proteins are the legumin-like seed storage proteins of the 11S size class [293-295]. The typical structure of an 11S storage protein consists of 3-6 monomers, which migrate into storage vacuoles (protein bodies) and generate the tri- and hexameric quaternary forms by hydrophobic interactions, with molecular weights of 150 - 400 kDa [67]. The rupture of the disulfide bonds in 11S monomers under reducing conditions releases the α (acidic) and β (basic) subunits [296]. *Coffea arabica* proteins, in absence of a reducing agent, showed subunit with molecular weight of 55 kDa, and in the presence of reducing agent (2-mercaptoethanol) consisted of two polypeptides with molecular weights of 33 and 24 kDa [293,295]. SDS PAGE was performed to evaluate these main protein compositions. The

scanned gels of samples from both processing options are presented in Figure 16. The Coomassie Blue staining shows two main bands of 34 and 20 kDa proteins in the de-pulped coffee beans. In both processing variants, an increase in the amount of higher molecular weight protein bands can be observed. These proteins result from crosslinking of different smaller proteins, or they are simply released better from the coffee bean matrix during the extraction. Further on-going experiments will encompass the nature of the composition of these protein bands. In the same context the nature of the main protein bands observed in the coffee pulp as well as in the fermentation wastewater (especially in the batch process) needs also to be addressed. No bands were found in parchment from the continuous process, whereas parchment from the batch process shows a first band of about 36 kDa and a second band with a molecular weight of 22 kDa. That corroborates with the results obtained from Lowry method where protein was detected in the parchment from batch process. The results of SDS-Page document some differences in the protein molecular composition. The following chapters show the structural changes occurring to the individual proteins, especially in those of the major 11S storage protein along the processing steps.



(a) (b)

Figure 16 Electrophoresis of coffee beans, by-products and wastewater

(a) batch and (b) continuous processing. With STD the protein ladder, DCB the de-pulped coffee beans, CP the coffee pulp, F0C the initial fermentation coffee beans, FFC the final fermentation coffee beans, WCB the washed coffee beans, PCB the parchment coffee beans, P the parchment, GCB the green coffee and FCW the fermented wastewater.

In addition to the protein content, the fluorescamine assay was used to quantify amounts of free amino acid groups of proteins and peptides, and amino acids for 2018 and 2019 production years in coffee bean samples (Figure 17). For both processing configurations, the concentration of free amino groups was higher in the coffee pulp, while looking at the by-products of the processing. This shows that most of the protein remained intact in the coffee bean (see also results of SDS-PAGE, Figure 16), while the free amino groups are lost into the coffee pulp.

An amount of free amino groups of 1.44 ± 0.09 g/100 g DW was obtained in green coffee beans 2018. Whereas in green coffee beans 2019, lower amount was found (0.93 ± 0.05

g/100g DW). Conversely, the free amino groups in the continuous processing remained more or less constant and the final significantly decreased value in green coffee bean 2018 (0.81 ± 0.03 g/ 100 g) was much lower than that obtained from the batch system. In 2019 coffee samples a significant decrease ($p < 0.0001$) was observed between final fermented and green coffee beans. However, values in green coffee beans 2019 (1.53 ± 0.08 g/100g DW) were higher compared to those in 2018. This demonstrates that circulating fresh water during fermentation and washing operations contributed to a progressive leaching of free amino groups. From the de-pulped coffee beans to the green coffee beans, free amino groups decreased to about 64% in the batch processing and to about 50% in the continuous processing for 2018 samples. Similar, for 2019 samples, a decrease to about 50% and 40% was observed for the batch and continuous process, respectively. Free amino groups in green coffee beans ranged between 0.8 to 1.5%.

Considering the wastewater samples, free amino groups were more pronounced in batch processing samples compared to those of the continuous processing. The results showed that free amino groups increased in wastewater with fermentation. Fermentation wastewater values in 2018 of 15,993.5 ± 170.9 and 153.5 ± 7.6 mg/L for the batch and continuous processing were obtained. While 2019 values were of 4,011.4 ± 371.3 mg/L and 254.8 ± 30.9 mg/L for the batch and continuous processing, respectively. Values were about 100 times higher in batch processing comparing to the continuous processing, keeping in mind that recirculation also increases the value documented. These data suggest that recirculation primarily leads to increased values for free amino groups. If a significant degradation of proteins in coffee beans occurred during the fermentation step, especially when operating in batch, cannot be interpreted from the observed values and still needs to be assessed more accurately. The results still document a significant contribution of free amino groups originating from the processing from the de-pulped to green coffee beans especially for the batch process. It can further be assumed that the increase may also result from the metabolic microbial turnover during the fermentation. This result reveals on one side the potential of reutilizing the wastewater with proper pre-treatment, but also gives the opportunity in changing the content of free amino groups in final green coffee bean. Knowing that this group of compounds is involved in the Maillard-Reaction during roasting, this possibility could be utilized to improve the coffee cup quality. Unfortunately, no detailed information is available to suggest that these free amino groups are either required or sufficient for the generation of the coffee aroma [6].

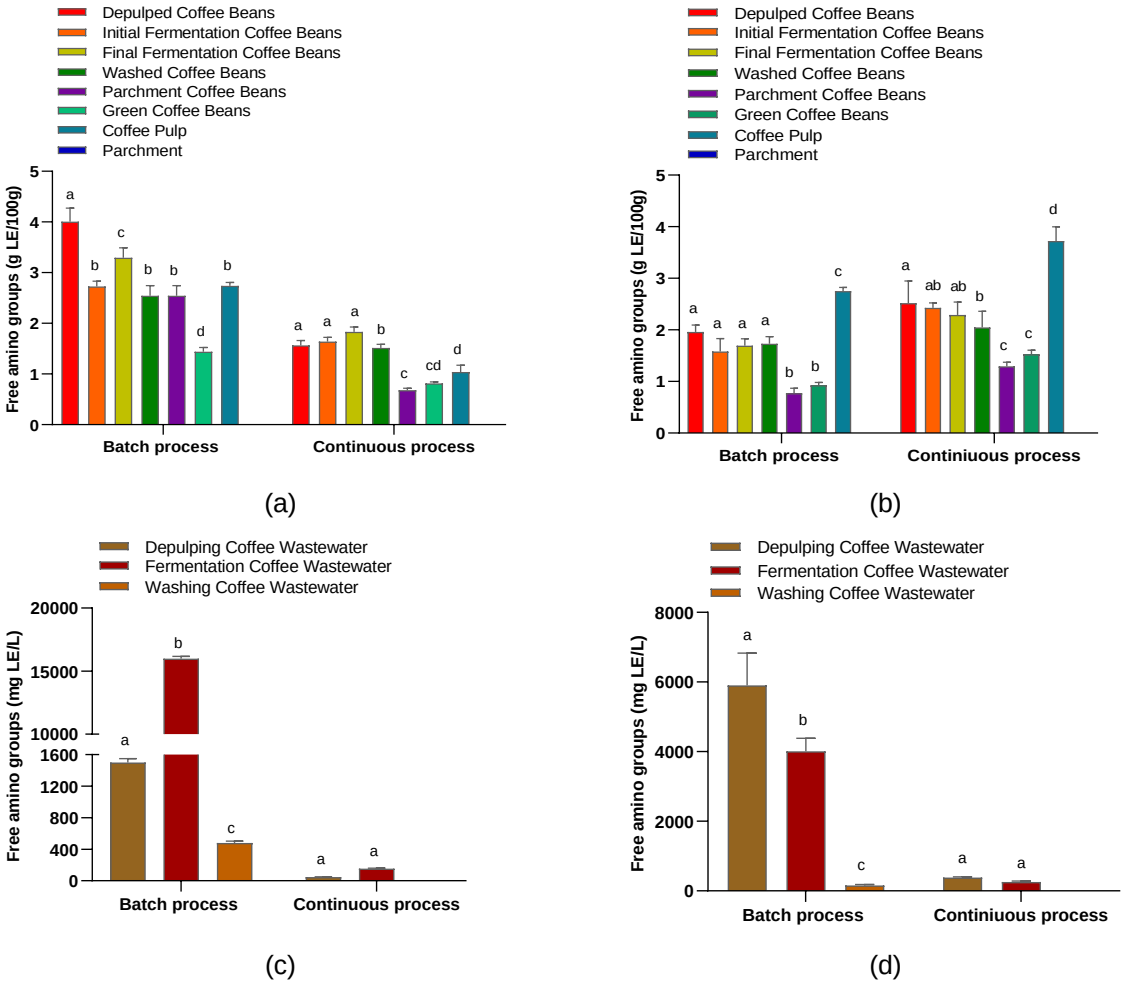


Figure 17 Free amino groups of coffee beans, by-products and wastewater

Amounts free amino groups of coffee beans, by-products and wastewater samples during the wet batch and continuous processing for production year 2018 (a, c) and 2019 (b, d). Data are expressed as means ± standard deviation. n = 3. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

Conclusion of the study 1 :

We hypothesized that the wet-coffee processing, whether in batch or continuous mode, can influence the physicochemical characteristics of green coffee beans, and thus later the roasting processing as well as the final cup quality. Thereafter, we found that the composition of green coffee beans from both batch and continuous production modes presented similarities. Further work will be directed towards the components that could participate in reactions taking part during the roasting. The by-products, especially during batch processing, revealed high concentrations of nutrients.

5.2. Study 2: Effect of the post-harvest processing on the protein modification in green coffee beans

The protein fraction, important for coffee cup quality, is modified during post-harvest treatment prior to roasting. Proteins may interact with phenolic compounds, which constitute the major metabolites of coffee, where the processing effects these interactions. This allows a hypothesis that the proteins are denatured and modified via enzymatic and/or redox activation steps. The present study was initiated to encompass the changes in the protein fraction. The investigations were limited to major storage protein of the green coffee beans. Fourteen *Coffea arabica* samples from various processing methods and countries were used. Different extraction protocols were compared to maintain the status quo of the protein modification. The extracts contained about 4-8 µg chlorogenic acid derivatives per mg extracted protein. High resolution chromatography with multiple reaction monitoring was used to detect lysine modifications in the coffee protein. Marker peptides were allocated for the storage protein of the coffee beans. Among these, the peptides modified K.FFLANGPQQGGK.E and R.LGGK.T of the α-chain and R.ITTVNSQK.I and K.VFDDEVK.Q of β-chain were detected. Results showed a significant increase ($p < 0.05$) of modified peptides wet processed green beans as compared to the dry ones. The present study contributes to a better understanding of the influence of the different processing methods on protein quality and its role in the scope of coffee cup quality and aroma.

5.2.1. Free thiol groups and amino nitrogen in the protein extracts

To measure the nitrogen compounds assimilated during coffee fermentation, free amino nitrogen was measured. Furthermore, free thiol groups were monitored to determine their reactivity and change during processing.

Free thiol groups and amino nitrogen were determined for coffee beans processed from two pilot wet processing companies from Guatemala and results are shown in Table 10. Exposed free thiol groups from coffee beans processed in batch process samples are found in high quantities. The values of 19.32 ± 1.23, 19.56 ± 1.25, 16.13 ± 1.03, 13.47 ± 0.86, and 5.91 ± 0.38 nmol/mg protein were obtained for depulped coffee beans, initial and final fermentation, washed, and green coffee beans, respectively. Conversely, with continuous process samples, values of 8.00 ± 0.51, 14.43 ± 0.92, 2.61 ± 0.17, 13.74 ± 0.88, and 4.93 ± 0.31 nmol/mg protein were obtained. After washing step, exposed free thiol groups decreased significantly ($p < 0.0001$). The amount of free thiol groups after denaturation reports significantly different values ($p < 0.05$) for each group in batch and continuous process samples. The values of 23.20 ± 0.39 and 7.65 ± 0.13; 4.83 ± 0.08 and 4.57 ± 0.076 nmol/mg protein were obtained for depulped and green coffee beans in batch and continuous process, respectively. These results allow the assumption that cysteine residues are less affected by the post-harvest treatment of coffee

beans from continuous process, where water is not recirculated, and fresh water is applied in the processing steps. In batch process, part of the water is returned to the beginning of the process and used again in the depulping and fermentation steps, eventually allowing an increase due to microbial load or a more pregnant denaturation of the coffee proteins via enzymatic and/or redox activation steps, which would allow a release of free thiol groups. The identification of the underlying mechanisms represents an element of future studies.

Values of the measurements of free amino nitrogen (FAN) ranged from 38.15 ± 1.14 and 48.62 ± 3.86 µg/mg protein, which were obtained for the green coffee and final fermentation coffee beans processed in continuous process. The content of FAN for green coffee beans processed in batch process sample decreased significantly (p = 0.148) compared to depulped coffee beans and values were 35.93 ± 4.14 and 46.97 ± 1.17 µg/mg protein for green and depulped coffee beans, respectively. High free amino nitrogen values indicate that samples analyzed have more unmodified lysine side chains that could be available for the initial Maillard reaction during roasting.

Table 10. Exposed free thiol groups, free thiol groups and free amino nitrogen in batch and continuous process

		DCB	IFCB	FFCB	WCB	GCB
Batch process	Exposed free thiol groups (nmol/mg)	19.33 ± 0.78^{a}	19.56 ± 0.12^{a}	16.13 ± 0.66^{b}	13.47 ± 0.42^{b}	5.91 ± 2.62^{c}
	Total free thiol groups (nmol/mg)	23.20 ± 0.24^{a}	14.52 ± 1.48^{b}	15.90 ± 0.21^{c}	10.13 ± 1.20^{d}	7.65 ± 1.20^{e}
	Free amino nitrogen (µg/mg)	46.97 ± 1.18^{a}	48.51 ± 3.80^{a}	57.52 ± 4.87^{b}	58.96 ± 6.86^{c}	35.94 ± 4.15^{d}
Continuous process	Exposed free thiol groups (nmol/mg)	8.00 ± 0.45^{a}	14.44 ± 0.48^{b}	2.61 ± 0.26^{c}	13.74 ± 1.55^{b}	4.93 ± 0.90^{c}
	Total free thiol groups (nmol/mg)	4.84 ± 1.65^{a}	12.18 ± 2.46^{b}	7.90 ± 1.41^{c}	10.66 ± 1.22^{d}	4.57 ± 2.23^{a}
	Free amino nitrogen (µg/mg)	47.17 ± 2.32^{ab}	51.35 ± 2.88^{a}	48.62 ± 3.86^{a}	45.48 ± 3.16^{ab}	38.15 ± 1.14^{b}

The proteins were extracted with option III without reduction and alkylation. For determination of exposed free thiol groups (A), the extracts were dissolved in 0.2 M Tris buffer, whereas to determine the total free thiol groups (B), 0.2 M Tris buffer containing SDS was used. Calibration was calculated using a series of concentration of reduced glutathione and N-acetylcysteine. Data is expressed as means ± standard deviation (n = 3) in nmol/mg protein. Different letters indicate significantly different values for each line ($p < 0.05$, ANOVA, Tukey's test).

5.2.2. Protein content

To compare the different extracted samples, protein content was determined according to Lowry et al. [297]. Table 11 shows the results of the protein content determined after extraction with PVPP (option I), SDS (option II), and the improved SDS extraction protocol (option III).

It is clearly observed that extraction using option II achieves significantly higher protein content for continuous process samples compared to extraction using option I ($p < 0.0001$). Protein content was found to be 1.33± 0.21, and 2.08± 0.08 mg/100 mg DW for continuous process samples extracted with option I and II, respectively. Values of 1.62 ± 0.04, and 1.73± 0.19 mg/100 g DW were obtained for batch process samples extracted with option I and II, respectively. Sample extracted with option III exhibited the highest content in protein, 3.75± 0.04, and 3.45 ± 0.13 mg/100 g DW for the continuous and batch process, respectively. The

results indicate that protein content using SDS in the extraction protocol was higher compared to those using PVPP and ascorbic acid.

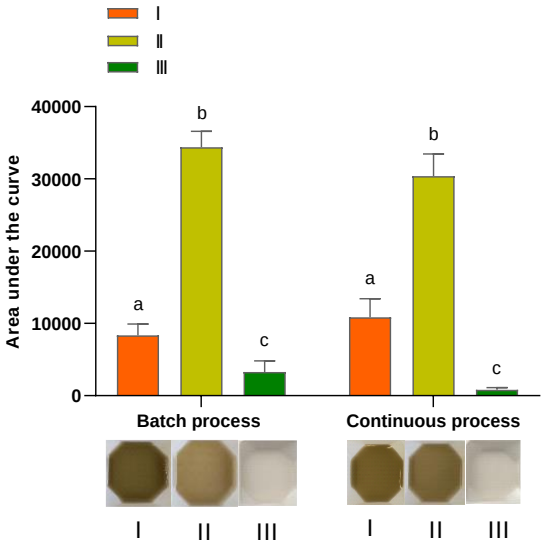
Table 11. Protein content after extraction option I, II, III.

	mg protein / 100 mg DW		
	Option I	**Option II**	**Option III**
Batch process	1.62 ± 0.04^{a}	1.73 ± 0.19^{a}	3.45 ± 0.13^{b}
Continuous process	1.33 ± 0.21^{a}	2.08 ± 0.08^{b}	3.75 ± 0.04^{c}

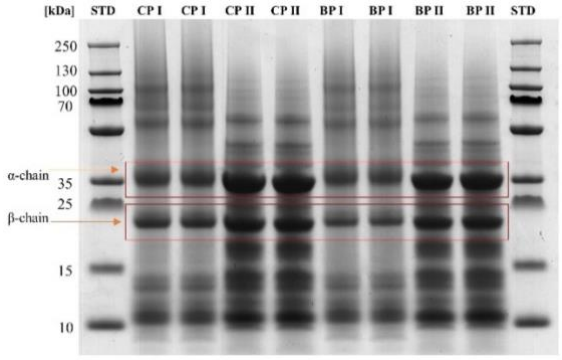
Option I: PVPP and ascorbic acid was used for the extraction; option II and III: SPS was used for the extraction. Data are expressed as means ± standard deviation (n = 3). Different letters indicate significantly different values within the lines ($p < 0.05$, ANOVA, Tukey's).

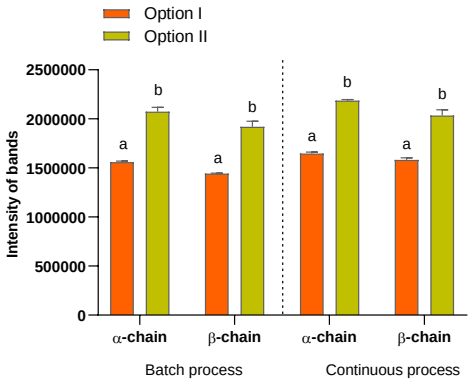
A fluorimeter was used to measure the visible fluorescence spectrum of the protein extracted with the three different option protocols. The dissolved extracts, especially after extraction with option I, show a darker green color compared to those of the option II and III. Protein extract with option III was colorless compared to option I and II (Figure 18). Bongartz et al. [298] reported a change of color when sunflower protein in an alkaline environment was incubated with CQA. An adduct e.g. with lysine results in a green benzacridine derivative as reported in [299,300] and confirmed with the aid of HPLC coupled with ESI-MS^n [301]. If the mechanism proposed by Namiki and colleagues is followed [299,300], a dimerization prior to the interaction with proteins seems to be precedent as documented for chlorogenic acid [302], and validated for the adduct formation with the amino group in a model system [222,301]. Thereafter, the present data suggests that the extraction with PVPP induces an oxidation of phenolic compounds during the extraction and give unexpected results by the added protein modifications. The extraction with SDS showed less influence on the analyzed protein fraction; therefore, option III was selected as a method for further analysis. Transmission measurements using a spectrophotometer from 325 to 480 nm wavelength were performed to quantify the different color appearances. The analysis of the variance showed that differences were significant ($p < 0.05$) for the two types of process. Option III, either for batch or continuous process showed the lowest emission values, therefore, it was chosen for further experiments. SDS-PAGE was used to exemplarily compare the extractions protocols (option I and II) and the results are presented in Figure 18b. The main coffee 11S storage protein was allocated toα- and β-chain bands as indicated in Figure 18b. The band intensity after extraction with option II was significant higher ($p < 0.05$) compared to the extraction with option I, indicating a higher efficiency of the protein extraction (Figure 18c). The typical structure of an 11S storage protein consists of 3-6 monomers, which migrate into storage vacuoles (protein bodies) and generate by hydrophobic interactions the tri- and hexameric quaternary forms, with molecular weights of 150− 400 kDa [303]. The rupture of the disulfide bonds in the 11S protein monomers under reducing conditions releases the α (acidic) and β (basic) subunits [296]. *Coffea arabica* proteins, in absence of a reducing agent, showed subunit with molecular weight of 55 kDa,

and in the presence of reducing agent (2-mercaptoethanol) consisted of two polypeptides with molecular weights of 33 and 24 kDa [304,305].



(a)





(b) (c)

Figure 18. Protein content

(a) protein content after extraction option I, II, III; (b) SDS-PAGE of continuous and batch process green coffee beans; (c) intensity of α and β chains. Data is expressed as means± standard deviation (n = 3). Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, p < 0.05, ANOVA, Tukey's test).

5.2.3. In-gel digestion

The gel bands of the α- and β-chain of the samples were cut and digestion either with trypsin or pepsin was performed. The sequence coverage of peptic and tryptic in-gel digestion is given in Figure 19. Fragment spectrum obtained by MALDI-TOF-MS analysis was compared to the sequence of the *in-silico* digested 11S. The fragment spectrum of the pepsin digestion with zero partials covers 3.6 and 4.3% for α-chain and β-chain, respectively. The sequence coverage increased significantly (p < 0.05) if a further partial is allowed in the digestion. Values

of 19.5 ± 1.6 and 22.8 ± 1.6% were obtained for α-chain and β-chain, respectively. If two partials are allowed, the sequence coverage increases significantly to 35.3 ± 11.0 and 33.2 ± 12.1%. The sequence coverage of the MALDI-TOF-MS fragment spectra of the trypsin-digested samples does not change significantly with an increase in the partials. Supplementary Figure 19b shows the sequence coverage of the samples after extraction with options I and II. There is no significant difference between the two extraction options. The sequence coverage of the α-chain shows lower values compared to those of β-chain for batch and continuous process. Samples were measured only one time therefore differences cannot be statistically checked for significance. These preliminary experiments indicate that, that the tryptic digestion is more effective and was therefore used for further experiments.

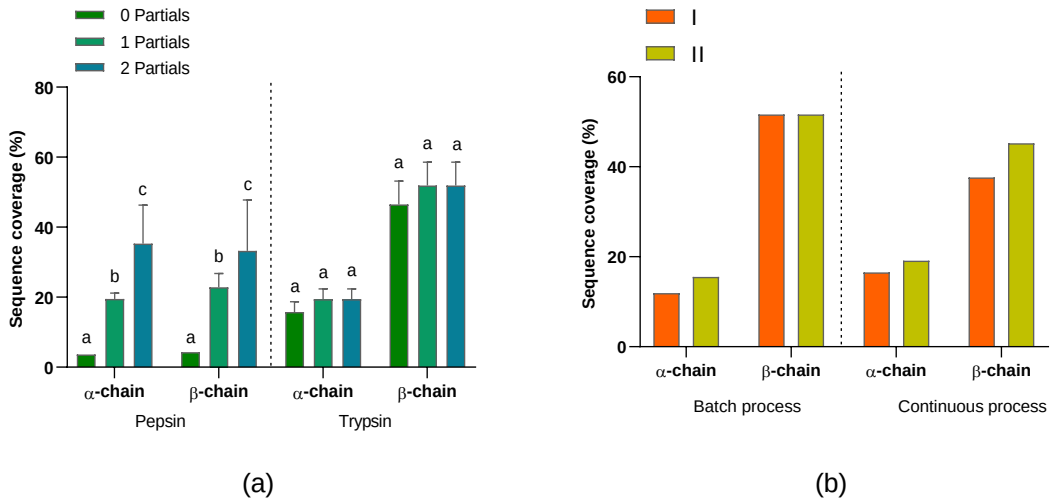


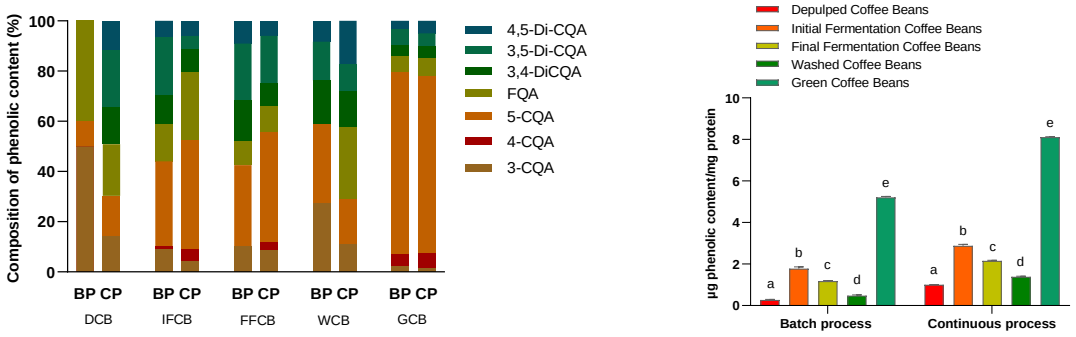
Figure 19. MALDI-TOF-MS.

(a) Sequence coverage of peptic and tryptic in-gel digestion; (b) sequence coverage after extraction with options I and II. Data is expressed as means ± standard deviation (n = 3). Partials describe the incompletely digestion peptide based on the number of unused interfaces that are contained in the fragment spectrum of the in-silico digestion. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test)

5.2.4. Phenolic compounds in protein extract by HPLC

Phenolic compounds in protein extracts were determined by HPLC to investigate the content of the phenolic substances as influenced by the post-harvest treatment. The percentage distribution of the seven detected phenolic compounds (Figure 20a) differs from the two coffee companies, especially between depulped coffee beans samples. The structures of these seven main compounds are given in our former work [306]. Recent studies reporting on the composition of coffee phenolics have documented more than 50 hydroxycinnamic acid derivatives being present [219,220]. The complexity of following up this type of reaction in coffee based food matrix arises from the fact that the major phenolic compounds present in

coffee beans are liable to isomerization and oxidation, thus producing themselves a series of reaction products, many of which have hardly been characterized [222]. The constituents 3,4-DiCQA, 3,5-DiCQA and 4,5-DiCQA were not detected in depulped coffee beans for batch process. Otherwise, similar composition was detected for the initial fermentation, final fermentation, washed and green coffee beans. The predominant constituent was 5-CQA with 72.3 and 70.7% for green coffee beans from batch and continuous process, respectively. The protein extraction indicates that the proteins were precipitated by addition of acetone and thereafter washed with methanol. This treatment can remove the loosely bound phenolic compounds, but only the subsequent treatment with urea (desolvation of the protein) and separation under conditions of the chromatographic conditions allowed the release of those molecules more tightly bound to the proteins as indicated in Figure 20. It can be inferred that these phenolic compounds detected in the protein extract entered in non-covalent interactions with the proteins. Composition of phenolic compounds of green coffee beans show similar composition for coffee beans processed in batch or continuous process. Individual constituents were added up to obtain an approximation of the total phenol content and data are shown in Figure 20b. The highest content of phenolic compounds is found in the green coffee beans with concentrations of 5.21 ± 0.04 and 8.11 ± 0.02 µg/mg protein for batch and continuous process, respectively. Thus, the drying step during the post-harvest treatments promotes a more stronger binding of the phenolic compounds. The results further indicate that coffee beans processed in continuous process triggers stronger binding of the proteins as compared to batch process. The post-harvest again seems to play a significant role, allowing a hypothesis that the proteins may undergo a more pregnant denaturation via enzymatic and/or redox activation steps due to re-use of the processing water for the beans from the batch process. This in turn could be responsible for structural changes in the storage protein resulting in loss of binding sites, thereby allowing a lower binding of the phenolic compounds.



(a) (b)

Figure 20. Phenolic compounds in protein extract.

(a) Composition of the major phenolic compounds; (b) amount of phenolic compounds. BP: Batch process; CP: Continuous process; DCB: depulped coffee beans; IFCB: initial fermentation coffee beans; FFCB: final fermentation coffee beans; WCB: washed coffee beans; GCB: green coffee beans. Data is expressed as percentage of total phenolic compounds (a) and the sum of means ± standard deviation (n = 3) of the detected phenolic compounds in µg/mg protein. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

5.2.5. Analysis of the protein modification using HPLC-MS/MS

Proteins can be modified by reaction with phenolic compounds. The bulk of interactions between these two fractions initially result in non-covalent interactions [307,308]. These can be based on hydrophobicity, hydrogen bonds, or ionic bonds [222]. Suryaprakash et al. [82] showed that proteins from sunflower seeds can act as non-covalent ligands for caffeic and quinic acids. In this context, these interactions often occur between caffeic acid and tryptophan, tyrosine, or lysine side chains [309]. If the interaction of protein and phenolic compound leads to a covalent bond, it is a protein modification. Such a modification can also occur in parallel with non-covalent reactions. Covalent bonds are catalyzed both enzymatically and non-enzymatically. Both reactions can be divided into two steps and require the presence of oxygen [222]. Generally, the first step involves the formation of an electrophilic reactive species of *o*-quinone. These are capable of undergoing a nucleophilic addition to proteins e.g. thiol and free amino groups, thereby covalently modifying the proteins. The green coffee beans have been shown to possess polyphenol oxidase (PPO) activity, therefore their proteins are liable to this type of modification [306]. In order to access such modifications, the proteins need to be broken down into peptides. Thus, a method was developed as indicated in section 4.2.10 to encompass such changes. The strategy includes a first step of identifying the unmodified peptides in the major 11S coffee storage protein and a second one that follows a modification by a single or dimerized chlorogenic acid molecule while applying targeted mass spectrometric analysis [306].

The percentage distribution of the detected masses for the α and β-chain of 11S protein can be seen in Figure 21. The peak areas of the fragment masses were added for each transition. It can be seen that the fragment K.LNAQEPSFR.F gave the strongest signal. The distribution values were between 57.3 and 72.8% for continuous process initial fermentation sample and batch process green coffee beans, respectively. The modified peptides are dominated by the peptide K.FFLAGNPQQQGGGK.E. This peptide was found modified with the CQA monomer. The corresponding proportion of the modified peptides of the α-chain ranges from 74.5% to 90.7% for batch process depulped coffee beans and continuous process final fermentation, respectively. The peptide R.LGGK.T was also detected in the modified state. This peptide was found modified with CQA and DiCQA.

