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EDITORIAL TEAM

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Comparative Study of the Protective Effect of *Cola anomala* and *Coffea arabica* Against Induced Toxicity in Rats

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Abstract

This work was aimed at evaluating the effects of *C. anomala* and *C. arabica on* Methotrexate (MTX) induced metabolic disorders. For this, the aqueous extract (AE) of *C. anomala* and *C. arabica* were prepared and their polyphenols, flavonoids and alkaloids contents determined as well as their antiradical and total antioxidant capacity. An animal experimentation using female rats was carried out for 14 days. Rats were divided into 6 groups; a negative control group receiving water; a positive control group receiving 12mg/Kg Bw of MTX; four tests groups receiving 12mg/Kg Bw of MTX and one of the extracts at a dose of 200mg/Kg Bw or 400mg/Kg Bw. At the end of the experiment, plasmas and hemolysates were prepared as well as liver and kidney homogenates for the evaluation of oxidative status (catalase, total protein and malondialdehyde (MDA)), liver toxicity (alanine amino transferase (ALT)) and renal toxicity (creatinine and urea) and lipid profile (triglycerides, total cholesterol and HDL-cholesterol). Weight gain in extract-treated rats was better with the *C. anomala*. Concerning oxidative status, MDA levels were generally lower in *C. anomala*-treated groups compared to *C. arabica* while catalase level was higher in *C. anomala*-treated rats. As for lipid profile, it is *C. arabica* that showed more or less better results. Both extracts led to an amelioration of toxicity markers compared to exclusive treatment with MTX. The results of this study suggest that *C. anomala* and *C. arabica* may reduce metabolic disorders associated with the intake of MTX during cancer treatment; *C. anomala* protecting better *C. arabica*.

Keywords: *Cola anomala*, *Coffea Arabica*, Methotrexate, metabolic disorders

1. Introduction

Caffeine is responsible for the stimulatory power of coffee and kola nut. The seeds of these two plants are highly consumed for this property. Traditionally, in western Africa, kola nut is consumed by mastication to fight physical and intellectual fatigue and to combat depression. It has also been recognised to ameliorate cognitive vigilance and performance (Bureau, 2013). The relationship between caffeine and cancer is very controversial. Some researchers have proven that the consumption of too much caffeine could affect the risk of developing cancer through various mechanisms. Still, other studies have shown the reduced risk of cancer with the consumption of coffee. The consumption of 3 to 4 cups of coffee per day is thought to reduce by 50% the risk of breast cancer in menopaused women and by 60% the risk of ovarian cancer. While for prostate cancer, this consumption could rather increase the risk of cancer development (Nehlig, 2012).

Recent research is geared at portraying the health benefits of these two plants that are attributable to the presence of bioactive secondary metabolites in their composition. It has been proven that the grains of *Coffea arabica* are an important source of biologically active metabolic molecules with many therapeutic properties. *C. arabica* contains phenolic compounds and their derivatives such as chlorogenic acid (Affonso *et al*., 2016) that are responsible for cardio protective, hepatoprotective, neuroprotective, nephroprotective properties. The presence of trigonelline in this plant has attributable antioxidant and antimicrobial properties (Najib, Ahmad & Labadjo, 2015). Plants of the family of Sterculiaceae (in which we find kola nut species) contain many bioactive molecules amongst which we find alkaloids, flavonoids and polyphenols (Fabunmi & Arotupin, 2015). Other species of kola nut have been proven to contain tannin that condenses into phlobaphenes that are responsible for the red color of the nuts. Xanthines (cafeine (triméthylxanthine) being the most abundant (2-3.5 %)), nitrogen

containing molecules are the most represented groups of alkaloids. These xanthines have been proven to be responsible for their antidiarrhetic properties. Their polyphenols provide their strong antioxidant potential. In Asia, some decaffeinated extracts are thought to be responsible for their anti-inflammatory and chemopreventive properties through their action on androgenic receptors (Bureau, 2013). Studies carried out on *Cola verticillata* showed, antiproliferative and anti-toxic properties of this species (Mbong *et al*., 2013, 2014).

Chemotherapy is one of the most used therapies in the treatment of cancers. However, side effects associated with this treatment are usually the worries of patients (Tecza, Pamula-Pilat, Lanuszewska & Grzybowska, 2015). Indeed, the toxicity of antineoplastic agents to rapidly replaceable cells such as bone marrow cells causes a large number of adverse effects in the body (Gorrini, Harris & Mak, 2013). Free radical generation and lipid peroxidation are expressed as some complications of these effects. Methotrexate (MTX) is used as a chemotherapeutic agent to treat certain cancers and auto-immune diseases. Many studies have demonstrated its toxic effects involving most body organs like the liver, kidney and blood parameters. Its side effects could be controversially related to the production of ROS (Boussios, Pentheroudakis, Katsanos & Pavlidi, 2012). Biologically, MTX causes lipid peroxidation indicated by high levels of Thiobarbituric acid reactive substances (TBARS). It also lowers levels of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase indicating oxidative stress and thus decreasing the antioxidant defense systems responsible for its many side effects (Montasser *et al*., 2017). Oxidative stress-induced lipid peroxidation leads to the deterioration of membrane permeability barrier and loss of functions. It has also been reported that oxidized lipids are markers of pathologic mechanisms in a variety of disease states (Volinsky& Kinnunen, 2013; Bochkov, Oskolkova & Birukov, 2010).

Treatment with MTX has been reported to induce in rats critical changes in biological, biochemical and hematological markers (Saka & Aouacheri, 2017).

Increasing attention is directed towards the role of plant bioactive molecules with antioxidants on the modulation of intracellular levels of reactive oxygen species resulting from cancer treatments (Saha *et al*., 2017). Plant bioactive compounds such as polyphenols, flavonoids and alkaloids can reduce oxidative stress through various mechanisms including trapping of these reactive oxygen species (Meher and Mishra, 2017). *Cola anomala* and *Coffea arabica*, two plants of the family of *Malvaceaes* and *Rubiaceaes* respectively, are widely produced in Cameroon and are used in particular for their stimulating properties. These plants have many bioactive compounds including polyphenols, flavonoids, alkaloids that give them their antioxidant properties (Najib, 2015). *C. anomala* is abundantly present in Cameroon but its consumption is more or less limited given that it is generally consumed by the elders and mainly during "traditional gatherings" compared to *C. arabica* that is more present in modern diets and is undoubtedly the most consumed beverage worldwide. Many biological activities have been attributed to both of these species and it was thought important to equally evaluate their possible biological benefits during treatment with a chemotherapeutic agent.

2. Methods

2.1 In vitro Study

This part of the study was carried out to determine the amounts of targeted bioactive molecules as well as the antioxidant potential of aqueous extracts of both plants.

2.1.1 Collection of Biological Material

The nuts of *C. anomala* were harvested in July 2017 in *Bandjoun*, Western region of Cameroon. The botanical identification was done in comparison to specimen N^{o} 113 collected in *Nkongmenek* corresponding to the sample *C. anomala K.Schum* at the Cameroon National Herbarium under voucher number 48706/HNC. After this, the nuts were washed, shade-dried, and powdered using a standard propeller crusher. The powder of roasted seeds of *C. arabica* was bought from a local seller in the Yaounde neighborhood in Cameroon.

2.2.2 Preparation of Aqueous Extracts

The extracts for this study were prepared depending on the plant sample. *C. anomala* was prepared by maceration. 300 g of powdered *C. anomala* nuts were macerated in 1.8L of distilled water for 12H at room temperature. After the marceration period, the filtrate was recuperated using Whatman paper no 3, Whatman International Limited, Kent, England. The filtrate was then oven-dried at 50°C for 72H. *C. arabica* on the other hand was prepared by infusion. 300 g of *C. arabica* powder was infused in hot water (90 °C) for 10 min. Following this, the mixture was filtered using Whatman paper N^{o} 3, Whatman International Limited, Kent, England. The obtained filtrate was equally oven-dried at 50°C for 72H. Both extracts were stored in polystyrene bags for further use.

2.2.3 Estimation of Bioactive Constituents and Antioxidant Potential

Determination of total polyphenols

The Folin-Ciocalteu reagent method described by Singleton & Rossi in 1965, was used to measure total phenolic content of the extracts. For this, 0.1 mL of extracts (4 mg/mL) was mixed with 0.75 mL of Folin-Ciocalteu (0.2N) in opaque tubes. After 5min at room temperature, 0.75 mL of sodium carbonate (6%) was added. The tubes were mixed and incubated for another 90min before measuring the optical densities at 725nm. Gallic acid was used as standard (0-1000 µg/mL). The test was done in triplicate for each extract and total phenol content was expressed in mg equivalent Gallic acid per gram dry weight of extract (mgGAE/g).

Determination of total flavonoids

The Aluminum chloride colorimetric method of Aiyegoro and Okoh, 2010 with slight modifications was exploited to assay the flavonoids content of the extracts. Briefly, 0.5 mL of various extracts (4 mg/mL) were added to 1.5 mL of methanol followed by addition of 0.1 mL of aluminum chloride ($AlCl_3$, 10%), 0.1 mL of potassium acetate (CH_3COOK, 1 M) and 2.8 mL of distilled water. The mixture was well shaken and incubated for 30 min at room temperature and its absorbance was measured at 415 nm. Quercetin was used as standard (0-1000 µg/mL) and the total flavonoids content were expressed as mg equivalent quercetin per gram dry weight of extract (mgQuerE/g).

Determination of alkaloids

The amount of alkaloids in the extracts was determined using a modified method described by Singh *et al* (Singh, Vats, Suri, Shyram, Rangganathan & Sriatharan, 2004). Summarily, 10mL of 95% ethanol were added to 100mg of extract powder after which the mixture was centrifuged at 5000 rpm for 10 min. 1 mL of Iron-III Chloride [$FeCl_3$ (0.025 M) + HCl (0.5M)] and 1 mL of ethanolic solution of 1,10 phenanthrolin (0.05 M) were added to 1 mL of the obtained supernatant. The mixture obtained was incubated for 30 min at 70°C. The absorbance of the red complex was measured at 510 nm. Quinine was used as standard at different concentrations (0-100 µg/mL) and the total alkaloids content was expressed as mg equivalent quinine per gram dry weight of extract (mg QuiE/g).

Free radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The free radical scavenging activity of the extracts of *C. anomala and C. arabica* was evaluated using the method described in 2004 by Katalinié collaborators (Katalinié, Milos, Modun & Boban, 2004). Briefly, 50 µL of sample solution at different concentrations (0.5 to 7.5 mg/mL) was added to 1.950 mL of fresh methanolic solution of DPPH (3.2 mg/100 mL), mixed and kept protected from light at 37 °C for 30 min. The decrease of absorbance was recorded at 517 nm. The percentage of DPPH inhibition was calculated as follows: DPPH Scavenging Effect (%) = $((A_0 - A_1)\ 100/\ (A_0))$, where A_0 was the absorbance of the DPPH control solution and A_1 was the absorbance of the assay (extract in presence of DPPH solution). The IC_{50} value was calculated by the table curve method.

Total antioxidant capacity

This activity was assessed by the phosphomolybdenum assay method as described by Prieto *et al* (Prieto, Pineda & Aguilar, 1999). Briefly, an aliquot of 100 µL of crude extract at different concentrations (0.5 to 7.5 mg/ml) was combined with 1 mL of reagent [(0.6M sulphuric acid (H_2SO_4), 28mM sodium phosphate (Na_3PO_4) and 4mM ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)]. The mixture was incubated at 95°C for 90 min after which it was cooled to room temperature. The variation in absorbance was measured at 765 nm. Ascorbic acid was used as standard and the total antioxidant capacity was expressed in mg equivalent ascorbic acid per gram dry weight of extract (mg AAE/g).

2.2 In vivo Study

2.2.1 Experimental Design and Protocol

Wistar rats used for this study were reared in the animal house of the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of Department of Biochemistry, University of Yaounde 1, Cameroon. Female albino *wistar* rats of average weight 175 ± 10 g were housed in a standard animal facility under controlled environmental conditions at room temperature. The rats were exposed to a 12h light-dark cycle and received a standard chow diet and water *ad libitum*. For the experimentation, rats were kept in stainless steel standard cages and were acclimatized for 2 weeks in this environment before the conduction of experiments. Distilled water was used for the oral administration of the drug and plant extracts. The study protocol for laboratory animal use and care was legitimated by the Animal Ethics Committee of the Faculty of Sciences, University of Yaounde I,

Cameroon.

Before the conduction of this experiment, the rats were weighed, divided into six groups of five rats each and labelled. Each group received a different treatment by oral administration (through esophageal gavage). The groups were:

Negative Control (NC): receiving distilled water by gavage

Positive Control (PC): receiving 12 mg/kg.Bw of MTX + distilled water by gavage

Assay 1 (E1): receiving 12 mg/kg.Bw of MTX + 200 mg/kg.Bw *C. anomala* by gavage

Assay 2 (E2): receiving 12 mg/kg.Bw of MTX + 400 mg/kg.Bw *C. anomala* by gavage

Assay 3 (E3): receiving 12 mg/kg.Bw of MTX + 200 mg/kg.Bw *C. arabica* by gavage

Assay 4 (E4): receiving 12 mg/kg.Bw of MTX + 400 mg/kg.Bw *C. arabica* by gavage

Both extracts as well as the drug were dissolved in distilled water. All groups received a daily treatment in the morning. The cancer drug (MTX) was administered once per week. The rats were weighed on Days 1, 4, 8 and 12 during the experimentation period using an electronic weighing balance. After 14 days of experimentation, overnight fasted rats were sacrificed by cervical dislocation under slight anesthesia with diethyl-ether and blood was collected in EDTA tubes for preparation of plasma and hemolysates of erythrocytes. The liver and kidneys of each rat was collected, washed and rinsed with ice-cold saline solution (0.9% NaCl). The homogenates were prepared at a concentration of 10% (w/v) in normal saline (0.9% NaCl). All prepared biological samples were stored at -20°C.

2.2.2 Biochemical Analysis

Measurement of lipid profile parameters

Total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-c) concentrations were estimated using Chronolab kits according to the methods described by the manufacturer. The concentration of LDL-cholesterol was calculated using Friedewald's formula: LDL-cholesterol (LDL-c) = TC- (HDL-c + TG/5) (Friedewald, Levy & Fridrickson, 1972).

Evaluation of oxidative stress markers

Lipid peroxidation

In this method, thiobarbituric acid reactive substances (TBARS) were estimated by measuring the pink chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) according to the method of Yagi (1976).

Catalase

The activity of catalase was assayed according to the method of Sinha (1972). The activity of this enzyme was expressed in mM hydrogen peroxide/min/mg of protein.

Total proteins

The level of total protein was assayed using the colorimetric method described by Gornall and collaborators (Gornall, Bardawill & David, 1949). The optical density was recorded at 540 nm. Bovine Serum Albumin (BSA) was used as standard at the concentration of 1 mg/mL.

Evaluation of hepatic and renal markers of toxicity

Hepatic marker

The activity of a transaminase (Alanine amino transferase (ALT) was determined using a CHRONOLAB kit according to the method described by the manufacturer. Pyruvate was used as standard and the activity was estimated using a standard curve. The results were expressed in IU/mL.

Renal markers

The levels of creatinine and urea in plasma were estimated using standard kits (Chronolab) according to the method described by the manufacturer. The obtained results were expressed in mg/dL.

2.2.3 Statistical Analysis

Results were expressed as means ± standard deviation. Statistical analysis was carried out using SPSS 20.0 for Windows. One way ANOVA (analysis of variance) followed by LSD (*Least Significant Difference*) and post hoc one factor was used to compare the means of different groups. Results were significant for $p \leq 0.05$. The

Microsoft office excel software was used to treat the data and plot the graphs.

3. Results

3.1 In vitro Study

This study revealed the presence of phenolic compounds, flavonoids and alkaloids in the aqueous extracts of *C. anomala* and *C. arabica* (Table 1). Alkaloid levels were found to be more important compared to the other two families of bioactive compounds. The amounts of phenolic compounds were similar in both extracts while flavonoid content was higher in *C. arabica* and alkaloids higher in *C. anomala*.

Table 1. Bioactive content of C. anomala and C. Arabica

Extracts	Polyphenols (mg GAE/g DM)	Flavonoids (mg Quer E/g DM)	Alkaloids (µg Qui E/g DM)
C. anomala	1910 ± 8.16	94.38 ± 0.17	614.0 ± 3.2
C. Arabica	1928.89 ± 5.67	159.58± 3.06	441.1 ± 5.6

The change in color from purple to yellow was found to be concentration dependent in both extracts for the scavenging of the DPPH free radical. Figure 1 is a representation of the inhibition percentages for both extracts. The extract of *C. anomala* exhibited best scavenging activity expressed by inhibition percentages varying from 7.34 to 64.14 % against inhibition percentages varying from 11.98 to 39.25% for *C. arabica*. The IC_{50} value of *C. anomala* was found to be 4.01 mg/mL while the IC25 value for *C. arabica* was found to be 2.33mg/mL.

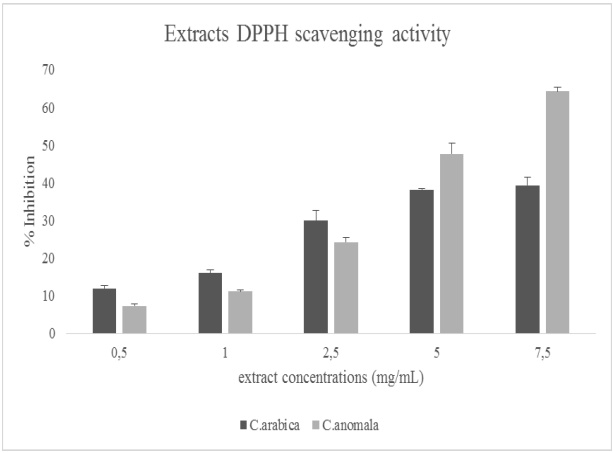

Figure 1. DPPH scavenging activity of extracts

As can be noticed in Figure 2, both extracts showed antioxidant activity and this was found to increase with increasing concentration of extract. For this study, the extract of *C. anomala* showed the highest reducing capacity at different concentrations compared to the extract of *C. arabica*. Depending on the extract concentration, this activity varied from 0.43 to 1.32 mg mgAAE/g DM for *C. anomala* extract against 0.16 to 1.17 mgAAE/g DM for *C. arabica* extract.

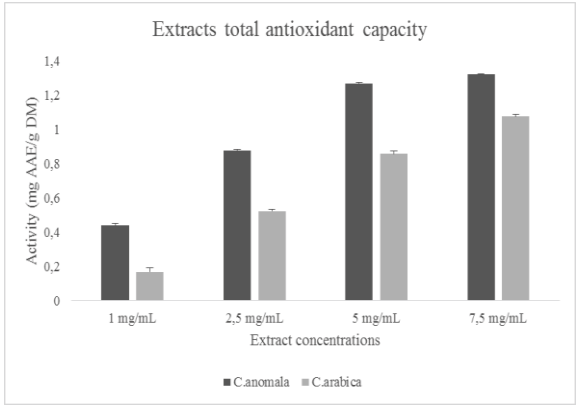

Figure 2. total antioxidant capacity of extracts plants

3.2 In vivo Study

The results obtained (Figure 3), revealed that feeding animals with chow diet in association with a treatment with MTX reduced weight gain in rats since weight gain in the positive control group was found to be lower than for the negative control (Group not receiving any treatment). Both extracts were found ameliorate weight gain in rat treated with MTX. The treated rats that also received *C. anomala* were found to gain weight better than rats those receiving MTX and the extract of *C. arabica*. The weight gain for both extracts was not dose-dependent.

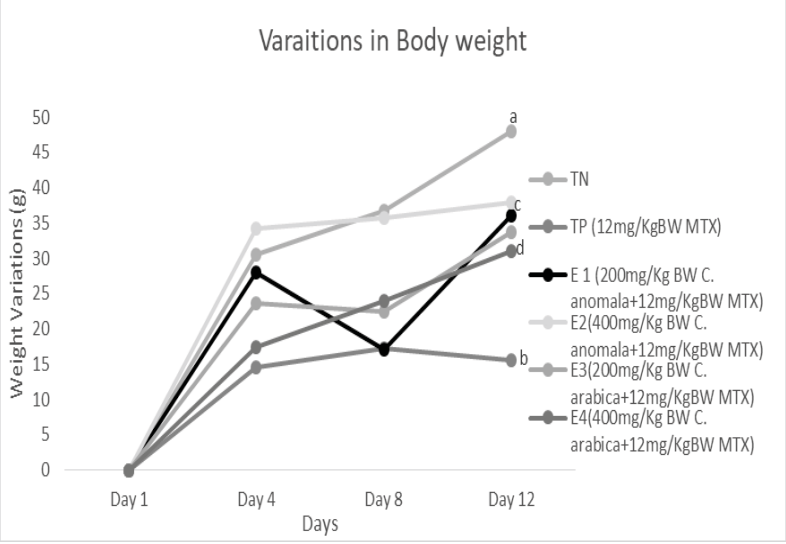

Figure 3. Effect of extracts on body weight of rats during Treatment

The results in Figure 4 show that there was no significant difference between the positive and negative control groups for the concentrations of TC, TG and HDL-c levels in plasma; only LDL-c was higher in the group receiving only MTX ($p< 0,005$). In the treated groups, a significant decrease of TC, HDL-c and TG and increase of LDL-c was observed in rats that were administered either of the aqueous extracts. Generally, so far as the lipid profile is concerned, treatment with MTX did not affect parameters while the administration of both aqueous extracts helped ameliorate these parameters especially for rats that received the higher dose of *C. arabica*.

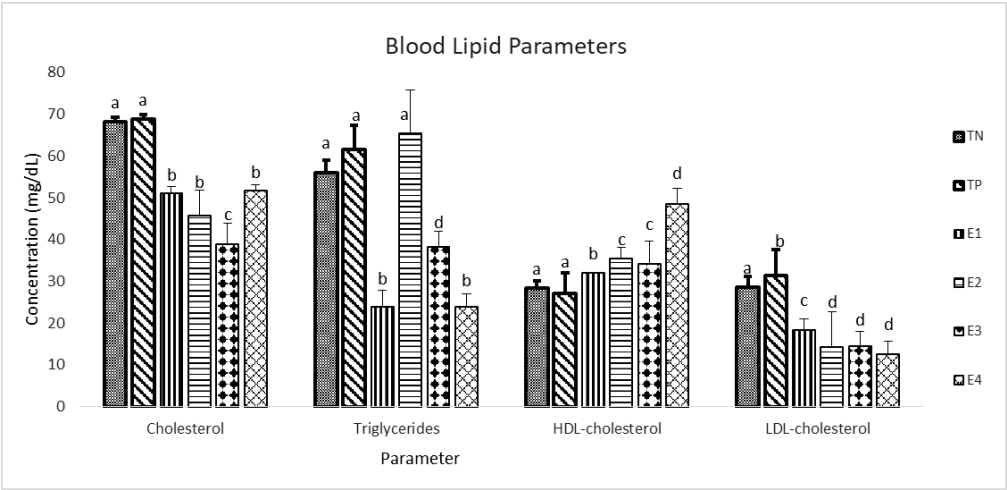

Figure 4. Effects of extracts of C. arabica and C. anomala on blood lipid paramters of experimental rats

The values are expressed as Mean ± Standard deviation. TN : Negative Control (normal rats) ; TP: Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. anomala ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg C.anomala ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C.arabica ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg C.arabica. The values affected with different letters (a, b, c, d) were significant.

It can be observed on Figure 5 an increase in MDA levels (p<0,05) in the positive control compared to the negative control group in kidney homogenates. In comparison with the positive control, the results obtained show (p<0,05) reduction of plasmatic, hepatic and kidney levels of MDA in treated groups that received the extract of *C. anomala*. In the kidney of rats treated with extracts, MDA levels were reduced to values comparable to that of the control group that received no treatment. These levels were also found to be lower in the plasma of rats treated with the aqueous extract of *C. anomala*. These results were similar in the liver but for the lower dose of the aqueous extract of *C. arabica*.

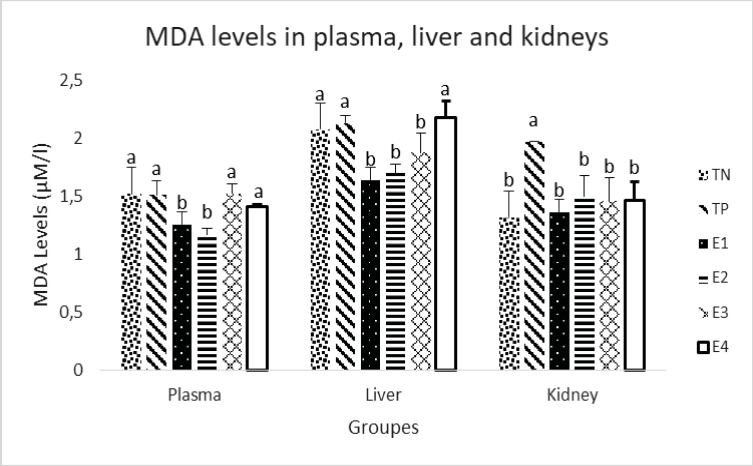

Figure 5. **E**ffects of extracts on antioxidants markers

The values are expressed as Mean ± Standard deviation. TN : Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. anomala ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg C. anomala ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. arabica ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg C. arabica. The values affected with different letters (a, b, c, d) were significant.

As concerns the activity of catalase as can be observed in Figure 6, treatment with MTX reduced the activity of catalase when compared to non-treated rats. Nonetheless, rats that received MTX treatment and either of the extracts at both doses saw their catalase activity boosted. It was at least noted that the extract of *C. anomala* showed better results when compared to *C. arabica* (p<0,05).

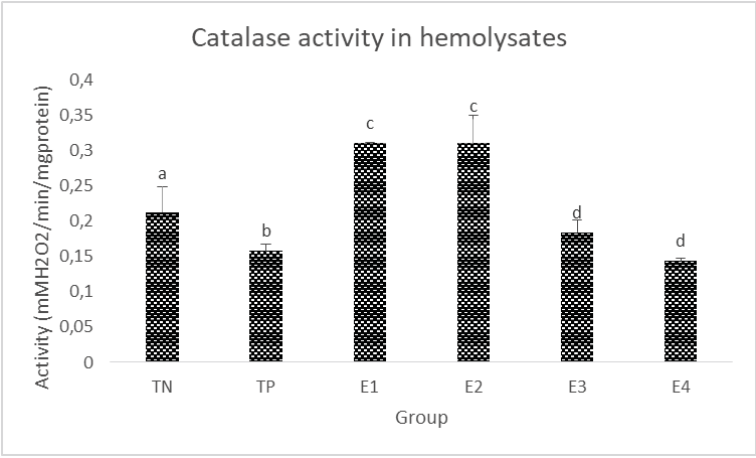

Figure 6. Effects of aqueous extracts of *C. anomala* and *C. arabica* on catalase activity in experimental rats

The values are expressed as Mean ± Standard deviation. TN : Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. anomala ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg C.anomala ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C.arabica ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg C.arabica. The values affected with different letters (a, b, c, d) were significant.

Figure 7 is a representation of plasma ALT activity in experimental rats. The activity of this enzyme was found to be higher in the positive compared to the negative control group. The combination of MTX with extracts reduced the activity of this enzyme to levels comparable to non-treated rats. This was not true for the 200mg dose of *C. anomala* where activity was found to be comparable to the activity of the positive control group.

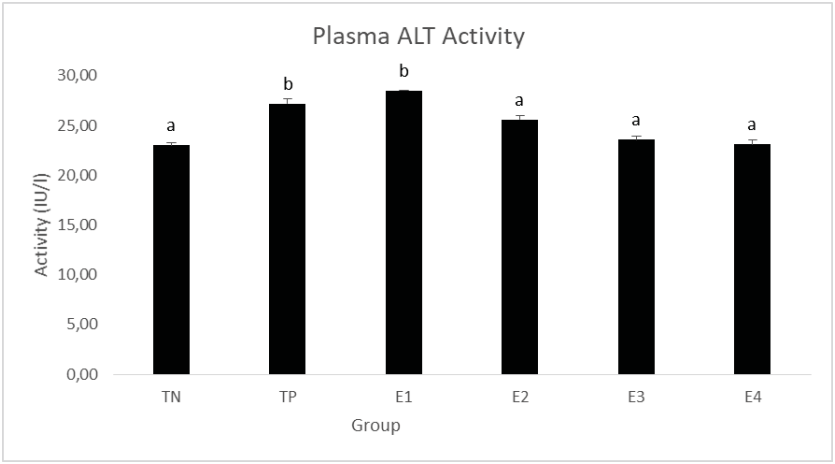

Figure 7. Effects of aqueous extracts of *C. arabica* and *C. anomala* on plasmatic Alanine amino-transferase (ALAT) levels in albino wistar rats

The values are expressed as Mean ± Standard deviation. TN : Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. anomala ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg C.anomala ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C.arabica ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg C.arabica. The values affected with different letters (a, b, c, d) were significant.