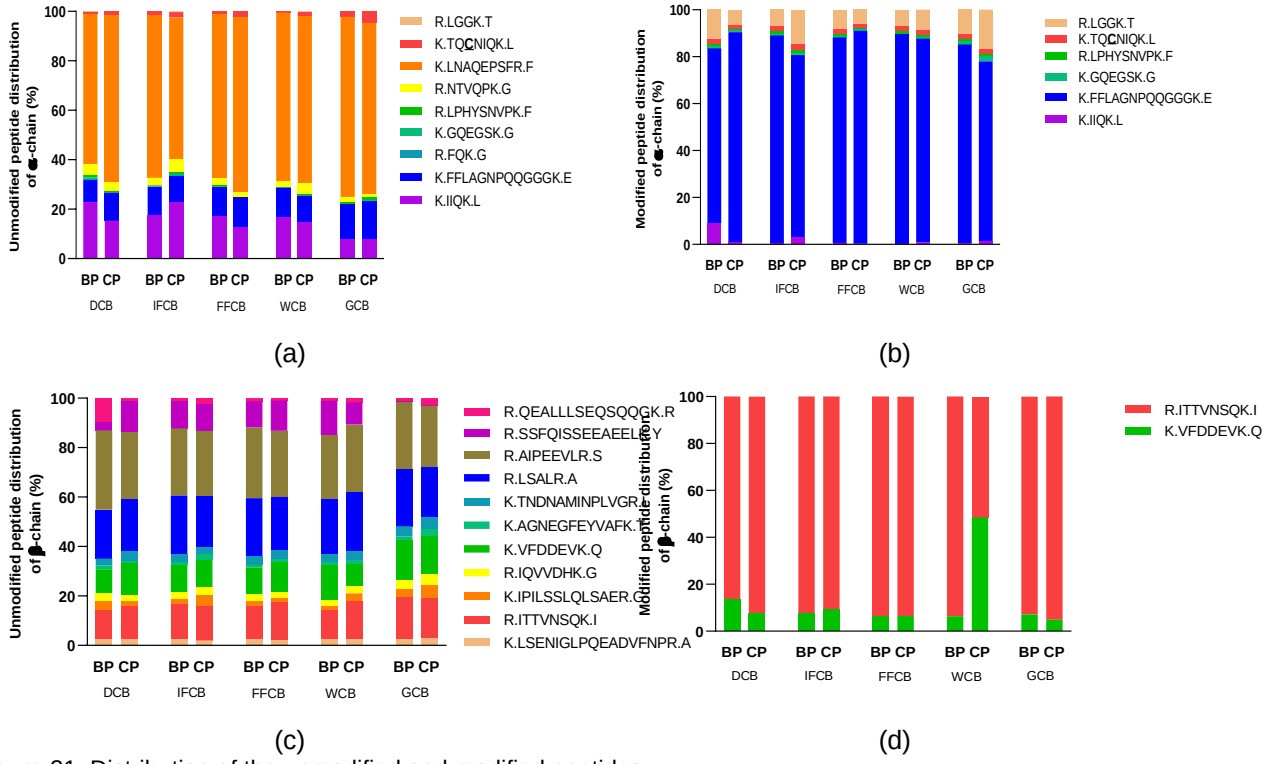


Figure 21. Distribution of the unmodified and modified peptides

Unmodified and modified peptides of the (a,b)α and (c,d) β-chain detected by HPLC-MS/MS from coffee samples from batch and continuous process. BP: Batch process; CP: Continuous process; DCB: depulped coffee beans; IFCB: initial fermentation coffee beans; FFCB: final fermentation coffee beans; WCB: washed coffee beans; GCB: green coffee beans. Data is expressed as means ± standard deviation (n = 2).

The distribution of the unmodified peptides of the β-chain is depicted in Figure 21b. More homogenous distribution is observed compared to the distribution in the α-chain. In fact the response for the β-chain was much stronger and more peptides could be allocated, in turn improving the overall sequence coverage.

From Figure 21b, it can be seen that only two modified peptides were detected. The peptide R.ITTVNSQK.I with DiCQA lysine modification is the predominant peptide in almost all the samples. Washed coffee beans in batch process show a proportion of 48.5% K.VFDDEVK.Q with a CQA-lysine adduct, differing significantly from the rest of the samples investigated. The reason for this observation is not yet clear and further experiments are needed to confirm this behavior.

The HPLC-MS/MS method was also applied to protein extracts from green *C. arabica* beans of various origins. The distribution of the unmodified and modified peptides of the α-chain are depicted in Figures 22 a and b. Among the modified peptides, K.IIQK.L was found to be predominant. The lysine-modified R.LGGK.T peptide was found in all the samples.

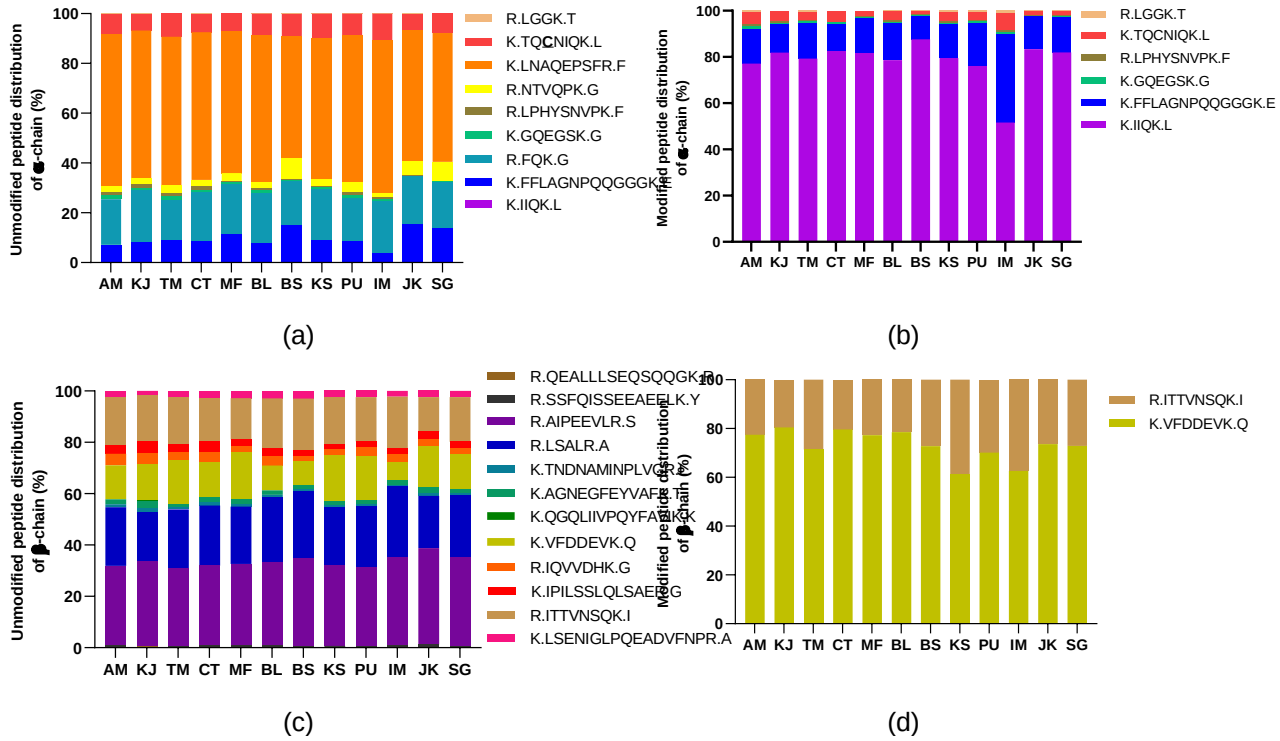


Figure 22. Distribution of the unmodified and modified peptides

Unmodified and modified peptides of the α-chain (a, b) and β-chain (c, d) of 11S by HPLC-MS/MS. AM: Maji Ethiopia; KJ: Jora Kenia; TM: Mbeye Peak Tanzania; CT: Terrazu Costa Rica; MF: Flaming Mexico; BL: Logoa Brazil; BS: Santos Brazil; KS: Supremo Colombia; PU: Urubamba Peru; IM: Malabar India; JK: Kayumas Indonesia; SG: Gayo Indonesia.

In the case of the proportion of modified peptides in the β-chain (Figure 22d), Brazil Santos green coffee beans (KS) is particularly marked by the peptide R.ITTVNSQK.I with 20.2%. In the case of Mexico Flamingo (BL), unmodified peptide K.VFDDEVK.Q shows percentage distribution of 18.3%. The most modified peptide of the β-chain in all the samples was K.VFDDEVK.Q. The proportions of the modified peptides of the β-chain are lower compared to that of the α-chain.

The proportions of modified peptides in the α-chain are shown in Figures 23 a & b as mean values for each type of coffee processing. The percentage of modified peptide R.LGGK.T in green coffee beans is significantly higher for the wet and monsoon compared to the dried and half-wet processing. The situation was similar with the modified peptide K.FFLAGNPQQGGK.E in green coffee beans. A significant increase in fraction of the peak area of the modified peptide was observed among the dried (13.0%), wet (19.5%), and monsoon processing (42.6%). In the case of β-chain (Figures 23 c and d), it can be seen that the proportion of the modified R.ITTVNSQK.I peptide is higher in the wet compared to the half-wet processing. In the case of the peptide K.VFDDEVK.Q, a significant different ($p < 0.05$) proportion is seen in monsoon compared to the dried, half-wet and wet processing.

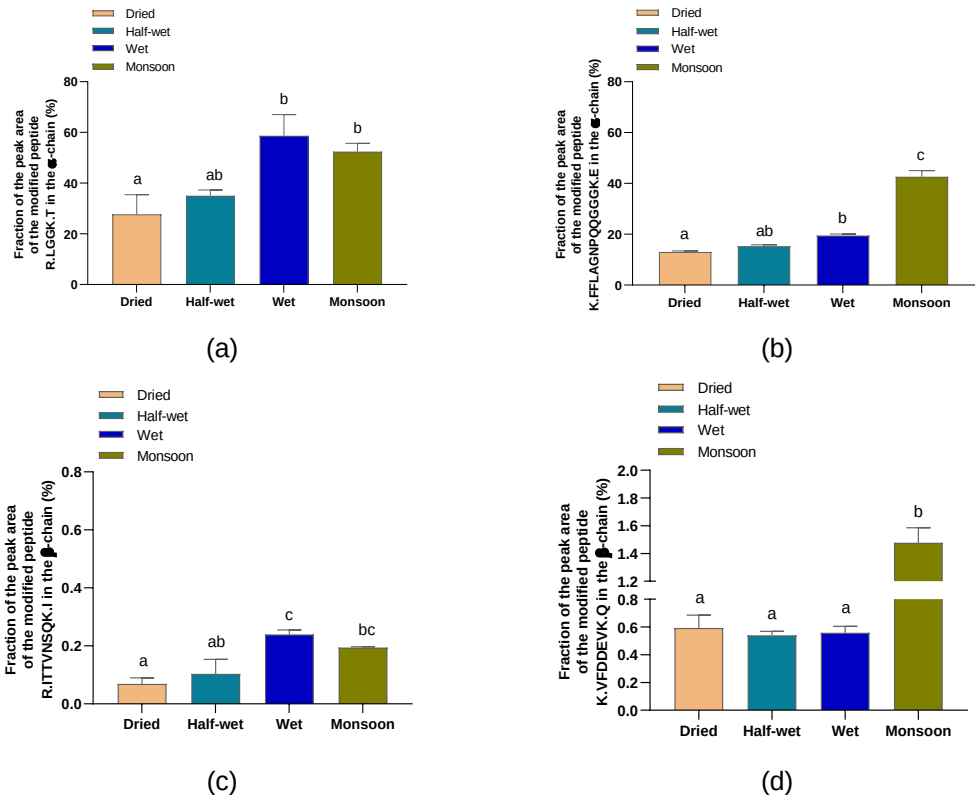


Figure 23. Proportion of the two most lysine-modified peptides in the α-chain and β-chain of the storage 11S protein.

Distribution of the most modified peptides of the α-chain (a,b) and β-chain (c,d) of 11S by HPLC-MS/MS. Data is expressed as means ± standard deviation (n = 2) in percentage of the HPLC-MS/MS peak area of the unmodified and modified peptide given as the sum of the peak areas. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

The peptides K.FFLANGPQQGGK.E and R.LGGK.T of the α-chain show a high level of lysine modifications in the samples analyzed. In the case of the β-chain, peptides were less lysine-modified. Schwenke et al. [310] and Rawel et al. [311] showed that the hydrophilic C-terminal region of the α-chain on the surface of the 11S contained a protective function against the internal β-chain, thereby being the preferred point of attack for chlorogenic acid. Green coffee samples produced by different processing methods showed connection with CQA-dependent lysine modification. Samples of the half-wet, wet and monsoon processing contain larger proportions of the lysine-modified target peptides. Presumably, the increased contact with water favors the oxidation reactions under consideration.

Conclu sion of the study 2:

Three different extraction protocols to isolate the protein fraction were compared. Subsequently a method to detect lysine and cystine modification in coffee protein was developed. Samples from five different processing steps from two coffee plantations in Guatemala were investigated. The developed method integrated analysis of 14 samples of green coffee beans from various processing methods and countries. The unmodified peptide K.LNAQEOSFR.F of the α-chain was detected with high signal intensity. The unmodified peptides of the β-chain showed a diverse spectrum and therefore several options for selecting

peptides as markers were available. The peptides K.FFLANGPQQGGK.E and R.LGGK.T of the α-chain showed a high level of lysine modifications in the samples analyzed. In the case of the β-chain, peptides were less lysine-modified. A recirculation of the water during coffee processing led to more lysine modification. Coffee processing that uses more water showed an increased proportion of the modified peptides. These in turn act in different ways with individual amino acids further increasing the diversity of the reaction products. This seems to be especially relevant for the α-chain on the surface of the 11S coffee storage protein, since many peptides for this fraction were also not found in the unmodified state and the overall response being much lower than that of the β-chain.

5.3. Study 3: Preparation of Activated Carbons from Spent Coffee Grounds and Coffee Parchment and Assessment of Their Adsorbent Efficiency

The valorization of coffee wastes through modification to activated carbon has been considered as a low-cost adsorbent with prospective to compete with commercial carbons. So far, very few studies have referred to the valorization of coffee parchment into activated carbon. Moreover, low-cost and efficient activation methods need to be more investigated. The aim of this study was to prepare activated carbon from spent coffee grounds and parchment, and to assess their adsorption performance. The co-calcination processing with calcium carbonate was used to prepare the activated carbons, and their adsorption capacity for organic acids, phenolic compounds and proteins was evaluated. Both spent coffee grounds and parchment showed yields after the calcination and washing treatments of around 9.0%. The adsorption of lactic acid was found to be optimal at pH 2. The maximum adsorption capacity of lactic acid with standard commercial granular activated carbon was 73.78 mg/g, while the values of 32.33 and 14.73 mg/g were registered for the parchment and spent coffee grounds activated carbons, respectively. The Langmuir isotherm showed that lactic acid was adsorbed as a monolayer and distributed homogeneously on the surface. Around 50% of total phenols and protein content from coffee wastewater were adsorbed after treatment with the prepared activated carbons, while 44, 43, and up to 84% of hydrophobic compounds were removed using parchment, spent coffee grounds and commercial activated carbon, respectively; the adsorption efficiencies of hydrophilic compounds ranged between 13 and 48%. Finally, these results illustrate the potential valorization of coffee by-products parchment and spent coffee grounds into activated carbon and their use as low-cost adsorbent for the removal of organic compounds from aqueous solutions.

5.3.1. Preparation of coffee by-products through pyrolysis

Spent coffee and parchment powders mixed with calcium carbonate at ratio 1:1 at 850° Cwere selected to test the effect of washing the materials after carbonization. Previous batch

experiments were performed to evaluate the effect of different preparation protocols. Figure 24 shows the effect of washing the sample with HCl and deionized water (P1-85 and SC1-85) and without washing (P1-85X and SC1-85X) after carbonization with 50 mg/L of Bromophenol Blue in the bulk solution. It can be clearly observed that samples after washing with HCl and deionized water were more efficient in Bromophenol Blue adsorption compared to those without washing treatment, therefore samples were prepared under same conditions for further analysis.

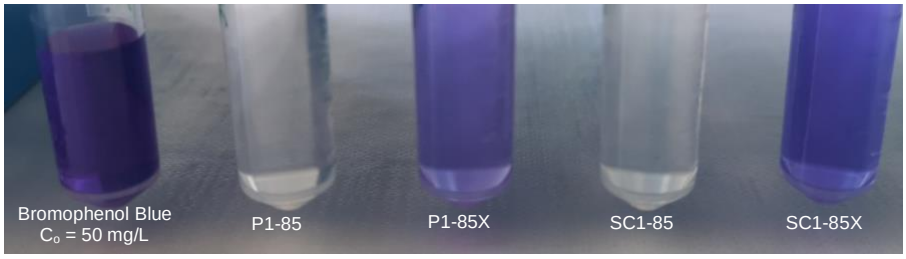


Figure 24 Effect of different preparation protocols on Bromophenol Blue adsorption

Activated carbon prepared under different protocols. P1-85X and SC1-85X were used without any further treatment after pyrolysis. P1-85 and SC1-85 were washed with 2 M HCl and deionized water after pyrolysis. Adsorbent dose of 0.2% (*w/v*) with 50 mg/L of Bromophenol Blue in the bulk solution without pH modification.

5.3.2. Activated carbon yield

Activated carbon yield is defined as final weight of activated carbon produced after carbonization, washing, and drying, divided by initial weight of raw material on a dry basis. Table 12 shows carbon yield after carbonization and washing step for the activated carbon varieties resulting from different ratios and carbonization temperatures. Decomposition of volatile compounds to residual carbon [312] and breaking down of calcium carbonate to calcium oxide [313] explains the initial weight loss in the carbonization step. The low yield after washing step indicates that calcium oxide was removed from activated carbon losing initial weight on a dry basis.

Table 12 Carbon yield for the activated carbon varieties

				Carbonization			Carbonization + Washing		
	Varieties	**$CaCO_3$: material**	**Temperature (° C)**	**w1 (g)**	**w2 (g)**	**Yield (%)**	**w1 (g)**	**w2 (g)**	**Yield (%)**
Parchment	P0-80	0:1	800	8.5	2.3	26.7%	-	-	-
	P0-85	0:1	850	10.1	2.6	25.3%	-	-	-
	P1-80	1:1	800	13.8	7.6	55.1%	13.8	1.2	8.6%
	P1-85	1:1	850	12.7	5.3	41.7%	12.7	1.3	9.9%
	P2-80	1:2	800	12.2	5.6	46.1%	12.2	1.6	12.9%
	P2-85	1:2	850	11.8	4.4	37.6%	11.8	1.3	10.9%
	P3-80	2:1	800	13.3	9.4	70.7%	13.3	0.8	5.8%
	P3-85	2:1	850	13.9	7.4	52.9%	13.9	0.5	3.7%

			Carbonization				Carbonization + Washing		
	Varieties	$CaCO_3$: material	Temperature (° C)	w1 (g)	w2 (g)	Yield (%)	w1 (g)	w2 (g)	Yield (%)
Spent coffee	SC0-80	0:1	800	12.5	3.1	24.8%	-	-	-
	SC0-85	0:1	850	12.4	3.0	24.2%	-	-	-
	SC1-80	1:1	800	18.9	10.6	56.0%	18.9	1.2	6.1%
	SC1-85	1:1	850	18.1	7.2	39.7%	18.1	1.6	8.7%
	SC2-80	1:2	800	17.3	7.5	43.1%	17.3	2.1	12.3%
	SC2-85	1:2	850	17.6	6.3	35.7%	17.6	2.2	12.4%
	SC3-80	2:1	800	17.0	11.7	68.5%	17.0	1.3	7.6%
	SC3-85	2:1	850	16.7	8.1	48.7%	16.7	0.7	4.0%

5.3.3. Characterization of activated carbon produced

Zeta potential measurements were performed to determine electrophoretic mobility of activated carbon particles in a diluted suspension. The experiments were conducted in a neutral solution (pH ~7) and the zeta potential was measured to be -16.9 ± 3.4, -18.6 ± 1.3, and -24.9 ± 0.23 mV for spent coffee, parchment and commercial activated carbons, respectively. Zeta potential < ± 30 mV indicates that the colloidal system is unstable and particles tend to attract each other [314], whereas the sign of the zeta potential shows that negative charges are predominant at the surface of the activated carbons [315].

SEM was used to determine the microstructure of the prepared activated carbons. Figure 25 shows the SEM images of raw materials, activated carbons obtained from spent coffee grounds, parchment and the commercially available activated carbon. It is clear from the images that co-calcination with $CaCO_3$ results in the development of a large number of pores on the surfaces of spent coffee grounds and coffee parchment activated carbons (Figure 25 b and d) compared to the raw materials (Figure 25 a and c). Calcium carbonate is decomposed at temperatures above 800° C producing calcium oxide and CO_2. The latter is normally used as oxidation reagent for physical activation. Arenas et al. [316] have demonstrated that CO_2 steam produces high surface areas due to the steam widened microporosity which allows carbon dioxide to reach new sites to develop new porosity. The development of pores is also related to yield decline, showing that development progress from outer to inner structures [317]. Section 8.12 shows the SEM images for all the parchment and spent coffee.

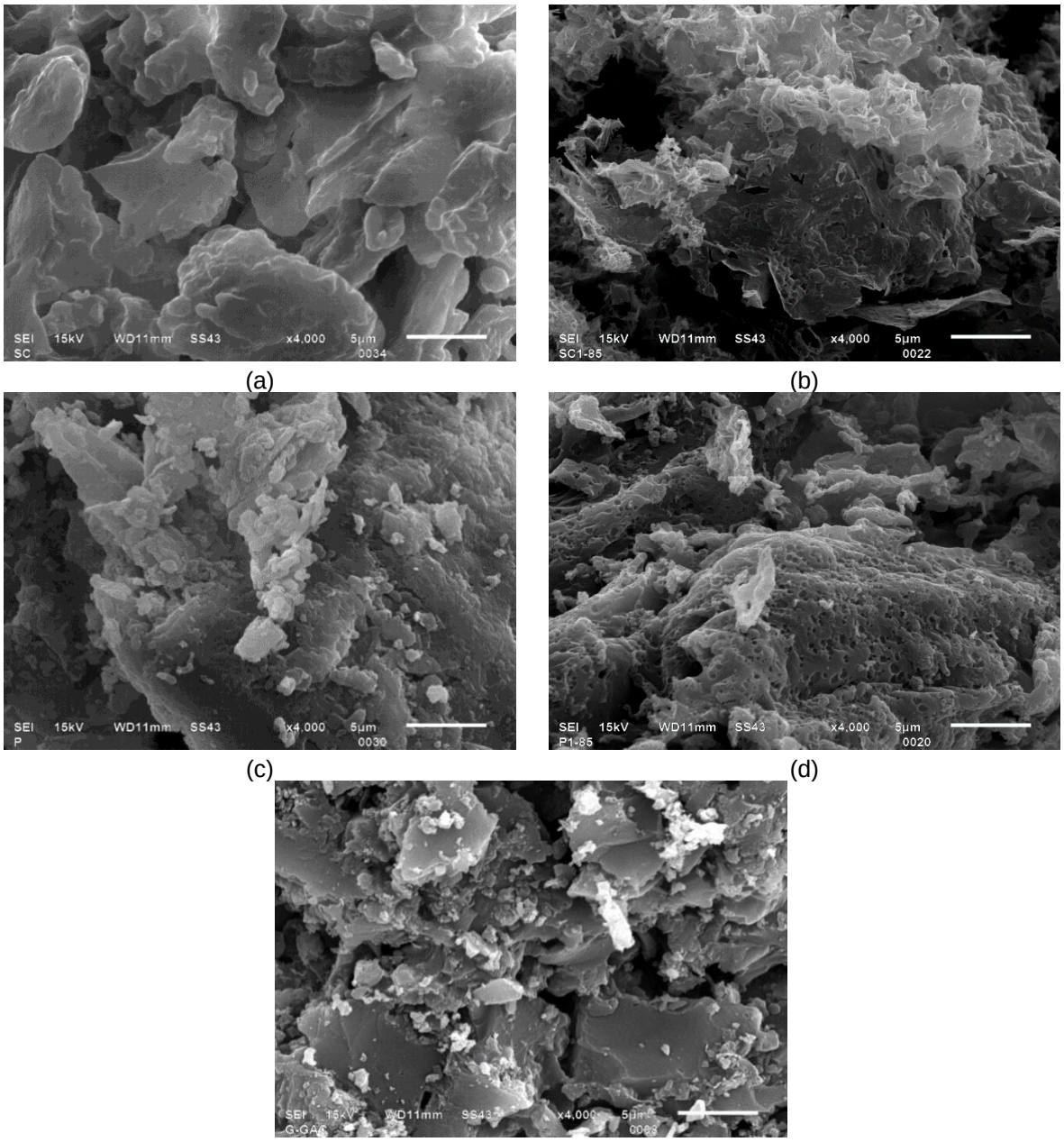


Figure 25 Scanning electron microscope images of activated carbon

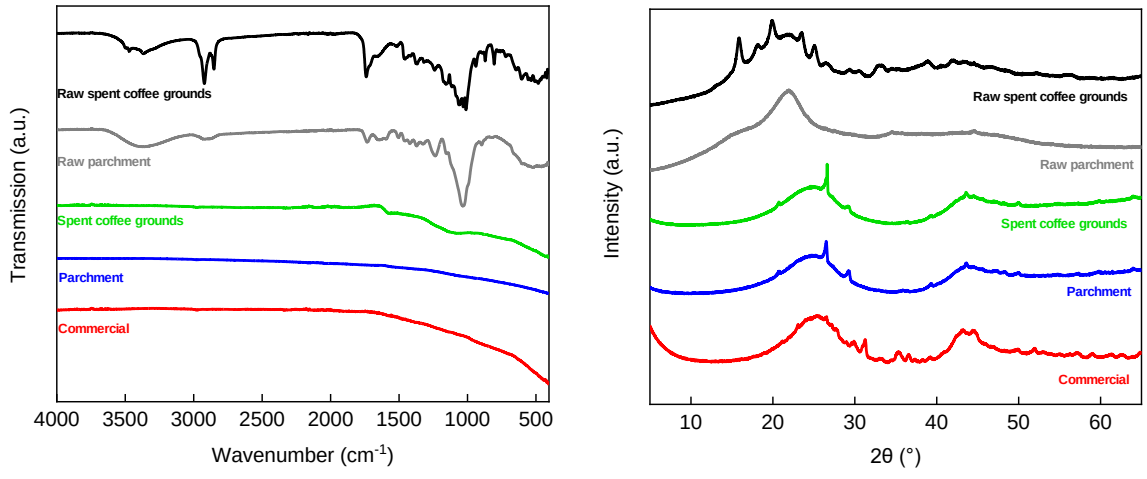
Produces from spent coffee and parchment (a) raw spent coffee, (b) spent coffee (c) raw coffee parchment, (d) parchment, (e) commercial

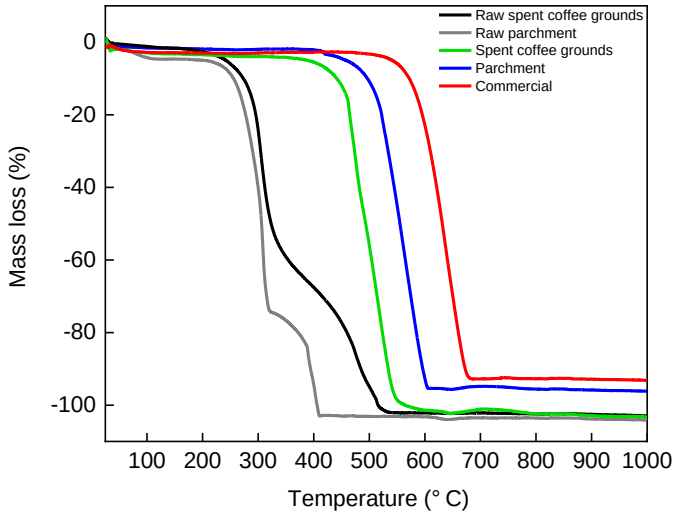
Figure 26a presents the IR spectrums of the materials. Bands from 1500 to 1000 cm^{-1} were obtained for the raw spent coffee and the parchment, while two bands at approximately 2900 cm^{-1} were recorded only for the raw spent coffee grounds. The bands exhibited by the raw materials are assigned to lignin/cellulose materials [318,319] This is consistent to the composition of spent coffee grounds and parchment rich in cellulose, lignin and hemicellulose [320,321]. For the co-calcinated samples, all the bands exhibited by its corresponding raw materials are no longer detected and spectrums were similar to commercial activated carbon. Elemental analysis data indicates that the raw spent coffee is composed of 56.3% carbon, 7.7% hydrogen, 1.8% nitrogen, and 0.47% sulfur. These values were 46.3, 6.4, 0.4, and 0.7%

for parchment. After co-calcination values of 84.0% carbon, 1.38% hydrogen, 2.6% nitrogen and 0.3% sulfur; 91.6% carbon, 0.9% hydrogen, 0.5% nitrogen, and 0.3% sulfur were obtained for the spent coffee and parchment activated carbons, respectively. High temperatures release volatile matter content, removing functional groups, therefore carbon content in activated carbon samples is higher when compared to those of the raw materials.

Structure of the materials analyzed using the XRD showed one main broad peak at approximately ~20° 2θ for both raw materials (Figure 26b). Those peaks are in agreement with diffraction peaks of amorphous cellulose containing material [322]. After co-calcination, the resulting activated carbons exhibited two main peaks at ~25° and ~43° 2θ, showing similar diffractograms compared to commercial activated carbon. Peaks around 20° and 40° Correspond to the reflection of the (002) and (100) planes of graphite structure of carbon black, respectively [323].

Thermogravimetric analysis was used to investigate the content of inorganic residues in the materials and results are presented in Figure 26c. The mass losses of raw spent coffee grounds were detected in the temperature range from 282° C to 324° C (54 % weight loss) and from 438° C to 520° C (29 % weight loss). These values were in the range from 286° C to 316° C (70% weight loss) and from 376° C to 410° C (29% weight loss) for raw parchment. Those weight losses were reported to be a thermal decomposition of the amorphous cellulose regions and oxidation of the carbon skeleton [324]. Similar thermogravimetric curves were previously reported from materials with hemicellulose and lignin content [322,325,326]. Weight losses of 98% in the range from 445° C to 546° C and of 93% from 504° C to 601° C were reported for spent coffee grounds and parchment activated carbons, respectively. Commercial activated carbon showed one decrease in sample mass in the temperature range from 582° C to 674° C constituting 90% of the total mass. The results for co-calcinated samples indicate an increase in material crystallinity therefore causing an improvement in thermal stability.



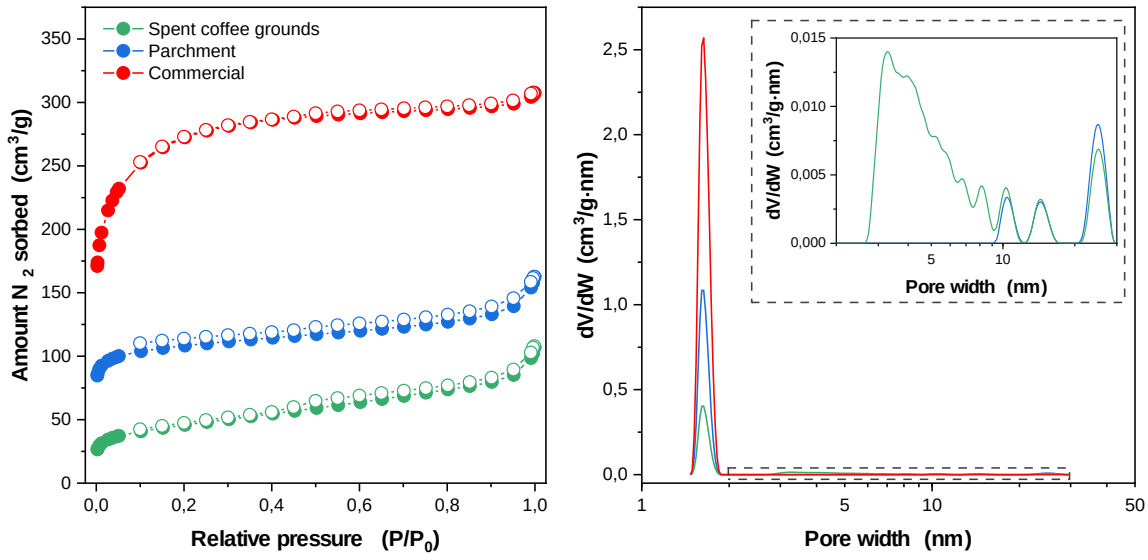


(c)

Figure 26 IR spectra, X-ray diffraction patterns and TGA data

(a) IR spectra, (b) X-ray diffraction patterns, and (c) TGA data of raw spent coffee grounds, raw parchment, spent coffee grounds, parchment and commercial activated carbons.