In Figure 8, it can be seen that the level of plasmatic creatinine was more important in the positive control group compared to the negative control (p<0,05). Treated rats that equally received the aqueous extract of *C. anomala* had creatinine levels that were lower than for the negative control (p<0,05) while *C. arabica* group values were more or less similar to the negative control group of rats (p<0,05).

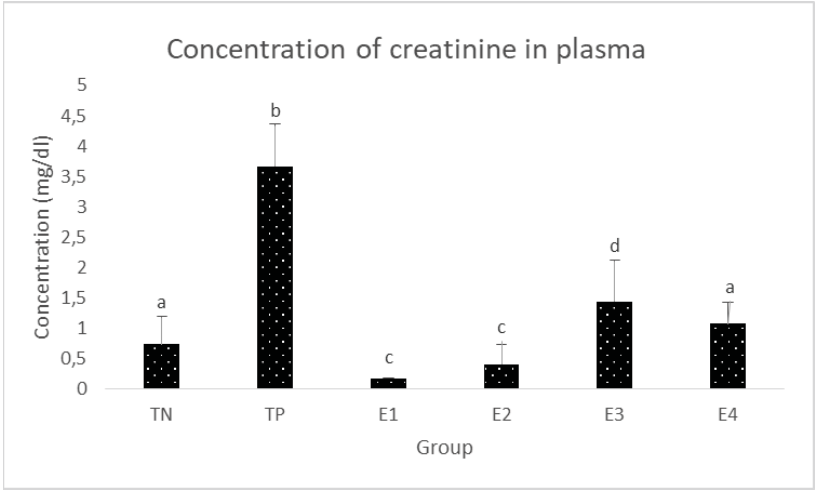

Figure 8. Effects of aqueous extracts of *C. anomala* and *C. arabica* on creatinine levels of plasma in experimental rats

The values are expressed as Mean ± Standard deviation. TN: Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. anomala ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg C. anomala ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. arabica ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg C. arabica. The values affected with different letters (a, b, c, d) were significant.

An increase in plasmatic urea levels was also observed after administration of MTX compared to the group that did not receive MTX (Figure 9). The extract-treated groups showed reduced levels of urea in the plasma of treated rats and the obtained values were comparable to those of the negative control. However, the dose 200 mg/kg.Bw of *C. arabica* showed a better reduction of urea level compared to all the other groups.

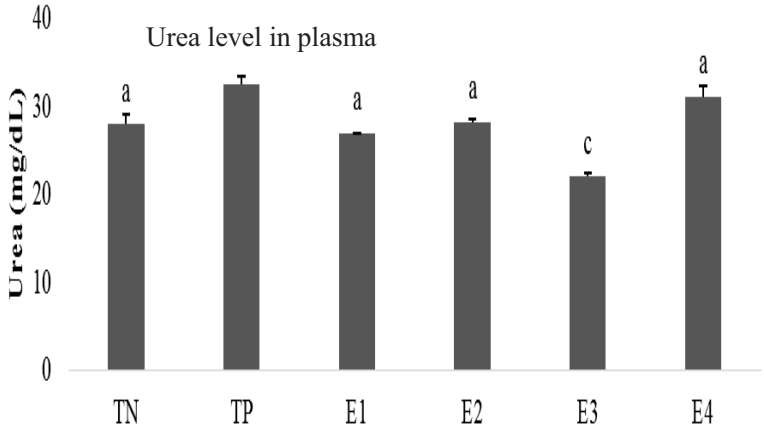

Figure 9. Effects of the aqueous extracts of *C. arabica* and *C. anomala* on the urea concentrations of the plasma of experimental rats

The values are expressed as Mean ± Standard deviation. TN: Negative Control (normal rats) ; TP: Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. anomala ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg C. anomala ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg C. arabica ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg C. arabica. The values affected with different letters (a, b, c, d) were significant.

4. Discussion

Weight loss is one of the most observed complications of chemotherapy due to an increase in treatment-related side effects (Bonomi, Batus, Fidler & Borgia, 2017). The results showed that even if weight gain in extract treated rats was not comparable to weight gain in non-treated rats, rats that received extracts in addition to MTX had better weight evolution compared to those that received MTX exclusively, suggesting a protective effect of the extracts against the limitations of weight gain induced by MTX. MTX is able to bind to neuropeptide Y (NPY) to decrease drug resistance by cancer cells (Böhme, Krieghoff & Beck-Sickinger, 2016). However NPY is a peptide neurotransmitter involved in the induction of food intake. Its association with MTX leads to a decrease in its activity resulting in a decrease in dietary intake, which is a direct consequence on weight gain in the positive control group compared to the negative control. On the other hand, the alkaloids present in our extracts with their stimulating action, could stimulate the neurotransmitter system and thus modulate the inhibitory action exerted by MTX on the NPY. Hussain *et al*. (2018) have shown that alkaloids thanks to their antioxidant and anti-amyloid as well as acetylcholine esterase inhibition, improve the functioning of the neurotransmitter system.

The antioxidant potential of each extract was evaluated using two methods: total antioxidant capacity (TAC) and scavenging of DPPH free radical. Both plant extracts were found to scavenge the DPPH free radical and reduce molybdate even if these activities varied according to the plant extracts. The activities observed could be linked to the presence of bioactive compounds that were found to be present in the prepared aqueous extracts. It has been demonstrated that polyphenols, flavonoids and alkaloids are important constituents that serve as powerful antioxidants due to the hydrogen-donating ability of their hydroxyl groups as well as their ability to donate electrons to stop the free radicals production involved in oxidative stress (Lin *et al*., 2016; Gulçin *et al*., 2010). For both tests, the extract of *C. anomala* was found to have a better antioxidant potential when compared to the extract of *C. arabica*. The amount of polyphenol was similar in both extracts while the amount of flavonoids was more important in the *C. arabica* extract and alkaloids more important in the extract of *C. anomala.* The activity of bioactive compounds depends more on the hydrogen and electron donating capacity than the amount of bioactive compound present in the sample. From this it could be thought that the antioxidant potential that was more important in *C. anomala* is due to the higher amount of alkaloids or due to bioactive compounds that are more active. This activity could also be due to the presence of other bioactive compounds that were not evaluated in this work. The above mentioned findings indicate that both *C. anomala* and *C. arabica* are sources

of phenolics, flavonoids and alkaloids and may therefore play a critical role as a singlet oxygen quencher and free radical scavenger to reduce molecular damage in the cell.

There is evidence that diseases such as cancer multiforms are highly related to oxidative stress. Also, oxidative stress may have a role in therapeutic mechanisms of anticancer agents like MTX. MTX decreases the effectiveness of the antioxidant defense system resulting in an increase in oxidative stress and MTX-induced oxidative stress has been observed in tumor tissues (Kolli, Nataraja, Isaac, Selvakumar &Abraham, 2014; El-sheikh, Morsy & Hamouda, 2016; Mahmoud, Hussein, Hozayen & El-Twab, 2017). Therefore, preventing oxidative damage and increasing antioxidants, could be an asset during cancer treatments. In this study, we investigated the influence of MTX treatment on the antioxidant defense system by evaluating the MDA levels and catalase activity in rats treated with MTX and MTX combined with either of our extracts. Associating both plants with MTX treatment reduced the MDA levels and increased catalase activity in experimental rats. It has been proven that the use of MTX induces lipid peroxidation with liberation of high levels of reactive oxygen and nitrogen species. The use of MTX has also been linked to the lowering of the activity of antioxidant enzymes such as catalase, superoxide dismutase and glutathione reductase (Montasser, Saleh, Ahmed-Farid, Saad & Mohamed-Assem, 2017). It can also cause an increase in the activity of these enzymes due to increased utilization for the scavenging of ROS generated due to oxidative stress (Saravanan, Ponmurugan, Sathiyavathi, Vadivukkarasi & Sengottuvelu, 2013). Catalase activity in this study was found to be lower in rats treated exclusively with MTX, meaning this treatment caused a reduction in its activity. The association of extracts to the MTX treatment protected the functioning of this enzyme as in this rats its activity was found to be higher. This mechanism can be further explained by the levels of MDA that was not found to be higher in rats that received MTX in combination with an extract compared to those that received only MTX. As a matter of fact, associating MTX with *C. anomala* provided lower levels of MDA levels for higher catalase activity (this was not the case with *C. arabica* where MDA levels were similar but catalase activity was higher than for the MTX exclusively treated group). From these results, the antioxidant defense system was found to be more protected by *C. anomala* than *C. arabica* in experimental rats. These results correspond to the *in vitro* results in this study where we found that the scavenging of DPPH free radical and the reduction of molybdate was more important with the aqueous extracts of *C. anomala* compared to the aqueous extract of *C. arabica.*

The role of bioactive molecules in the protection of the antioxidant defense system as is the case with *C. anomala* and *C. arabica* is of great importance in protecting biological systems from the deleterious effects of reactive oxygen and nitrogen species that are generated during cancer treatment with a chemotherapeutic drug. These reactive species have been found to increase cell damage by influencing the activity of the mitochondrial respiratory chain, causeing inflammation, all leading to the damage of other biological molecules, like proteins and DNA (Lea, Tung & Zhou, 2015; Halliwell, 2002). All these are capable of leading to disorders in metabolic pathways. In this study was also evaluated the effect of our extracts on MTX-induced lipid metabolism disturbances by measuring some parameters of the lipid profile. MTX is a drug that is known to cause hepatic steatosis (Shetty *et al*., 2017) that leads to modifications in blood lipid parameters. Treatment with MTX did not affect these parameters except for LDL-cholesterol that was found to be increased ($p < 0.05$). It could be that the treatment was not long enough for disorders in these parameters to be observed. This interpretation can be supported by the fact that rats that received MTX in combination with extracts were found to have parameters that were better than those of rats that did not receive any treatment. The decrease in TG and LDL-cholesterol as can be observed in Figure 4 may be due to the activation of hepatic lipase which hydrolyzes TG-rich lipoproteins (LDL-c, VLDL-c) thus causing an increase in HDL-c (Abdulazeez, 2011). Also, decrease in total cholesterol may be due to a possible inhibition of the enzyme HMG-CoA reductase; the inhibition of this enzyme has been reported to prevent the production of hepatic cholesterol (Ahmadi, Ghorbanhagho & Argani, 2017). Comparing both extracts, showed that the aqueous extract of *C. arabica* ameliorated lipid parameters better than *C. anomala* especially with the 400mg dose($p < 0.05$).

The evaluation of protein, urea and creatinine levels and the activity of ALT were carried out to determine the level of damage that MTX treatment could have caused on the liver and kidneys of experimental rats.

The results showed that the total protein level was not different between groups. However, a significantly ($p < 0.05$) elevated plasma ALT activity was observed in the group that received only MTX. Montasser and collaborators in 2017 also showed that treatment with MTX elevated plasma levels of ALT indicating liver toxicity since the release of this enzyme into the blood stream is an indicator of liver damage due to the destruction of liver cells through the loss of the functional integrity of hepatocytes leading to a leakage of its content into the blood stream (Montasser, Saleh, Ahmed-Farid, Saad & Mohamed-Assem, 2017). Both doses of *C. arabica* and the 400mg dose of *C. anomala* helped reduce ALT to levels that were comparable to that of

non-treated rats. It can thus be suggested that the aqueous extract of *C. arabica* protected the liver more than *C. anomala*. In this experimentation, MTX also increased the levels of creatinine and urea both indicators of renal toxicity. Harms *et al*. (2017) also carried out a study where plasma creatinine and urea levels were high after treatment with MTX (Harms, Khawaja, Taylor, Han & Mrug, 2017). As can be observed in Figures 8 and 9, the levels of these metabolic wastes were found to be lowered by associating MTX with either of the extracts. From these results, it can be suggested that both extracts improved glomerular filtration there by reducing the deleterious effects of MTX on the kidney. It is thought that this drop could be promoted by phenolic compounds contained in these extracts through their ability to protect the kidney from MTX-induced oxidative stress (Ahmed, Zaki & Nabil, 2015). Creatinine was lower in *C. anomala*-treated groups while urea was lower only for the 200mg dose of *C. arabica*.

5. Conclusion

The aim of this work was to compare the ability of *C. anomala* and *C. arabica* to protect rats against MTX-induced toxicity. Concerning the parameters evaluated in this study, both aqueous extracts of *C. anomala* and *C. arabica* protected against metabolic disorders that were generated by MTX. It appears that, these extracts contain important amounts of bioactive compounds with good scavenging and reducing capacity. These compounds helped increase antioxidant status of experimental rats; the consequence of this being the protection of rats exposed to MTX from renal and liver damage as well as improved lipid metabolism parameters. To a certain level, *C. anomala* protected the rats better against these deleterious effects compared to *C. arabica* at least for the two doses (200 and 400 mg/kg.Bw) and for the parameters investigated. All doses exhibited hepatoprotective and nephroprotective effects.