N_2 sorption measurements were done to determine the specific surface area and porosity of the activated carbon materials, and the resulting isotherms are presented in the Figure 27a. The adsorption branch of the isotherms of the activated carbon materials showed rapid N_2 uptake at low relative pressure ($P/P_0 < 0.1$), followed by a gradual uptake at higher relative pressures. Their sorption behavior indicates that these materials are largely microporous (IUPAC classification Type Ib) [327]. The activated carbon materials from parchment and spent coffee grounds exhibited open hysteresis, as seen from the non-closing of the desorption branch at low P/P_0. The resulting activated carbon from parchment and spent coffee ground exhibited a BET specific surface area (SSA_{BET}) of 416 and 167 m^2/g, respectively, while the commercial activated carbon had the highest SSA_{BET} of 1010 m^2/g (Table 13). The commercial activated carbon also had the highest pore volume of 0.46 cm^3/g, followed by parchment and spent coffee grounds with 0.22 and 0.14 cm^3/g, respectively (Table 13). The pore size distribution (PSD) analysis (Figure 46b) of the adsorption branch using HS-NLDFT calculation revealed that all three materials exhibited a large proportion of micropores (< 2 nm pore width), with the commercial activated carbon having only micropores. On the other hand, the activated carbons from parchment and spent coffee grounds also have mesopores (2-30 nm pore width; Figure 46b inset). The parchment activated carbon has a more defined mesopore distribution compared to the spent coffee grounds.



(a) (b)

Figure 27 N_2 sorption isotherms

(a) N2 sorption isotherms, and (b) differential pore size distribution of spent coffee grounds, parchment and commercial activated carbons.

Table 13. Porosity data derived from N2 sorption measurements at 77 K.

Activated carbon material	**SSA_{BET} [a] (m^2/g)**	**V_{pore} [b] (cm^3/g)**
Parchment	416	0.22
Spent coffee grounds	167	0.14
Commercial	1010	0.46

Note: [a] specific surface area (SSA_{BET}) calculated using the BET equation in the pressure range of 0.1-0.3 for the raw materials and 0.05-0.1 for the activated carbons; [b]pore volume (V_{pore}) calculated using HS-NLDFT with a minimum pore width of 1.47 nm.

5.3.4. Effect of calcination temperature, pH and initial concentration on Bromophenol Blue adsorption

Carbonization temperature influences the adsorption capacity of the adsorbent, therefore a careful selection of the carbonization process can enrich the carbon content and porosity in the char [328]. Bromophenol Blue was used as adsorbate to evaluate the effect of carbonization temperature and calcium carbonate to material ratios on their adsorption capacity. Figure 28 presents the adsorption capacity of Bromophenol Blue of spent coffee and parchment for different carbonization temperatures and ratio mixtures. When carbonization temperature increases the adsorption capacity also increases. When the mass ratio of calcium carbonate to material increased, the adsorption capacity increased whether at 800° C or 850° C. Likewise, when the mass ratio of calcium carbonate to material decreases, the adsorption capacity also decreases. Spent coffee and parchment without adding calcium carbonate whether at 800° C or 850° C do not show adsorption efficiency for Bromophenol Blue. High values of adsorption efficiency were obtained in SC1-85 (99%).

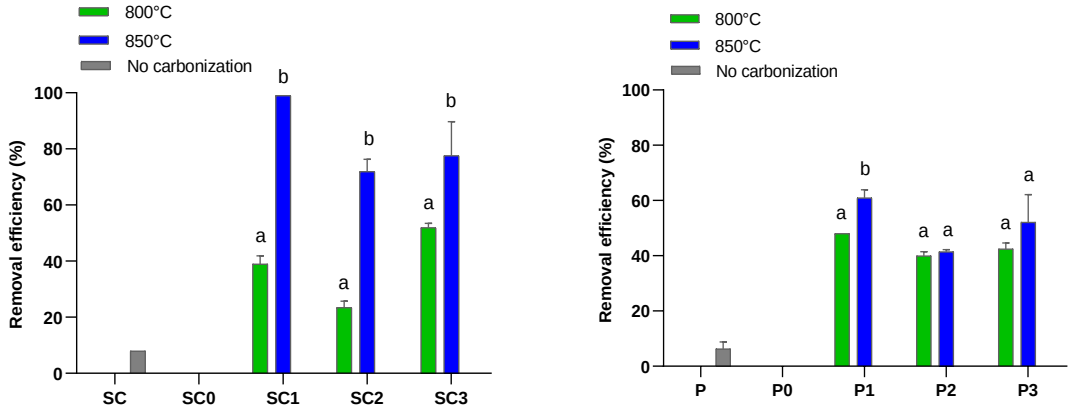
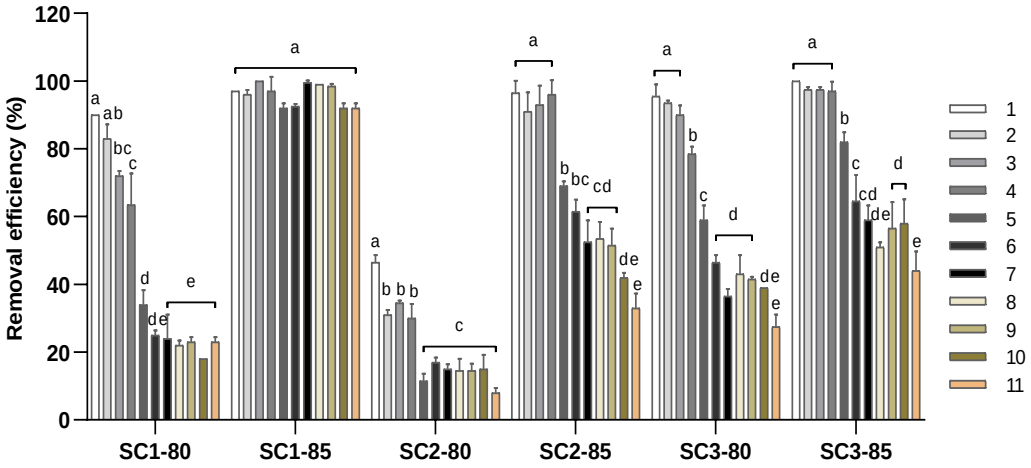


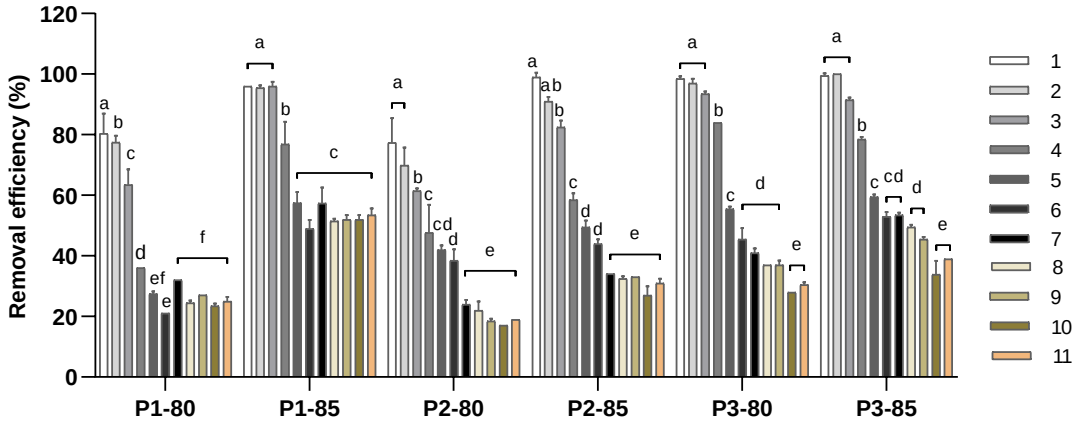
Figure 28 Effect of carbonization temperatures on Bromophenol Blue adsorption

Activated carbon prepared from (a) spent coffee or (b) parchment at different mass ratios (calcium carbonate: material) and different carbonization temperatures (800 and 850° C). Adsorbent dose of 0.2% *(w/v)* with 50 mg/L of Bromophenol Blue in the bulk solution without pH modification. Data are expressed as means ± standard deviation. $n = 3$. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Šidák's test)

The effect of pH from 1 to 11 on the adsorption capacity with 4 mg of AC in 2 mL of aqueous Bromophenol Blue dye solution of 50 mg/L at a contact time of 1 hour was investigated. pH plays an important role in aqueous chemistry and considerably affects the adsorption capacity. Figure 29a illustrates the removal efficiencies of Bromophenol Blue for SC1-80, SC1-85, SC2-80, SC2-85, SC3-80, and SC3-85. Figure 28b shows the removal efficiencies of Bromophenol Blue for P1-80, P1-85, P2-80, P2-85, P3-80, and P3-85. The removal efficiency decreased as pH increased. Because Bromophenol Blue is slightly negative charged when dissolved in aqueous solution, electrostatic attraction could favor adsorption when pH value is between 2 and 3. Nevertheless, when the pH value of the aqueous solution increases, the positively charged surface decreases, meaning a reduction of electrostatic attraction and removal efficiency. If pH value surpasses 5 or 6 the surface is negatively charged resulting in an electrostatic repulsion and therefore decreasing in removal efficiency. pH at point of zero charged (pH_{PZC}) was determined from the intersection of the curve initial pH vs Δ pH (initial pH - final pH) for each sample. The surface of the AC is positively charged when pH values are below pH_{PZC} and negatively charged when are above pH_{PZC}. Results of pH_{PZC} are compiled in Table 14.



(a)



(b)

Figure 29 Effect of pH on Bromophenol Blue adsorption

Effect of pH on Bromophenol Blue adsorption on (a) spent coffee or (b) parchment. Activated carbon prepared with different mass ratios (Calcium carbonate: material) and carbonization temperature (800 and 850° C). Adsorption experiments performed with adsorbent dose of 0.2% *(w/v)* with 50 mg/L of Bromophenol Blue in the bulk solution at different pH (1-11). Data are expressed as means ± standard deviation. n = 3. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

Table 14 Point of zero charged of spent coffee and parchment.

	AC	pH_{PZC}
Spent Coffee	SC1-80	6.75
	SC1-85	6.88
	SC2-80	5.88
	SC2-85	6.30
	SC3-80	5.77
	SC3-85	6.25

	AC	pH_{PZC}
Coffee parchment	P1-80	7.87
	P1-85	6.80
	P2-80	5.84
	P2-85	6.42
	P3-80	6.84
	P3-85	7.01

Adsorption experiments with 4 mg adsorbent in 2 mL adsorbate of Bromophenol Blue solution of 50 mg/L, pH range of 1 to 11, 1 hour contact time at room temperature

The effect of various initial dye concentrations (10 – 100 mg/L) on adsorption efficiency at fixed pH 4 for 1 hour contact time was evaluated. Tables 15 and 16 illustrate the adsorption efficiency and amount of dye adsorbed by spent coffee and parchment activated carbon. The adsorption efficiencies for SC2-85, SC3-80, and SC3-85 with initial dye concentration 10 – 30 mg/L were about 99 – 82%. SC2-80 shows lower percentage of adsorption, a maximum of adsorption efficiency of 70% in an initial dye concentration of 10 mg/L. Likewise, adsorption efficiency of parchment activated carbon was greater at low initial dye concentration, ranging between 99-70%. Activated carbon SC1-85 shows greater percentage of adsorption between 100-60 % in a wider initial dye concentration range (between 10-150 mg/L). The uptake decreased when initial dye concentration was between 50-60 mg/L with exception of sample SC1-85, which shows an uptake decreasing at initial dye concentration between 150-200 mg/L. A decrease in the adsorption efficiency with the increase in dye concentration in the solution was observed. The adsorption uptake increases until the material reaches the maximum uptake, where thereafter the uptake decreases. The maximum uptake of spent coffee activated carbon samples was shown by SC1-85 with 46.33 mg/g. Whereas, for parchment activated carbon the maximum uptake was 20.58 mg/g by P1-85.

Table 15 Adsorption efficiency of Bromophenol Blue onto spent coffee and parchment activated carbon

Initial dye concentration (mg/L)	Adsorption efficiency (%)											
	Spent coffee activated carbon						Parchment activated carbon					
	SC1-80	SC1-85	SC2-80	SC2-85	SC3-80	SC3-85	P1-80	P1-85	P2-80	P2-85	P3-80	P3-85
10	98.04 ± 0.00^{a}	96.28 ± 0.00^{a}	70.45 ± 0.11^{a}	99.38 ± 0.00^{a}	99.59 ± 0.00^{a}	98.04 ± 0.01^{a}	95.97 ± 0.01^{a}	96.69 ± 0.01^{a}	95.04 ± 0.01^{a}	96.38 ± 0.00^{a}	98.66 ± 0.01^{a}	97.21 ± 0.00^{a}
20	95.56 ± 0.02^{a}	99.54 ± 0.00^{a}	27.74 ± 0.03^{b}	98.92 ± 0.00^{a}	98.71 ± 0.00^{a}	94.99 ± 0.01^{a}	95.71 ± 0.01^{a}	98.55 ± 0.00^{a}	89.15 ± 0.00^{b}	97.26 ± 0.01^{a}	97.93 ± 0.00^{ab}	97.62 ± 0.00^{a}
30	80.44 ± 0.03^{b}	98.83 ± 0.00^{a}	20.01 ± 0.05^{b}	96.87 ± 0.01^{a}	92.60 ± 0.01^{a}	93.84 ± 0.00^{a}	80.37 ± 0.02^{b}	99.28 ± 0.00^{a}	69.70 ± 0.04^{c}	83.44 ± 0.02^{b}	92.67 ± 0.00^{b}	75.45 ± 0.05^{b}
40	62.19 ± 0.01^{c}	99.61 ± 0.00^{a}	10.38 ± 0.02^{c}	84.40 ± 0.02^{b}	81.82 ± 0.04^{b}	96.57 ± 0.01^{a}	69.83 ± 0.04^{c}	97.24 ± 0.00^{a}	58.65 ± 0.03^{d}	67.30 ± 0.01^{c}	84.99 ± 0.01^{c}	59.66 ± 0.04^{c}
50	47.73 ± 0.01^{d}	99.88 ± 0.00^{a}	4.46 ± 0.02^{d}	71.82 ± 0.07^{c}	70.58 ± 0.01^{b}	84.28 ± 0.05^{b}	59.94 ± 0.01^{d}	82.83 ± 0.01^{b}	52.07 ± 0.01^{e}	56.74 ± 0.02^{d}	73.22 ± 0.01^{d}	35.39 ± 0.03^{d}
60	42.06 ± 0.01^{d}	99.55 ± 0.00^{a}	2.63 ± 0.03^{d}	64.46 ± 0.08^{c}	54.49 ± 0.08^{c}	74.59 ± 0.07^{c}	49.90 ± 0.00^{e}	57.77 ± 0.02^{c}	44.54 ± 0.03^{f}	39.36 ± 0.02^{e}	62.90 ± 0.02^{e}	11.90 ± 0.01^{e}
80	24.69 ± 0.01^{e}	96.16 ± 0.00^{a}	-	33.61 ± 0.01^{d}	35.76 ± 0.02^{d}	46.37 ± 0.06^{d}	25.76 ± 0.00^{f}	28.46 ± 0.04^{d}	26.87 ± 0.00^{g}	18.61 ± 0.02^{f}	36.51 ± 0.01^{f}	-
100	11.57 ± 0.02^{f}	89.18 ± 0.00^{a}	-	19.19 ± 0.05^{e}	24.01 ± 0.06^{e}	32.12 ± 0.09^{e}	17.18 ± 0.01^{g}	2.49 ± 0.02^{e}	20.34 ± 0.02^{h}	5.40 ± 0.00^{g}	22.41 ± 0.01^{g}	-
150	2.33 ± 0.00^{g}	59.68 ± 0.00^{b}	-	2.09 ± 0.01^{f}	14.77 ± 0.02^{e}	19.37 ± 0.03^{f}	-	-	-	-	-	-
200	-	25.60 ± 0.00^{c}	-	-	-	-	-	-	-	-	-	-

Bromophenol Blue at different initial dye concentrations (10-200 mg/L), 4 mg adsorbent in 2 mL adsorbate, and 1 hour contact time at room temperature. Data are expressed as means ± standard deviation. n = 3. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

Table 16 Amount of Bromophenol Blue adsorbed onto spent coffee and parchment activated carbon

Initial dye concentration (mg/L)	Amount adsorbed (mg/g)											
	Spent coffee activated carbon						Parchment activated carbon					
	SC1-80	SC1-85	SC2-80	SC2-85	SC3-80	SC3-85	P1-80	P1-85	P2-80	P2-85	P3-80	P3-85
10	4.90 ± 0.01^{a}	481 ± 0.02^{a}	3.52 ± 0.56^{a}	4.97 ± 0.01^{a}	4.98 ± 0.01^{a}	4,90 ± 0.03^{a}	4.80 ± 0.06^{a}	4.83 ± 0.07^{a}	4.75 ± 0.07^{a}	4.82 ± 0.01^{a}	4.93 ± 0.03^{a}	4.86 ± 0.00^{a}
20	9.56 ± 0.17^{b}	9.95 ± 0.03^{b}	2.77 ± 0.32^{a}	9.89 ± 0.03^{b}	9.87 ± 0.00^{bc}	9.50 ± 0.15^{b}	9.57 ± 0.12^{b}	9.86 ± 0.04^{b}	8.92 ± 0.04^{b}	9.73 ± 0.07^{b}	9.79 ± 0.01^{b}	9.76 ± 0.01^{b}
30	12.04 ± 0.45^{c}	14.82 ± 0.07^{c}	3.00 ± 0.69^{a}	14.53 ± 0.12^{ce}	13.89 ± 0.18^{cd}	14.08 ± 0.04^{c}	12.06 ± 0.32^{c}	14.89 ± 0.02^{c}	10.46 ± 0.54^{c}	12.52 ± 0.28^{c}	13.90 ± 0.04^{c}	11.31 ± 0.78^{c}
40	12.44 ± 0.30^{c}	19.92 ± 0.06^{d}	2.08 ± 0.42^{a}	16.88 ± 0.31^{cde}	16.36 ± 0.90^{d}	19.31 ± 0.28^{d}	13.97 ± 0.74^{d}	19.45 ± 0.05^{d}	11.75 ± 0.52^{c}	13.46 ± 0.29^{d}	17.00 ± 0.10^{d}	11.93 ± 0.73^{c}
50	11.93 ± 0.15^{c}	24.97 ± 0.01^{ei}	1.12 ± 0.43^{a}	17.95 ± 1.76^{cd}	17.64 ± 0.37^{d}	21.07 ± 1.31^{d}	14.99 ± 0.17^{d}	20.71 ± 0.18^{d}	13.02 ± 0.37^{d}	14.19 ± 0.54^{d}	18.30 ± 0.20^{d}	8.84 ± 0.77^{d}
60	12.62 ± 0.29^{c}	29.87 ± 0.02^{fi}	1.49 ± 0.18^{a}	19.34 ± 2.32^{d}	16.35 ± 2.37^{de}	22.38 ± 2.11^{d}	14.99 ± 0.01^{d}	17.31 ± 0.65^{e}	13.39 ± 0.83^{d}	11.79 ± 0.61^{e}	18.88 ± 0.60^{d}	3.58 ± 0.40^{a}
80	9.88 ± 0.24^{b}	38.47 ± 0.18^{g}	-	13.45 ± 0.50^{e}	14.30 ± 0.99^{def}	18.55 ± 2.31^{de}	10.29 ± 0.09^{e}	11.38 ± 1.56^{f}	10.73 ± 0.07^{c}	7.40 ± 0.61^{f}	14.55 ± 0.49^{e}	-
100	5.79 ± 1.02^{b}	44.59 ± 0.50^{h}	-	9.60 ± 2.52^{e}	12.00 ± 2.83^{ef}	16.06 ± 4.57^{e}	8.72 ± 0.09^{f}	1.23 ± 0.84^{g}	10. 25 ± 0.68^{c}	2.80 ± 0.04^{g}	11.18 ± 0.66^{f}	-
150	1.75 ± 0.20^{d}	44.76 ± 2.21^{h}	-	2.06 ± 0.50^{f}	11.07 ± 1.18^{f}	14.53 ± 2.24^{e}	-	-	-	-	-	-
200	-	25.65 ± 5.46^{i}	-	-	-	-	-	-	-	-	-	-

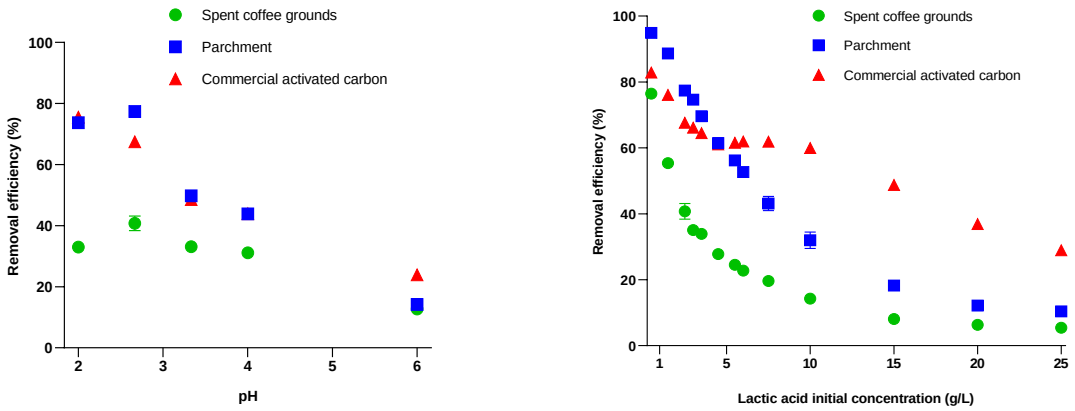
Bromophenol Blue at different initial dye concentrations (10-200 mg/L), 4 mg adsorbent in 2 mL adsorbate, and 1 hour contact time at room temperature. Data are expressed as means ± standard deviation. n = 3. Different letters indicate significantly different values for each group (different superscript letters indicate a significant different, $p < 0.05$, ANOVA, Tukey's test).

5.3.5. Lactic acid adsorption

5.3.5.1. Effect of pH and concentration on lactic acid adsorption

The effect of pH on the lactic acid adsorption capacity was investigated. Five different initial pH were tested, 2.0, 2.85, 3.86, 4.0 and 6.0 and the results are presented in Figure 30a. Data indicates that the three activated carbons exhibit selectivity at a pH below pK_a (3.86) of lactic acid. Removal efficiency clearly decreased from 74 ± 0.17 to 14 ± 0.64% for parchment, from 33 ± 0.84 to 13 ± 0.26% for spent coffee, and from 75 ± 0.99 to 24% ± 1.09% for commercial activated carbon. Similar values of lactic acid adsorption on different commercial activated carbon were previously reported under similar experimental conditions [275,329]. Lactic acid adsorption onto activated carbon was reported as physical adsorption which depends on electronic polarization, decrease of lactic acid at pH above pK_a is owed to a decrease on dipole-dipole interactions [330]. The shown experimental data agrees with the above as the activated carbon samples exhibit adsorption affinity below pK_a. Data also showed that lactic acid adsorption is higher for the commercial activated carbon and parchment compared to the spent coffee efficiencies.

Adsorption capacity of lactic acid was investigated by modifying the initial lactic acid concentration in the range of 0.5 to 25 g/L, and the results are presented in Figure 30b. The removal efficiency significantly decreased when the lactic acid increased from 0.5 to 25 g/L. The values ranged from 94.92 ± 0.003 to 10.43 ± 0.004% ($p < 0.0001$), from 76.46 ± 0.006 to 5.45 ± 0.002% ($p < 0.0001$), and from 82.88 ± 0.005 to 21.60 ± 0.018% ($p < 0.0001$) for parchment, spent coffee and commercial activated carbons, respectively. Nevertheless, the adsorption capacity increased until it reached adsorption equilibrium with values ranging from 4.75 ± 0.01 to 26.07 ± 0.94 mg/g, from 3.82 ± 0.03 to 13.62 ± 0.54 mg/g, and from 4.14 ± 0.01 to 72.36 ± 2.61 mg/g for parchment, spent coffee and commercial activated carbons. The experimental data showed that adsorption of lactic acid onto activated carbon is affected by lactic acid initial concentration. Saturation of available pore sites on the adsorbent has been reported as the initial concentration increased [331]. Difference of adsorption efficiencies between activated carbon samples is therefore related to the amount of pore sites available on the adsorbent.



(a) (b)

Figure 30 Effect of lactic acid adsorption on activated carbon

(a) Different initial pH (2 – 6) with 2.5 g/L of lactic acid in the bulk solution, adsorbent dose of 10% (*w/v)* and 4 h contact time at room temperature; (b) Different initial lactic acid concentrations (0.5 – 25 g/L) without pH correction, adsorbent dose 10% (*w/v)* and 4 h contact time at room temperature.

5.3.5.2. Lactic acid adsorption isotherm

Langmuir and Freundlich isotherms were applied to find equilibrium adsorption characteristics. Figure 31 shows the non-linear plots for Langmuir and Freundlich isotherm of lactic acid at room temperature. Table 17 exhibits the isotherm parameters and regression coefficients for the two isotherms plots. The maximum adsorption capacity (q_m) was 13.99 ± 0.45, 29.48 ± 1.31, 93.48 ± 5.60 mg/g for parchment, spent coffee grounds, and commercial activated carbons, respectively. Langmuir constant K_L is related to the sorption energy and represents the affinity of the adsorbent to the adsorbate. The values calculated were 3.07 x 10^{-4} for commercial activated carbon, 4.9 x 10^{-3} for parchment, and 2.4 x 10^{-3} for spent coffee. Coefficient of determination (R^2) was compared from Langmuir and Freundlich isotherm. Langmuir isotherm was found to be more appropriate in interpreting the adsorption behavior. The shown experimental data agrees with another study where lactic acid adsorption follows the Langmuir isotherm [275]. Langmuir isotherm model states that the adsorbate was adsorbed as monolayer and therefore allocated homogeneously on the surface with restricted interaction between adsorbate molecules. Langmuir data parameters indicate that parchment has more affinity to lactic acid compared to spent coffee and commercial activated carbon.

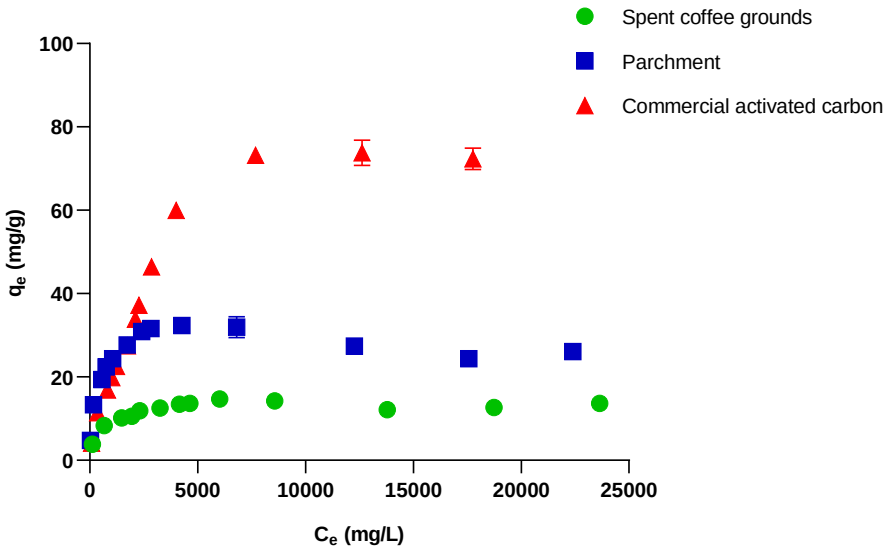


Figure 31 Adsorption capacity for lactic at equilibrium

Adsorption capacity (q_e) for lactic acid versus lactic acid concentration at equilibrium in liquid phase (C_e) for spent coffee, parchment, and commercial activated carbon, without pH corrections, adsorbent dose 10% *(w/v)* and 4 h contact time at room temperature.

Table 17 Langmuir and Freundlich isotherm model parameters

Isotherms	Coeffi cients	Units	Spent coffee	Parchment	Commercial activated carbon
Langmuir	q_m	mg/g	13.99	29.89	93.48
	K_L	L/mg	0.0024	0.0049	3.07×10^{-4}
	R^2	-	0.89	0.87	0.96
Freundlich	n	-	7.07	9.62	2.39
	K_F	mg/g $(L/g)^n$	3.63	9.62	1.41
	R^2	-	0.63	0.54	0.88

Langmuir and Freundlich isotherm parameters obtained from the nonlinear fitting for commercial activated carbon, spent coffee, and parchment.

5.3.6. Adsorption of main organic acids present in wet-coffee processing

Organic acids are constituents of importance in wet-coffee processing. During coffee processing, to guarantee the proper drying of green coffee, fermentation takes place so the mucilage layer can be degraded. In previous work, citric acid, malic acid, and lactic acid were found to be present in coffee beans and by-products while in coffee wastewater the main organic acids were lactic acid and propionic acid [266]. Adsorption efficiency of citric, malic, lactic, acetic, propionic, and butyric acid was evaluated by preparing a model fermentation broth mixture of 20 g/L containing the six organic acids. The results indicate that all six organic acids were adsorbed onto the three activated carbons. Among the activated carbon samples, parchment showed the highest adsorption removal efficiency for malic acid 73.83 ± 0.065%, lactic acid 52.80 ± 0.063%, acetic acid 85.01 ± 0.002%, propionic acid 96.86 ± 0.001%, and butyric acid 99.52 ± 0.000%, while commercial activated carbon exhibited the highest removal efficiency for citric acid 84.69 ± 0.006% (Figure 32). In complex systems, competition between compounds and activated carbon surface takes place, binding compounds with more

selectivity and affinity to the adsorbent. Silva et al. [37] reported that adsorption of organic acids onto activated carbon is related to the size of the fatty acid chain and hydrophobicity of the compounds.

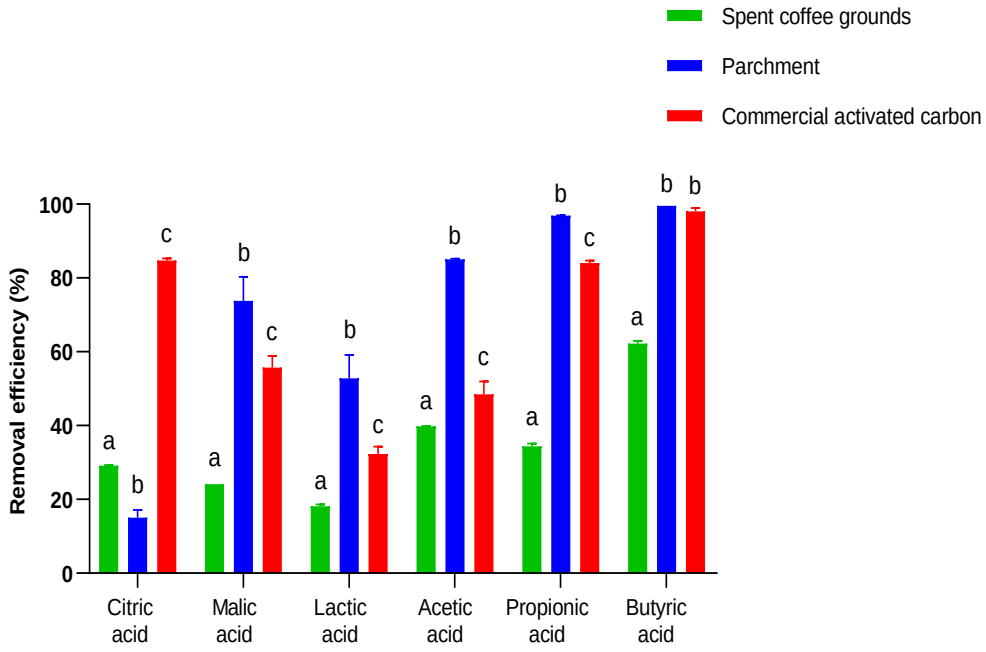


Figure 32 Effect of organic acids adsorption on activated carbon

Effect of citric, malic, lactic, acetic, propionic, and butyric acid adsorption on spent coffee, parchment, and commercial activated carbon. 200 g/L in the bulk solution, adsorbent dose of 10% (*w/v)* and 4 h contact time at room temperature.