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Efficacy of the Aqueous Extracts of *Justicia Galeopsis* Leaves on the Improvement of Hematological Parameters in Anemic Rats

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Abstract

Antianemc potential of aqueous of *Justicia galeopsis* leaves was studied using *Wistar* Albino rats after induction of anemia by phenylhydrazine hydrochloride. Forty rats (20 male and 20 female) subdivided into five groups of eight rats were used. There was a group as control (not anemic) and four other anemic groups which had received by gavage respectively 1 ml/kg of distilled water, 1 ml/kg of body weight of Vitafer (reference drug commonly used to treat anemia), 100 mg/kg of body weight of extract of *J. galeopsis* leaves cooked during 30 min and 30 mg/kg of body weight of extract of *J. galeopsis* leaves cooked during 45 min. Hematological parameters (red blood cells, hemoglobin and hematocrit) were analyzed as indices of anemia and the weights of specific organs (liver, spleen and kidney) were evaluated. The results of this investigation had showed that aqueous extract of *J. galeopsis* leaves cooked improved red blood cells, hemoglobin and hematocrit levels. These extracts were not toxic for liver, spleen and kidney. The administration of 100 mg/kg/day of extract of leaves cooked leaves during 30 min promotes a better recovery rate of the number of red blood cells (94.80 %), hemoglobin level (159.53 %) and hematocrit (117.72 %) than Vitafer and the extract of leaves cooked for 45 min. This is suggestive that aqueous extracts of *Justicia galeopsis* leaves cooked during 30 min may be exploited during 2 weeks in the treatment of anemia.

Keywords: *galeopsis* leaves, water cooking, anemia, hematological parameters

1. Introduction

Anemia is a disease that affects all countries in the world, whether industrialized or not (Ponmozhi, & Ramya, 2015). It is also a public health problem that affects almost a quarter of the world's population. This disease affects people of all ages but the most affected are children, pregnant women and the elderly (Senou et al., 2016). There are many causes of anemia (McLean et al., 2007). The most important causes are nutritional deficiencies, then the pregnancy and the high prevalence of blood parasites such as Plasmodium, Trypanosomes (Sènou et al., 2016). Among nutritional deficiencies, iron deficiency is the main cause of anemia (Ramakrishnan 2002). There is also a deficiency of folic acid and / or vitamin B 12 which causes megaloblastic anemia (Green, 2017).

Some pharmaceutical drugs are used to treat anemia. These include iron, vitamin B12, folic acid etc. The search for other alternatives to overcome the problem of the high cost of pharmaceutical drugs is encouraged by WHO (Sènou et al., 2016). Thus, *Justicia secunda* leaves (Tossou et al., 2008), *Alchornea cordifolia* leaves, *Coco nucifera* milk, *Hibiscus sabdarifa* flowers (Séguéna et al., 2013), and *Jatropha tanjorensis* leaves (Idu et al., 2014) were studied and their anti-anemia properties were discovered. Beyond these trees and shrubs, studies have also shown that the consumption of some leafy vegetables, in particular *Ipomoea batatas* (Osime et al., 2008), *Spinacia oleracea* (Luka et al., 2014), *Moringa olifera* (Madukwe et al., 2013) improve hematological parameters. These plants could therefore be used in place of pharmaceutical drugs (Tossou et al., 2008).

Justicia galeopsis is a spontaneous plant whose leaves are eaten as leafy vegetables in Nigeria (Obichi et al., 2015) and Côte d'Ivoire (Kouakou, 2015). In Côte d'Ivoire, the leaves of this plant are very appreciated by populations of Abengourou who like their taste. The cooked dishes of these leaves are offered to distinguished guests or consumed during the festivities (Kouakou, 2015). These leaves contain, in significant quantity, many nutrients in particular iron, vitamin B12, Folic acid, calcium and magnesium (Afolaby et al., 2012; Kouakou,

2015; Obichi et al., 2015; Loukou et al., 2018).

This plant has advantageous nutritional profile for the health of populations. It could be used like the leaves of *Ipomoea batatas*, *Spinacia oleracea* and *Moringa olifera* in the treatment of anemia. However, it remains little known in the other regions of Côte d'Ivoire. The literature provides information only about their medical uses.

The objective of this study is to test the anti-anemic properties of the aqueous extracts of the leaves of *Justicia galeopsis* on Wistar rats, after induction of anemia by phenylhydrazine hydrochloride.

2. Materials and Methods

2.1 Collection and Authentication of Plant

Justicia galeopsis leaves, commonly known in the local language "Agni" as *Assiaploua* were collected fresh and at maturity from cultivated farmlands located at Abobo. Abobo is one of thirteen communes of the district of Abidjan, Côte d'Ivoire. It is located in Abidjan north between 5°42 north latitude and -4°02 west longitude and at an altitude of 105 meters above sea level (Aka et al., 2013). Plants were identified and authenticated by National Floristic Center (University Felix Houphouët Boigny, Abidjan-Côte d'Ivoire).

2.2 Processing of Plant

The fresh leaves were separated from the stem, washed with distilled water and drained at ambient temperature. Collected leaves were divided into three lots. The first lot is that of the fresh leaves obtained by Agbemafle et al. (2012) method and lyophilized. The other two were cooked at 100 °C respectively during 30 and 45 min by using the method of Randrianatoandro (2010) in the proportion 40 g of leafy vegetables immerged in 200 mL of boiled water. Boiled samples were cooled, crushed with a laboratory crusher (Culatti, France) and lyophilized. All lyophilized samples were ground in fine powder and store in a clean dry air-tight bottle in a refrigerator (4 °C) until required for analyses.

2.3 Preparation of Aqueous Plant Extracts

Fifty grams of powdered plant material was extracted in 1.5 l of distilled water for 24 hours. At the end of extraction time, the mixture was filtered twice through folded cotton and whatman filter grade 1. The filtrate was evaporated at 50 °c for 24 hours in a ventilated oven. The dried powder was then stored in containers at -20 °c until used for bioassay. The powder obtained was dissolved in distilled water (1 ml for 100 g body weight) and administrated by gavage to rats.

The amount of residue administered to a rat is proportional to the amount of leaves consumed by an adult who weighs about 60 kg.

2.4 Animal Handling and Grouping

Eight-week-old male and female *Wistar* albino rats bred in animal house of higher normal school (E.N.S.) at Felix Houphouet Boigny University were used for this study. The rats weighed on average 127.7 g. The animals were housed in locally fabricated cage. They were allowed to acclimatize to the new environment for seven days before the beginning of the experiment. The rats were fed with animal feed (Ivograin) and water *ad libitum*. The experimental protocol and the animal handling procedures were conducted according to the guidelines of the ethics committee of Nangui Abrogoua University (Côte d'Ivoire).

2.5 Experimental Protocol

Forty *Wistar* albino rats were used in this study. The rats were randomly divided into five groups made of eight animals (four male and four female) each. Group 1 was not anemic and served as negative control. The rats of other groups were anemic with group 2 as positive control. Groups 3, 4 and 5 were treated with respectively the Vitafer (1 mg/kg of body weight / day (D)), plant extract cooked during 30 min (100 mg/kg of body weight / day) and plant extract cooked during 45 min (30 mg/ kg of body weight / day) from D2 to D15. The plant extract and Vitafer were administered by gavage using a gastric tube. Vitafer is reference drug commonly used to treat anemia.

The detail of the protocol is presented as follows:

Group 1: Non-anemic control, consisting of rats given 1 ml/kg of body weight / day of distilled water on D0 to D15.

Group 2: Anemic control consisting of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 1 ml/kg of body weight / day of distilled water from D2 to D15.

Group 3: Control reference, made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1)

and 1 ml / kg / day of Vitafer, from D2 to D15.

Group 4: Test 1, made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 100 mg / kg /day of the *Justicia galeopsis* aqueous extract cooked during 30 min from D2 to D15.

Group 5: Test 2, made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 30 mg / kg /day of the *Justicia galeopsis* aqueous extract cooked during 45 min from D2 to D15.

The administration of the extracts had begun at D2 (the third day after induction) after ascertainment of the anemia.

2.5 Induction of Anemic Condition

Anemia was induced by phenylhydrazine Chloridrate. Phenylhydrazine was previously dissolved in sterilized normal saline. It was administered to rats intraperitoneally (IP) at a dose of 40 mg/ kg of body weight / day (Naughton al., 1995) for two days (D0 and D1). Rats that were observed to have hemoglobin levels less than 11 g/dL were considered anemic.

2.6 Collection and Preparation of Blood Samples

Blood was collected early in the morning thanks to the technique of amputation of the tail tip (5 mm from the end), previously disinfected with the alcohol 96 °C (Kraus, 1980). Blood was stored in the EDTA (Ethylene-Diamine-Tetra-Acetic acid) tubes for the determination of red blood cell count, hemoglobin and hematocrit. Blood samples were taken on days D0, D2, D7 and D15, the morning after the weighing.

2.7 Evaluation of Hematological Parameters

The hematological parameters included hemoglobin (Hb), red blood cells (RBC) and hematocrit were determined using the automatic analyzer URIT- 2900® (Guilin, China).

2.8 Determination of Body Absolute Organ Weights

The body weight of each rat was assessed during the acclimatization period, once before beginning of experience, during the test period (D0, D2, D7, and D15) and on the day of sacrifice. On the day of sacrifice, all the animals were euthanized using diethyl ether in a desiccator. Different organs including the heart, liver, spleen, and kidney were carefully dissected out and weighed.

2.9 Statistical Analysis

Statistical tests of hematological parameters were carried out using software R. The results were expressed in the form of mean ± standard deviation. The statistical significance of the differences between the experimental groups is determined by the Paired t test. Results of the organs are presented as the average ± standard deviation, and the differences among test groups were assessed by one-way analysis of variance followed by Duncan's New Multiple Range Test using Statistica 7.1 (StatSoft).

3. Results

3.1 Effects of Justicia Galeopsis Leaves Aqueous Extract on Red Blood Cells Number

Table 1 shows the effects of oral administration of aqueous extract of *Justicia galeopsis* leaves cooked during 30 and 45 min on red blood cells number. The administration of phenylhydrazine had caused on day D2 a significant reduction ($p<0.05$) in the red blood cells level in the rats of the four groups (2;3;4 and 5) by about -20.71 to -48.08 %. After treatment with 100 mg/kg of *Justicia galeopsis* aqueous extract cooked during 30 min (extract 1), a gradual recovery was obtained on the following days (+ 10.91 % on D7 and + 94.94 % on D15). On the other hand, in anemic rats that received distilled water, Vitafer and 30 mg/kg of *Justicia galeopsis* aqueous extract cooked during 45 min (extract 2), there was an increase in the number of red blood cells only on day 15 with a recovery rate of respectively 55 %, + 64.69 % and+ 60.52 %.˙The results had showed that extract 1 promotes better recovery than Vitafer, which had a higher recovery rate than extract 2.

Table 1. Effects of Oral Administration of Aqueous Extract of *Justicia Galeopsis* Leaves Cooked during 30 and 45 Min on Red Blood Cells Number

Groups	Red blood cells (10^6/µl)			
	D0	D2	D7	D15
Group 1 (Control)	5.00 ± 2.12	4.50 ± 2.48	2.92 ± 1.70	5.36 ± 1.50
Group 2 (Positive control)	5.12 ± 1.77	3.10 ± 1.11	2.72 ± 1.00	4.81 ± 1.10
Variation		-39.45 % [a*]	-120.25 % [b]	+55.16 % [b*]
Group 3 (+1 ml/kg of Vitafer)	6.34 ± 1.85	3.51 ± 1.40	1.73 ± 0.89	5.78 ± 1.32
Variation		-44.64 % [a*]	-50.71 % [b*]	+64.67 % [b*]
Group 4 (+100 mg/kg of extract 1)	4.95 ± 1.88	2.57 ± 0.93	2.85 ± 0.74	5.01 ± 1.27
Variation		-48.08 % [a*]	+108.9 % [b]	+94.94 % [b**]
Group 5 (+30 mg/kg of extract 2)	4.25 ± 2.33	3.37 ± 0.86	2.19 ± 1.07	5.40 ± 1.20
Variation		-20.71 % [a]	-35.01 % [b*]	+60.23 % [b**]

[a]Variation percentage from day D0; [b]Variation percentage from day D2; *Significant difference ($p<0.05$; **Highly significant difference ($p<0.01$); extract 1: *Justicia galeopsis* aqueous extract cooked during 30 min; extract 2: *Justicia galeopsis* aqueous extract cooked during 45 min

3.2 Effects of Justicia Galeopsis Leaves Aqueous Extract on Hemoglobin Rate

Table 2 shows the effects of oral administration of aqueous extract of *Justicia galeopsis* leaves cooked during 30 and 45 min on hemoglobin rate. The administration of phenylhydrazine had caused on day D2 a significant reduction ($p<0.05$) in the hemoglogin level in the rats of the four groups (2;3;4 and 5) by about -27.56 % to -58.77 % After treatment with distilled water, Vitafer, 30 mg/kg of *Justicia galeopsis* aqueous extract cooked during 45 min (extract 2) and 100 mg/kg of *Justicia galeopsis* aqueous extract cooked during 30 min (extract 1), a gradual recovery was obtained on the following days (D7 and D15). The results had showed that after 15 days of treatment, the reduction in the hemoglobin level was completely corrected in the rats which received Vitafer and those which received the extract of leaves cooked for 30 min, with a recovery rate greater than 100 %. However, the recovery rate of extract 1 is better than that of Vitafer.

Table 2. Effects of Oral Administration of Aqueous Extract of *Justicia Galeopsis* Leaves Cooked during 30 and 45 Min on Red Blood Cells Number on Hemoglobin Rate

Groups	Hemoglobin rate (g/dl)			
	D0	D2	D7	D15
Group 1 (Control)	11.57 ± 2.47	9.08 ± 3.23	8.05 ± 2.62	13.23 ± 1.64
Group 2 (Positive control)	11.81 ± 2.09	7.55 ± 1.99	8.65 ± 1.32	13.17 ± 1.98
Variation		-36.07 % [a**]	+14.57 % [b]	+74.44 % [b***]
Group 3 (+ 1ml/kg of Vitafer)	11.00 ± 3.14	7.06 ± 2.69	7.54 ± 2.45	14.45 ± 2.12
Variation		-35.82 % [a*]	+6.80 % [b]	+104.67 % [b***]
Group 4 (+ 100 mg/kg of extract 1)	13.05 ± 2.66	5.38 ± 1.73	7.63 ± 2.10	13.97 ± 1.20
Variation		-58.77 % [a***]	+41.82 % [b]	+159.67 % [b***]
Group 5 (+ 30 mg/kg of extract 2)	9.65 ± 2.23	6.99 ± 1.60	8.65 ± 2.30	13.05 ± 1.15
Variation		-27.56 % [a*]	+23.75 % [b]	+86.70 % [b***]

[a]Variation percentage from day D0; [b]Variation percentage from day D2; *Significant difference ($p<0.05$; **Highly significant difference ($p<0.01$); [***]Very highly significant difference ($p<0.001$); extract 1: *Justicia galeopsis* aqueous extract cooked during 30 min; extract 2: *Justicia galeopsis* aqueous extract cooked during 45 min

3.3 Effects of Justicia Galeopsis Leaves Aqueous Extract on Hematocrit Rate

The effects of oral administration of aqueous extract of *Justicia galeopsis* leaves cooked during 30 and 45 min on hematocrit rate are presented in the table 3. The administration of phenylhydrazine had caused on day D2 a significant reduction ($p<0.05$) in the hematocrit level in the rats of the four groups (2;3;4 and 5) by about -21.33 % to -47.90 %.