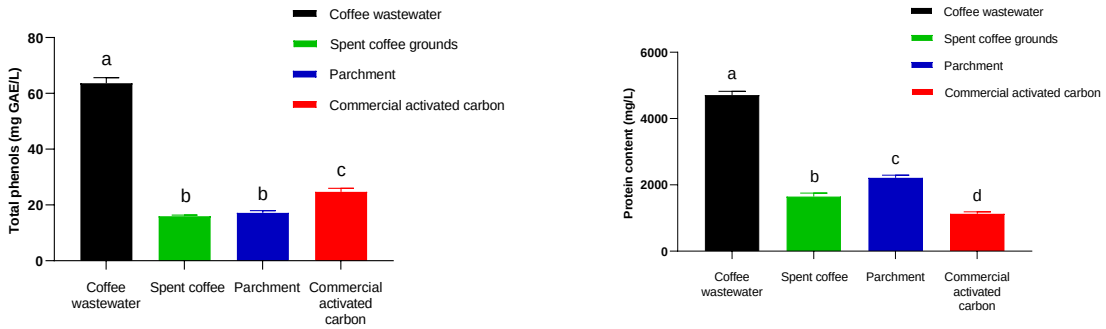
5.3.7. Adsorption of organic compounds from wet-coffee processing wastewater

Figure 33 presents the concentration of total phenols, and protein content of wastewater before and after treatment with parchment, spent coffee and granular activated carbon. A reduction from 100 to 30% and 100 to 20% of total phenols and protein content can be observed, respectively. Total phenol content in wastewater was 63.63 ± 2.01 mg GAE/L, and after treatment with parchment, spent coffee and granular activated carbon the values were 17.18 ± 0.81, 15.97 ± 0.42, and 24. 68 ± 1.34 mg GAE/L, respectively. These results indicate that up to 60% of total phenols are removed from wastewater after treatment with the three types of activated carbon. The efficiency of polyphenol adsorption onto activated carbon has been reported to be dependent to the type of carbon, carbon surface functionalities, pH of the bulk solution, and oxygen availability [332].

The results showed that after treatment, protein content decreased when compared with coffee wastewater. The values of 2,212.50 ± 80.57, 1,647.5 ± 104.04, and 1,127.5 ± 61.31 mg/L were obtained for parchment, spent coffee and granular activated carbon, respectively. It was reported that adsorption of proteins by the activated carbons was more efficient for lower molecular weight proteins (13-66 kDa) when the pH of the bulk solution was close to the protein

isoelectric point [333,334]. The overall protein charge is weakened and electrostatic repulsion forces between the adsorbent and protein take place [334]. Moreover, the hydrophobic nature of the activated carbons results in effective adsorption of hydrophobic segments of the protein [335].

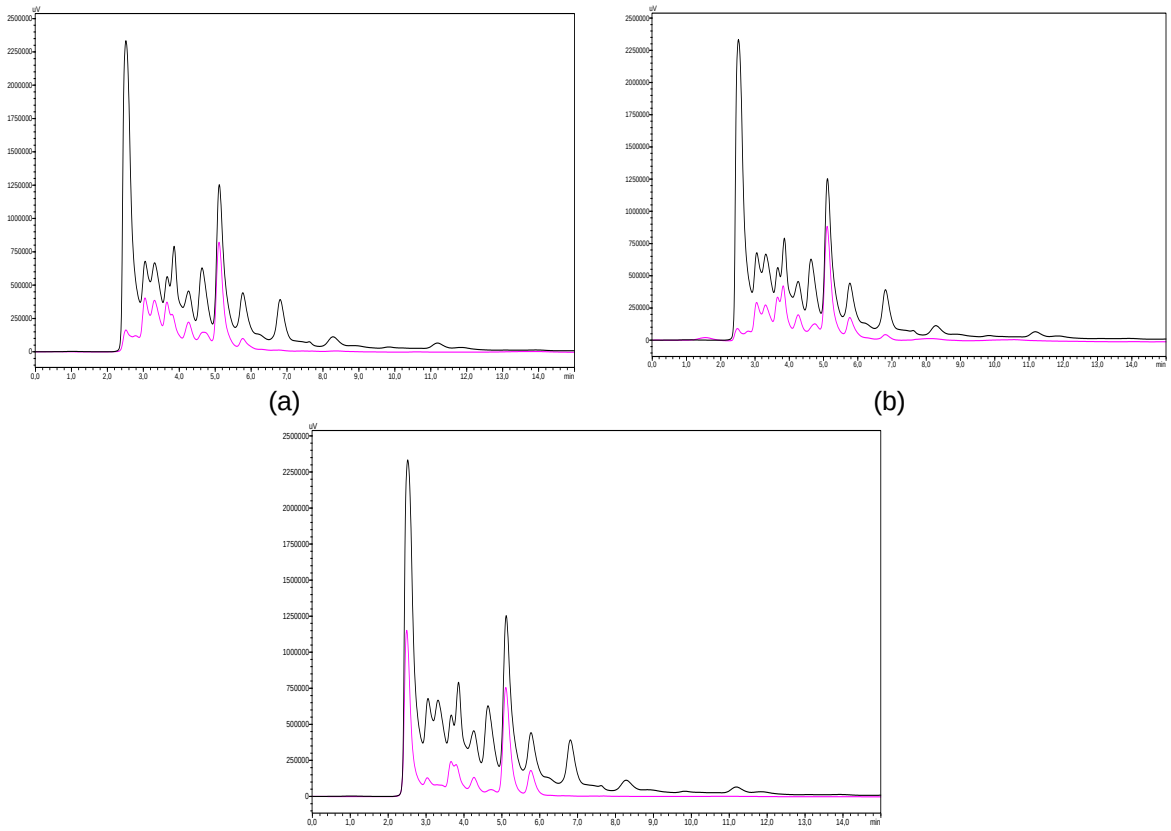
The organic acids in coffee wastewater were analyzed by using HPLC. Figure 34 shows the chromatograms comparing coffee wastewater before and after treatment with spent coffee (a), parchment (b), and commercial activated carbon (c). It appeared that the total area of coffee wastewater chromatogram decreased after treatment. Removal efficiencies of all adsorbents were up to 50%. Coffee wastewater was also investigated using HPLC-MS/MS. The total ion current spectrum in negative and positive mode from wastewater shows a reduction in compounds after batch experiments. In the ion spectrum from spent coffee and parchment (Figure 35 a and b) similar trends can be observed. Adsorption efficiency results indicate that up to 40% of hydrophobic compounds are removed by spent coffee and parchment whereas up to 80% is removed onto commercial granular activated carbon. This percentage decreases with the hydrophilic compounds to a value of 12.45 ± 3.32%, 14.35 ± 1.44% and 47.90 ± 1.19% for spent coffee, parchment and commercial activated carbon, respectively (Table 18). Activated carbon is mainly hydrophobic, giving more affinity for non-polar compounds such as hydrocarbons. Besides this activated carbon property, the presence of mesopores might facilitate diffusion process and resulting in higher adsorption rates [336]. It has been investigated that a mesoporous structure and narrow pore size distribution makes carbon better adsorbents in wastewater treatment than a heterogeneous pore structure [337]. The decrease in removal efficiency on activated carbon surface is associated to the competitive interaction between compounds on the pore structures.



(a) (b)

Figure 33 Adsorption of total phenols and protein content on activated carbon

Adsorption of (a) total phenols and (b) protein content from coffee wastewater on parchment, spent coffee and commercial activated carbon. Adsorbent dose of 10% (*w/v)* and 4 h contact time at room temperature.

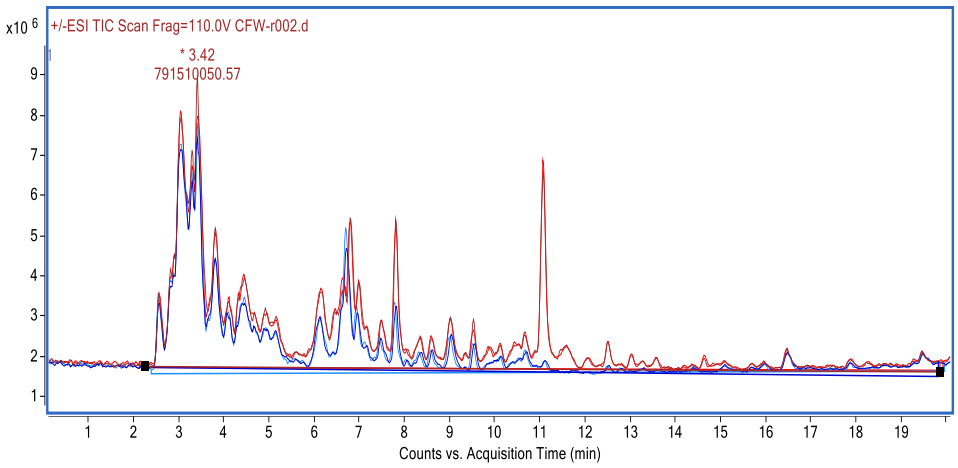


(a) (b)

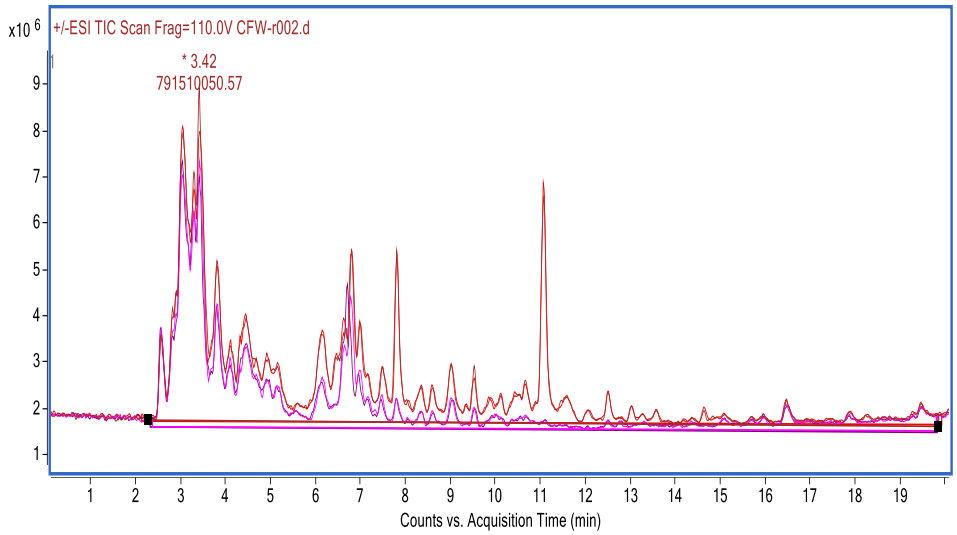
(c)

Figure 34 HPLC-MS chromatogram for organic acids of wastewater after adsorption onto activated carbon.

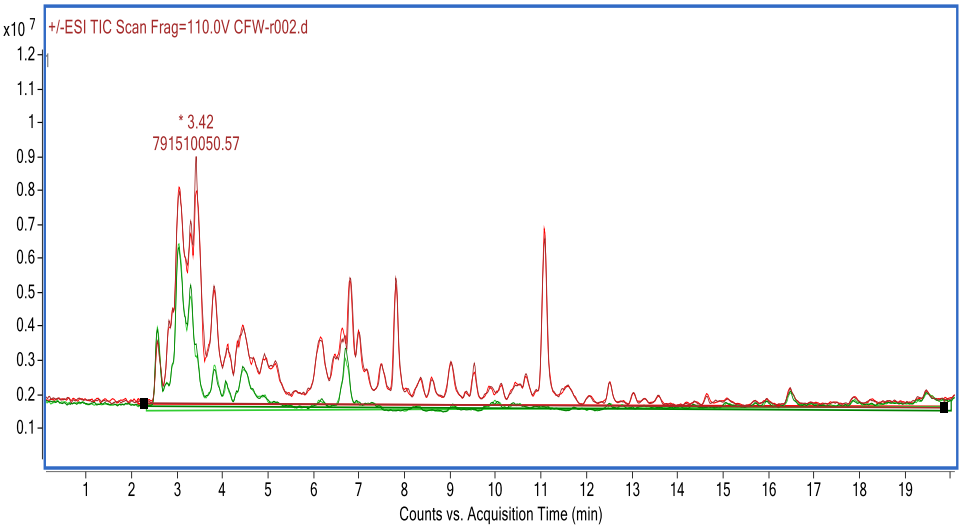
HPLC-MS chromatogram for organic acids comparing coffee wastewater after adsorption onto parchment (a), spent coffee grounds (b), and commercial activated carbon (c). Adsorbent dose of 10% (*w/v*) and 4 hours contact time at room temperature.



(a)



(b)



(c)

Figure 35 HPLC-MS/MS total ion current spectrum

HPLC-MS/MS total ion current spectrum comparing coffee wastewater after adsorption onto parchment (a), spent coffee grounds (b), and commercial activated carbon (c). Hydrophilic compounds from 2 – 6 min and hydrophobic compounds from 6 – 19 min. Adsorbent dose of 10% (*w/v*) and 4 hours contact time at room temperature

Table 18 Adsorption efficiency of hydrophilic and hydrophobic compounds from coffee wastewater on parchment, spent coffee, and commercial activated carbon.

Activated carbon	**Adsorption efficiency of hydrophilic components**	**Adsorption efficiency of hydrophobic components**
Parchment	12.45 ± 3.32%	44.43 ± 2.61%
Spent coffee	14.35 ± 1.44%	43.04 ± 1.99%
Commercial activated carbon	47.90 ± 1.19%	83.57 ± 1.96%

Hydrophilic compounds from 2 – 6 min and hydrophobic compounds from 6 – 19 min. Adsorbent dose of 10% (*w/v*) and 4 hours contact time at room temperature

Conclu sion of the study 3:

Activated carbons were prepared from spent coffee grounds and coffee parchment by co-calcination with calcium carbonate, and their efficiency in absorbing organic compounds was compared to one commercially available activated carbon. Both spent coffee grounds and parchment showed yields of 9.0% after calcination and washing treatments. SEM of the activated carbon materials confirmed that co-calcination with calcium carbonate improved pore structures compared to the raw materials. We found that characteristics of the prepared activated carbon presented similarities to the commercially available form. Further work will be directed towards the optimization of the activation methods to improve the quality of the materials produced. The adsorption of lactic acid occurred via a monolayer, with restricted interaction between adsorbate molecules. Parchment activated carbon showed greater adsorption efficiency for the main monocarboxylic acids present in wet-coffee processing when compared to those of spent coffee and commercial activated carbons. Spent coffee grounds and parchment proved to have similar adsorption efficiency to commercial activated carbon for the removal of total phenols and protein content from coffee wastewater. All adsorbents showed greater affinity to remove hydrophobic than hydrophilic compounds. This study showed that coffee parchment and spent coffee grounds can be valorized via co-calcination with calcium carbonate to produce activated carbons.

6. Summary and outlook – Schlussfolgerung und Ausblick

6.1. Summary and outlook

Countries processing raw coffee beans are burdened with low economical incomes to fight the serious environmental problems caused by the by-products and wastewater that is generated during the wet-coffee processing. The aim of this work was to develop alternative methods of improving the waste by-product quality and thus making the process economically more attractive with valorization options that can be brought to the coffee producers. In order to overcome the pollution potential of the process a clear understanding of its constitution is necessary to design a feasible treatment. The effects of wet-coffee processing methods on characteristics and quality of green coffee beans will also provide a broad range of improvement possibilities.

The type of processing influences not only the constitution of green coffee but also of by-products and wastewater. Therefore, coffee bean samples as well as by-products and wastewater collected at different production steps of two major processing options (batch and continuous wet processing) were analyzed in terms of their content in total phenols, antioxidant capacity, caffeine content, organic acids, reducing sugars, free amino groups, and protein content. Green coffee beans showed the highest concentration of organic acids and sucrose for both batch and continuous processing compared to the coffee beans along the processing steps. Batch green coffee beans contained higher amount of phenols than continuous green coffee beans. The results showed that 40% of caffeine was removed with pulp. Batch green coffee beans contained higher amounts of phenols, with 5-caffeoylquinic acid being the main constituent. Similar values of protein content were reported in the green coffee bean in batch and continuous processing. A significant decrease for free amino acid groups during processing was observed in processed beans. Coffee pulp, especially during batch processing, revealed high concentration of nutrients.

In the case of the wastewater samples, organic constitutes analyzed were more pronounced in batch processing samples compared to those of the continuous processing. The caffeine content was up to five times higher in the wastewater resulting from the fermentation step of the batch processing compared to the continuous processing. Free amino groups increased in wastewater with fermentation. Values were about 100 times higher in batch processing compared to the continuous processing, keeping in mind that recirculation also increases the value documented. These results clearly show that the composition of wastewater is dependent on how much and how often the wastewater is recycled in the processing, since those are the major differences between batch and continuous wet processing. Considering the coffee beans, results indicate that the proteins or their structures might be affected during both processing options and a positive effect of the fermentation on the solubility and

accessibility of proteins seems to be probable, while regarding the steps of de-pulping to initial fermentation, especially while considering batch process.

Oxidation reactions of present phenolic compounds induce the modification of certain amino acid side chains. These reactions have an influence on coffee quality and particularly on the coffee aroma. A protein extraction protocol using SDS, PVPP and ascorbic acid was first developed. The comparison of these extracts revealed possible differences in content of phenolic compounds and associated proteins. Furthermore, an MRM method for HPLC-MS/MS was developed. The method allows the identification of potential marker peptides as well as lysine-modified peptides of the main storage protein 11S. Results showed the influence of different processing methods on protein modifications related to chlorogenic acid when batch and continuous processing were compared. Samples of semi-wet, wet, and monsooned processing methods showed higher concentration of CQA and DiCQA lysine adducts compared with the dry method. The steps of coffee processing influence the different constituents of green coffee beans which, during roasting, give rise to aroma compounds and express the characteristics of roasted coffee beans. Thus, the process has an influence on coffee cup quality. These results give the opportunity in changing the content of free amino groups in final green coffee beans. Knowing that this group of compounds is involved in the Maillard reaction during roasting, this possibility could be utilized for the coffee producers to improve the quality of green coffee beans and finally the coffee cup quality.

The valorization of coffee wastes through modification to activated carbon has been considered as a low-cost option creating an adsorbent with prospective to compete with commercial carbons. An activation protocol via co-calcination using a mixture of spent coffee grounds or parchment with calcium carbonate at 850° C under an argon atmosphere was first developed. Activated carbons were prepared to assess their adsorption capacity for organic acids, phenolic compounds, and proteins. The materials were characterized using IR spectra, X-ray diffraction patterns, TGA, and SEM. The data confirmed that co-calcination with calcium carbonate improved pore structures compared to the raw materials. The characteristics of the prepared active carbon presented similarities to the ones commercially available. Adsorption of lactic acid occurred via monolayer, with restricted interaction between adsorbate molecules. The adsorption of lactic acid was affected by lactic acid initial concentration and pH. Activated carbon samples exhibited adsorption affinity below pK_a of lactic acid. Data also showed that lactic acid adsorption is higher for parchment and commercial activated carbon efficiencies, compared to that of the spent coffee grounds. In complex systems, competition between compounds and activated carbon surface was observed, binding compounds with more selectivity and affinity to the adsorbent. Parchment activated carbon showed greater adsorption efficiency for the main monocarboxylic acids present in wet-coffee processing when compared to those of spent coffee grounds and commercial activated carbons. Spent coffee

grounds and parchment proved to have similar adsorption efficiency to commercial activated carbon for the removal of total phenols and protein content from coffee wastewater. All adsorbents showed greater affinity to remove hydrophobic than hydrophilic compounds. Activated carbon is mainly hydrophobic, giving more affinity for non-polar compounds such as hydrocarbons. Besides, the presence of micropores facilitate the diffusion process and result in higher adsorption rates. The decrease in removal efficiency on activated carbon surface is associated to the competitive interaction between compounds on the pore structure. Further work needs to be directed to the optimization of the activation methods to improve the quality of the materials produced and the viability of applying such experiments in-situ to bring the coffee producer further valorization opportunities with environmental perspectives.

The results of this study document a significant information originating from the processing of the de-pulped to green coffee beans. Furthermore, it showed that coffee parchment and spent coffee grounds can be valorized as low-cost option to produce activated carbons. Coffee producers would profit in establishing appropriate simple technologies to improve green coffee quality, re-use coffee by-products, and wastewater valorization.

6.2. Schlussfolgerung und Ausblick

Die Länder, die Rohkaffee verarbeiten, haben nur ein geringes wirtschaftliches Einkommen, um die ernsten Umweltprobleme zu bekämpfen, die durch die bei der Nasskaffeeverarbeitung anfallenden Nebenprodukte und Abwässer verursacht werden. Ziel dieser Arbeit war es, alternative Methoden zu entwickeln, um die Qualität der Nebenprodukte zu verbessern und so den Prozess wirtschaftlich attraktiver zu machen, indem den Kaffeeproduzenten weitere Valorisierungsoptionen zur Verfügung gestellt werden. Um das Verschmutzungspotenzial des Prozesses zu überwinden und eine praktikable Behandlung zu entwickeln, ist ein klares Verständnis seiner Zusammensetzung erforderlich. Die Auswirkungen der Nasskaffee-Verarbeitungsmethoden auf die Eigenschaften und die Qualität der grünen Kaffeebohnen werden ebenfalls eine breite Palette von Verbesserungsmöglichkeiten bieten.

Die Art der Verarbeitung beeinflusst nicht nur die Zusammensetzung des Rohkaffees, sondern auch die der Nebenprodukte und des Abwassers. Daher wurden Proben von Kaffeebohnen sowie Nebenprodukte und Abwässer, die bei verschiedenen Produktionsschritten von zwei Hauptverarbeitungsoptionen (diskontinuierliche und kontinuierliche Nassaufbereitung) gesammelt wurden, auf ihren Gehalt an Gesamtphenolen, antioxidativer Kapazität, Koffein, organischen Säuren, reduzierenden Zuckern, freien Aminogruppen und Proteinen hin untersucht. Grüne Kaffeebohnen wiesen sowohl bei der diskontinuierlichen als auch bei der kontinuierlichen Verarbeitung die höchsten Konzentrationen an organischen Säuren und Saccharose auf, verglichen mit den Kaffeebohnen aus den einzelnen Verarbeitungsschritten. Rohkaffeebohnen, die im Batch-Verfahren verarbeitet wurden, enthielten eine höhere Menge an Phenolen als Rohkaffeebohnen, die kontinuierlich verarbeitet wurden. Die Ergebnisse zeigten, dass 40% des Koffeins mit dem Fruchtfleisch entfernt wurden. Rohkaffeebohnen aus dem Batch-Verfahren enthielten höhere Mengen an Phenolen, wobei 5-Coffeoylchinasäure der Hauptbestandteil war. Der Proteingehalt der grünen Kaffeebohnen war bei der kontinuierlichen und der diskontinuierlichen Verarbeitung ähnlich hoch. Bei den verarbeiteten Bohnen wurde ein signifikanter Rückgang der freien Aminosäuregruppen während der Verarbeitung festgestellt. Das Fruchtfleisch der Kaffeekirsche wies, insbesondere bei der diskontinuierlichen Verarbeitung, eine hohe Konzentration an Nährstoffen auf.

Bei den Abwasserproben waren die analysierten organischen Bestandteile in den Proben aus der Batch-Verarbeitung stärker ausgeprägt als in denen aus der kontinuierlichen Verarbeitung. Der Koffeingehalt war im Abwasser aus der Fermentationsstufe der Batch-Verarbeitung bis zu fünfmal höher als bei der kontinuierlichen Verarbeitung. Die freien Aminogruppen im Abwasser nahmen mit der Fermentation zu. Die Werte waren bei der Batch-Verarbeitung etwa 100-mal höher als bei der kontinuierlichen Verarbeitung, wobei zu berücksichtigen ist, dass die Rezirkulation den dokumentierten Wert ebenfalls erhöht. Diese Ergebnisse zeigen deutlich, dass die Zusammensetzung des Abwassers davon abhängt, wie viel und wie oft das Abwasser

bei der Verarbeitung recycelt wird, da dies die Hauptunterschiede zwischen der diskontinuierlichen und der kontinuierlichen Nassverarbeitung sind. In Bezug auf die Kaffeebohnen deuten die Ergebnisse darauf hin, dass die Proteine oder ihre Strukturen bei beiden Verarbeitungsoptionen beeinträchtigt werden könnten, und eine positive Auswirkung der Fermentierung auf die Löslichkeit und Zugänglichkeit der Proteine scheint wahrscheinlich zu sein, wobei die Schritte vom Entpulpen bis zur anfänglichen Fermentierung, insbesondere beim Batch-Verfahren, berücksichtigt werden.

Die Oxidationsreaktionen der vorhandenen phenolischen Verbindungen führen zur Veränderung bestimmter Aminosäureseitenketten. Diese Reaktionen haben einen Einfluss auf die Kaffeequalität und insbesondere auf das Kaffeearoma. Zunächst wurde ein Proteinextraktionsprotokoll unter Verwendung von SDS, PVPP und Ascorbinsäure entwickelt. Der Vergleich dieser Extrakte ergab mögliche Unterschiede im Gehalt an phenolischen Verbindungen und assoziierten Proteinen. Außerdem wurde eine MRM-Methode für HPLC-MS/MS entwickelt. Die Methode ermöglicht die Identifizierung potenzieller Markerpeptide sowie Lysin-modifizierter Peptide des Hauptspeicherproteins 11S. Die Ergebnisse zeigten den Einfluss verschiedener Verarbeitungsmethoden auf Proteinmodifikationen im Zusammenhang mit Chlorogensäure, wenn Batch- und kontinuierliche Verarbeitung verglichen wurden. Proben aus halbnassen, nassen und Monsun-Verarbeitungsmethoden wiesen im Vergleich zur trockenen Methode eine höhere Konzentration von CQA- und DiCQA-Lysinaddukten auf. Die einzelnen Schritte der Kaffeeverarbeitung beeinflussen die verschiedenen Bestandteile der grünen Kaffeebohnen, die beim Rösten zu Aromastoffen werden und die Eigenschaften der gerösteten Kaffeebohnen zum Ausdruck bringen. Somit hat das Verfahren einen Einfluss auf die Qualität des zubereiteten Kaffees. Diese Ergebnisse bieten die Möglichkeit, den Gehalt an freien Aminogruppen in den fertigen grünen Kaffeebohnen zu verändern. Da diese Gruppe von Verbindungen an der Maillard-Reaktion während des Röstens beteiligt ist, könnte diese Möglichkeit von den Kaffeeproduzenten genutzt werden, um die Qualität der grünen Kaffeebohnen und schließlich die Qualität des zubereiteten Kaffees zu verbessern.

Die Aufwertung von Kaffeeabfällen durch Modifizierung zu Aktivkohle wurde als kostengünstige Option zur Schaffung eines Adsorptionsmittels betrachtet, das mit kommerziellen Kohlen konkurrieren könnte. Zunächst wurde ein Aktivierungsprotokoll durch Co-Kalzinierung unter Verwendung einer Mischung aus verbrauchtem Kaffeesatz oder Pergament mit Kalziumcarbonat bei 850° C unter Argonatmosphäre entwickelt. Die Aktivkohlen wurden hergestellt, um ihre Adsorptionskapazität für organische Säuren, phenolische Verbindungen und Proteine zu bewerten. Die Materialien wurden anhand von IR-Spektren, Röntgenbeugungsmustern, TGA und SEM charakterisiert. Die Daten bestätigten, dass die Co-Kalzinierung mit Kalziumcarbonat die Porenstrukturen im Vergleich zu den Rohmaterialien verbesserte. Die Eigenschaften der hergestellten Aktivkohle wiesen Ähnlichkeiten mit den im

Handel erhältlichen auf. Die Adsorption von Milchsäure erfolgte über eine Monoschicht mit eingeschränkter Wechselwirkung zwischen den adsorbierten Molekülen. Die Adsorption von Milchsäure wurde durch die anfängliche Milchsäurekonzentration und den pH-Wert beeinflusst. Aktivkohleproben wiesen eine Adsorptionsaffinität unterhalb des pKa-Wertes der Milchsäure auf. Die Daten zeigten auch, dass die Adsorption von Milchsäure bei Pergament und handelsüblicher Aktivkohle höher ist als bei gebrauchtem Kaffeesatz. In komplexen Systemen wurde eine Konkurrenz zwischen den Verbindungen und der Aktivkohleoberfläche beobachtet, wobei die Verbindungen mit größerer Selektivität und Affinität an das Adsorptionsmittel gebunden wurden. Pergament-Aktivkohle zeigte eine höhere Adsorptionseffizienz für die wichtigsten Monocarbonsäuren, die bei der Verarbeitung von Nasskaffee vorkommen, im Vergleich zu gebrauchtem Kaffeesatz und handelsüblicher Aktivkohle. Bei der Entfernung von Gesamtphenolen und Proteingehalt aus Kaffeeabwasser erwiesen sich Kaffeesatz und Pergament als ähnlich effizient wie kommerzielle Aktivkohle. Alle Adsorptionsmittel zeigten eine größere Affinität zur Entfernung hydrophober als hydrophiler Verbindungen. Aktivkohle ist hauptsächlich hydrophob, was zu einer höheren Affinität für unpolare Verbindungen wie Kohlenwasserstoffe führt. Außerdem erleichtert das Vorhandensein von Mikroporen den Diffusionsprozess und führt zu höheren Adsorptionsraten. Die Abnahme der Abscheideleistung auf der Aktivkohleoberfläche ist auf die konkurrierende Wechselwirkung zwischen den Verbindungen auf der Porenstruktur zurückzuführen. Weitere Arbeiten müssen sich mit der Optimierung der Aktivierungsmethoden befassen, um die Qualität der hergestellten Materialien zu verbessern und die Durchführbarkeit solcher Experimente in-situ zu prüfen, um den Kaffeeproduzenten weitere Verwertungsmöglichkeiten mit ökologischen Perspektiven zu bieten.

Die Ergebnisse dieser Studie dokumentieren eine bedeutende Information, die aus der Verarbeitung der entpulpten zu grünen Kaffeebohnen stammt. Darüber hinaus wurde gezeigt, dass Kaffeepergament und verbrauchter Kaffeesatz als kostengünstige Option zur Herstellung von Aktivkohle verwertet werden können. Kaffeeproduzenten würden von der Einführung geeigneter einfacher Technologien zur Verbesserung der Rohkaffeequalität, der Wiederverwendung von Kaffeenebenprodukten und der Aufwertung von Abwässern profitieren.