After treatment with 100 mg/kg of extract of *Justicia galeopsis* leaves cooked during 30 min (extract 1), a gradual recovery was obtained on the following days +27.53 % on D7 and +117.66 % on D15). In anemic rats that had received distilled water, Vitafer and 30 mg/kg of *Justicia galeopsis* aqueous extract cooked during 45

min (extract 2), there was an increase in the level of hematocrit only on day 15 with a recovery rate of respectively +68.40 %, +81.74 % and +84.64 %.

The reduction in the hematocrit level was completely corrected only in the rats which received the extract of leaves cooked for 30 min, with a recovery rate greater than 100 % (+117.66 %). The recovery rate obtained with the extract is higher than that of Vitafer. The results had showed that extract 1 promotes better recovery than Vitafer. The recovery rate of extract 2 (84.64 %) is higher than that of Vitafer (81.74 %).

Table 3. Effects of Oral Administration of Aqueous Extract of *Justicia Galeopsis* Leaves Cooked during 30 and 45 Min on Red Blood Cells Number on Hematocrit Rate

Groups	Hematocrit rate (%)			
	D0	D2	D7	D15
Group 1 (Control)	32.50 ± 12.63	26.82 ± 14.83	17.02 ± 9.75	34.31 ± 6.66
Group 2 (Positive control)	32.03 ± 9.46	20.03 ± 5.76	20.02 ± 7.97	33.73 ± 6.32
Variation		-37.45 % [a*]	-0.08 % [b]	+68.36 % [b**]
Group 3 (+ 1 ml/kg of Vitafer)	34.12 ± 13.97	20.48 ± 8.37	13.16 ± 7.47	37.22 ± 10.02
Variation		-39.98 % [a]	-3574 % [b]	+8174 % [b*]
Group 4 (+ 100 mg/kg of extract 1)	29.98 ± 9.41	15.62 ± 5.55	19.92 ± 3.94	34.00 ± 7.00
Variation		-47.90 % [a**]	+27.53 % [b]	+117.66 % [b***]
Group 5 (+ 30 mg/kg of extract 2)	24.66 ± 9.26	19.40 ± 4.86	16.25 ± 8.89	35.82 ± 8.89
Variation		-21.33 % [a]	-16.23 % [b]	+84.64 % [b**]

[a]Variation percentage from day D0; [b]Variation percentage from day D2; *Significant difference ($p<0.05$; **Highly significant difference ($p<0.01$); [***]Very highly significant difference ($p<0.001$); extract 1: *Justicia galeopsis* aqueous extract cooked during 30 min; extract 2: *Justicia galeopsis* aqueous extract cooked during 45 min

3.4 Effects of Justicia Galeopsis Leaves Aqueous Extract on Liver, Spleen, and Kidney

As depicted in tables 4, oral administration of 100 mg/kg body weight of extract 1 (aqueous extract of *Justicia galeopsis* leaves cooked during 30 min) and 30 mg/kg body weight of extract 2 (aqueous extract of *J. galeopsis* leaves cooked during 30 min) in rats at a dose of daily had no significant effect on liver and kidney weights. However, the spleen weight was significantly greater in the anemic rats untreated and treated with Vitafer and the extracts of *J. galeopsis* leaves when compared to the non-anemic group.

Table 4 Effects of Oral Administration of Aqueous Extract of *Justicia Galeopsis* Leaves Cooked during 30 and 45 Min on Organ Weights

Groups	Organ weights (g)		
	Liver	Spleen	Kidney
Group 1 (Control)	0.79 ± 0.13^{a}	0.37 ± 0.14^{a}	4.11 ± 0.40^{a}
Group 2 (Positive control)	0.79 ± 0.10^{a}	0.53 ± 0.19^{ab}	3.97 ± 0.65^{a}
Group 3 (+ 1 ml/kg of Vitafer)	0.83 ± 0.11^{a}	0.43 ± 0.10^{ab}	4.05 ± 0.46^{a}
Group 4 (+ 100 mg/kg of extract 1)	0.87 ± 0.15^{a}	0.55 ± 0.14^{b}	3.80 ± 0.62^{a}
Group 5 (+ 30 mg/kg of extract 2)	0.86 ± 0.09^{a}	0.60 ± 0.19^{b}	4.28 ± 0.70^{a}

Results are expressed as Mean ± standard deviation (SD) for eight animals per group; means with different superscript letters in the columns are significantly different using the Student's t-test $p < 0.05$ was considered statistically significant

4. Discussion

Subacute intoxication of rats with 40 mg per kilogram body weight of Phenylhydrazine (PHZ) for two days resulted in decreasing of red blood cell count, hemoglobin and hematocrit values caused by hemolytic anemia. Administration of PHZ to rats has been reported to result in the production of both aryl and hydroxyl radicals which produce oxidative stress on the red cell membrane resulting in hemolysis by lipid peroxidation Cighetti et al. (1999). According to Ferrali et al. (1997) this lipid peroxidation increase methaemoglobinemia in the blood. These Similar results were reported by Tossou et al. (2008) in rats administered with PHZ to induce anemia.

The group of positive control rats (anemia rats receiving no treatment) had a recovery rate of red blood cells (+ 55 %), hemoglobin (+ 74.46 %) and hematocrit (+ 68.36 %) lower than that of the other groups which also

received PHZ. Indeed, the body had tried to correct the losses caused by the injection of PHZ but this correction was incomplete until D15. According to Nancy (2011) under normal conditions, the body generates new red blood cells to replace the lost ones but this takes longer; as observed in the untreated anemic rats. So, these rats will need a treatment favoring the increase in hematological parameters following disorders leading to their decrease.

The decrease in red blood cells was almost completely corrected (+ 94.94 %) in the group of rats which had received 100 mg/kg of *Justicia galeopsis* leaves extract cooked during 30 min in contrary to those which had received Vitafer (+ 64.67 %) and 30 mg/kg of extract 2 (+ 60.23 %). The extract of leaves cooked during 30 min allows a better stimulation of the synthesis of red blood cells. These results are better than those of Tossou et al. (2008) who had showed that the administration of 1000 mg/kg of *Justicia secunda* leaf extract increased the number of red blood cells with a recovery of + 98.11 %.

The decrease in hemoglobin induced by phenylhydrazine was completely corrected in the group of rats which received extract 1 and the group of those which received Vitafer. Hemoglobin is the key element in the structure of red blood cells, stimulating their synthesis will also stimulate the production of red blood cells. Therefore, massive production of hemoglobin will cause more red blood cells. Thus, the hemoglobin recovery rate of rats having received the extract of cooked leaves for 30 min being higher (+ 159.53 %) than that of rats having received Vitafer (+ 104.67 %), their red blood cell recovery rate will also be higher than those of rats having received Vitafer and the extract 2 as indicated by le results. The speedy and progressive recovery of anemic rats on treatment with extracts 1 could be due to increased erythropoiesis. According to Koury and Ponka (2004), Folate, vitamin B, and iron are required for optimal erythropoiesis. In fact, according to Loukou et al. (2018), *Justicia galeopsis* uncooked leaves are very rich in vitamin B12 (4173.09 mg/kg) and vitamin C (892.17 mg/kg). Their leaves cooked during 30 min contain more iron (458.98 mg/kg) and vitamin C (4.75±1.60 mg/100g) than the leaves cooked during 45 min which contain 453.93 mg/kg of iron and 3.80 mg/100g of vitamin C (Loukou et al., 2020). This composition of *Justicia galeopsis* leaves extract cooked during 30 min may account for the faster reversal of PHZ induced anemia.

The decrease in hematocrit level observed was completely corrected in rats which received the extract of leave cooked during 30 min. The hematocrit corresponds to the volume occupied by red blood cells in the blood volume, its value depends on the number of red blood cells in the blood volume. However, this explains the fact that the hematocrit recovery rate of rats having received the extract of the leaves cooked during 30 min is higher than that of the other groups of rats because of its highest recovery rate of red blood cells. This result is better than that observed by Tossou et al. (2008) with the administration of 1000 mg/kg/day of extract of *Justicia secunda* leaves in Benin.

There was no significant difference in the liver and kidney weight for all groups relative to the non-anemic, anemic control and treated with Vitafer and *J. galeopsis* extracts. This indicates that the extracts of *J. galeopsis* leaves were not toxic for those organs at the doses administered. The relative spleen weight was significantly greater in the anemic control and anemic rats untreated and treated when compared to the non-anemic group. This effect is mainly the consequence of the metabolism of phenylhydrazine, as had been suggested earlier. The spleen serves to cleanse the body of damaged old particles transported by the blood (Australasian Society of Clinical Immunology and Allergy 2014). This suggests that an increase in the relative spleen weight might be attributed to the spleen fighting foreign particles due to the anemic condition of the rats. This is also consistent with authors who suggested that the rate of erythropoiesis and 2'5'-A polymerase activity increases after a dose of phenylhydrazine (Orlic et al., 1982).

5. Conclusion

In conclusion, the in vivo study had confirmed the hematinic activity of aqueous extract of *Justicia galeopsis* leaves cooked during 30 and 45 min. The administration of 100 mg / kg / day of extract of leaves cooked during 30 min promotes a better recovery rate of the number of red blood cells (94.80 %), hemoglobin level (159.53 %) and hematocrit (117.72 %) than Vitafer and the extract of leaves cooked for 45 min. The leaves of *Justicia galeopsis* therefore have anti-anemic properties. Therefore, this plant could be used in the management and the treatment of anemia.

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Rapid Detection and Identification Systems for the Microbiological Assessment of Processed Soy Foods: A Review

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Abstract

Plant-based diets are gaining interest in promoting physical and environmental health worldwide. The widely growing consumption of processed soy foods results in an increased demand for safe and high quality soy foods. Many of the rapid bacterial detection methods currently available are inhibited by components in the food matrixes. In recent years, high-throughput devices have been developed, which aid in the enumeration and evaluation of microorganisms in processed soy foods (automated fluorescent filter method, high-throughput identification using matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and automated most probable number method). These methods are more rapid and convenient compared to the conventional culture method. This review discusses alternate reliable methods for the microbiological assessment of processed soy foods, which guarantees the safety of the food delivered for consumption.

Keywords: soy foods, microorganisms, risk assessment

1. Introduction

Microorganisms are one of the most important food quality indicators. The consistent manufacture of safe, high-quality foods requires a well-planned hygiene program aimed at controlling and reducing bacterial contamination during and post-processing.

The Japanese food culture has been added to the UNESCO's intangible cultural heritage list (UNESCO, 2013). Soy foods are one of the representatives of the Japanese "Washoku" culture. Therefore, development of analytical methods for the microbiological risk assessment of soy foods is a great importance.

Soybean is one of the most prominent protein sources, and the balance of essential amino acids is similar to that of milk and eggs. Various kinds of soy foods are commercially available. Soy protein isolate is a highly refined powdered protein that has good gelation and emulsifying properties (Hettiarachchy & Kalapathy, 1997; Tsumura, 2009). It is also used as an ingredient in a healthy diet, targeting those with high cholesterol levels. Additionally, soybeans are attracting the most attention among plant proteins, for use as a sustainable protein source (Thrane et al., 2017).

On the other hand, there is little microbial information on processed soy foods (Table 1). Soil-derived *Bacillus*, found in soybean, is the main flora in processed soy foods, and is one of the targets in the sanitation management program (Zhou et al., 2017).

Table 1. Main bacteria isolated from processed soy foods

Items of foods	Detected bacteria	Reference
Tofu	*Streptococcus, Pediococcus, Lactobacillus, Pseudomonas, Pseudomonas, Enterobacter, Enterobacter*	Tuitemwong & Fung, 1991
Tofu	Coliforms, *Pseudomonas, Escherichia, Enterococcus,* Lactic acid bacteria, *Bacillus, Staphylococcus*	Ananchaipattana et al., 2012
Tempeh	*Enterobacteriaceae*, Lactic acid bacteria, *Staphylococcus, Bacillus, Escherichia*	Samson et al., 1987
Kinema	*Bacillus, Enterococcus*	Sarkar et al., 1994
Sufu	Mesophilic aerobic bacteria, Lactic acid bacteria	Han et al., 2001
Doenjang	*Leuconostoc, Tetragenococcus, Enterococcus, Bacillus*	Kim et al., 2009
Meju	*Bacillus, Enterococcus, Pediococcus*	Kim et al., 2011
Cheonggukjang	*Bacillus,* Lactic acid bacteria	Nam et al., 2012
Denjang-meju	*Bacillus*	Jung et al., 2014
Stinky tofu	*Streptococcus, Lactococcus*	Gu et al., 2018

Traditionally, microorganisms in processed soy foods and other retail foods have been evaluated by standard plate count on selective media (International Organization for Standardization, 2003; Official methods of Analysis Online, 2005). However, these methods usually take more than a day to detect the microorganisms (Vasavada & White, 1993). Therefore, a rapid, accurate and simple method is desirable.

Several rapid detection methods are used in the food industry (Gracias & McKillip, 2004). Polymerase chain reaction (PCR) is one of the most commonly used techniques due to its high sensitivity. In particular, reverse transcription PCR is a valuable method for detecting viable bacteria. However, this method needs to extract nucleic acids from the food samples. In addition, lipids and proteins in food can often interfere with PCR, necessitating the development of other methods (Gadkar & Filion, 2013; Schrader et al., 2012). Fluorescence staining method is widely used for microbial detection without culturing (Miyanaga et al., 2007; Yamaguchi et al., 2007), and flow cytometry is used to count viable bacterial cells (Diaper et al., 1992; Jepras et al., 1995; Khan et al., 2010).