7. References

1. Favaro, C.P.; Baraldi, I.J.; Casciatori, F.P.; Farinas, C.S. beta-Mannanase Production Using Coffee Industry Waste for Application in Soluble Coffee Processing. *Biomolecules* **2020**, *10*, doi:10.3390/biom10020227.
2. Rattan, S.; Parande, A.K.; Nagaraju, V.D.; Ghiwari, G.K. A comprehensive review on utilization of wastewater from coffee processing. *Environ Sci Pollut Res Int* **2015**, *22*, 6461-6472, doi:10.1007/s11356-015-4079-5.
3. Rubayiza, A.B.; Meurens, M. Chemical Discrimination of Arabica and Robusta Coffees by Fourier Transform Raman Spectroscopy. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 4654-4659.
4. Ramalakshmi, K.; Raghavan, B. Caffeine in coffee: its removal. Why and how? *Crit Rev Food Sci Nutr* **1999**, *39*, 441-456, doi:10.1080/10408699991279231.
5. Poltronieri, P.; Rossi, F. Challenges in Specialty Coffee Processing and Quality Assurance. *Challenges* **2016**, *7*, 19, doi:10.3390/challe7020019.
6. Montavon, P.; Mauron, A.F.; Duruz, E. Changes in green coffee protein profiles during roasting. *J Agric Food Chem* **2003**, *51*, 2335-2343, doi:10.1021/jf020832b.
7. de Merlo Pereira, G.V.; Thomaz Soccol, V.; Kaur Brar, S.; Neto, E.; Soccol, C.R. Microbial Ecology and Starter Culture Technology in Coffee Processing. *Critical Reviews in Food Science and Nutrition* **2015**, 10.1080/10408398.2015.1067759, doi:10.1080/10408398.2015.1067759.
8. Avallone, S.G., Bernard; Brillouet, Jean-Marc; Olguin, Eugenia; Guiraud, Joseph-Pierre. Microbiological and Biochemical Study of Coffee Fermentation. *Current Microbiology* **2001**, *42*, 252-256, doi:10.1007/s002840110213.
9. Payel, G.N., Venkatachalapathy. Processing and Drying of Coffee – A Review. *International Journal of Engineering Research & Technology (IJERT)* **2014**, *3*.
10. Seninde, D.R.; IV, E.C. Coffee Flavor: A Review. *Beverages* **2020**, *6*.
11. Dong, W.; Tan, L.; Zhao, J.; Hu, R.; Lu, M. Characterization of Fatty Acid, Amino Acid and Volatile Compound Compositions and Bioactive Components of Seven Coffee (Coffea robusta) Cultivars Grown in Hainan Province, China. *Molecules* **2015**, *20*, 16687-16708, doi:10.3390/molecules200916687.
12. Dong, W.; Cheng, K.; Hu, R.; Chu, Z.; Zhao, J.; Long, Y. Effect of Microwave Vacuum Drying on the Drying Characteristics, Color, Microstructure, and Antioxidant Activity of Green Coffee Beans. *Molecules* **2018**, *23*, doi:10.3390/molecules23051146.
13. Mullen, W.; Nemzer, B.; Stalmach, A.; Ali, S.; Combet, E. Polyphenolic and Hydroxycinnamate Contents of Whole Coffee Fruits from China, India, and Mexico. *Journal of Agricultural and Food Chemistry* **2013**, *61*, 5298-5309.
14. Napolitano, A.; Fogliano, V.; Tafuri, A.; Ritieni, A. Natural Occurrence of Ochratoxin A and Antioxidant Activities of Green and Roasted Coffees and Corresponding Byproducts. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 10499-10504.
15. Kleinwächter, M.; Bytof, G.; Selmar, D. Coffee Beans and Processing. **2015**, 10.1016/b978-0-12-409517-5.00009-7, 73-81, doi:10.1016/b978-0-12-409517-5.00009-7.
16. Galanakis, C.M. Handbook of Coffee Processing By-products Sustainable Applications *Elsevier* **2017**.
17. Chala, B.; Oechsner, H.; Müller, J. Introducing Temperature as Variable Parameter into Kinetic Models for Anaerobic Fermentation of Coffee Husk, Pulp and Mucilage. *Applied Sciences* **2019**, *9*, 412, doi:10.3390/app9030412.
18. von Enden, J.C.; Calvert, K.C. *Review of coffee waste water characteristics and aproaches to treatment.*; PPP Project „Improvement of Coffee Quality and Sustainability of Coffee Production in Vietnam". German Technical Cooperation Agency (GTZ) Khe Sanh, Huong Hoa, Quang Tri, SR Vietnam / CEO Renertech Consulting. 159 St. Andrew Street, Invercargill, 9501. New Zealand, 2002.
19. Iriondo-DeHond, A.; Iriondo-DeHond, M.; Castillo, M.D.d. Applications of Compounds from Coffee Processing By-Products. *Biomolecules* **2020**, *10*.

20. Obruca, S.; Benesova, P.; Petrik, S.; Oborna, J.; Prikryl, R.; Marova, I. Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds. *Process Biochemestry* **2014**, *49*, 1409-1414.
21. Goncalves, M.; Guerreiro, M.C.; Ramos, P.H.; Oliveira, L.C.A.d.; Sapag, K. Activated carbon prepared from coffee pulp: potential adsorbent of organic contaminants in aqueous solution *Water Science & Technology* **2013**, *68*, 1085-1090.
22. Ramón-Gonçalves, M.; Alcaraz, L.; León-González, S.P.-F.M.E.; Rosales-Conrado, N.; López, F.A. Extraction of polyphenols and synthesis of new activated carbon from spent coffee grounds. *Scientific Reports* **2019**, *9*.
23. Iakovleva, E.; Sillanpaa, M.; Maydannik, P.; Liu, J.T.; Allen, S.; Albadarin, A.B.; Mangwandi, C. Manufacturing of novel low-cost adsorbent: Co-granulation of limestone and coffee waste. *J Environ Manage* **2017**, 10.1016/j.jenvman.2017.05.039, 1-8, doi:10.1016/j.jenvman.2017.05.039.
24. Rosson, E.; Garbo, F.; Marangoni, G.; Bertani, R.; Lavagnolo, M.C.; Moretti, E.; Tolon, A.; Mozzon, M.; Sgarbossa, P. Activated Carbon from Spent Coffee Grounds: A Good Competitor of Commercial Carbons for Water Decontamination. *applied sciences* **2020**, *10*.
25. Ribeiro, H.; Marto, J.; Raposo, S.; Agapito, M.; Isaac, V.; Chiari, B.G. From coffee industry waste materials to skin-friendly products with improved skin fat levels. *European Journal of Lipid Science and Technology* **2013**, *115*, 330-336.
26. Rani, M.; Appaiah, A. Production of bacterial cellulose by Gluconacetobacter hansenii UAC09 using coffee cherry husk. *Journal of Food Science and Technology* **2013**, *50*, 755-762.
27. Chavan, A.A.; Pinto, J.; Liakos, I.; Bayer, I.S.; Lauciello, S.; Athanassiou, A.; Fragouli, D. Spent Coffee Bioelastomeric Composite Foams for the Removal of Pb2+and Hg2+from Water. *ACS Sustainable Chemistry & Engineering* **2016**, *4*, 5495-5502, doi:10.1021/acssuschemeng.6b01098.
28. Chwastowski, J.; Bradło, D.; Zukowski, W. Adsorption of Cadmium, Manganese and Lead Ions from Aqueous Solutions Using Spent Coffee Grounds and Biochar Produced by Its Pyrolysis in the Fluidized Bed Reactor. *Materials* **2020**, *13*.
29. Azouaou, N.; Sadaoui, Z.; Djaafri, A.; Mokaddem, H. Adsorption of cadmium from aqueous solution onto untreated coffee grounds: Equilibrium, kinetics and thermodynamics. *J. Haz. Mater* **2010**, *184*, 129-134.
30. Oliveira, L.S.; Franca, A.S.; Alves, T.M.; Rocha, S.D.F. Evaluation of untreated coffee husks as potential biosorbents for treatment of dye contaminated waters. *J. Haz. Mater* **2008**, *155*, 507-512.
31. Plaza, M.G.; Gonzáles, A.S.; Pevida, C.; Pis, J.J.; Rubiera, F. Valorisation of spent coffee grounds as CO2 adsorbents for postcombustion capture applications. *Applied Energy* **2012**, *99*, 272-279.
32. Kante, K.; Nieto-Delgado, C.; Rangel-Mendez, J.R.; Bandosz, T.J. Spent coffee-based activated carbon: specific surface features and their importance for H2S separation process. *J. Hazard. Mater.* **2012**, *201-202*, 141-147.
33. Kemp, K.C.; Baek, S.B.; Lee, W.-G.; Meyyappan, M.; Kim, K.S. Activated carbon derived from waste coffee grounds for stable methane storage. *Nanotechnology* **2015**, *26*.
34. Farah, A.; Santos, T.F.d. Chapter 1 - The Coffee Plant and Beans: An Introduction. *Coffee in Health and Diseases Prevention* **2015**, 5-10.
35. Janissen, B.; Huynh, T. Chemical composition and value-adding applications of coffee industry by-products: A review. *Resources, Conservation and Recycling* **2018**, *128*, 110-117.
36. DaMatta, F.M.; Ronchi, C.P.; Maestri, M.; Barros, R.S. Ecophysiology of coffee growth and production. *Brazilian Plant Physiol.* **2007**, *19*.
37. Miljkovic, D.; Duell, B.; Miljkovic, V. Methods for coffee cherry products. *US-Pat.* **2010**, *7*, 254-263.

38. Murthy, P.S.; Madhava Naidu, M. Sustainable management of coffee industry by-products and value addition—A review. *Resources, Conservation and Recycling* **2012**, *66*, 45-58, doi:10.1016/j.resconrec.2012.06.005.
39. Miedaner, T. Kaffee – der Genuss Arabiens. In *Genusspflanzen*, Springer, 2018; pp. 155-177.
40. Farah, A. Coffee: Emerging Health effects and Disease Prevention. In *Coffee: Emerging Health Effects and Disease Prevention*, Chu, Y.-F., Ed. Willey-Blackwell: 2012; pp. 22-58.
41. Clifford, M.N. Chlorogenic acids and other cinnamates – nature, occurrence and dietary burden†. *Journal of the Science of Food and Agriculture* **1999**, *79*, 362-372.
42. Mason, H.S.; Peterson, E. The reaction of quinones with protamine and nucleoprotamine: N-terminal proline. *Journal of Biological Chemistry* **1955**, *212.1*, 485-493.
43. Belitz, H.-D.; Grosch, W. Food Chemistry. *Springer* **1999**, *Second Edition*
44. Esquivel, P.; Jiménez, V.M. Functional properties of coffee and coffee by-products. *Food Research International* **2012**, *46*, 488-495, doi:10.1016/j.foodres.2011.05.028.
45. Favaro, C.P.; Baraldi, I.J.; Casciatori, F.P.; Farinas, C.S. β-Mannanase Production Using Coffee Industry Waste for Application in Soluble Coffee Processing. *biomolecules* **2020**, *10*.
46. Murthy, P.S.; Naidu, M.M. Sustainable management of coffee industry by-products and value addition-A review. *Resour Conserv Recy* **2012**, *66*, 45-58, doi:10.1016/j.resconrec.2012.06.005.
47. Ferrão, J.d.M. *O café: a bebida negra dos sonhos claros*; Chaves Ferreira: 2009.
48. Ramalakshmi, K.; Rao, L.J.M.; Takano-Ishikawa, Y.; Goto, M. Bioactivities of low-grade green coffee and spent coffee in different in vitro model systems. *Food Chem* **2009**, *115*, 79-85, doi:10.1016/j.foodchem.2008.11.063.
49. Pavlović, M.D.; Buntić, A.V.; Šiler-Marinković, S.S.; Antonović, D.G.; Milutinović, M.D.; Radovanović, N.R.; Dimitrijević-Branković, S.I. Spent Coffee Grounds as Adsorbents for Pesticide Paraquat Removal from its Aqueous Solutions. *Internation Conference on Civil, Biological and Environmental Engineering (CBEE)* **2014**, 60-65.
50. Variyar, P.S.; Ahmad, R.; Bhat, R.; Niyas, Z.; Sharma, A. Flavoring components of raw monsooned arabica coffee and their changes during radiation processing. *J Agric Food Chem* **2003**, *51*, 7945 - 7950.
51. Murthy, P.S.; Manonmani, H.K. Physico-chemical, antioxidant and antimicrobial properties of Indian monsooned coffee. *European Food Research and Technology* **2009**, *229*, 645-650.
52. Rawel, H.M.; S, R.; Kroll, J. Characterisation of 11S Protein Fractions and Phenolic Compounds from Green Coffee Beans under Special Consideration of their Interactions–A Review. *Deutsche Lebensmittel-Rundschau* **2005**, 148-160.
53. Ali, M.; T, H.; J, K.; M, K.; R, P.; Kruse, H.P.; Rawel, H. Characterization and Modeling of the Interactions between Coffee Storage Proteins and Phenolic Compounds. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 11601-11608.
54. Macrae, R. coffee. In *Nitrogenous Components*, R., v.C.R.J.a.M., Ed. Springer: 1985; pp. 115-152.
55. Clifford, M.N. Chemical and Physical Aspects of Green Coffee and Coffee Products. In *Coffee. Botany, Biochemistry and Production of Beans and Beverage*, Springer: von Clifford M N and Willson K C., 1985; pp. 305-374.
56. Montavon, P.; Duruz, E.; Rumo, G.; Pratz, G. Evolution of Green Coffee Protein Profiles with Maturation and Relationship to Coffee Cup Quality. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 2328-2334.
57. Acuña, R.; Bassüner, R.; Beilinson, V.; Cortina, H.; Cadena-Gómez, G.; Montes, V.; Nielsen, N.C. Coffee seeds contain 11S storage proteins. *Physiologia Plantarum* **1999**, *105*, 122-131.
58. Montavon, P.; Mauron, A.-F.; Duruz, E. Changes in Green Coffee Proteinn Profiles during Roasting *J. Agr. Food Chem.* **2003**, *51*, 2335-2343.

59. Whitfield, F.B. Volatiles from interactions of Maillard reactions and lipids. *Crit Rev Food Sci Nutr.* **1992**, *31*, 1-58.
60. Rogers, W.J.; Bézard, G.; Deshayes, A.; Meyer, I.; Pétiard, V.; Marraccini, P. Biochemical and molecular characterization and expression of the 11S-type storage protein from Coffea arabica endosperm. *Plant Physiology and Biochemistry* **1999**, *37*.
61. Acuña, R.; assuener, R.; Beilinson, V.; Cotina, H.; Cadena-Gómez, G.; Montes, V.; Nielsen, N.C. Coffee seeds contain 11S storage proteins *Physiologia Plantarum* **1999**, *105*, 122-131.
62. Thaler, H.; Arneth, W. Untersuchungen an Kaffee und Kaffee-Ersatz. *XII. Mitteilung Polysaccharide des gerösteten Arabica-Kaffees* **1968**, *138*, 137-145.
63. UniProt. P93079-Sequenz des 11S-Speicherproteins. Availabe online: https : / / www.uniprot.org/uniprot/P93079. (accessed on 22.01.2021).
64. Schwenke, K.D. Reflections about the functional potential of legume proteins A Review. *Food / Nahrung* **2001**, *45*, 377-381, doi:doi.org/10.1002/1521-3803(20011001)45:6<377::AID-FOOD377>3.0.CO;2-G.
65. Fukushima, D. Structures of plant storage proteins and their functions. *Food Reviews International* **2009**, *7*, 353-381.
66. Baú, S.M.T.; Mazzafera, P.; Santoro, L.G. Seed storage proteins in coffee. *Revista Brasileira de Fisiologia Vegetal* **2001**, *13*.
67. Shutov, A.D.; Vaintraub, I.A. Degradation of storage proteins in germinating seeds. *Phytochemistry* **1987**, *26*, 1557-1566.
68. Bandil, G.B.; Etto, R.M.; Galvão, C.W.; Ramos, H.J.O.; Souza, E.M.; Pedrosa, F.O.; Chaves, D.F.S.; Huergo, L.F.; Ayub, R.A. Comparative proteomic analysis between early developmental stages of the Coffea arabica fruits. *Genet Mol Res.* **2013**, *12*, 5102-5110.
69. Arnold, U.; Ludwig, E.; Kühn, R.; Möschwitzer, U. Analysis of free amino acids in green coffee beans *Lebensmittle-Untersuchung und -Froschung* **1994**, *199*, 22-25.
70. Rawel, H.M.; Meidtner, K.; Kroll, J. Binding of Selected Phenolic Compounds to Proteins. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 4228-4235.
71. Li, A.-N.; Li, S.; Zhang, Y.-J.; Xu, X.-R.; Chen, Y.-M.; Li, H.-B. Resources and biological activities of natural polyphenols. *Nutrients* **2014**, *6*, 6020-6047.
72. Tsao, R. Chemistry and biochemistry of dietary polyphenols. *Nutrients* **2010**, *12*, 1231-1246.
73. Sęczyk, Ł.; Świeca, M.; Kapusta, I.; Gawlik-Dziki, U. Protein–Phenolic Interactions as a Factor Affecting the Physicochemical Properties of White Bean Proteins. *Molecules* **2019**, *24*.
74. Gonthier, M.-P.; Verny, M.-A.; Besson, C.; Rémésy, C.; Scalbert, A. Chlorogenic Acid Bioavailability Largely Depends on Its Metabolism by the Gut Microflora in Rats. *The Journal of Nutrition* **2003**, *133*, 1853-1859.
75. Rawel, H.M.; Kulling, S.E. Nutritional contribution of coffee, cacao and tea phenolics to human health. *Journal für Verbraucherschutz und Lebensmittelsicherheit* **2007**, *2*, 399-406.
76. Clifford, M.N. Diet-Derived Phenols in Plasma and Tissues and their Implications for Health. *Planta Medica* **2004**, *70*.
77. Li, A.N.; Li, S.; Zhang, Y.J.; Xu, X.R.; Chen, Y.M.; Li, H.B. Resources and biological activities of natural polyphenols. *Nutrients* **2014**, *6*, 6020-6047, doi:10.3390/nu6126020.
78. Scalbert, A.; Manach, C.; Morand, C.; Rémésy, C.; Jiménez, L. Dietary polyphenols and the prevention of diseases. *Critical reviews in food science and nutrition* **2005**, *45*, 287-306.
79. D'Archivio, M.; Filesi, C.; Benedetto, R.D.; Gargiulo, R.; Giovannini, C.; Masella, R. Polyphenols, dietary sources and bioavailability. *Annali dell'Istituto Superiore di Sanità* **2007**, *43*, 348-361.
80. Rawel, H.M.; Rohn, S. Nature of hydroxycinnamate-protein interactions. *Phytochemistry Reviews* **2009**, *9*, 93-109.

81. Czubinski, J.; Dwiecki, K.; Siger, A.; Neunert, G.; Lampart-Szczapa, E. Characterisation of different digestion susceptibility of lupin seed globulins. *Food Chemistry* **2014**, *15*, 418-426.
82. Suryaprakash, P.; R P Kumar, V.P. Thermodynamics of interaction of caffeic acid and quinic acid with multisubunit proteins. *International Journal of Biological Macromolecules* **2000**, *27*, 219-228.
83. Prigent, S.V.E.; Gruppen, H.; Visser, A.J.W.G.; Koningsveld, G.A.V.; Jong, G.A.H.D.; Voragen, A.G.J. Effects of non-covalent interactions with 5-O-caffeoylquinic acid (chlorogenic acid) on the heat denaturation and solubility of globular proteins. *Journal of Agricultural and Food Chemistry* **2003**, *51*, 5088-5095.
84. Cilliers, J.J.L.; Singleton, V.L. Characterization of the products of nonenzymic autoxidative phenolic reactions in a caffeic acid model system. *Journal of Agricultural and Food Chemistry* **1991**, *39*, 1298-1303.
85. Nicolas, J.; Richard-Forget, F.; Goupy, P.; Amiot, M.J.; Aubert, S. Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition* **1994**, ^*34*, 109-1057.
86. Guo, A.; Jiang, J.; True, A.D.; Xiong, Y.L. Myofibrillar Protein Cross-Linking and Gelling Behavior Modified by Structurally Relevant Phenolic Compounds. *Agricultural and Food Chemistry* **2021**, *69*, 1308-1317.
87. Kroll, J.; Rawel, H.M.; Rohn, S. Reactions of Plant Phenolics with Food Proteins and Enzymes under Special Consideration of Covalent Bonds. *Food Science and Technology* **2003**, *9*, 205-218.
88. Jakobek, L. Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry* **2015**, *175*, 556-567.
89. Rawel, H.M.; Rohn, S.; Kruse, H.-P.; JürgenKroll. Structural changes induced in bovine serum albumin by covalent attachment of chlorogenic acid. *Food Chemistry* **2002**, *78*, 443-455.
90. Rawel, H.M.; Rohn, S.; Kroll, J. Influence of a sugar moiety (rhamnosylglucoside) at 3-O position on the reactivity of quercetin with whey proteins. *International Journal of Biological Macromolecules* **2003**, *32*, 109-120.
91. A.Gerrard, J. Protein–protein crosslinking in food: methods, consequences, applications. *Trends in Food Science & Technology* **2002**, *13*, 391-399.
92. McKerchar, H.J.; Clerens, S.; Dobson, R.C.J.; Dyer, J.M.; Maes, E.; Gerrard, J.A. Protein-protein crosslinking in food: Proteomic characterisation methods, consequences and applications. *Trends in Food Science & Technology* **2019**, *86*, 217-229.
93. Hanft, F.; Koehler, P. Studies on the effect of glucose oxidase in bread making. *Journal of the Science of Food and Agriculture* **2006**, *86*, 1699-1704.
94. Friedman, M. Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *Journal of Agricultural and Food Chemistry* **1999**, *47*, 1295-1319.
95. Lund, M.N.; Ray, C.A. Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. *Journal of Agricultural and Food Chemistry* **2017**, *65*, 4537-4552.
96. Singh, H. Modification of food proteins by covalent crosslinking. *Trends in Food Science & Technology* **1991**, *2*, 196-200.
97. Rombouts, I.; Lagrain, B.; Brunnbauer, M.; Koehler, P.; Brijs, K.; Delcour, J.A. Identification of isopeptide bonds in heat-treated wheat gluten peptides. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 1236-1243.
98. Rohn, S. Possibilities and limitations in the analysis of covalent interactions between phenolic compounds and proteins. *Food Research International* **2014**, *65*, 13-19.
99. Rawel, H.M.; Kroll, J.; Hohl, U.C. Model studies on reactions of plant phenols with whey proteins. *Food/Nahrung* **2001**, *45*, 72-81.
100. Kroll, J.; Rawel, H.M.; Seidelmann, N. Physicochemical Properties and Susceptibility to Proteolytic Digestion of Myoglobin−Phenol Derivatives. *Journal of Agricultural and Food Chemistry* **2000**, *48*, 1580-1587.

101. Rawel, H.M.; Czajka, D.; Rohn, S.; Kroll, J. Interactions of different phenolic acids and flavonoids with soy proteins. *International Journal of Biological Macromolecules* **2002**, *30*, 137-150.
102. J.EO'Connell; P.FFox. Proposed mechanism for the effect of polyphenols on the heat stability of milk. *International Dairy Journal* **1999**, *9*, 523-536.
103. Petzke, K.J.; Rohn, S.S.S.; Rawel, H.M.; Kroll, J. Chlorogenic Acid Moderately Decreases the Quality of Whey Proteins in Rats. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 3714-3720.
104. Boekel, M.A.J.S.v. Flavor Compounds Formed during the Maillard Reaction. *Biotechnology Advances* **2006**, *24*, 230-233.
105. Wei Lee, L.W.C., Mun; Curran, Philip; Yu, Bin; Quan Liu, Shao. Coffee fermentation and flavor - An intricate and delicate relationship. *Food Chemistry* **2015**, *185*, 182-191, doi:10.1016/j.foodchem.2015.03.124.
106. Liu, E.-H.; Qi, L.-W.; Li, P. Structural relationship and binding mechanisms of five flavonoids with bovine serum albumin. *Molecules* **2010**, *15*, 9092-9103.
107. Kluger, R.; Alagic, A. Chemical cross-linking and protein-protein interactions-a review with illustrative protocols. *Bioorganic Chemistry* **2004**, *32*, 451-472.
108. Liu, F.; Ma, C.; McClements, D.J.; Gao, Y. A comparative study of covalent and non-covalent interactions between zein and polyphenols in ethanol-water solution. *Food Hydrocolloids* **2017**, *63*, 625-634.
109. Gallo, M.; Vinci, G.; Graziani, G.; Ferranti, P.; Simone, C.D. The interaction of cocoa polyphenols with milk proteins studied by proteomic techniques. *Food Research International* **2013**, *54*, 406-415.
110. Poltronieri, P.; Rossi, F. Challenges in Specialty Coffee Processing and Quality Assurance. *challenges* **2016**, *7*.
111. Coffee Taster´s Flavor Wheel. Available online: https://sca.coffee/research/coffee-tasters-flavor-wheel. Availabe online: (accessed on 28 July).
112. Shemekite, F.; Gomez-Brandon, M.; Franke-Whittle, I.H.; Praehauser, B.; Insam, H.; Assefa, F. Coffee husk composting: An investigation of the process using molecular and non-molecular tools. *Waste Manage* **2014**, *34*, 642-652, doi:10.1016/j.wasman.2013.11.010.
113. Velmourougane, K.; Bhat, R.; Gopinandhan, T.N. Composting coffee wastes, a potential source of ochratoxigenic fungi and ochratoxin A contamination. *World Mycotoxin J* **2012**, *5*, 373-376, doi:10.3920/Wmj2012.1386.
114. Salakinkop, S.R.; Shivaprasad, P.; Raghuramulu, Y. Manurial value and economics of composting of coffee (Coffea canephora) wastes. *Indian J Agr Sci* **2005**, *75*, 814-816.
115. Raphael, K.; Velmourougane, K. Chemical and microbiological changes during vermicomposting of coffee pulp using exotic (Eudrilus eugeniae) and native earthworm (Perionyx ceylanesis) species. *Biodegradation* **2011**, *22*, 497-507, doi:10.1007/s10532-010-9422-4.
116. Nogueira, W.A.; Nogueira, F.N.; Devens, D.C. Temperature and pH control in composting of coffee and agricultural wastes. *Water Sci Technol* **1999**, *40*, 113-119, doi:Doi 10.1016/S0273-1223(99)00371-6.
117. Nascimento, A.F.; Pires, F.R.; Czepak, M.P.; Fernandes, A.A.; Rodrigues, J.D. Characterization of Vermicompost Produced with Straw Coffee and Cattle Manure. *Rev Caatinga* **2015**, *28*, 1-9.
118. Fujii, K.; Takeshi, K. Penicillium strains as dominant degraders in soil for coffee residue, a biological waste unsuitable for fertilization. *J Appl Microbiol* **2007**, *103*, 2713-2720, doi:10.1111/j.1365-2672.2007.03529.x.
119. Dzung, N.A.; Dzung, T.T.; Khanh, V.T.P. Evaluation of Coffee Husk Compost for Improving Soil Fertility and Sustainable Coffee Production in Rural Central Highland of Vietnam. *Resources and Environment* **2013**, *3*, 77-82, doi:doi: 10.5923/j.re.20130304.03.

.

120. Zibetti, V.K.; Nachtigal, G.D.; de Lima, D.L.; Schiedeck, G. Growth and Reproduction of Earthworm in Organic Waste Mixtures and Effects on Chemical and Microbial Properties of Vermicompost. *Interciencia* **2015**, *40*, 57-62.
121. Prakongkep, N.; Gilkes, R.J.; Wiriyakitnateekul, W. Forms and solubility of plant nutrient elements in tropical plant waste biochars. *J Plant Nutr Soil Sc* **2015**, *178*, 732-740, doi:10.1002/jpln.201500001.
122. Orozco, F.H.; Cegarra, J.; Trujillo, L.M.; Roig, A. Vermicomposting of coffee pulp using the earthworm Eisenia fetida: Effects on C and N contents and the availability of nutrients. *Biol Fert Soils* **1996**, *22*, 162-166.
123. Morikawa, C.K.; Saigusa, M. Recycling coffee grounds and tea leaf wastes to improve the yield and mineral content of grains of paddy rice. *J Sci Food Agr* **2011**, *91*, 2108-2111, doi:10.1002/jsfa.4444.
124. Morikawa, C.K.; Saigusa, M. Recycling coffee and tea wastes to increase plant available Fe in alkaline soils. *Plant Soil* **2008**, *304*, 249-255, doi:10.1007/s11104-008-9544-1.
125. Escudero, C.; Gabaldon, C.; Marzal, P.; Villaescusa, I. Effect of EDTA on divalent metal adsorption onto grape stalk and exhausted coffee wastes. *J Hazard Mater* **2008**, *152*, 476-485, doi:10.1016/j.jhazmat.2007.07.013.
126. Ciesielczuk, T.; Rosik-Dulewska, C.; Wisniewska, E. Possibilities of Coffee Spent Ground Use as a Slow Action Organo-mineral Fertilizer. *Rocz Ochr Sr* **2015**, *17*, 422-437.
127. Valero, D.; Montes, J.A.; Rico, J.L.; Rico, C. Influence of headspace pressure on methane production in Biochemical Methane Potential (BMP) tests. *Waste Manage* **2016**, *48*, 193-198, doi:10.1016/j.wasman.2015.11.012.
128. Neves, L.; Oliveira, R.; Alves, M.M. Anaerobic co-digestion of coffee waste and sewage sludge. *Waste Manage* **2006**, *26*, 176-181, doi:10.1016/j.wasman.2004.12.022.
129. Malave', A.C.L.; Bernardi, M.; Fino, D.; Ruggeri, B. Multistep anaerobic digestion (MAD) as a tool to increase energy production via H-2 + CH4. *Int J Hydrogen Energ* **2015**, *40*, 5050-5061, doi:10.1016/j.ijhydene.2015.02.068.
130. Kostenberg, D.; Marchaim, U. Anaerobic-Digestion and Horticultural Value of Solid-Waste from Manufacture of Instant Coffee. *Environ Technol* **1993**, *14*, 973-980.
131. Kostenberg, D.; Marchaim, U. Solid-Waste from the Instant Coffee Industry as a Substrate for Anaerobic Thermophilic Digestion. *Water Sci Technol* **1993**, *27*, 97-107.
132. Hughes, S.R.; Lopez-Nunez, J.C.; Jones, M.A.; Moser, B.R.; Cox, E.J.; Lindquist, M.; Galindo-Leva, L.A.; Riano-Herrera, N.M.; Rodriguez-Valencia, N.; Gast, F., et al. Sustainable conversion of coffee and other crop wastes to biofuels and bioproducts using coupled biochemical and thermochemical processes in a multi-stage biorefinery concept. *Appl Microbiol Biot* **2014**, *98*, 8413-8431, doi:10.1007/s00253-014-5991-1.
133. Houbron, E.; Larrinaga, A.; Rustrian, E. Liquefaction and methanization of solid and liquid coffee wastes by two phase anaerobic digestion process. *Water Sci Technol* **2003**, *48*, 255-262.
134. Houbron, E.; Gonzalez-Lopez, G.I.; Cano-Lozano, V.; Rustrian, E. Hydraulic retention time impact of treated recirculated leachate on the hydrolytic kinetic rate of coffee pulp in an acidogenic reactor. *Water Sci Technol* **2008**, *58*, 1415-1421, doi:10.2166/wst.2008.492.
135. Hernandez, M.A.; Susa, M.R.; Andres, Y. Use of coffee mucilage as a new substrate for hydrogen production in anaerobic co-digestion with swine manure. *Bioresource Technol* **2014**, *168*, 112-118, doi:10.1016/j.biortech.2014.02.101.
136. de Oliveira, J.L.; da Silva, J.N.; Pereira, E.G.; Oliveira, D.; Carvalho, D.R. Characterization and mapping of waste from coffee and eucalyptus production in Brazil for thermochemical conversion of energy via gasification. *Renew Sust Energ Rev* **2013**, *21*, 52-58, doi:10.1016/j.rser.2012.12.025.
137. Corro, G.; Paniagua, L.; Pal, U.; Banuelos, F.; Rosas, M. Generation of biogas from coffee-pulp and cow-dung co-digestion: Infrared studies of postcombustion emissions. *Energ Convers Manage* **2013**, *74*, 471-481, doi:10.1016/j.enconman.2013.07.017.

138. Chaiklangmuang, S.; Kurosawa, K.; Li, L.Y.; Morishita, K.; Takarada, T. Thermal degradation behavior of coffee residue in comparison with biomasses and its product yields from gasification. *J Energy Inst* **2015**, *88*, 323-331, doi:10.1016/j.joei.2014.08.001.
139. Abouelenien, F.; Namba, Y.; Kosseva, M.R.; Nishio, N.; Nakashimada, Y. Enhancement of methane production from co-digestion of chicken manure with agricultural wastes. *Bioresource Technol* **2014**, *159*, 80-87, doi:10.1016/j.biortech.2014.02.050.
140. Mussatto, S.I.; Machado, E.M.S.; Carneiro, L.M.; Teixeira, J.A. Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. *Appl Energ* **2012**, *92*, 763-768, doi:10.1016/j.apenergy.2011.08.020.
141. Menezes, E.G.T.; do Carmo, J.R.; Alves, J.G.L.F.; Menezes, A.G.T.; Guimaraes, I.C.; Queiroz, F.; Pimenta, C.J. Optimization of alkaline pretreatment of coffee pulp for production of bioethanol. *Biotechnol Progr* **2014**, *30*, 451-462, doi:10.1002/btpr.1856.
142. Gurram, R.; Al-Shannag, M.; Knapp, S.; Das, T.; Singsaas, E.; Alkasrawi, M. Technical possibilities of bioethanol production from coffee pulp: a renewable feedstock. *Clean Technol Envir* **2016**, *18*, 269-278, doi:10.1007/s10098-015-1015-9.
143. Kondamudi, N.; Mohapatra, S.K.; Misra, M. Spent coffee grounds as a versatile source of green energy. *J Agric Food Chem* **2008**, *56*, 11757-11760, doi:10.1021/jf802487s.
144. Zanella, E.; Della Zassa, M.; Navarini, L.; Canu, P. Low-Temperature Co-pyrolysis of Polypropylene and Coffee Wastes to Fuels. *Energ Fuel* **2013**, *27*, 1357-1364, doi:10.1021/ef301305x.
145. Tsai, W.T.; Liu, S.C. Effect of temperature on thermochemical property and true density of torrefied coffee residue. *J Anal Appl Pyrol* **2013**, *102*, 47-52, doi:10.1016/j.jaap.2013.04.003.
146. Soares, L.D.; Moris, V.A.D.; Yamaji, F.M.; de Paiva, J.M.F. Use of Waste Coffee Grounds and Sawdust in Briquettes Molding and Evaluation of Properties. *Materia-Brazil* **2015**, *20*, 550-560, doi:10.1590/S1517-707620150002.0055.
147. Zerbinatti, O.E.; da Silva, A.B.; Pereira, A.J.; Miranda, J.M. Briquetting of wastes from coffee plants conducted in zero harvest system. *Semin-Cienc Agrar* **2014**, *35*, 1143-1152, doi:10.5433/1679-0359.2014v35n3p1143.
148. Saenger, M.; Hartge, E.U.; Werther, J.; Ogada, T.; Siagi, Z. Combustion of coffee husks. *Renew Energ* **2001**, *23*, 103-121, doi:Doi 10.1016/S0960-1481(00)00106-3.
149. Rincon, S.L.; Gomez, A. Comparative Behaviour of Agricultural Biomass Residues during Thermochemical Processing. *Global Nest J* **2012**, *14*, 111-117.
150. Protasio, T.D.; de Melo, I.C.N.A.; Guimaraes, M.; Mendes, R.F.; Trugilho, P.F. Thermal Decomposition of Torrefied and Carbonized Briquettes of Residues from Coffee Grain Processing. *Cienc Agrotec* **2013**, *37*, 221-228.
151. Protasio, T.D.; Bufalino, L.; Tonoli, G.H.D.; Guimaraes, M.; Trugilho, P.F.; Mendes, L.M. Brazilian Lignocellulosic Wastes for Bioenergy Production: Characterization and Comparison with Fossil Fuels. *Bioresources* **2013**, *8*, 1166-1185.
152. Mokhtar, N.M.; Omar, R.; Idris, A. Microwave Pyrolysis for Conversion of Materials to Energy: A Brief Review. *Energ Source Part A* **2012**, *34*, 2104-2122, doi:10.1080/15567036.2010.493923.
153. Jeguirim, M.; Bikai, J.; Elmay, Y.; Limousy, L.; Njeugna, E. Thermal characterization and pyrolysis kinetics of tropical biomass feedstocks for energy recovery. *Energy Sustain Dev* **2014**, *23*, 188-193, doi:10.1016/j.esd.2014.09.009.
154. Fernandez, Y.; Menendez, J.A. Influence of feed characteristics on the microwave-assisted pyrolysis used to produce syngas from biomass wastes. *J Anal Appl Pyrol* **2011**, *91*, 316-322, doi:10.1016/j.jaap.2011.03.010.
155. Becidan, M.; Varhegyi, G.; Hustad, J.E.; Skreiberg, O. Thermal decomposition of biomass wastes. A kinetic study. *Ind Eng Chem Res* **2007**, *46*, 2428-2437, doi:10.1021/ie061468z.
156. Esquivel, P.; Jimenez, V.M. Functional properties of coffee and coffee by-products. *Food Res Int* **2012**, *46*, 488-495, doi:10.1016/j.foodres.2011.05.028.

157. Murthy, P.S.; Naidu, M.M. Recovery of Phenolic Antioxidants and Functional Compounds from Coffee Industry By-Products. *Food Bioprocess Tech* **2012**, *5*, 897-903, doi:10.1007/s11947-010-0363-z.
158. Murthy, P.S.; Naidu, M.M. Production and Application of Xylanase from Penicillium sp Utilizing Coffee By-products. *Food Bioprocess Tech* **2012**, *5*, 657-664, doi:10.1007/s11947-010-0331-7.
159. Yusriah, L.; Sapuan, S.M.; Zainudin, E.S.; Mariatti, M. Underutilized Malaysian Agro-wastes Fiber as Reinforcement in Polymer Composites: Potential and Challenges. *J Polym Mater* **2012**, *29*, 201-216.
160. Rattan, S.; Parande, A.K.; Nagaraju, V.D.; Ghiwari, G.K. A comprehensive review on utilization of wastewater from coffee processing. *Environ Sci Pollut R* **2015**, *22*, 6461-6472, doi:10.1007/s11356-015-4079-5.
161. Ravindran, R.; Jaiswal, A.K. Exploitation of Food Industry Waste for High-Value Products. *Trends Biotechnol* **2016**, *34*, 58-69.
162. Bravo, J.; Juaniz, I.; Monente, C.; Caemmerer, B.; Kroh, L.W.; De Pena, M.P.; Cid, C. Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. *J Agric Food Chem* **2012**, *60*, 12565-12573, doi:10.1021/jf3040594.
163. Zuorro, A.; Lavecchia, R. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. *J Clean Prod* **2012**, *34*, 49-56, doi:10.1016/j.jclepro.2011.12.003.
164. Obruca, S.; Benesova, P.; Petrik, S.; Oborna, J.; Prikryl, R.; Marova, I. Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds. *Process Biochem* **2014**, *49*, 1409-1414, doi:10.1016/j.procbio.2014.05.013.
165. Manna, L.; Bugnone, C.A.; Banchero, M. Valorization of hazelnut, coffee and grape wastes through supercritical fluid extraction of triglycerides and polyphenols. *J Supercrit Fluid* **2015**, *104*, 204-211, doi:10.1016/j.supflu.2015.06.012.
166. Zuorro, A. Optimization of polyphenol recovery from espresso coffee residues using factorial design and response surface methodology. *Sep Purif Technol* **2015**, *152*, 64-69, doi:10.1016/j.seppur.2015.08.016.
167. Kyzas, G.Z. Commercial Coffee Wastes as Materials for Adsorption of Heavy Metals from Aqueous Solutions. *Materials* **2012**, *5*, 1826-1840, doi:10.3390/ma5101826.
168. Imessaoudene, D.; Hanini, S.; Bouzidi, A.; Ararem, A. Kinetic and thermodynamic study of cobalt adsorption by spent coffee. *Desalin Water Treat* **2016**, *57*, 6116-6123.
169. Wu, C.H.; Kuo, C.Y.; Guan, S.S. Adsorption of heavy metals from aqueous solutions by waste coffee residues: kinetics, equilibrium, and thermodynamics. *Desalin Water Treat* **2016**, *57*, 5056-5064.
170. Nowicki, P.; Skibiszewska, P.; Pietrzak, R. NO2 removal on adsorbents prepared from coffee industry waste materials. *Adsorption* **2013**, *19*, 521-528, doi:10.1007/s10450-013-9474-y.
171. Yang, C.Y.; Liu, Y.Y.; Ma, C.Y.; Norton, M.; Qiao, J.L. Preparing Desirable Activated Carbons from Agricultural Residues for Potential Uses in Water Treatment. *Waste Biomass Valori* **2015**, *6*, 1029-1036, doi:10.1007/s12649-015-9408-x.
172. Jutakridsada, P.; Prajaksud, C.; Kuboonya-Aruk, L.; Theerakulpisut, S.; Kamwilaisak, K. Adsorption characteristics of activated carbon prepared from spent ground coffee. *Clean Technol Envir* **2016**, *18*, 639-645.
173. Nowicki, P.; Kazmierczak-Razna, J.; Skibiszewska, P.; Wisniewska, M.; Nosal-Wiercinska, A.; Pietrzak, R. Production of activated carbons from biodegradable waste materials as an alternative way of their utilisation. *Adsorption* **2016**, *22*, 489-502.
174. Popovici, D.; Dusescu, C.; Neagu, M. Removal of Phenol from Aqueous Solutions on Activated Carbon Obtained from Coffee Grounds. *Rev Chim-Bucharest* **2016**, *67*, 751-756.
175. Kyzas, G.Z. A Decolorization Technique with Spent "Greek Coffee" Grounds as Zero-Cost Adsorbents for Industrial Textile Wastewaters. *Materials* **2012**, *5*, 2069-2087, doi:10.3390/ma5112069.

176. Kyzas, G.Z.; Lazaridis, N.K.; Mitropoulos, A.C. Removal of dyes from aqueous solutions with untreated coffee residues as potential low-cost adsorbents: Equilibrium, reuse and thermodynamic approach. *Chem Eng J* **2012**, *189*, 148-159, doi:10.1016/j.cej.2012.02.045.
177. Chinmai, K.; C., H.B.; Dsouza, K.D.; R., M.C.B.; S., S.B. Feasibility Studies on Spent Coffee Grounds Biochar as an Adsorbent for Color Removal. *International Journal of Application or Innovation in Engineering & Management (IJAIEM)* **2014**, *3*, 9-13.
178. dos Santos, D.C.; Adebayo, M.A.; Pereira, S.D.P.; Prola, L.D.T.; Cataluna, R.; Lima, E.C.; Saucier, C.; Gally, C.R.; Machado, F.M. New carbon composite adsorbents for the removal of textile dyes from aqueous solutions: Kinetic, equilibrium, and thermodynamic studies. *Korean J Chem Eng* **2014**, *31*, 1470-1479, doi:10.1007/s11814-014-0086-3.
179. Nitayaphat, W.; Jintakosol, T.; Engkaseth, K.; Wanrakakit, Y. Removal of Methylene Blue from Aqueous Solution by Coffee Residues. *Chiang Mai J Sci* **2015**, *42*, 407-416.
180. Lafi, R.; Hafiane, A. Removal of methyl orange (MO) from aqueous solution using cationic surfactants modified coffee waste (MCWs). *J Taiwan Inst Chem E* **2016**, *58*, 424-433.
181. Knob, A.; Fortkamp, D.; Prolo, T.; Izidoro, S.C.; Almeida, J.M. Agro-residues as Alternative for Xylanase Production by Filamentous Fungi. *Bioresources* **2014**, *9*, 5738-5773.
182. Dias, M.; Melo, M.M.; Schwan, R.F.; Silva, C.F. A new alternative use for coffee pulp from semi-dry process to beta-glucosidase production by Bacillus subtilis. *Lett Appl Microbiol* **2015**, *61*, 588-595, doi:10.1111/lam.12498.
183. Battista, F.; Fino, D.; Mancini, G. Optimization of biogas production from coffee production waste. *Bioresource Technol* **2016**, *200*, 884-890.
184. Dohlert, P.; Weidauer, M.; Enthaler, S. Spent coffee ground as source for hydrocarbon fuels. *J Energy Chem* **2016**, *25*, 146-152.
185. Sakuragi, K.; Li, P.; Otaka, M.; Makino, H. Recovery of Bio-Oil from Industrial Food Waste by Liquefied Dimethyl Ether for Biodiesel Production. *Energies* **2016**, *9*.
186. Yang, L.X.; Nazari, L.; Yuan, Z.S.; Corscadden, K.; Xu, C.B.; He, Q. Hydrothermal liquefaction of spent coffee grounds in water medium for bio-oil production. *Biomass Bioenerg* **2016**, *86*, 191-198.
187. Iyer, K.A.; Zhang, L.H.; Torkelson, J.M. Direct Use of Natural Antioxidant-rich Agro-wastes as Thermal Stabilizer for Polymer: Processing and Recycling. *Acs Sustain Chem Eng* **2016**, *4*, 881-889.
188. Acchar, W.; Dultra, E.J.V. Thermal analysis and X-ray diffraction of untreated coffee's husk ash reject and its potential use in ceramics. *J Therm Anal Calorim* **2013**, *111*, 1331-1334, doi:10.1007/s10973-012-2478-0.
189. Acchar, W.; Dultra, E.J.V.; Segadaes, A.M. Untreated coffee husk ashes used as flux in ceramic tiles. *Appl Clay Sci* **2013**, *75-76*, 141-147, doi:10.1016/j.clay.2013.03.009.
190. Acchar, W.; Avelino, K.A.; Segadaes, A.M. Granite waste and coffee husk ash synergistic effect on clay-based ceramics. *Adv Appl Ceram* **2016**, *135*, 236-242.
191. Rani, M.U.; Appaiah, K.A.A. Production of bacterial cellulose by Gluconacetobacter hansenii UAC09 using coffee cherry husk. *J Food Sci Tech Mys* **2013**, *50*, 755-762, doi:10.1007/s13197-011-0401-5.
192. Bondesson, E. A nutritional analysis on the by-product coffee husk and its potential utilization in food production. Swedish University of Agricultural Sciences. Swedish University of Agricultural Sciences, Uppsala, 2015.
193. Ribeiro, H.; Marto, J.; Raposo, S.; Agapito, M.; Isaac, V.; Chiari, B.G.; Lisboa, P.F.; Paiva, A.; Barreiros, S.; Simoes, P. From coffee industry waste materials to skin-friendly products with improved skin fat levels. *Eur J Lipid Sci Tech* **2013**, *115*, 330-336, doi:10.1002/ejlt.201200239.
194. Marto, J.; Gouveia, L.F.; Chiari, B.G.; Paiva, A.; Isaac, V.; Pinto, P.; Simoes, P.; Almeida, A.J.; Ribeiro, H.M. The green generation of sunscreens: Using coffee industrial sub-products. *Ind Crop Prod* **2016**, *80*, 93-100.

195. Arulrajah, A.; Maghoolpilehrood, F.; Disfani, M.M.; Horpihulsuk, S. Spent coffee grounds as a non-structural embankment fill material: engineering and environmental considerations. *J Clean Prod* **2014**, *72*, 181-186, doi:10.1016/j.jclepro.2014.03.010.
196. Gama, N.V.; Soares, B.; Freire, C.S.R.; Silva, R.; Neto, C.P.; Barros-Timmons, A.; Ferreira, A. Bio-based polyurethane foams toward applications beyond thermal insulation. *Mater Design* **2015**, *76*, 77-85, doi:10.1016/j.matdes.2015.03.032.
197. Arulrajah, A.; Kua, T.A.; Phetchuay, C.; Horpibulsuk, S.; Mahghoolpilehrood, F.; Disfani, M.M. Spent Coffee Grounds-Fly Ash Geopolymer Used as an Embankment Structural Fill Material. *J Mater Civil Eng* **2016**, *28*.
198. Velasco, P.M.; Mendivil, M.A.; Morales, M.P.; Munoz, L. Eco-fired clay bricks made by adding spent coffee grounds: a sustainable way to improve buildings insulation. *Mater Struct* **2016**, *49*, 641-650.
199. van Dam, J.E.G.; Harmsen, P. *Coffee residues utilization*; Wageningen UR Food & Biobased Research: Wageningen, 2010.
200. Marchaim, U.; Kostenberg, D.; Epstein, E. Auxins and phenols in anaerobic thermophilic digestion of coffee wastes and their synergistic effect in horticulture. *Microbiology+* **1997**, *66*, 578-582.
201. Machado, C.M.M.; Soccol, C.R.; de Oliveira, B.H.; Pandey, A. Gibberellic acid production by solid-state fermentation in coffee husk. *Appl Biochem Biotech* **2002**, *102*, 179-191, doi:Doi 10.1385/Abab:102-103:1-6:179.
202. Reis, K.C.; Pereira, L.; Melo, I.C.N.A.; Marconcini, J.M.; Trugilho, P.F.; Tonoli, G.H.D. Particles of Coffee Wastes as Reinforcement in Polyhydroxybutyrate (PHB) Based Composites. *Mater Res-Ibero-Am J* **2015**, *18*, 546-552, doi:10.1590/1516-1439.318114.
203. Goncalves, M.; Souza, V.C.; Galhardo, T.S.; Mantovani, M.; Figueiredo, F.C.A.; Mandelli, D.; Carvalho, W.A. Glycerol Conversion Catalyzed by Carbons Prepared from Agroindustrial Wastes. *Ind Eng Chem Res* **2013**, *52*, 2832-2839, doi:10.1021/ie303072d.
204. Rufford, T.E.; Hulicova-Jurcakova, D.; Zhu, Z.H.; Lu, G.Q. Nanoporous carbon electrode from waste coffee beans for high performance supercapacitors. *Electrochem Commun* **2008**, *10*, 1594-1597, doi:10.1016/j.elecom.2008.08.022.
205. Murthy, P.S.; Naidu, M.M.; Srinivas, P. Production of alpha-amylase under solid-state fermentation utilizing coffee waste. *J Chem Technol Biot* **2009**, *84*, 1246-1249, doi:10.1002/jctb.2142.
206. Macedo, G.A.; Matsuda, L.K.; Battestin, V. Screening of tanase producing fungi present in rich tannin vegetable residues. *Cienc Agrotec* **2005**, *29*, 833-838, doi:Doi 10.1590/S1413-70542005000400016.
207. Eliche-Quesada, D.; Martinez-Garcia, C.; Martinez-Cartas, M.L.; Cotes-Palomino, M.T.; Perez-Villarejo, L.; Cruz-Perez, N.; Corpas-Iglesias, F.A. The use of different forms of waste in the manufacture of ceramic bricks. *Appl Clay Sci* **2011**, *52*, 270-276, doi:10.1016/j.clay.2011.03.003.
208. Eliche-Quesada, D.; Perez-Villarejo, L.; Iglesias-Godino, F.J.; Martinez-Garcia, C.; Corpas-Iglesias, F.A. Incorporation of coffee grounds into clay brick production. *Adv Appl Ceram* **2011**, *110*, 225-232, doi:10.1179/1743676111y.0000000006.
209. Espindola-Gonzalez, A.; Martinez-Hernandez, A.L.; Angeles-Chavez, C.; Castano, V.M.; Velasco-Santos, C. Novel Crystalline SiO2 Nanoparticles via Annelids Bioprocessing of Agro-Industrial Wastes. *Nanoscale Res Lett* **2010**, *5*, 1408-1417, doi:10.1007/s11671-010-9654-6.
210. Alfaro, M.E.; Barrantes, M.F.C.; Villalta, G.A.J.; Amador, K.R. Coffee wastes as potential source of nanocellulose. *Abstr Pap Am Chem S* **2014**, *247*.
211. Akasaka, H.; Takahata, T.; Toda, I.; Ono, H.; Ohshio, S.; Himeno, S.; Kokubu, T.; Saitoh, H. Hydrogen storage ability of porous carbon material fabricated from coffee bean wastes. *Int J Hydrogen Energ* **2011**, *36*, 580-585, doi:10.1016/j.ijhydene.2010.09.102.

212. Alstrom, S. Antibacterial Activity of Tea and Coffee Wastes against Some Plant Pathogenic Pseudomonas-Syringae Strains. *J Phytopathol* **1992**, *136*, 329-334, doi:DOI 10.1111/j.1439-0434.1992.tb01315.x.
213. Rojas, J.B.U.; Verreth, J.A.J.; Amato, S.; Huisman, E.A. Biological treatments affect the chemical composition of coffee pulp. *Bioresource Technol* **2003**, *89*, 267-274, doi:10.1016/S0960-8524(03)00070-1.
214. BelloMendoza, R.; Sanchez, J.E. Anaerobic filter treatment of wastewater from mushroom cultivation on coffee pulp. *World J Microb Biot* **1997**, *13*, 51-55.
215. Ramya, P.M.; Jayasravanthi, M.; Dulla, B.J.; Venkata, N.R. Chemical Oxygen Demand Reduction from Coffee Processing Waste Water - a Comparative Study on Usage of Biosorbents Prepared from Agricultural Wastes. *Global Nest J* **2015**, *17*, 291-300.
216. Belitz, H.-D.; Grosch, W.; Schieberle, P. *Food Chemistry*, 3 ed.; springer: Berlin, Heidelberg, , 2004; pp. 941-942.
217. Rawel, H.M.; Rohn, S.; Kroll, J. Characterisation of 11S Protein Fractions and Phenolic Compounds from Green Coffee Beans under Special Consideration of their Interactions – A Review. *Deutsche Lebensmittel-Rundschau* **2005**, *101*, 148-160.
218. Farah, A.; Monteiro, M.C.; Calado, V.; Franca, A.S.; Trugo, L.C. Correlation between cup quality and chemical attributes of Brazilian coffee. *Food Chem* **2006**, *98*, 373-380.
219. Jaiswal, R.; Kuhnert, N. Hierarchical scheme for liquid chromatography/multi-stage spectrometric identification of 3,4,5-triacyl chlorogenic acids in green Robusta coffee beans. *Rapid Commun Mass Spectrom* **2010**, *24*, 2283-2294, doi:10.1002/rcm.4639.
220. Jaiswal, R.; Kuhnert, N. Identification and characterization of five new classes of chlorogenic acids in burdock (Arctium lappa L.) roots by liquid chromatography/tandem mass spectrometry. *Food Funct* **2011**, *2*, 63-71, doi:10.1039/c0fo00125b.
221. Robards, K.; Prenzler, P.D.; Tucker, G.; Swatsitang, P.; Glover, W. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem* **1999**, *66*, 401-436.
222. Rawel, H.M.; Rohn, S. Nature of hydroxycinnamate-protein interactions. *Phytochem Rev* **2010**, *9*, 93-109, doi:10.1007/s11101-009-9154-4.
223. Schilling, S.; Sigolotto, C.-I.; Carle, R.; Schieber, A. Characterization of covalent addition products of chlorogenic acid quinone with amino acid derivatives in model systems and apple juice by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **2008**, *22*, 441-448, doi:10.1002/rcm.3381.
224. Bongartz, V.; Brandt, L.; Gehrmann, M.L.; Zimmermann, B.F.; Schulze-Kaysers, N.; Schieber, A. Evidence for the Formation of Benzacridine Derivatives in Alkaline-Treated Sunflower Meal and Model Solutions. *Molecules* **2016**, *21*, doi:ARTN 91 10.3390/molecules21010091.
225. Chapagain, A.K.; Hoekstra, A.T. Virtual water flows between nations in relation to trade in livestock and livestock products. *UNESCO-IHE* **2003**.
226. Chapagain, A.K.; Hoekstra, A.Y. The water needed to have the Dutch drink Coffee. *Value of Water Research Report Series UNESCO-IHE* **2003**.
227. Chanakya, H.N.; De Alwis, A.A.P. Environmental Issues and Management in Primary Coffee Processing. *Process Safety and Environmental Protection* **2004**, *82*, 291-300, doi:10.1205/095758204323162319.
228. Ijanu, E.M.; Kamaruddin, M.A.; Norashiddin, F.A. Coffee processing wastewater treatment: a critical review on current treatment technologies with a proposed alternative. *Applied Water Science* **2020**, *10*.
229. Woldesenbet, A.G.; Woldeyes, B.; Chandravanishi, B.S. Characteristics of Wet Coffee Processing Waste and Its Environmental Impact in Ethiopia. *International Journal of Research in Engineering and Science* **2014**, *2*, 01-05.
230. Siddiqui, W.A.; Wassem, M. A Comparative Study of Sugar Mill Treated and Untreated Effluent- A Case Study. *Oriental Journal of Chemistry* **2012**, *28*, 1899-1904.
231. Haddis, A.D.R. Effect of effluent generated from coffee processing plant on the water bodies and human health in its vicinity. *Journal of Hazardous Materials* **2008**, *152*, 259-262, doi:10.1016/j.jhazmat.2007.06.094.

232. Woldesenbet, A.G.; Woldeyes, B.; Chandravanshi, B.S. Wet coffee processing waste management practice in Ethiopia. *Asian Journal of Science and Technology* **2015**, *6*, 1476-1471.
233. Hue N.V; Bittenbender H.C; M, O.-E. Managing coffee processing water in Hawaii. **2016**.
234. Olguin, E.J.R., D; Sanchez, G; Hernandez, E; Ramirez, M. E. Productivity, Protein Content and Nutrient Removal from Anaerobic Effluents of Coffee Wastewater in Salvinia minima Ponds, under Subtropical Conditions. *Acta Biotechnol* **2003**, *23*, 259-270.
235. De Puala e Melo, A.C.d.P.S., A; de Carvalho, J.J; Caixeta Salomao, L. UTILIZACAO DE AGUA RESIDUARIA DO PROCESSO POS-COLHEITA DO CAFÉ NA PRODUCAO DE MUDAS DE CAFEEIRO. *Irriga, Botucatu* **2011**, *16*, 413-423.
236. Kulandaivelu, V.; Bhat, R. Changes in the physico-chemical and biological quality attributes of soil following amendment with untreated coffee processing wastewater. *European Journal of Soil Biology* **2012**, *50*, 39-43, doi:10.1016/j.ejsobi.2011.11.011.
237. Batista, R.O.; Matos, A.T.d.; Cunha, F.F.d.; Mônaco, P.A.L.; Santos, D.B.d. Hydraulic performance of drip irrigation subunits using wastewater from coffee fruit processing. *Water Resources and Irrigation Managment* **2012**, *1*.
238. Oliveira Batista, R.; Teixeira de Matos, A.; França da Cunha, F.; Alfonsa Lo Mônaco, P.; Batista de Santos, D. Hydraulic performance of drip irrigation subunits using wastewater from coffee fruit processing. *Water Resources and Irrigation Managment* **2012**, *1*, 1-6.
239. Bello-Mendoza, R.C.-R., M.F. Start-up of an Anaerobic Hybrid (UASB/Filter) Reactor Treating Wastewater from Coffee Processing Plant *Anaerobe Environmental Microbiology* **1998**.
240. Guardia Puebla, Y.R.P., S; Jimenez Herandez, J; Sanchez-Giron Renedo, V. Performance of a UASB reactor treating coffee wet wastewater. *Revista Ciencias Tecnicas Agropecuarias* **2013**, *22* 35-41.
241. Affam, A.C. Operational performance of vertical upflow roughing filter for pre-treatment of leachate using limestone filter media. *Journal of Urban and Environmental Engineering* **2013**, *7*, 117-125, doi:10.4090/juee.2013.v7n1.117125.
242. Perez Rodriguez, S.P., S; Fernandez Boizan, M. Estudio de la Biodegradabilidad Anaerobia de las Aguas Residuales del Beneficio Humdo del Cafe. *Interciencia* **2000**, *25 No.8*, 386-390.
243. Selvamurugan, M.; Doraisamy, P.; Maheswari, M. An integrated treatment system for coffee processing wastewater using anaerobic and aerobic process. *Ecological Engineering* **2010**, *36*, 1686-1690, doi:10.1016/j.ecoleng.2010.07.013.
244. Cavanagh, D.; Fitzgerald, G.F.; McAuliffe, O. From field to fermentation: The origins of Lactococcus lactis and its domestication to the dairy environment. *Food Microbiology* **2015**, *47*, 45-61.
245. Ferreira Silva, C.M.V., Danille; de Souza Cordeiro, Cecilial; Ferreira Duarte, Whasley; Ribeiro Dias, Disney; Freitas Schwan, Rosane. Evaluation of a potencial starter culture for enhance quality of coffee fermentation. *World J Microbiol Biotechnol* **2013**, *29*, 235-247, doi:10.1007/s11274-012-1175-2.
246. Pereira, G.V.d.M.; Vale, A.d.S.; Neto, D.P.d.C.; Muynarsk, E.S.; Soccol, V.T.; Socool, C.R. Lactic acid bacteria: what coffee industry should know? *Current Opinion in Food Science* **2020**, *31*, 1-8.
247. Calle, V.H. Activadores bioquimicos para la fermentación del café. *Cenicafé* **1957**, *8*, 94-101.
248. Frank, H.A.; De la Cruz, A.S. Role of incidental microgolora in natural decomposition of mucilage layer in kona coffee cherries. *Journal of Food Science* **1964**, *29*, 850-853.
249. Frank, H.A.; Lum, A.N.; De La Cruz, A.S. Bacteria responsible for mucilage layer decomposition in Kona cffee cherries. *Applied Microbiology* **1965**, *13*, 201-207.
250. Avallone, S.B., Jean M.; Guyot, Bernard; Olguin, Eugenia; Guiraud, Joseph P. Involvemnt of pectolytic micro-organisms in coffee fermentation. *International Journal of Food Science and Technology* **2002**, *37*, 191-198.

251. Jackels, S.C.J., Charles F. Characterization of the Coffee Mucilage Fermentation Process Using Chemical Indicators: A field Study in Nicaragua. *Journal of Food Science* **2005**, *70*.
252. Pereira, G.V.d.M.; Beux, M.; Pagnoncelli, M.G.B.; Soccol, V.T.; Rodrigues, C.; Soccol, C.R. Isolation, selection and evaluation of antagonistic yeasts and lactic acid bacteria against ochratoxigenic fungus Aspergillus westerdijkiae on coffee beans. *Letters in Applied Microbiology* **2015**, *62*, 96-101.
253. Cruz-Salomón, A.; Ríos-Valdovinos, E.; Pola-Albores, F.; Lagunas-Rivera, S.; Meza-Gordillo, R.; Ruíz-Valdiviezo, V. Evaluation of Hydraulic Retention Time on Treatment of Coffee Processing Wastewater (CPWW) in EGSB Bioreactor. *Sustainability* **2017**, *10*, 83, doi:10.3390/su10010083.
254. Bo, Z.; Wei-min, C.; Pin-jing, H. Influence of lactic acid on the two-phase anaerobic digestion of kitchen wastes. *Journal of Environmental Sciences* **2007**, *19*, 244-249.
255. Swenson, H.; Stadie, N.P. Langmuir's Theory of Adsorption: A Centennial Review. *Langmuir* **2019**, *35*, 5409-5426.
256. Bernal, V.; Giraldo, L.; Moreno-Piraján, J.C. Physicochemical Properties of Activated Carbon: Their Effect on the Adsorption of Pharmaceutical Compounds and Adsorbate–Adsorbent Interactions. *Journal of Carbon Research* **2018**, *4*.
257. A.Ahmadpour; D.D.Do. The preparation of active carbons from coal by chemical and physcial activation *Carbon* **1996**, *34*, 471-479.
258. Bernardo, M.; Lapa, N.; Matos, I.; Fonseca, I. Critical discussion on activated carbons from bio - wastes - environmental risk assessment. *Grupo Español Carbón* **2016**.
259. Kong, L.; Su, M.; Peng, Y.; Hou, L.a.; Liu, J.; Li, H.; Diao, Z.; Shih, K.; Xiong, Y.; Chen, D. Producing sawdust derived activated carbon by co-calcinations with limestone for enhanced Acid Orange II adsorption. *Journal of Cleaner Production* **2017**, *168*, 22-29.
260. Boonamnuayvitaya, V.; Sae-ung, S.; Tanthapanichakoon, W. Preparation of activated carbons from coffee residue for the adsorption of formaldehyde. *Separation and Purification Technology* **2005**, *42*, 159-168.
261. Khenniche, L.; Aissani, F. Preparation and Characterization of Carbons from Coffee Residue: Adsorption of Salicylic Acid on the Prepared Carbons. *Journal of Chemical & Engineering Data* **2010**, *55*, 728-734.
262. Rothfos, B. *Coffee production* Gordian-Max-Rieck: Germany, 1980.
263. Singleton, V.L.; Rudolf Orthofer; Lamuela-Raventós, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology* **1999**, *299*, 152-178.
264. Laing, W.; Christeller, J. Extraction of Proteins from Plant Tissues. *Current Protocols in Protein Science* **2004**, *38*.
265. Wang, W.; Vignani, R.; Scali, M.; Cresti, M. A universal and rapid protocol for protein extraction from recalcitrant plant tissues for proteomic analysis. *Electrophoresis* **2006**, *27*, 2782-2786.
266. Campos, G.A.F.; Sagu, S.T.; Celis, P.S.; Rawel, H.M. Comparison of Batch and Continuous Wet-Processing of Coffee: Changes in the Main Compounds in Beans, By-Products and Wastewater. *Foods* **2020**, *9*.
267. M.Stoscheck, C. Quantitation of protein. *Methods in Enzymology* **1990**, *182*, 50-68.
268. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*.
269. Bereman, M.S.; MacLean, B.; Tomazela, D.M.; Liebler, D.C.; MacCoss, M.J. The development of selected reaction monitoring methods for targeted proteomics via empirical refinement. *Proteomics* **2012**, *12*, 1134-1141.
270. Tchewonpi, S.S.; Zimmermann, L.; Landgräber, E.; Homann, T.; Huschek, G.; Özpinar, H.; Schweigert, F.J.; Rawel, H.M. Comprehensive Characterization and Relative Quantification of α-Amylase/Trypsin Inhibitors from Wheat Cultivars by Targeted HPLC-MS/MS. *foods* **2020**, *9*.
271. Pearson, W.R.; Lipman, D.J. Improved tools for biological sequence comparison. *Proceedings of the National Academy of Sciences of the United States of America* **1988**, *85*, 2444-2448.