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been shown to be a rapid and reliable method for bacterial identification (Lay, 2001; Mazzeo et al., 2006). The speed and accuracy of MALDI-TOF MS is widespread as a routine bacterial identification method in food microbiology (Angelakis et al., 2011; Böhme et al., 2011; Hochel et al., 2012; Kuda et al., 2014). Bacterial identification using MALDI-TOF MS eliminates the shortcomings of conventional culture methods because the time required for bacterial identification is very short. However, it is difficult to detect and identify bacteria in high lipid-containing food. Moreover, little information is available on non-culture based bacterial identification in food samples using the MALDI-TOF MS (Barreiro et al., 2012; Ferreira et al., 2010; Furukawa et al., 2013; Katase & Tsumura, 2014).

There are many alternatives methods that have been validated by protocols based on the international certification ISO 16140 standard. Petrifilm method is one of the widely applied methods (Freitas et al., 2009; Silva et al., 2005; de Sousa et al., 2005). This method is convenient and reliable, but is labor intensive. Therefore, a simple and automated method is required for quality assurance in the food industry.

In this review, alternate rapid methods of microbial assessment for the safety of processed soy foods, are discussed.

2. Automated Microbial Cell Counting System with Fluorescent Staining in Processed Soy Foods

Microbiological contamination of processed foods is a key issue in the food industry. Traditional methods serve as a reference for the microbiological quality control of food products, as they are reliable and easy to use for microorganism identification. However, these methods are time consuming and labor intensive. Moreover, they depend on the ability of microorganisms to form visible colonies after an incubation period of typically one to three days. This long time-to-result is an issue for food industries, as process improvement requires the availability of faster methods to control microbiological quality.

Consequently, a lot of technologies have been developed to reduce the time to result. These alternate technologies need to be accurate, sensitive and cost-effective. One of the most representative technologies is the fluorescent filter method (Boulos et al., 1999; Lahtinen et al., 2006; Ootsubo et al., 2003; Pettipher & Rodrigues,

1982).

Bioplorer (Koyo Sangyo Co., Ltd), an optical device designed for measuring microbial count in cosmetics, toiletries and foods, has been reported (Arai et al., 2006; Masakiyo et al., 2010; Nishimura et al., 2006; Nishimura et al., 2008; Shimakita et al., 2006; Shimakita et al., 2007). This device consists of a charge-coupled device camera, an optical unit, a light emitting diode (LED) light source, and a driving stage (Figure 1). Fluorescent dyes such as 4, 6-diamidino-2-phenylindole or propidium iodide are used for microbial staining on a sampling tip, followed by placing the tip on the instrument for automated counting. The advantage of an automated cell counting system is that, it requires much less time compared to microscopy, which consumes more than one hour even if measured by a skilled person (Yamaguchi et al., 2003). Although previous studies have demonstrated that this system could successfully enumerate bacterial cells in various food samples (Shimakita et al., 2006), colloidal and proteinaceous fluid such as soymilk and soy protein isolate solutions are exceptions, owing to filtration problems. To separate microorganisms from a proteinaceous fluid, a conventional isoelectric precipitation method, which is commonly used for the production of soy protein isolates (Petenate & Glatz, 1983; Tsumura et al., 2004).

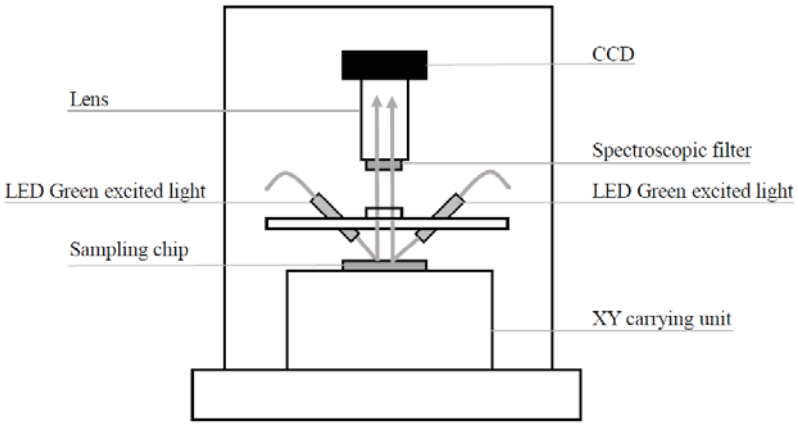

Figure 1. Schematic of the LED-illuminated detection apparatus

Tsumura and Tsuboi (2012) demonstrated that the total bacterial cells in solubilized soymilk could be enumerated using the automated cell counting system. However, for accurate viable cell count, the separation of microorganisms from the complex food matrix is essential (Araki et al., 2010; Benoit & Donahue, 2003; Fukushima et al., 2007; Stevens & Jaykus, 2004). On removal of the food matrix, this system can be implemented and the viable cell count can be obtained by staining with 6-carboxyfluorescein diacetate. Katase et al. (2013) demonstrated the use of this system, combined with a bacterial cell recovery method, in the enumeration of coliforms present in processed soy products. Bacilli are sometimes present as contaminants in processed soy foods (Fang et al., 1999; Pascall et al., 2006). For preferential coliform detection, culture enrichment of coliforms with brilliant green and sodium deoxycholate is performed within 6 h. This method was faster than other conventional methods for detecting trace levels of coliforms in soymilk. An in-house verification test has also been performed, according to the AOAC guidelines (Feldsine et al., 2002), to verify the applicability of this method for the detection of coliforms. This method did not give false positive or false negative results, resulting in higher sensitivity and specificity. A chi square value well below the threshold indicated that the method under investigation, and the reference method, were not statistically different. This alternate technique is more rapid and convenient than the conventional plate count method. Thus, it can be used to quickly detect coliform contamination in processed soy foods such as soymilk. Further improvements are needed for actually detecting specific bacteria by fluorescent *in-situ* hybridization staining (Nishimura et al., 2008; Yamaguchi et al., 2009).

3. MALDI-TOF MS Identification of Bacteria in Processed Soy Foods

In recent years, a rapid method for bacterial identification in processed soy foods using the MALDI-TOF MS system has been generalized (Table 2). However, the fat matrixes in foods interfere with accurate identification of bacteria using the MALDI-TOF MS system. Katase & Tsumura (2014) found that the identification accuracy of MALDI-TOF MS was lower when the fat content of the soymilk sample was ≥ 50%, indicating that the residual fat matrixes might have interfered with the raw mass spectrum and affected the spectrum matching.

Table 2. MALDI-TOF MS bacterial identification of processed soy foods

Items of foods	Identified bacteria	Reference
Korean fermented foods	*Weissella*	Kim et al., 2017
Korean soybean paste (doenjang)	*Bacillus, Paenibacillus, Tetragenococcus, Stapylococcus, Clostridium*	Woo et al., 2015
Soy sauce	*Tetragenococcus*	Kuda et al., 2014
Fried-Tofu, Soy milk	*Enterobacter, Klebsiella, Escherichia, Enterobacter*	Katase & Tsumura, 2014
Soy milk, Tofu, Frozen tofu, Dried tofu	*Leuconostoc, Serratia, Bacillus, Raoultella*	Furukawa et al., 2013
Tofu	*Cronobacter*	Hochel et al., 2012

Other rapid detection methods, such as PCR, are also inhibited by fat matrixes (Gadkar & Filion, 2013; Schrader et al., 2012). According to the International Organization for Standardization (2010), in the bacterial inspection of butter, it is essential to remove the fatty phase.

Katase & Tsumura (2014) demonstrated the separation of coliforms in processed soymilk, using an isoelectric precipitation method, practically applied to produce soy protein isolates (Petenate & Glatz, 1983; Tsumura et al., 2004), in combination with surfactant (sodium dodecyl sulfate) treatment. As a result of this treatment, accurate identification scores were obtained in soymilk samples with high fat content, using the MALDI-TOF MS system.

Contamination of processed foods is not always due to a single bacterial species. However, the coliform concentration in commercially available processed foods is relatively low. The dominant bacterial species in the food product can be identified by selective enrichment methods. The coliforms isolated from commercial soy foods belong to the *Enterobacteriaceae* family (Ananchaipattana et al., 2012; Préstamo et al., 2000). Currently, confirmation test is required following the successful detection of coliforms cultured in brilliant green-bile-lactose broth. On the other hand, since MALDI-TOF MS can directly identify bacterial cells, these confirmation tests are not required. Due to the discriminatory capabilities of MALDI-TOF MS, this direct bacterial identification method can be used in the food industry for quality control, process monitoring, and for confirming the presence of bacteria detected by other tests.

4. Automated Enumeration System Based on the Most Probable Number (MPN) Method

Most probable number (MPN) is the primary method for the enumeration of food borne bacteria since it can detect low bacterial levels. However, MPN method is labor intensive and time consuming. TEMPO is an automated enumeration system based on the MPN method for bacterial enumeration in food samples (Kobayashi et al., 2008; Kunicka, 2007; Owen et al., 2010; Paulsen et al., 2006; Paulsen et al., 2008: Torlak et al., 2008;). It is an internationally recognized method validated by ISO and the AOAC, and evaluated in various foods (Crowley et al., 2009; Crowley et al., 2010; Jasson et al., 2010).

Artificially contaminated processed soy products (soy protein isolate, soybean soluble polysaccharides, soymilk and tofu) were evaluated using different TEMPO kits (total aerobic bacteria; total coliform; *Enterobacteriaceae*; yeast and mold; *Staphylococcus aureus*). A high Pearson correlation coefficient was seen between the TEMPO and standard plate method, and no statistically significant difference was observed between the two methods (Katase & Tsumura, 2011).

The standard plate method requires time, materials, and is labor intensive (Vasavada & White, 1993). The confidence that the method has been executed accurately often depends on the skills of the staff. A non-technical staff may not be able to distinguish between a microbial colony and a food particle while performing the bacterial count.

Accurate results were obtained for majority of the processed soy food samples using the TEMPO method. Occasionally, in richly colored soy foods (soy sauce) and fermented soy foods (natto), some results were anomalous. This system provides improved standardization and minimizes economic loss, in terms of labor, by eliminating dilutions, preparation of medium, and plate counting. Therefore, TEMPO is a reliable alternate method for the microbial testing of processed soy foods.

5. Next Generation Sequencing Technique for Microbiological Risk Assessment

In recent years, the next generation sequencer has brought about innovation in microbial analysis (Table 3). In Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC), routine whole genome sequencing (WGS) has successfully identified the sources of food-borne pathogen contamination

(Rantsiou et al., 2018). Without relying on WGS, the analysis of the flora contained in food samples has been successfully examined using the 16S ribosomal RNA sequencing method (Jagadeesan et al., 2019). The 16S metagenomic analysis can be used routinely to evaluate the bacterial quality of food ingredients (Patro et al., 2016). At present, the hurdles are high in terms of cost, for the food companies to use these techniques easily. However, this technology will be used actively in the future for microbiological risk assessment.

Table 3. 16S amplicon sequencing approach for microbial assessment of processed soy foods

Items of foods	Target bacteria	Reference
Fermented soybean products	*Lactobacillus, Enterococcus, Fructobacillus, Staphylococcus, Carnobacterium*	Xie et al., 2019
Soy-daddawa, fermented indigenous food	*Bacillus, Staphylococcus*	Ezeokoli et al., 2018
Da-jiang, fermented soybean condiment	*Staphylococcus, Leuconostoc*	Wu et al., 2018
Fermented soybean foods	*Bacillus, Tetragenococcus, Enterococcus*	Lee et al., 2017
Soybean pastes	predominant phyla (*Firmicutes*, *Proteobacteria*, *Actinobacteria*)	Sun et al., 2018
Fermented soy bean paste (doenjang)	*Tetragenococcus, Enterococcus, Leuconostoc, Lactobacillus*	Kim et al., 2016

6. Conclusion

In the clinical field, microbial measurement is being accelerated and automated. However, in the food sector, rapid microbial evaluation technologies are not widespread. This is because the samples to be tested are not simple like water and blood. Foods contain many ingredients such as pigments, proteins, and lipids, which inhibit microbial measurement. Moreover, since the cost of the conventional medium-based method is low, introduction of a new method with high cost is often not preferred. Low cost, simple pre-treatment methods such as protein removal by isoelectric precipitation, and oil removal using surfactants has achieved rapid detection, identification and automation of microbial measurements in processed soy foods. For food companies, the ability to quickly judge the results of microbial tests means enhancing the microbial management system. Compared to the conventional culture method that relies heavily on human labor, automated methods enable each person to obtain similar results, aiding in the advancement of microbial test standardization. Consequently, food manufacturers can focus on making safer products. These findings are considered to contribute greatly in guaranteeing the delivery of safe and high-quality processed soy foods.

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Drivers and Solutions for Food Waste in the Restoration Sector: A Case Study

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Abstract

The aim of this research is to examine the main drivers of food waste in restaurants, the main tools used by the sector to reduce waste and to forward a number of solutions to waste generation. The paper uses a case study in a municipality of Madrid, Spain, where a number of restaurants' managers were interviewed. The work collects information on how restaurants manage waste, investigate what are the perceptions and attitudes of managers, and determine what kind of solutions could be put in place to reduce in the restauration sector. Findings show that although restaurant managers are aware of the footprint that food waste create, excuses for inaction —going from putting the blame on clients or the space of their premises— were commonly used in the restaurants analysed for this research.

Keywords: food waste, sustainability, restoration sector, behaviour, climate change, reduction of food waste, restaurants, sustainable practices

1. Introduction

Finding ways to reduce food waste has become in the last years an increasingly important topic for researchers and the international community. Food waste occurs throughout the entire food supply chain, from agricultural production, to postproduction procedures, processing, distribution, and consumption (FAO, 2011; Lipinski et al. 2013; Papargyropoulou et al., 2014). Food waste and food loss have been studied from the perspectives of agricultural production (Delgado et al., 2017; Rao & Rathod, 2018; Chang & Li, 2019); of consumers and households' perspective (Schanes, 2018; Boschini et al. 2020; Mattila et al., 2019; Cerciello et al., 2018; Schmidt et Matties, 2016); and of innovation, in the way food waste may be transformed into energy (Yeo et al. 2019).