272. Brunauer, S.; Emmett, P.H.; Teller, E. Adsorption of Gases in Multimolecular Layers. *J. Am. Chem. Soc.* **1938**, *60*, 309-319.
273. Rouquerol, J.; Llewellyn, P.; Rouquerol, F. Is the bet equation applicable to microporous adsorbents? *Studies in Surface Science and Catalysis* **2007**, *160*, 49-56, doi:https://doi.org/10.1016/S0167-2991(07)80008-5.
274. Maziarka, P.; Wurzer, C.; Arauzo, P.J.; Dieguez-Alonso, A.; Mašek, O.; Ronsse, F. Do you BET on routine? The reliability of N2 physisorption for the quantitative assessment of biochar's surface area. *Chemical Engineering Journal* **2021**, *418*.
275. Pradhan, N.; Rene, E.R.; Lens, P.N.L.; Dipasquale, L.; D'Ippolito, G.; Fontana, A.; Panico, A.; Esposito, G. Adsorption Behaviour of Lactic Acid on Granular Activated Carbon and Anionic Resins: Thermodynamics, Isotherms and Kinetic Studies. *Energies* **2017**, *10*.
276. Evangelista, R.L.; Mangold, A.J.; Nikolov, Z.L. Recovery of Lactic Acid by Sorption *Applied Biochemistry and Biotechnology* **1994**, *45*, 131-144.
277. Moldes, A.B.; Alonso, J.L.; Parajó, J.C. Recovery of lactic acid from simultaneous saccharification and fermentation media using anion exchange resins *Bioprocess Biosyst Engenieering* **2003**, *25*, 357-363.
278. Franca, A.S.; L.S., O.; Mendonça, J.C.F.; Silva, X.N.A. Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry* **2005**, *90*, 89-94.
279. Ramalakshmi, K.; Kubra, I.R.; Rao, L.J.M. Physicochemical characteristics of green coffee: Comparison of graded and defective beans. *Journal of Food Science* **2007**, *72*, S333-S337.
280. dePaula, J.; Farah, A. Caffeine Consumption through Coffee: Content in the Beverage, Metabolism, Health Benefits and Risks. *Beverages* **2019**, *5*, 37, doi:10.3390/beverages5020037.
281. Rawel, H.M.; Rohn, S.; Kroll, J. Characterisation of 11s protein fractions and phenolic compounds from green coffee beans under special consideration of their interactions: A review. *Deutsche Lebensmittel-Rundschau* **2005b**, *101*.
282. Rodriguez-Gomez, R.; Vanheuverzwjin, J.; Souard, F.; Delporte, C.; Stevigny, C.; Stoffelen, P.; De Braekeleer, K.; Kauffmann, J.M. Determination of Three Main Chlorogenic Acids in Water Extracts of Coffee Leaves by Liquid Chromatography Coupled to an Electrochemical Detector. *Antioxidants* **2018**, *7*, doi:10.3390/antiox7100143.
283. Lopez, C.I.; D, B.; D, M.; E, D. Factors related to the formation of ''overfermented coffee beans'' during the wet processing method and storage of coffee. *ASIC, 13 Colloque, Paipa* **1989**.
284. Sivetz, M. Coffee processing technology *AVIC, Westport Connecticut* **1963**, *2*.
285. Jham, G.M.; Fernandes, A.S.; C.F., G.; A.A., S. Comparison of GC and HPLC for the Quantification of Organic Acids in Coffee. *Phytochem Anal* **2002**, *13*, 99-104.
286. Vásquez Morera, R. Influencia de la recirculación de las aguas del despulpado del cafe sobre su calidad. San Pedro Sula, Honduras.
287. Jacquet, M. Alternativas tecnológicas del beneficiado húmedo en relación con la conservación del medio ambiente. San Pedro Sula, Honduras.
288. Knopp, S.; Bytof, G.; Selmar, D. Influence of processing on the content of sugars in green Arabica coffee beans. *Eur Food Res Technol* **2006**, *223*, 195-201.
289. Borém, F.M.; Figueiredo, L.P.; Ribeiro, F.C.; Taveira, J.H.S.; Giomo, G.S.; Salva, T.J.G. The relationship between organic acids, sucrose and the quality of specialty coffees. *African journal of agricultural research* **2016**, *11*, 709-717.
290. Acidri, R.; Sawai, Y.; Sugimoto, Y.; Handa, T.; Sasagawa, D.; Masunaga, T.; Yamamoto, S.; Nishihara, E. Phytochemical Profile and Antioxidant Capacity of Coffee Plant Organs Compared to Green and Roasted Coffee Beans. *Antioxidants* **2020**, *9*, doi:10.3390/antiox9020093.
291. Kinyua, A.W.; Kpkorrir, R.; B., M.; C., K. Effect of Different Fermentation Methods on Physicochemical Composition and Sensory Quality of Coffee (Coffea arabica). *Journal of Environmental Science, Toxicology and Food Technology* **2017**, *11*, 31-36.

292. Shimizu, M.M.; Mazzafera, P. Compositional changes of proteins and amino acids in germinating coffee seeds. *Brazilian Archives of Biology and Technology* **2000**, *43*, 259-265, doi:10.1590/s1516-89132000000300003.
293. Acuña, R.; Bassüner, R.; Beilinson, V.; Cortina, H.; Cadena-Gómez, G.; Montes, V.; Nielsen, N.C. Coffee seeds contain 11S storage proteins. *Physiologia Plantarum* **1999**, *105*, 122-131, doi:10.1034/j.1399-3054.1999.105119.x.
294. Baú, S.M.T.; Mazzafera, P.; Santoro, L.g. Seed storage proteins in coffee. *Revista Brasileira de Fisiologia Vegetal* **2001**, *13*, 33-40.
295. Rogers, W.J.; Bézard, G.; Deshayes, A.; Meyer, I.; Pétiard, V.; Marraccini, P. Biochemical and molecular characterization and expression of the 11S-type storage protein from Coffea arabica endosperm. *Plant Physiology and Biochemistry* **1999**, *37*, 261-272, doi:http://dx.doi.org/10.1016/S0981-9428(99)80024-2.
296. Fukushima, D. Structures of plant storage proteins and their functions. *Food Reviews International* **1991**, *7*, 353-381, doi:10.1080/87559129109540916.
297. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. *J Biol Chem* **1951**, *193*, 265-275.
298. Bongartz, V.; Brandt, L.; Gehrmann, M.L.; Zimmermann, B.F.; Schulze-Kaysers, N.; Schieber, A. Evidence for the Formation of Benzacridine Derivatives in Alkaline-Treated Sunflower Meal and Model Solutions. *Molecules* **2016**, *21*, 91, doi:10.3390/molecules21010091.
299. Namiki, M.; Yabuta, G.; Koizumi, Y.; Yano, M. Development of free radical products during the greening reaction of caffeic acid esters (or chlorogenic acid) and a primary amino compound. *Biosci Biotechnol Biochem* **2001**, *65*, 2131-2136, doi:10.1271/bbb.65.2131.
300. Yabuta, G.; Koizumi, Y.; Namiki, K.; Hida, M.; Namiki, M. Structure of green pigment formed by the reaction of caffeic acid esters (or chlorogenic acid) with a primary amino compound. *Biosci Biotechnol Biochem* **2001**, *65*, 2121-2130, doi:10.1271/bbb.65.2121.
301. Schilling, S.; Sigolotto, C.I.; Carle, R.; Schieber, A. Characterization of covalent addition products of chlorogenic acid quinone with amino acid derivatives in model systems and apple juice by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom* **2008**, *22*, 441-448, doi:10.1002/rcm.3381.
302. Prigent, S.V.; Gruppen, H.; Visser, A.J.; Van Koningsveld, G.A.; De Jong, G.A.; Voragen, A.G. Effects of non-covalent interactions with 5-O-caffeoylquinic acid (chlorogenic acid) on the heat denaturation and solubility of globular proteins. *J Agric Food Chem* **2003**, *51*, 5088-5095, doi:10.1021/jf021229w.
303. Shutov, A.D.; Vaintraub, I.A. Degradation of Storage Proteins in Germinating-Seeds. *Phytochemistry* **1987**, *26*, 1557-1566, doi:Doi 10.1016/S0031-9422(00)82245-1.
304. Acuna, R.; Bassuner, R.; Beilinson, V.; Cortina, H.; Cadena-Gomez, G.; Montes, V.; Nielsen, N.C. Coffee seeds contain 11S storage proteins. *Physiologia Plantarum* **1999**, *105*, 122-131, doi:DOI 10.1034/j.1399-3054.1999.105119.x.
305. Rogers, W.J.; Bezard, G.; Deshayes, A.; Meyer, I.; Petiard, V.; Marraccini, P. Biochemical and molecular characterization and expression of the 11S-type storage protein from Coffea arabica endosperm. *Plant Physiology and Biochemistry* **1999**, *37*, 261-272, doi:Doi 10.1016/S0981-9428(99)80024-2.
306. Ali, M.; Homann, T.; Kreisel, J.; Khalil, M.; Puhlmann, R.; Kruse, H.P.; Rawel, H. Characterization and Modeling of the Interactions between Coffee Storage Proteins and Phenolic Compounds. *Journal of Agricultural and Food Chemistry* **2012**, *60*, 11601-11608, doi:10.1021/jf303372a.
307. Czubinski, J.; Dwiecki, K. A review of methods used for investigation of protein-phenolic compound interactions. *Int J Food Sci Tech* **2017**, *52*, 573-585, doi:10.1111/ijfs.13339.
308. Rawel, H.M.; Meidtner, K.; Kroll, J. Binding of selected phenolic compounds to proteins. *J Agric Food Chem* **2005**, *53*, 4228-4235, doi:10.1021/jf0480290.
309. Suryaprakash, P.; Kumar, R.P.; Prakash, V. Thermodynamics of interaction of caffeic acid and quinic acid with multisubunit proteins. *Int J Biol Macromol* **2000**, *27*, 219-228, doi:10.1016/s0141-8130(00)00119-7.

310. Schwenke, K.D. Reflections about the functional potential of legume proteins - A review. *Nahrung-Food* **2001**, *45*, 377-381, doi:Doi 10.1002/1521-3803(20011001)45:6<377::Aid-Food377>3.0.Co;2-G.
311. Rawel, H.M.; Rohn, S.; Kruse, H.P.; Kroll, J. Structural changes induced in bovine serum albumin by covalent attachment of chlorogenic acid. *Food Chemistry* **2002**, *78*, 443-455, doi:Pii S0308-8146(02)00155-3
312. Gu, X.; Ma, X.; Li, L.; Liu, C.; Cheng, K.; Li, Z. Pyrolysis of poplar wood sawdust by TG-FTIR and Py–GC/MS. *Journal of analytical and applied pyrolysis* **2013**, *102*, 16-23.
313. Wang, C.B.; Chen, L.; Jia, L.F.; Tan, Y.W. Simultaneous calcination and sulfation of limestone in CFBB. *Appl. Energ.* **2015**, *155*, 478-484.
314. Sis, H.; Birinci, M. Effect of nonionic and ionic surfactants on zeta potential and dispersion properties of carbon black powders. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2009**, *341*, 60-67.
315. H.Ridaoui; A.Jada; L.Vidal; J.-B.Donnet. Effect of cationic surfactant and block copolymer on carbon black particle surface charge and size. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2006**, *278*, 149-159.
316. E.Arenas; Chejne, F. The effect of the activating agent and temperature on the porosity development of physically activated coal chars. *Carbon* **2004**, *42*, 2451-2455.
317. Baek, J.; Hye-Min Lee; Roh, J.-S.; Lee, H.-S.; Kang, H.-S.; Kim, B.-J. Studies on preparation and applications of polymeric precursor-based activated hard carbons: I. Activation mechanism and microstructure analyses. *Microporous and Mesoporous Materials* **2016**, *219*, 258-264.
318. Kim, M.-S.; Kim, J.-G. Adsorption Characteristics of Spent Coffee Grounds as an Alternative Adsorbent for Cadmium in Solution. *environments* **2020**, *7*.
319. Kante, K.; Nieto-Delgado, C.; Rangel-Mendez, J.R.; Bandosz, T.J. Spent coffee-based activated carbon: specific surface features and their importance for H2S separation process. *J Hazard Mater* **2012**, *201-202*, 141-147, doi:10.1016/j.jhazmat.2011.11.053.
320. Klingel, T.; Kremer, J.I.; Gottstein, V.; Rezende, T.R.d.; Schwarz, S.; Lachenmeier, D.W. A Review of Coffee By-Products Including Leaf, Flower, Cherry, Husk, Silver Skin, and Spent Grounds as Novel Foods within the European Union. *foods* **2020**, *9*.
321. Scully, D.S.; Jaiswal, A.K.; Abu-Ghannam, N. An Investigation into Spent Coffee Waste as a Renewable Source of Bioactive Compounds and Industrially Important Sugars. *bioengineering* **2016**, *3*.
322. Reis, R.S.; Tienne, L.G.P.; Souza, D.d.H.S.; Marques, M.d.F.V.; Monteiro, S.N. Characterization of coffee parchment and innovative steam explosion treatment to obtain microfibrillated cellulose as potential composite reinforcement. *Journal of Materials Research and Technology* **2020**, *9*, 9412-9421.
323. Li, Z.Q.; Lu, C.J.; Xia, Z.P.; Zhou, Y.; Luo, Z. X-ray diffraction patterns of graphite and turbostratic carbon. *Carbon* **2005**, *45*, 1686-1695.
324. Biegun, M.; Dymerska, A.; Chen, X.; Mijowska, E. Study of the Active Carbon from Used Coffee Grounds as the Active Materialfor a High-Temperature Stable Supercapacitorwith Ionic-Liquid Electrolyte. *Materials* **2020**, *13*.
325. Chen, X.; Yu, J.; Zhang, Z.; Lu, C. Study on structure and thermal stability properties of cellulose fibers from rice straw. *Carbohydrate Polymers* **2011**, *85*, 245-250.
326. Block, I.; Günter, C.; Rodrigues, A.D.; Paasch, S.; Hesemann, P. Carbon Adsorbents from Spent Coffee for Removal of Methylene Blue and Methyl Orange fromWater. *Materials* **2021**, *14*.
327. Thommes, M.; Kaneko, K.; Neimark, A.V.; Olivier, J.P.; Rodriguez-Reinoso, F.; Rouquerol, J.; Sing, K.S.W. Physisorption of gases, with special reference to the evaluation of surface area and pore size distribution (IUPAC Technical Report). *Pure Appl. Chem* **2015**, *87*, 1051-1069.
328. Ma, H.T.; Ho, V.T.T.; Pham, N.B.; Nguyen, D.C.; Vo, K.T.D.; Ly, H.C.; Phan, T.D. Effect of the carbonization and activation processes on the adsorption capacity of rice husk activated carbon. *Vietnam Journal of Science and Technology* **2017**, *55*, 485-493.

329. Seankham, S.; Novalin, S.; Pruksasri, S. Kinetics and Adsorption Isotherm of Lactic Acid from fermentation broth onto activated choracoal *Chemical Industry & Chemical Engineergin Quarterly* **2017**, *23*, 515-521.
330. Gao, M.-T.; Shimamura, T.; Ishida, N.; Takahashi, H. pH-uncontrolled lactic acid fermentation with activated carbon as an adsorbent. *Enzyme and Microbial Technology* **2011**, *48*, 526-530.
331. Bayazit, S.S.; Inci, I.; Uslu, H. Adsorption of Lactic Acid from Model Fermentation Broth onto Activated Carbon and Amberlite IRA-67. *Journal of Chemical & Engineering Data* **2011**, *56*, 1751-1754.
332. Dabrowski, A.; Podkoscielny, P.; Hubicki, Z.; Barczak, M. Adsorption of phenolic compounds by activated carbon—a critical review. *Chemosphere* **2003**, *58*, 1049-1070.
333. Stone, M.T.; Kozlov, M. Separating Proteins with Activated Carbon. *Langmuir* **2014**, *30*, 8046-8055.
334. sekaran, G.; Mariappan, M.; Raghavan, K.V. Adsorption of Bovine Serum Albumin from salt solution onto activated carbon *Bioprocess Engineering* **1996**, *15*, 165-169.
335. Seredych, M.; Mikhalovska, L.; Mikhalovsky, S.; Gogotsi, Y. Adsorption of Bovine Serum Albumin on Carbon-Based Materials. *Journal of Carbon Research* **2017**, *4*.
336. Piai, L.; Dykstra, J.E.; Adishakti, M.G.; Blokland, M.; Langenhoff, A.A.M.; Wal, A.v.d. Diffusion of hydrophilic organic micropollutants in granular activated carbon with different pore sizes. *Water Research* **2019**, *162*, 518-527.
337. Lian, F.; Chang, C.; Du, Y.; Zhu, L.; Xing, B.; Liu, C. Adsorptive removal of hydrophobic organic compounds by carbonaceous adsorbents: A comparative study of waste-polymer-based, coal based activated carbon, and carbon nanotubes. *Journal of Environmental Sciences* **2012**, *24*, 1549-1558.

Curriculum vitae

The pages 125 - 126 (curriculum vitae) contain personal information and are therefore not part of the online publication.

List of publications

- Figueroa Campos, G.A. et al (2021) *Preparation of Activated Carbons from Spent Coffee Grounds and Coffee Parchment and Assessment of Their Adsorbent Efficiency.* Processes 2021, 9, 1396. https://doi.org/10.3390/pr9081396
- Figueroa Campos, G.A. et al (2020) *Comparison of Batch and Continuous Wet-Processing of Coffee: Changes in the main compounds in Beans, By-Products and Wastewater.* Foods 9, 1135. https://doi.org/10.3390/foods9081135
- Figueroa Campos, G.A. (2016). *Proposed Integrated Solid Waste Management for the City of Jalapa.* Magazine of the School of Postgraduate studies, Engineering School, University of San Carlos of Guatemala.
- Figueroa Campos, G.A. (2015). *Energetic content of municipal solid waste in the City of Jalapa, Guatemala*. Magazine of the School of Postgraduate studies, Engineering School, University of San Carlos of Guatemala.
- Figueroa Campos, G.A. (2015). *Guidelines for final disposal of municipal solid waste, in the city of Jalapa*. Water, sanitation and environment Magazine of the Regional School of Sanitary Engineering and Water Resources of the University of San Carlos of Guatemala.
- Figueroa G.A. (2015). *Pollution load of municipal solid waste in the City of Jalapa, Guatemala*. Technology and Health magazine of the General Directorate of Research of the University of San Carlos of Guatemala.

Acknowledgements

The page 129 (Acknowledgements) contains personal information and is therefore not part of the online publication.

Affidavit

I hereby certify that I have written this scientific paper independently and without the help of help of third parties. Other than the indicated sources and aids were not used. The sections taken verbatim or in terms of content from the sources used are marked as such.

This scientific work has not been submitted to any examination authority in the same or a similar form and has not been published.

Gustavo A. Figueroa Campos

Potsdam, 29.11.2021

8. Appendix

8.1. List of chemicals

Name	Manufacturer
Acetonitrile	VWR International, Pennsylvania, United States
Formic acid	Acros Organics B.V.B.A., New Jersey, United States
Ammonium acetate	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Bovine Serum albumin	Sigma-Aldrich, Buchs, Switzerland
Chromabond® Sorbent C_{18}	Macherey-Nagel GmbH & Co., Düren, Germany
Coomassie Brilliant Blue G-250	SERVA Electrophoresis, Heidelberg, Germany
Coomassie Brilliant Blue R-250	GE Healthcare, Illinois, United States
DTT sample buffer	Thermo Fisher Scientific, Massachusetts, United States
Ethanol 96%	VWR International, Pennsylvania, United States
Acetic acid	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Folin & Ciocalteu´s phenol reagent	Sigma-Aldrich, Buchs, Switzerland
GWGG (H-Gly-Trp-Gly-Gly-OH)	Bachem AG, Bubendorf, Switzerland
Urea	Merck Group, Darmstadt, Germany
Iodoacetamide	Sigma-Aldrich, Buchs, Switzerland
Iron III chloride	Sigma-Aldrich, Buchs, Switzerland
Potassium iodide	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Copper sulfate (II) sulfate pentahydrate	Merk Group, Darmstadt, Germany
L-cysteine	Sigma-Aldrich, Buchs, Switzerland
L-Glutathione, reduced	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Methanol	VWR, Pennsylvania, United States
N-Acetylcysteine	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
n-Hexane	Avantor, Pennsylvania, United States

Name	Manufacturer
Natrium hydroxide	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Ninhydrin	SERVA Electrophoresis, Heidelberg, Germany
NuPAGE™ LDS-sample buffer (4x)	Thermo Fisher Scientific, Massachusetts, United States
NuPAGE™ LDS-sample buffer (20x)	Thermo Fisher Scientific, Massachusetts, United States
PageRuler™ Plus Protein-Standard (10-250 kDa)	Thermo Fisher Scientific, Massachusetts, United States
Pepsine	Promega GmbH, Walldorf, Germany
Peptide calibration standard II	Buker, Massachusetts, United States
Pronase (Streptomyces protease griseus)	Sigma-Aldrich, Buchs, Switzerland
Polyvinyl polypyrrolidone	Sigma-Aldrich, Buchs, Switzerland
Hydrochloric acid	Merck Group, Darmstadt, Germany
SDS	SERVA Electrophoresis, Heidelberg, Germany
Trichloroacetic acid	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Trifluoroacetic acid (TFA)	Acros Organics B.V.B.A., New Jersey, United States
Tris-(hydroxymethyl)-phosphine Hydrocholoride (Tris)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Trypsin	Amresco, Ohio, United States
TPTZ (2,4,6-Tris(2-pyridyl)-s-Triazine)	Sigma-Aldrich, Buchs, Switzerland
Caffeine	Sigma-Aldrich, Buchs, Switzerland
Chlorogenic acid hemihydrate	Sigma-Aldrich, Buchs, Switzerland
Citric acid	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Acetic acid	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Gallic acid monohydrate	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Malic acid	Sigma-Aldrich, Buchs, Switzerland
Propionic acid	Sigma-Aldrich, Buchs, Switzerland
Lactic acid	Alfa Aesar, Massachusetts, United States

Name	Manufacturer
Butyric acid	Alfa Aesar, Massachusetts, United States
Calcium carbonate	VWR, Pennsylvania, United States
Commercial carbon Norit®GAC 1240EN	CABOTCORP, Boston, Massachusetts, United States
Commercial carbon Merk	Merck Group, Darmstadt, Germany

8.2. List of buffer solutions

Total phenolic compounds by Folin -Ciocalteu

Folin solution:

Folin-Ciocalteu reagent	11.25 mL
NaOH 0.01 M	9.00 mL

Reducing sugars by dinitrosalicylic method

Acetate buffer (0.05 M):

Sodium acetate	0.41 g
Dist. H_2O	60 mL

Adjust to pH 4.8 with glacial acetic acid

Fill up to 100 mL with distilled H_2O

DNS reagent :

3,4-dinitrosalicylic acid	1.0 g
Potassium sodium tartrate tetrahydrate	40.3 g
NaOH	1.6 g
Dist. H_2O	100 mL

Antioxidant capacity by FRAP method

FRAP reagent:

Acetate buffer (0.25 M):

Glacial acetic acid	1.5 g

Adjust to pH 3.6 with NaOH

Fill up to 100 mL with distilled H_2O

$FeCl_3.6H_2O$ (20 mM):

Iron (III)-chloride	54.06 mg
Acetate buffer	10 mL

TPTZ (10 mM):

TPTZ	31.33 mg
40 mM Hydrochloric acid (HCl)	10.00 mL

For FRAP reagent mix 10 mL of $FeCl_3.6H_2O$ (20 mM) with 10 mL of TPTZ (10 mM)

Free amino groups by Fluorescamine assay

Fluorescamine solution:

Fluorescamine	30 mg
Dimethyl sulfoxide	10 mL

PBS buffer (5 mM):

Dipotassium hydrogen phosphate	0.714 g
Potassium dihydrogen phosphate	0.123 g
Sodium Chloride (NaCl)	8.766 g
Dist. H_2O	1000 mL

pH (7.2- 7.4)

Protein content by Lowry method

Copper tartrate complex (CTC):

Solution A:

Copper sulfate	0.1 g
Sodium tartrate	0.2 g
Sodium carbonate	10 g
Dist. H_2O	40 mL

Solution B:

Sodium carbonate	10 g
Dist. H_2O	40 mL

For CTC solution mix solution A and solution B

Analytic solution:

Reagent A:

CTC solution	1 part
0.8 mol/L NaOH	2 parts

Reagent B:

Folin-Cioalteu reagent	1 part
Dist. H_2O	5 parts

SDS-PAGE

SDS sample buffer:

SDS	2 g (0.139 M)
Tris	305 mg (50.247 M)
Dist. H_2O	30 mL
HCl/NaOH	adjust pH to 6.8
DTT-sample reducing reagent	5 mL (0.005 M)
Fill up to 50 mL with dist. H_2O	

Running buffer:

MES SDS running buffer	50 mL
Dist. H_2O	950 mL

Coomassie color solution:

Coomassie brilliant blue R-250	1 pellet
Acetic acid 10%	2 L

In-gel digestion

Colloidal Coomassie Blue color reagent:

Solution A:

$(NH_4)_2SO_4$	8 g
Dist. H_2O	76.8 mL
Phosphoric acid (85%)	1.6 mL

Solution B:

Coomassie brilliant blue G 250	0.5 g
Dist. H_2O	9.5 mL

Mix solution A with 1.6 mL solution B and add 20 mL methanol

Destaining solution:

Ammonium bicarbonate	80 mg
Acetonitrile	20 mL
Dist. H_2O	20 mL

Activated Trypsin:

Trypsin storage solution:

Dist. H_2O	915 µL
37 % (v/v) HCl	85 µL

Trypsin stock solution:

Trypsin	20 µg
Trypsin storage solution	20 µL

Trypsin working solution:

Trypsin stock solution	20 µL
Dist. H_2O	45 µL
Activated Trypsin:	
Trypsin working solution	1 µL
Digestive buffer	9 µL
Activated Pepsin:	
Pepsin storage solution:	
Dist. H_2O	915 µL
37 % (v/v) HCl	85 µL
Pepsin stock solution:	
Pepsin	20 µg
Pepsin storage solution	20 µL
Pepsin working solution:	
Pepsin stock solution	20 µL
Dist. H_2O	45 µL
Activated pepsin:	
Pepsin working solution	1 µL
0.1 M HCl	9 µL
Digestive buffer (25 mM):	
Ammonium bicarbonate	10 mg
Dist. H_2O	5 mL
Reduction buffer:	
TCEP	2 mg
Digestive buffer (25 mM)	1189 µL
Alkylation buffer:	
IAA	20 mg
Digestive buffer (25 mM)	1500 mL

MALDI-TOF-MS

Matrix solution:	
0.1 % TFA	700 µL
Acetonitrile	300 µL
HCCA	20 mg

Free amino nitrogen

Glycine stock solution (2 mg/L):	
Glycine	2 mg

Dist. H_2O	1870 µL
Dilute 20 µL with 1980 µL with Dist. H_2O	
Ninhydrin color reagent:	
Na_2HPO_4	2 mg
KH_2PO_4	0.6 g
Ninhydrin	50 mg
Fructose	30 mg
Dist. H_2O	10 mL
Potassium iodide solution:	
Potassium iodide	20 mg
Ethanol 96 %	4 mL
Dist. H_2O	6 mL

Free thiol groups

Tris-buffer (0.2 M)	
Tris	1.2114 g
Dist. H_2O	40 mL
Modify with 1 M HCl to pH 8	
Fill up to 50 mL with distilled H_2O	
0.2 M Tris-SDS buffer:	
Tris	1.2114 g
SDS	0.5 g
Dist. H_2O	40 mL
Modify with 1 M HCl to pH 8	
Fill up to 50 mL with distilled H_2O	
DTNB buffer:	
DTNB	50 mg
Ethanol 96 %	50 mL

In-solution digestion

Trypsin solution:	
Trypsin	40 mg
Dist. H_2O	10 mL
Digestive buffer (0.1 M):	
Ammonium carbonate	1.304 g
Dist. H_2O	140 mL
Digestive buffer (50 mM):	

Ammonium carbonate	0.396 g
Dist. H_2O	100 mL
Modify with 1 M HCl to pH 8.5	
Pronase solution:	
Pronase	1 mg
Digestive buffer (50 mM)	100 mL

Solid phase extraction

SPE buffer A:	
Acetonitrile	100 mL
Dist. H_2O	100 mL
0.1 % Formic acid	200 µL
SPE buffer B:	
Acetonitrile	200 mL
0.1 % Formic acid	200 µL

8.3. List of software

Name	**Manufacturer**
BioTools 3.2	Bruker, Massachusetts, United States
flexAnalysis 3.3	Bruker, Massachusetts, United States
flexControl 3.4	Bruker, Massachusetts, United States
GraphPad Prism 6.01	GraphPad Software, San Diego, California, United States
Origin Pro 2019b	OriginLab Corporation, Massachusetts, United States
ImageLab 6.01	Bio-Rad Laboratories Ltd., Hertfordshire, United Kingdom
Skyline 20.2	MacCoss Lab, Department of Genome Science, University of Washington, Washington, United States
UniProt	European Bioinformatics Institute, Hinxton, United Kingdom

8.3.1. FlexControl software parameters

Parameter	Condition
Rage of masses	300-6200 Da
Mode	Reflect mode
Sample rate	0.5 GS/s
Laser intensity	30-40%

8.3.2. FlexAnalysis software parameters

Parameter	Condition
Peak detection algorithm	Snap
Signal to noise ratio upper limit	6
Baseline sub action algorithm	TopHat
Smoothing algorithm	SavitzkyGolay
Calibration mode	Expanded cubically

8.3.3. Parameters of Mascot database compared with BioTools

Parameter	Condition
Search title	Coffea
Taxonomy	Other green plants
Data bank	Swiss Prot
Enzyme	Trypsin or Pepsin A
Partials	0-2
Global modification	Carbamidomethyl (N-terminal)
Variable modification	Carboxymethyl (C)
Mass tolerance	500 ppm
Mass units	MH^+

8.4. Lists of instruments

Instrument / device	Manufacturer
Blender	Moulinex, Écully, France
Centrifuge 3K20	Sigma-Aldrich, St. Louis, Missouri, United States
Photometer BioRAD iMark ™ – Microplate Reader	Bio-RAD Laboratories GmbH, Hercules, California, United Sates
Photometer BioTek Power Wave XS2	BioTek, Winooski, Vermont, United States
pH- Meter SevenEase S20	Mettler Toledo, Columbus, Ohio, United States
Scale Sartorius Bais	Sartorius AG, Göttingen, Germany
Shaker device neolab® Intellimixer iMX	neoLab Migge GmbH, Heidelberg, Germany
Rotator neoVortex® 0-2500 UpM (D-6012)	neoLab Migge GmbH, Heidelberg, Germany
Electrophoresis chamber	Thermo Fisher Scientific, Waltham Massachusetts, United Sates
Visiprep™ extraction device	Merck Group, Darmstadt, Germany
Freeze Dreyer Alpha 1-4	Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany
Gel-Scanner	SERVA Electrophoresis GmbH, Heidelberg, Germany
Non-returnable Cuvettes	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Magnetic stirrer with hot plate	IKA Werke GmbH & Co. KG, Staufen, Germany
NuPAGETM 12% Bis-Tris-Gel	Thermo Fisher Scientific, Waltham Massachusetts, United Sates
Pipettes (0.5 – 10 µL; 10 – 100 µL; 1000µL)	Eppendorf, Hamburg, Germany
Multi-Channel Pipette (20- 200 µL)	Corning HTL SA, Warszawa, Poland
5 mL Pipette	VWR International, Pennsylvania, United States
Ultrasonic bath	BANDELIN electronic GmbH & Co. KG, Berlin, Germany
Vortex mixer TX4 Digital IR	VELP Scientifica Srl, Usmate Velate MB, Italy
Vortex mixer D-6012	neoLab Migge GmbH, Heidelberg, Germany
Dryer oven Memmert Modell 400	Memmert GmbH + Co.KG, Büchenbach, Germany

Instrument / device	Manufacturer
Pyrolysis oven Linn High Therm LM-412	Linn High Therm GmbH, Hirschbach, Germany
Scanning Electron Microscopy JEOL JSM-6510	JEOL Ltd. Tokyo, Japan

8.4.1. Lists of HPLC instruments

Instrument / device	Manufacturer
Autoinjector, SIL 10A	Shimadzu, Kyoto, Japan
Control unit, SCL 10A VP	Shimadzu, Kyoto, Japan
Degasser, DGU 20A 5	Shimadzu, Kyoto, Japan
UV-Vis detector, SPD-M20A	Shimadzu, Kyoto, Japan
Column oven, CTO 10ASVP	Shimadzu, Kyoto, Japan
Pump, LC 10AD	Shimadzu, Kyoto, Japan
Column, 250 x 3mm PerfectSil 300 C8 5µm	MZ-Analysentechnik GmbH, Mainz, Germany
Column, 250 x 4.6mm 6% crosslinked, SUPELCOGEL H	Sigma-Aldrich, St. Luis, Missouri, United States

8.5. Conditions for HPLC

8.5.1. HPLC analysis of phenolic compounds

Parameter	Conditions
Mobile phase	0.1 %TFA (A), CH_3OH (B)
Column temperature	40° C
Flowrate	1 mL/min
Running time	45 min
Injection volume	10 µL

8.5.2. HPLC analysis of phenolic compounds in protein extraction

Parameter	Conditions
Mobile phase	0.1 %TFA (A), CH_3OH (B)
Column temperature	37° C
Flowrate	0.6 mL/min
Running time	20 min
Injection volume	10 µL

8.5.3. Gradient A of HPLC

Time (min)	A (0.1 % TFA) (%)	B (Methanol) (%)
0	80	20
3	80	20
20	65	35
37	32	68
40	32	68
43	80	20
45	80	20

8.5.4. Gradient 1 of HPLC

Time (min)	A (0.1 % TFA) (%)	B (Acetonitrile) (%)
0	90	20
22	82	20
30	20	35
33	20	68
35	90	68
42	90	20

8.5.5. Gradient 2 of HPLC

Time (min)	A (0.1 % TFA) (%)	B (Acetonitrile) (%)
0	90	10
7	85	15
15	20	80
18	20	80
19	90	10
28	90	10

8.5.6. Gradient 3 of HPLC

Time (min)	A (0.1 % TFA) (%)	B (Acetonitrile) (%)
0	90	10
5	85	15
10	20	80
12	20	80
12.5	90	10
20	90	10

8.5.7. Gradient 4 of HPLC

Time (min)	A (0.1 % TFA) (%)	B (Acetonitrile) (%)
0	90	10
6	87.5	12.5
10	60	40
15	50	50
18	50	50
19	90	10
26	90	10

8.5.8. Gradient 5 of HPLC

Time (min)	A (0.1 % TFA) (%)	B (Acetonitrile) (%)
0	100	0
3	100	0
7	60	40
10	60	40
11	20	80
13	20	80
14	100	0
17	100	0

8.5.9. Gradient 6 of HPLC

Time (min)	A (0.1 % TFA) (%)	B (Acetonitrile) (%)
0	100	0
3	100	0
7	60	40
10	60	40
11	20	80
13	20	80
14	100	0
20	100	0

8.5.10. Conditions of the HPLC analysis of organic acids

Parameter	Conditions
Mobile phase	0.01 N H_2SO_4 (isocratic)
Column temperature	70° C
Flowrate	0.5 mL/min
Running time	15 min
Injection volume	50 µL

8.6. MALDI-TOF-MS instrument

Instrument / device	Manufacturer
Autoflex speed	Bruker, Massachusetts, United States
Smartbeam2 Laser, version 20110	Bruker, Massachusetts, United States
MALDI plate MTP 384 polished steel	Bruker, Massachusetts, United States
MALDI plate holder, T_0209520_0021715_0	Bruker, Massachusetts, United States

8.7. Standard peptide of the peptide calibration standard II

Peptide	Masse[1] $[M+H]^+$ [Da]
Bradykinin 1-7	757.3992
Angiotensin II	1046.5418
Angiotensin I	1296.6848
Substance P	1347.7354
Bombesin	11619.8223
Renin substance	1758.93326
ACTH 1-17	2093.0862
ACTH 18-39	2465.1983
Somatostatin 28	3147.470

[1]monoisotopic

8.8. HPLC-MS/MS parameters

Instrument / device	Manufacturer
HPLC Agilent Infinity 1260 system service	Agilent Technologies Sales Co. KG, Waldbronn, Germany
Kinetex C_8 column 150 x 4.6mm; 2.6 µm	Phenomenex, Torrance, California, United States
Triple Quadrupole Agilent G6470A series services GmbH	Agilent Technologies Sales Co. KG, Waldbronn, Germany

8.8.1. HPLC conditions of the HPLC-MS/MS I

Parameter	Condition
Mobile phase	0.1% formic acid (A), acetonitrile (B)
Column temperature	30° C
Flowrate	0.5 mL/min
Running time	28 min
Injection volume	10 µL

8.8.2. HPLC conditions of the HPLC-MS/MS II

Parameter	Condition
Mobile phase	0.1% formic acid (A), acetonitrile (B)
Column temperature	30° C
Flowrate	0.5 mL/min

Parameter	Condition
Running time	15 min
Injection volume	0.5 µL

8.8.3. Gradient I of HPLC-MS/MS mobile phase

Time (min)	A [%] 0.1 % formic acid	B [%] acetonitrile
0	100	0
5	100	0
20	50	50
21	5	95
24	5	95
25	100	0
28	100	0

8.8.4. Gradient II of HPLC-MS/MS mobile phase

Time (min)	A [%] 0.1 % formic acid	B [%] acetonitrile
0	100	0
4	100	0
10	0	100
13	0	100
14	100	0
15	100	0

8.8.5. ESI conditions I

Parameter	Condition
Ionization mode	Positive
Temperature	275° C
Gas	Nitrogen
Gas flowrate	11.0 L/min
Capillary tension	5425 nA
Nebulizing pressure	35.0 psi

8.8.6. ESI conditions II

Parameter	Condition
Ionization mode	Positive and negative
Temperature	275° C

Parameter	Condition
Gas	Nitrogen
Gas flowrate	11.0 L/min
Capillary tension	5425 nA
Nebulizing pressure	35.0 psi

8.8.7. MS/MS conditions

Parameter	Condition
Collision gas	Nitrogen
Pressure	5,1326 mPa
Detection mode	MRM
Fragmentary tension	130 V
Collision energy	Depends on the transition
Cell accelerator voltage	5 kV
Dwell time	20 ms

8.9. Analyzed mass transition of the P93079 sequence of the 11S protein

8.9.1. α-chain

Peptide sequence	Q1 (m/z)	Q3 (m/z)	Retention time (min)
Without modification of the lysine side chain			
R.LGGK.T	187.6	261.1	4.2
		204.1	
		147.1	
K.TQCNIQK.L	446.2	662.3	11.5
		502.3	
		388.3	
		275.2	
		147.1	
K.LNAQEPSFR.F	531.3	834.4	14.2
		763.4	
		635.3	
		506.3	
		409.2	
		322.2	
R.NTVQPK.G	343.7	572.3	11.1

Peptide sequence	Q1 (m/z)	Q3 (m/z)	Retention time (min)
		471.3	
		372.2	
R.LPHYSNVPK.F	527.8	941.5	13.3
		707.4	
		544.3	
		457.3	
		343.2	
K.GQEGSK.G	303.1	548.3	12.2
		420.2	
		291.2	
		234.1	
		147.1	
R.FQK.G	211.6	275.2	8.8
		147.1	
K.FFLAGMPQQGGK.E	660.8	785.4	15.4
		671.3	
		574.3	
		446.2	
		318.2	
K.IIQK.L	251.2	388.3	11.2
		275.5	
		147.1	
CQA-Lysin adducts			
R.LGG**K**.T	363.7	613.2	12.4
		556.2	
		499.2	
R.LPHYSNVP**K**.F	703.8	1293.6	15.8
		896.4	
		809.4	
		695.3	
K.GQEGS**K**.G	479.1	772.3	16.3
		643.2	
		586.2	
		499.2	
K.FFLAGNPQQGG**K**.E	836.9	1137.5	20.4

Peptide sequence	Q1 (m/z)	Q3 (m/z)	Retention time (min)
		1023.4	
		926.4	
		798.3	
		670.3	
K.IIQ**K**.L	427.2	740.3	14.3
		627.3	
		499.2	
diCQA-Lysin adducts			
R.LGG**K**.T	528.7	943.3	15.4
		886.3	
		829.2	
K.TQCNIQ**K**.L	787.3	1344.4	14.9
		1184.4	
		1070.4	
		957.3	
		829.2	

8.9.2. β-chain

Peptide sequence	Q1 (m/z)	Q3 (m/z)	Retention time (min)
Without modification of the lysine side chain			
K.LSEMIGLPQEADVFNPR.A	950.0	1172.6	16.8
		632.4	
		386.2	
R.ITTVNSQK.I	445.8	676.4	11.9
		575.3	
		476.2	
		362.2	
		529.3	
		616.3	
K.IPILSSLQLSAER.G	713.9	1313.7	17.4
		903.5	
		816.5	

Peptide sequence	Q1 (m/z)	Q3 (m/z)	Retention time (min)
		703.4	
		575.3	
		462.2	
R.IQVVDHK.G	419.7	597.3	12.4
		498.3	
		399.2	
		440.3	
		555.3	
K.VFDDEVK.Q	426.2	605.3	13.8
		490.3	
		375.2	
		477.2	
		606.2	
K.AGNEGFEYVAFK.T	666.3	903.5	16.2
		756.4	
		627.4	
		464.4	
		365.2	
K.TNDNAMINPLVGR.L	707.9	899.5	15.9
		768.4	
		655.4	
		541.3	
R.LSALR.A	280.2	446.3	13.3
		359.2	
		175.1	
R.AIPEEVLR.S	463.8	742.4	15.1
		645.4	
		516.3	
R.SSFQISSEEAEELK.Y	792.4	934.4	15.4
		847.4	
		718.3	
		589.3	
		518.2	
		389.2	
R.QEALLLSEQSQQGK.R	779.9	891.4	15.0

Peptide sequence	Q1 (m/z)	Q3 (m/z)	Retention time (min)
		804.4	
		675.3	
		547.3	
		460.3	
		442.2	
		668.4	
CQA-Lysin adducts			
K.VFDDEV**K**.Q	602.3	727.3	17.0
		598.3	
		499.2	
		477.2	
		606.2	
diCQA-Lysin adducts			
R.ITTVNSQ**K**.I	786.8	1044.3	14.9
		957.3	
		829.3	
		529.3	
		626.3	

8.10. Physicochemical characteristics of coffee wastewater

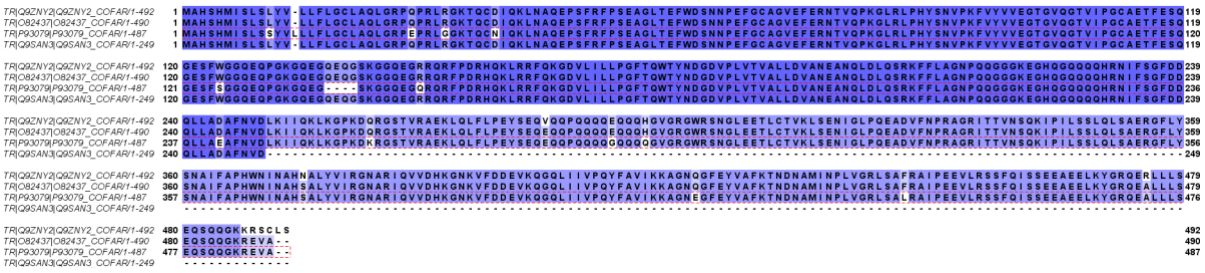
Batch process	T (° C)	pH	ORP (mV)	COD (mg/L)	BOD (mg/L)	COD/BOD	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Suspended solids (mg/L)	Dissolved solids (mg/L)	Dissolved oxygen (mg/L)
Sampling year 2018											
Depulping coffee wastewater	16.2	4.4	192.1	17,290.0	8,440.0	2.1	18.8	147.5	2,421.5	451.4	1.8
Fermentation coffee wastewater	17.8	4.4	190.3	26,780.0	12,181.0	2.2	51.1	29.0	5,250.0	1,062.2	1.7
Washing coffee wastewater	16.8	4.8	293.3	16,260.0	6,410.0	2.5	27.5	158.5	4,475.0	196.6	2.3
Sampling year 2019											
Depulping coffee wastewater	20.4	4.3	328.0	64,800.0	40,650.0	1.6	24.5	785.0	13,000.0	1,632.0	1.7
Fermentation coffee wastewater	20.4	4.3	334.0	73,050.0	45,900.0	1.6	33.5	765.0	14,150.0	1,627.0	1.6
Washing coffee wastewater	19.1	4.4	319.9	7,120.0	3,635.0	2.0	7.5	260.0	7,900.0	276.0	2.6

Temperature, pH, ORP, and dissolved oxygen along both processes were measured in-situ using a portable multiparameter HI98194 (HANNA instruments, Woonsocket, RI, USA). Analysis of COD, BOD, total phosphorus, total nitrogen, suspended solids, dissolved solids along both processes were performed in the laboratory facilities of the Regional School of Sanitary Engineering and Water Resources (ERIS), University of San Carlos of Guatemala, Guatemala City, Guatemala.

Continuous process	T (° C)	pH	ORP (mV)	COD (mg/L)	BOD (mg/L)	COD/BOD	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Suspended solids (mg/L)	Dissolved solids (mg/L)	Dissolved oxygen (mg/L)
Sampling year 2018											
Depulping coffee wastewater	18.4	6.0	235.3	10,925.0	5,615.0	1.9	112.0	71.0	2,775.0	347.0	5.8
Fermentation coffee wastewater	17.5	6.2	249.4	10,220.0	5,505.0	1.9	106.0	70.0	1,210.0	251.0	6.3
Washing coffee wastewater	17.1	4.6	270.0	5,860.0	1,975.0	3.0	93.0	61.0	1,070.0	247.0	6.0
Sampling year 2019											
Depulping coffee wastewater	16.2	6.3	295.4	14,060.0	6,030.0	2.3	571.0	73.0	680.0	221.1	4.2
Fermentation coffee wastewater	15.8	6.4	292.6	15,240.0	6,300.0	2.4	361.0	101.0	862.0	197.0	3.9
Washing coffee wastewater	14.4	6.3	165.3	1,210.0	566.0	2.1	50.0	22.0	218.0	142.4	4.3

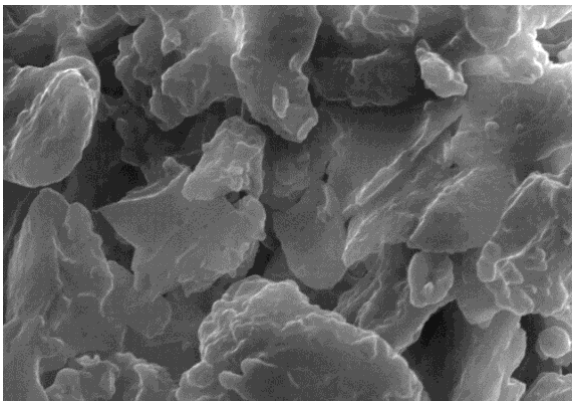
Temperature, pH, ORP, and dissolved oxygen along both processes were measured in situ using a portable multiparameter HI98194 (HANNA instruments, Woonsocket, RI, USA). Analysis of COD, BOD, total phosphorus, total nitrogen, suspended solids, dissolved solids along both processes were performed in the laboratory facilities of the Regional School of Sanitary Engineering and Water Resources (ERIS), University of San Carlos of Guatemala, Guatemala City, Guatemala

8.11. FASTA sequence from the UniProt database of the 11S

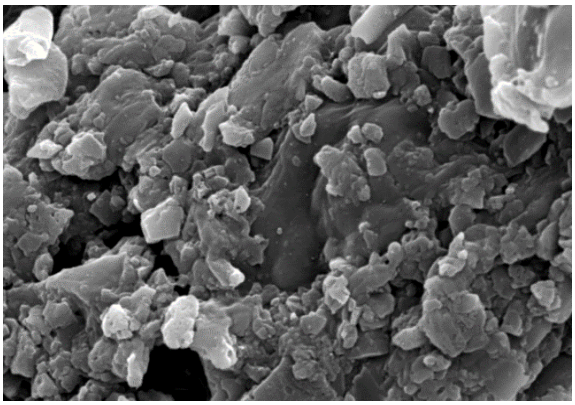


8.12. Scanning Electron Microscope images of activated carbon materials

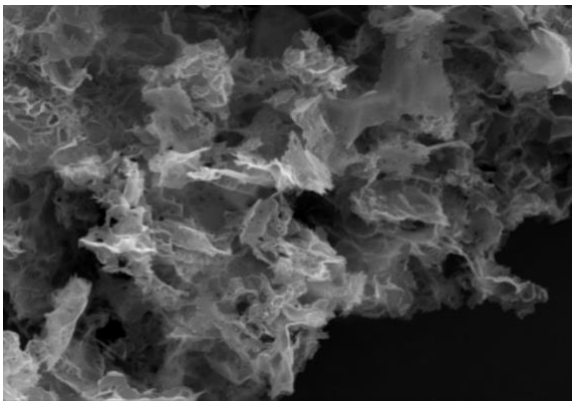
8.12.1. SEM of spent coffee grounds



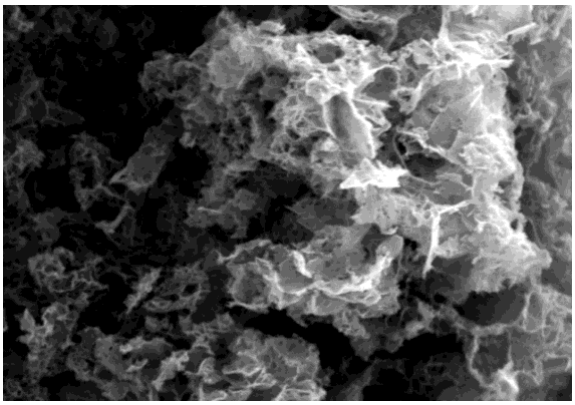
(a)



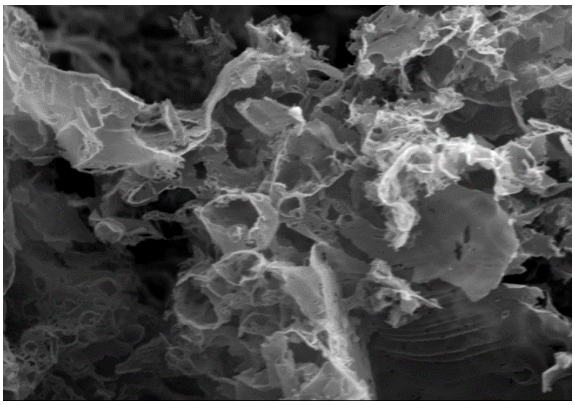
(b)



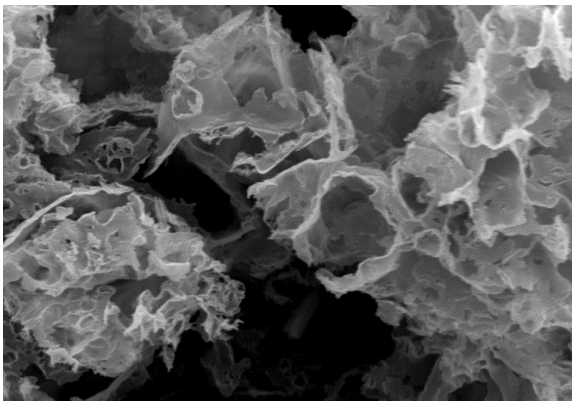
(c)



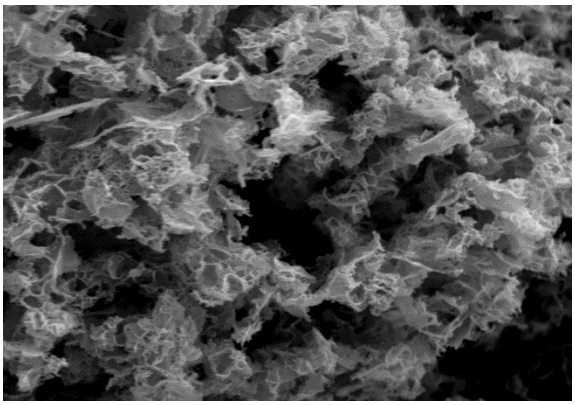
(d)



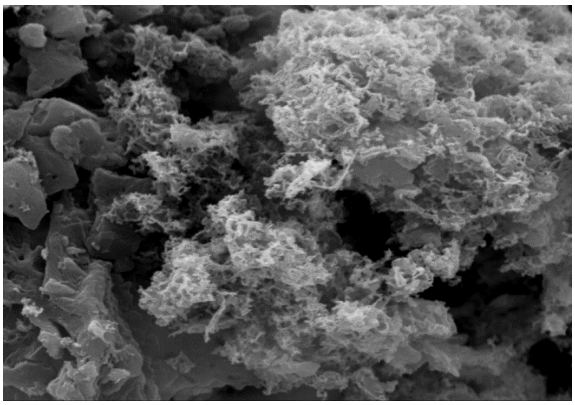
(e)



(f)



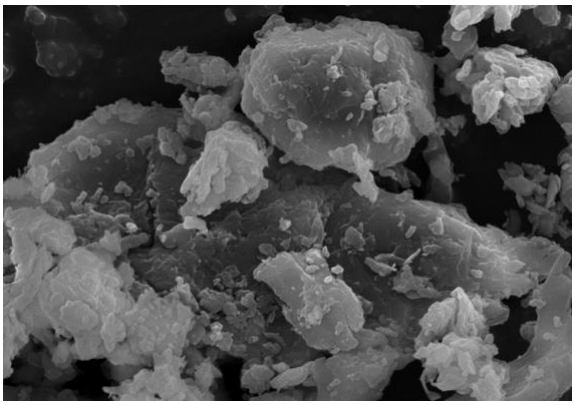
(g)



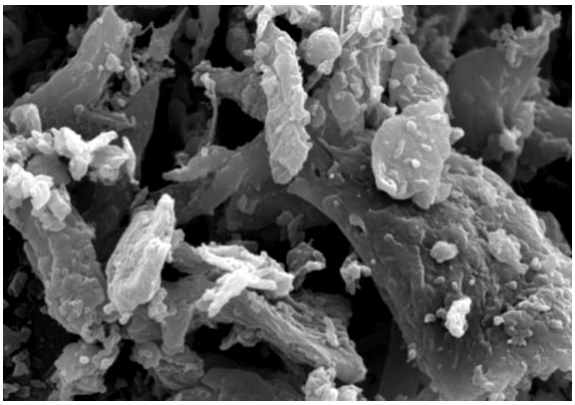
(h)

Scanning electron microscope (SEM) images of activated carbon produces from (a) raw SC, (b) SC0-80, (c) SC1-80, (d) SC1-85, (e) SC2-80, (f) SC2-85, (g) SC3-80, and (h) SC3-85. Images were amplified 4000 times.

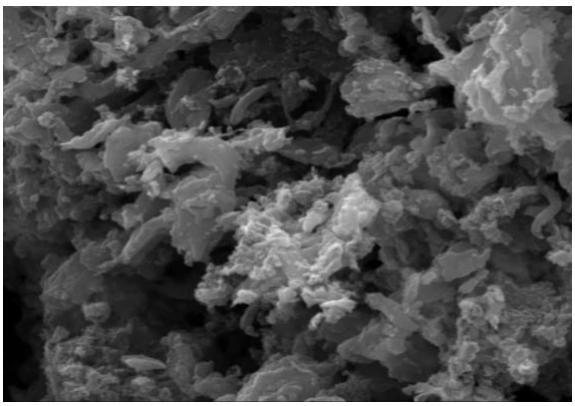
8.12.2. SEM of parchment



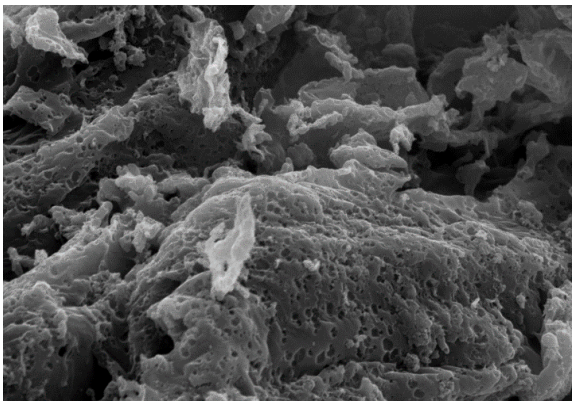
(a)



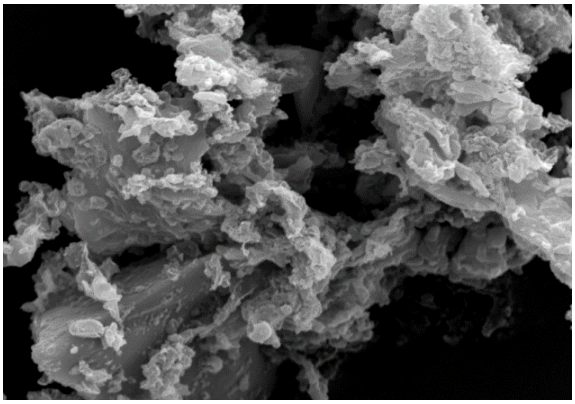
(b)



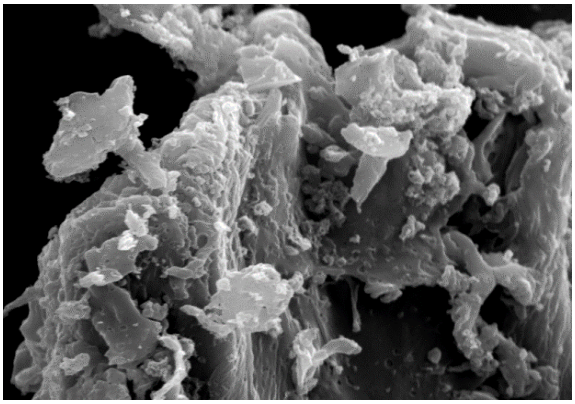
(c)



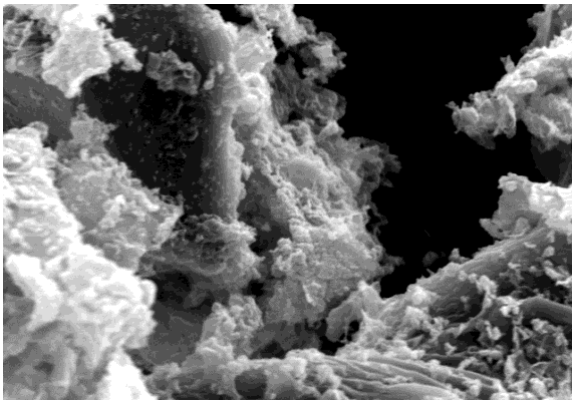
(d)



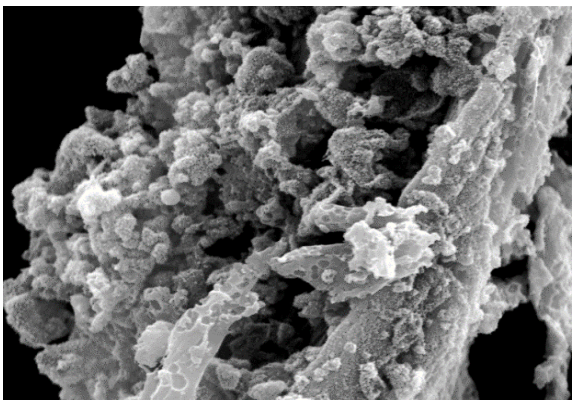
(e)



(f)



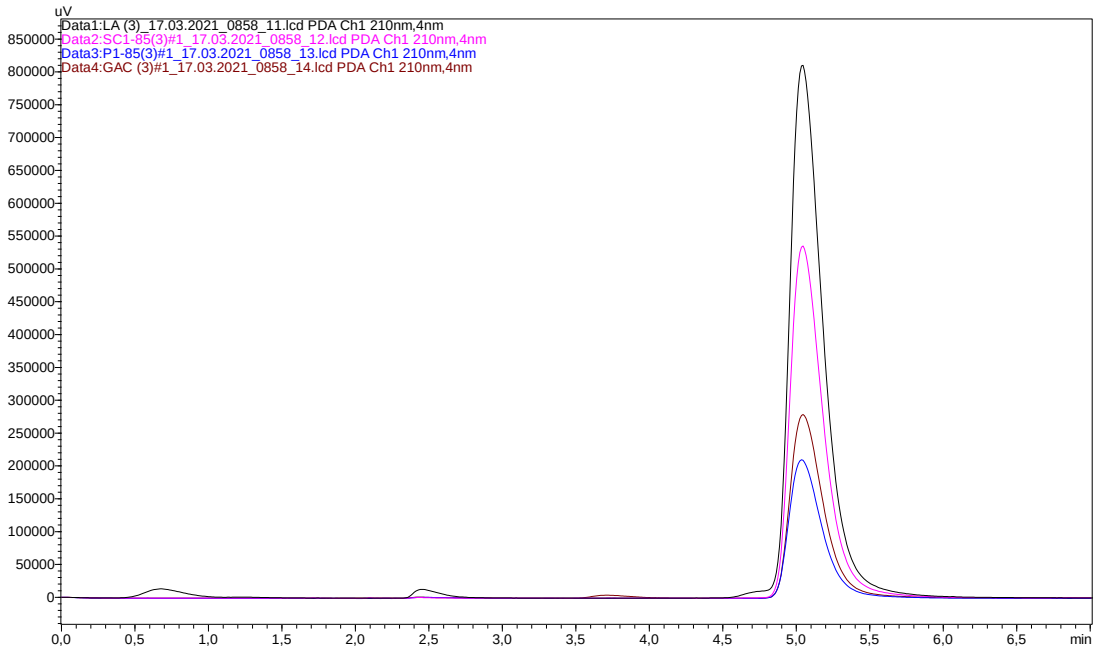
(g)



(h)

Scanning electron microscope (SEM) images of activated carbon produces from (a) raw P, (b) P0-80, (c) P1-80, (d) P1-85, (e) P2-80, (f) P2-85, (g) P3-80, and (h) P3-85. Images were amplified 4000 times.

8.12.3. Adsorption of lactic acid on activated carbon chromatogram



HPLC chromatograms of lactic acid adsorption onto spent coffee grounds, parchment, and commercial activated carbon (GAC 1240EN). No pH modification with 3.0 g/L of lactic acid in the bulk solution, adsorbent dose of 10% (*w/v*) and 4 h contact time at room temperature.