While food waste levels at the end of the food chain (processing, distribution and consumption) are greater in high-income regions (FAO, 2013; Papargyropoulou et al., 2014), the waste of food incurs in economic implications for everyone participating in the food supply chain, from the farmer to the food producer and the consumer in both developed and developing countries (Papargyropoulou et al., 2014; Baldwin, 2015). Although there are several published articles on food waste in the food service industry (Betz et al., 2015; Baldwin, 2015), the extraordinary amount of food waste produced every year leaves room for additional empirical research whose objective is to contribute towards promoting and increasing sustainability in the food chain sector, and consequently, to increasing global sustainability. According to the Food and Agriculture Organization (FAO), approximately one-third of all food produced in the world is lost or wasted (FAO, 2011), with an associated cost of USD 936 billion per year, the equivalent to the GDP of Indonesia or the Netherlands (FAO, 2015). One of the advantages of food waste is that it is relatively easy to reduce, since each of us can opt to consume more responsibly, thereby contributing to the reduction of greenhouse gases. With regards to food waste, it is necessary to also mention other types of waste (non-food waste) that are generated along with the food elements, such as cardboard, plastics or glass.

In Spain, the Ministry of Agriculture, Fisheries and Food (MAPA by its current acronym in Spanish) published in 2016 a guide where it placed Spain in the thirteenth position in the amount of wasted food per inhabitant, with 176 kg of wasted food per year per inhabitant, most of which was generated in the stages of distribution and consumption. The MAPA identified the European Union´s catering sector as responsible for 14% of the overall

amount of food wasted in Europe (MAGRAMA, 2017). Furthermore, considering these figures, FAO estimated that there were 2 billion people suffering from food insecurity in 2019 (FAO, 2019). In October 2015, Boadilla del Monte, a municipality of Madrid, joined the Covenant of Mayors for Climate and Energy, thus committing the municipality to reduce its greenhouse gases (GHG) emissions. This paper shows part of the results of an analysis of waste management in the restoration sector promoted by Boadilla's city council with the aim of contributing to improving their waste management strategy and thus their GHG emissions.

In the European Union (EU) alone, in 2012, 88 million tons of food (the equivalent of 173 kg per person) were generated with an associated cost of 143,000 million euros (Stenmark et al., 2016). The European Union, through the circular economy concept, seeks to promote a new type of economic development, in which waste reduction plays a fundamental role. There are multiple causes of food waste, which exists at all levels (manufacturing, catering, domestic...), and among them are the size of the cooked portions in restaurants, the labelling legislation, the lack of planning in restaurants, food handling, procurement management or the own quality requirements of the restaurants, among others (Plascarb, 2013). The reduction of waste and the improvement of the management of the latter are of paramount importance in the reduction of greenhouse gases, and comply with Sustainable Development Goal (SDG) 12, which commits the international community to guarantee sustainable consumption and production at all levels between 2015 and 2030 (United Nations, 2015).

The reduction of food and non-food waste is aligned with Sustainable Development Goal 12, aiming to ensure sustainable consumption and production habits which will contribute towards the promotion of efficient energy and resource use and in the improvement of everyone´s quality of life. Moreover, it will also promote good practices among companies and consumers with respects to the reduction in the use of resources. Specifically, Sustainable Goal 12.3 focuses on halving the global food waste per capita at the consumer level (United Nations, 2015). In short, it can be said that waste reduction has a threefold objective: (1) to reduce the environmental impact, (2) to contribute to an economy with a reduced use of resources and (3) to promote more sustainable consumption patterns.

However, very often, greenhouse gas emissions or unsustainable consumption patterns are almost exclusively linked to energy consumption, transport, or the manufacturing industry. As an example, during our data collection more than 26% of the respondents did not know the impact of food waste on climate change or on sustainability, which should probably serve as a base for more targeted information campaigns in the restoration sector. Emissions from fuel combustion (including those of transport) accounted for 79% of the total greenhouse gas emissions in Europe in 2017, followed by agriculture (10%), industrial processes (8%) and waste management (3%) (Eurostat, 2019a). The amount of waste per capita has been reduced since 2009 going from 510 to 492kg per capita (Eurostat, 2019b), showing that much more should be done to reduce the amount of waste generated at the European level.

This study provides an overview of how restaurants in Boadilla del Monte (Madrid) manage waste, deep on the perceptions and attitudes of managers, examines the reasons they state for those attitudes and determine what kind of solutions could be put in place to reduce waste (food and non-food) based on the findings of this study. The research questions for this study are: What are the main drivers of food waste in restaurants? What are the main tools used by the sector to reduce waste? What are the main obstacles to reducing waste? How can waste be reduced?

The argument of the paper is that although restaurant managers are aware of the footprint that food waste create, excuses for inaction are commonly used in the restoration sector. The goal of this research is thus to endeavour a diagnosis on the motivations and drivers of food waste as well as a series of recommendations on waste management in the restoration sector, thus contributing to increase the sustainability of this type of industry.

2. Method

The data used in this report has been collected through online surveys and semi-structured one-to-one interviews that were carried out in restaurants of Boadilla del Monte between May and August 2018. Both the surveys and the interviews enabled the collection of a large amount of data, which helped us determine what are the different realities of waste management in the restaurants of the municipality. The interview consisted in 29 questions, which covered perceptions and behaviours about waste management, food waste management, non-food waste management and general awareness on the impact of waste on sustainability. A total of 23 restaurants, fast food, non-fast food, participated in the survey.

The analysis has been completed with an extensive literature search consisting of specialised reports and papers on food waste as well as information from European and Spanish official sources to compare and contrast our local findings with those more general. To have a better perspective on the problems with waste management in

the municipality, the authors interviewed representatives of the company in charge of the waste collection, as well as the town council environmental representative, who is currently the municipality mayor.

The paper is organized in three sections. The first one provides an overview on the perceptions of the interviewees on the contribution of food waste to climate change and unsustainability and on the main drivers of food waste in the daily running of restaurants. The second section focusses on the tools restaurants have in place to reduce waste generation within their premises and the main obstacles they identify to reduce waste, and the third section proposes a number of solutions to diminish the amount of waste generation. The paper ends with some conclusions on the analysis carried out.

3. Results

Most of the restaurants surveyed (87%) perceived food waste as a real problem that should be treated at different societal levels (i.e. from individuals, to regional and national governments), and 73.9% of respondents believed that food waste contributes to climate change. These results are in line with the information published by the FAO (FAO, 2017) stating that food losses and waste represent a considerable waste of land, water, energy and agricultural inputs, and cause the emission of millions of tonnes of greenhouse gases. Almost all the interviewees (95.7%) agreed that food waste generated economic losses; however, some restaurants pointed out that they considered those economic losses associated to food waste as something "normal", which was more dependent on customer behaviour and attitude than on a bad practice from the part of the restaurants.

According to the FAO (2013), 1,300 million meals annually end up in the trash. In developed countries, most of the food is lost in the last phase of the supply chain, at consumption, as the results of the survey point to. While consumers over-buy or overreact to expiration dates and to product preferential consumption, retailers reject large quantities of food due to the present quality and aesthetic standards. The result of all this is that up to 46% of food ends up wasted at the processing, distribution, and final consumption stages (FAO, 2013). The restaurant managers were asked on the stage in which waste was produced to have a better understanding of the extend of the problem and to elaborate adequate proposals to diminish the waste.

Many of the restaurants claimed that they had an effective food management system, and that they thereby only bought the food needed. Furthermore, 9% of the restaurants interviewed responded that most of the food wasted derived from the unused stored food and 4% said that most food waste came from the preparation of the dishes. Interestingly, most of them blamed the clients for the food waste. On the one hand, all the restaurants surveyed said they had the habit of advising customers about the quantities of food they ordered, which is an important step towards minimizing food waste levels. But on the other, 70% of the restaurants interviewed considered that it was the clients that generated most of the food waste, which leads us to question whether adequately-sized portions of food are actually served in the restaurants that participated in the surveys, and whether "diners" are properly advised. In line with this, a total of 87% of the restaurants surveyed considered that they served proportionally correct sized food portions, which contracts with the previous affirmation and with previous research that point that serving excessive food portions increases the likelihood of eating more than we need (Benton, 2015; Brunstrom, 2011) and at the same time generates greater food waste.

Some restaurants said that they hardly generated any waste and considered themselves efficient with respect to the use of food in the preparation of their dishes. Furthermore, they said that the food at their restaurant was prepared at the time of the order, thereby minimizing the amount of food stored as well as the potential to generate waste. Considering that almost 70% of those interviewed said that most of the food waste came from the diner leftovers, as it has just been pointed out, it is interesting to examine a bit further the types of food waste that the restaurant clientele leaves. According to the interviewees, these include side dishes such as fries and salads, bread and rice and legumes, what gives us information on what kind of food may be reduced to avoid waste. This information, however, does not go in line with that published by the Spanish Ministry of Agriculture, Fisheries and Food (MAGRAMA, 2017), where only 30% of the food waste came from the leftovers of the clients. Once again, it seems that restaurant managers tend to account the consumers for most of the food waste and not on the amount of side dishes served.

We found important to consider the type of clientele that restaurants have and the expectations of the latter with regards to quality and variety of the food. What the authors observed in their interviews was that while some restaurants claimed that their clientele would "eat until they burst", other restaurants' clients seemed to appreciate high quality dishes, and that had an influence on the attitudes of managers towards food waste management, since the former tended to understand food waste not as the consequence of an excess of food on the plate but as the gluttony of clients. Therefore, the perception of the restaurant managers on their customers' expectations and needs seem to determine how much food will be served and, subsequently, how meals and food

orders will be planned for the short term (i.e. days or weeks).

As pointed out above, one of the aims of SDG 12 is to promote more sustainable consumption patterns. In line with this, 82.6% of the surveys and interview respondents said that they would like to be recognized for their sustainable policies and practices, which is an important point as it could potentially bring value to these restaurants by differentiating them from competitors. However, as many of them pointed to, sustainability in the restoration sector remains a vague concept that can range from using ecological cleaning products in restaurants to promoting the use of the Tupperware among the restaurants´ clientele. Indeed, even those restaurants that want to be recognized as more sustainable find problems in the process. For example, some respondents recognized that they experienced difficulties in knowing where the food supplies come from. Some of the restaurants said they really did not know the origin of the food served at their restaurant. They obviously knew the suppliers but had no real knowledge of the origin of their products. This contrasts considerably with other countries, such as in some Scandinavian countries where restaurant owners even know the name of the farm from which each of the products, they serve their customers.

At present, there is no national brand or standard that recognizes sustainable catering establishments in Spain, and future research could deep in the role of national governments in the promotion of sustainable practices through the creation of standards or the promotion of tools such as blockchain, a technology increasingly used to enhance transparency in agricultural and livestock supply chains (Beef Central, 2018).

3.1 Perceptions on Waste Management

A set of the questions of the survey was aimed to assess how restaurants manage their non-food waste, mainly paper, plastic, and glass. The first thing that the restaurants were asked was to self-assess themselves as regards to their recycling habits and give themselves a grade out of 10. The average score for this self-assessment was almost 8, which shows that restaurants participating in this study considered themselves quite efficient with respect to their recycling behaviour. The pie chart below displays the type of waste restaurants said they generated the most, the most popular answer being glass and organic waste, followed by paper and plastic.

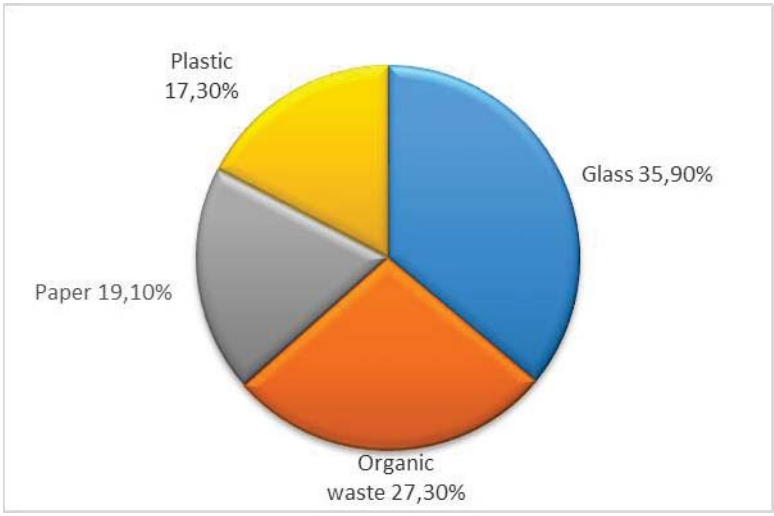

Figure 1. Percentage of answers to the question: How would you distribute the total amount of waste produced in your restaurant (total waste = 100%)

As seen in the pie chart, glass (i.e. wine, water end beer bottles) is the largest type of waste generated by restaurants surveyed (35.9%), followed by organic waste (27.3%). Based on these results, we understand that bigger efforts should be made to reduce both the amount of glass used (maybe considering the option of giving preference to tap water served in jugs over bottled water) and the amount of organic waste produced. In this regard, restaurants were asked on their management and on the different tools they had in place to reduce these amounts of waste.

As a way of reducing the amount of glass and plastic generated in restaurants by the bottles of water, interviewees were asked whether or not restaurants would serve tap water in jugs, to what 56.5% of respondents said that they served jugs of water to their customers if they asked for them, while 34.8% responded that they did not offer the option of serving water in jugs even though they knew that it would contribute to reduce waste.

Although serving a glass of tap water seems easy, simple and, theoretically does not suppose an additional cost to the restaurant, the biggest claim of some restaurants was the economic part, arguing that serving tap water via a glass or a jug meant a cost for them, even if it was very small. One of the arguments given was that all restaurants pay for the water they consume like any of us in our homes, so why give it to customers for free when it costs restaurants? All restaurants serve bottled water, either in glass or plastic bottles, and their profit margin is considerable, so serving tap water in their restaurants would mean economic losses that they would not be willing to assume. The restaurants also referred to the problem of space that involves having jugs of water available to customers as a reason to deny the option of serving water in jugs. Normally, the space that restaurants have in the kitchen and bar is quite limited, so storing jugs implies the use of a space that some establishments do not have, and the limited space that they do have is used for other more priority purposes. However, managers interviewed did not seem to find any space problem in storing hundreds of bottles in their establishments.

A second set of questions were directed to the management of the organic waste produced in the restaurants. Something that the authors specifically wanted to know was whether restaurants did something with their leftovers (i.e. we need to highlight that we are referring to here to the food that does not leave the kitchen) and how they organised the separation of waste within their premises. As regards the first question, 69.6% of respondents said they did try to take advantage of their food leftovers through some of these initiatives: allowing workers to take home their leftovers; using leftovers for employees' breakfast, lunch or dinner; taking restaurant leftovers to a nursing home; preparing small snacks and “tapas” for customers when ordering a beer or a glass of wine; preparing new recipes with leftover food, such as salmorejo with the bread from the previous day.

Some interviewees told us that although these initiatives were great, there were a few elements that needed to be taken into account such as the compliance with specific food quality and safety standards and regulations, which are very important when giving leftovers to others, such as in the preparation of new snacks or recipes. This is in line with some authors that indicate that in developed nations the compliance with high health and hygiene standards are one of the main causes of food waste (Waarst, 2011), thereby the potential of rendering more flexible the current standards should perhaps be considered in order to reduce the amount of food that ends up in the trash.

Another fundamental aspect to understand waste management is related to how residue separation is carried out in the participating establishments: Although some of the interviewees admitted that during moments of elevated stress levels while serving meals, staff might not recycle properly since in those circumstances they prioritized the quick service to the clients before recycling, however, overall, 85.7% of the respondents said that they separated all of their residues in different containers. This result is very much aligned with the self-assessment mark that the restaurants gave themselves with respect to their recycling behaviour (i.e. average score of 7.98). In this regard, the company in charge of collecting organic waste and packaging in the municipality, confirmed that most restaurants did a good job in depositing their garbage in the different recycling containers; however, they pointed out some important aspects that could be improved: for example, how some restaurants sometimes left containers and paper / cardboard out of the recycling containers, and they blamed this firstly on how quickly the workers needed to throw away the waste, and secondly on the possible lack of training of some of the restaurant staff on the importance of recycling properly.

In this regard, during the surveys, data was also compiled regarding the policy and training of the restaurant staff in waste management. Although 70% of the restaurants affirmed that their staff received training in waste separation, by this most interviewees referred to the food handling course that all catering staff need to carry out to work in the restoration sector in Spain. This type of course usually lasts 10 hours, many of them are taught online and includes among the subjects to be covered a wide variety of topics, from food preservation and handling to the hygiene of the premises and staff. Therefore, amongst the knowledge acquired, there is very little that concerns the existing legislation on food waste handling and recycling or on the actual impact that recycling has on the proper functioning of the restaurants or even on its profits and losses.

We also found out that restaurants considered an especially important and determining element for recycling properly the size of their premises to store waste. Several restaurants mentioned that it would be ideal to have all four types of recycling bins in their kitchen, however the space they had did not allow it. Some of the restaurants answered that they had two of the four containers because of this reason. Other restaurants said that, logistically, they had the public recycling containers remarkably close to the street, so it was not necessary to have bins in the kitchen for the four waste types. For example, cardboard waste often takes up a lot of space, and they therefore did not store but just took it down to the nearest paper recycling container. On many occasions, restaurants receive food and other items in large cardboard boxes that take up a lot of space, even folded, therefore taking

them outside is just easier.

Another element to consider when evaluating waste management is knowing how the latter is carried out inside the restaurant. Of the restaurants surveyed, 17% had one or more people in charge of waste separation. In some of the restaurants interviewed, it was the waiters that oversaw the separation of the waste and the throwing out the garbage, while in others the tasks were separated amongst the kitchen staff and the waiters. In 70% of the restaurants surveyed the separation of waste was everyone´s tasks, and this according to some restaurant managers contributed to being efficient in the restaurant´s recycling activities, while for some other respondents, it leads to inefficient recycling endeavours.

In any case, from the interviews it is impossible to deduce whether there was a person in charge of the supervision or whether the waste separation was done correctly by the restaurant workers, thereby relegating the task to the goodwill of the workers. It must be remembered here that the final responsibility is of the restaurant managers and not of the workers, therefore properly training the workers in waste management and having one or two people responsible for managing waste properly is very important. Numerous publications, for example, point out the advantages of training staff as regards to the benefits of recycling in achieving more efficient waste management in restaurants, which leads, among others, to the saving of raw materials, thereby reducing costs and improving the service that restaurants give to the clients (Norden, 2012). Some reports estimate that every tonne of food waste produced costs of €2000 (Creedon, 2010). Similarly, a more recent report by WRAP (2018) pointed out that establishments that trained staff in waste management can recover 64% of the investment after the first years and that percentage increased to 80% in two years.

Restaurants were finally asked on the initiatives they had in place to promote sustainably within the daily run of their restaurants. Although it cannot be said that the restaurants surveyed have an internal sustainability policy, 18% said they used recycled paper napkins in their establishments, although some admitted that they bought the recycled napkins because they were cheaper and not because they were more environmentally friendly. However, 37% of restaurants did say that they bought local and seasonal products as a way of contributing towards sustainability, while 18% of the restaurants interviewed informed that they chose to use cloth tablecloths as these generated less waste.

4. Discussion

The role of the food service sector in the reduction of food waste in developed countries is important to shift to more sustainable consumption patterns and practices and to increase awareness on the impact that food waste has on the environment or the economy (Papargyropoulou et al., 2014). While there is no single practice of reducing food waste (Mattila, 2019), below there are a set of recommendations to reduce food waste that authors propose based on the results of the analysis and after the reviewing of specialised literature, the interviews with the restaurants participating in this study, with Boadilla´s environmental representative and the representatives of the company in charge of collecting organic waste and packaging in the Municipality.

4.1 Recommendations on Training

Sustainable restaurants not only try to offer fresh, local and seasonal products, they also efficiently control waste and manage the use of water and energy. Thus, among the most important issues in waste prevention is the training and motivation of staff (Creedon et al., 2010; Filimonau et al., 2020). This is very necessary to ensure the correct disposal of the waste. As pointed out above, although waste separation training is included in the food handling courses that people have to take to work in the restoration sector, it would be advisable to train employees with regards to the benefit of sustainability and waste recycling both for the environment and for the restaurants themselves. Another recommendation is to appoint at least one person in charge of the restaurant´s waste management (ReFED, 2018). In practically all of the restaurants that were surveyed, there was normally no-one in charge of ensuring that waste disposal is carried out correctly within the restaurant and that the waste is properly separated in the premises, and deposited in the outside municipal recycling containers.

According to a Unilever report (Unilever, 2016), a 50% reduction in food waste is potentially achievable for most restaurants. Regularly conducting a waste audit would be another way of determining restaurant waste levels, thereby potentially leading to new behaviour as regards to the purchasing, storage, preparation and the serving of rations.

Some researchers also point to the behavioural drivers behind food waste (Setti et al., 2018; Mondéjar-Jiménez et al., 2016), among of which is the lack of consumer awareness with respect to the impact of their food in the economy or the environment (Neff et al., 2015; Stancu et al., 2016; Kibler et al., 2018). The restoration sector can help improve the information customers receive in different ways. For instance, restaurants may improve

their communication regarding and promoting the flexibility of their menu options, by giving different serving size options. Clear communication in this respect will help clients make better choices regarding how much they want to eat. Part of the communication restaurants can offer may well include the setting up of posters and placards to remind clients of the benefits of sustainable practices and of the importance of saving water or energy in restaurants.

4.2 Recommendation on the Size of the Servings

There are studies that point to the lack of creativity as regard to the use leftovers as a potential cause of food waste (Cappellini and Parsons, 2012; Schimidt & Matthies, 2016; Kibler et al., 2018; Närvänen et al., 2018). Some of the restaurants surveyed made use of most leftovers by offering them as appetizers or transforming them into new dishes. Getting creative and turning leftover vegetables into tasty soups, using leftover breakfast toast for fish cakes or leftover chicken or pork to make paté are all options for reducing food waste.

Around 70% of the restaurants that participated in this study claimed that they had a policy where waiters advised customers to take their leftover food in a doggy bag. However, in many cases, restaurants did not anticipate themselves to the clients´ requests but instead just offered them a doggy back when the clients said that they wanted to take their leftovers home. Perhaps this is one of the areas that may be improved to easily avoid and reduce to a minimum the cost of food waste. Catering chains, such as Starbucks, already contribute to waste reduction by giving a small discount to customers who bring their own cup for coffee (Starbucks, 2018), which could be another option to consider especially for take away restaurants. These are small gestures that may be endeavoured depending on the restaurant type (it seems a good idea for fast-food restaurants).

As Porpino (2016) points out when analysing household food waste behaviour, consumers tend to enjoy serving big portion sizes, but retailers are increasingly aware of their responsibility in reducing food waste caused by big portions. Therefore, another recommendation for restaurants is to adapt the size of their menus and servings. Adapting the size of the servings according to what the customers of each restaurant tends to consume will help to both save money and reduce food waste. The options to determine adequate portion sizes range from serving the food in front of the diners, which gives them the say regarding how much they want to eat, to offering different size offerings of the same dish. Other recommendation would include the possibility of having less extensive menus with a selection of fewer dishes, which will help restaurants be much more accurate when buying and managing food products.

The donation of food to local authorities can also be a measure to reduce food waste or to use as food for animals (Betz et al., 2015; Baldwin, 2015). Some restaurants surveyed told us that they gave part of their leftovers to charities, such as nursing homes, food banks or NGOs. Other restaurants said that the current health regulations in Spain made these initiatives difficult. Perhaps it may be possible from the part of the public administrations in Spain to encourage or facilitate the transfer of restaurant leftovers to those that need it most. In line with this there are initiatives that put restaurants or supermarkets in contact with costumers so the former can sell unconsumed food at a lower price at the end of a working day, like “11th Hour” from Singapore, “No Food Wasted” (Netherlands), “FoodCloud” (Ireland and United Kingdom) or “Too Good to Go” (Europe and the United States).

Another measure is to use the leftover organic remains of fruits and vegetables from prepared meals, etc., as compost for soil amendment, anaerobic digestion for energy or producing meals or tallow (Baldwin, 2015). Some city councils have already progressed in this respect and are presently part of the Composting Network (Composta en Red), a Spanish national network of local entities whose aim is to promote the development of public policies that are in favour of composting.

The philosophy of take-away restaurants goes hand in hand with a huge generation of waste. In these cases, establishments can also use environmentally friendly measures such as opting for recycled materials, paper / cardboard (versus plastic) (Rosa, 2018), avoiding the use of straws, compensating customers who bring their own containers or facilitating the separation of waste through the implementation of recycling containers in the restaurant premises. Alternative measures can be the use of dispensers to serve sauces such as ketchup, mayonnaise, oil, etc., instead of using individual packets.

Finally, as described in this report, some restaurant owners had a negative perspective as regards to offering free jugs of water to their clients, since there are several associated costs involved (i.e. the water, the ice, the cleaning, etc.). However, the authors of this paper consider this a good alternative to the big ecological footprint that bottled water has on the environment. An intermediate solution may be for restaurants to serve jugs of filtered water. This water could be sold like bottled water, but at a lower price (Diario de Navarra, 2018), which will help reduce the number of plastic and glass bottles used, thereby reducing the amount of waste generated.

Furthermore, by having a filtered water system, restaurants will also be able to save on space that is currently occupied by glass and/or plastic bottles.

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Impact of Industrial Revolutions on Food Machinery - An Overview

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Abstract

The industrial revolutions occurring at specific periods impacted differently on food industry machinery, including analytical and innovative scientific equipment, and had significant effects on food manufacturing. The fourth industrial revolution (4IR) unlike the other industrial revolutions (1IR, 2IR, and 3IR) has a faster significant impact and niches on artificial intelligence (AI), machine learning (ML), blockchain, robotics, the internet of things (IoT), digitalization, big data, autonomous vehicles, additive manufacturing, nanotechnology, biotechnology, and 3D food printing technologies. Going forward would require more mechatronic engineers as process technicians, particularly in paperless processes and automation of the digital rich future.

Keywords: industrial revolution, machines, food, industry, 4IR, digitalization, artificial intelligence, machine learning, robotics, blockchain

1. Introduction

Different forms of technologies have been enablers over the centuries in the food industry. Each emerging technology is characterized by the introduction of transformational new technologies into manufacturing. The four industrial revolutions (IR) occurring at a specific period in history impacted differently on food industry machinery, including analytical and scientific equipment, and had significant effects on food processing and products. Technology has been an enabler in the food industry. The industrial revolution began around 1780 with the introduction of mechanical production plants powered by liquid water or steam. The second industrial revolution (2IR) came after about 30 years when the first mechanical assembly line powered by electricity was constructed. Around the 1960s, the third industrial revolution (3IR) with new technologies, internet, and the renewable energies changed history (Strozzi et al., 2017). The fourth industrial revolution (4IR), coined as late as 2016 by Schwab during the World Economic Forum in Dovos, makes use of automation, deep mechanism, and digitalization (Prisecaru, 2016) niching on artificial intelligence (AI), machine learning (ML) and robotics. The use of novel techniques in the food industry over the centuries has seen increasing high-pressure processing usage around the globe, including the development of powered machines such as automated extrusion. North America and Mexico (54%) are ahead in high pressure (HP) usage for high-value products compared to Europe (25%), Asia (12%), and Africa (1%). It is possible that industrial revolution periods slightly overlap (Tables 1) and not strictly distinct for start and end dates. Hence, merging of technologies over the centuries evolved into the new technology of the period under consideration. Such merging will enable having an insight into 5IR in every process, including innovative printed reactors. The fourth industrial revolution (4IR) unlike the other industrial revolutions (1IR, 2IR, and 3IR) has a faster significant impact and niches on artificial intelligence (AI), machine learning (ML), blockchain, robotics, the internet of things (IoT), digitalization, big data, autonomous vehicles, additive manufacturing, nanotechnology, biotechnology, and 3D food printing technologies.

2. The First Industrial Revolution and the Impact on Food Machinery

The first industrial revolution (1IR) was the transition to new manufacturing processes in the period from about 1760 to sometime between 1820 and 1900 (Strozzi et al., 2017). This transition included going from hand production methods to machines for processing with mechanical production equipment driven by water and steam power (Crafts, 2006) but without digitalization(Tables 1). Important technological developments involved mechanized cotton spinning powered by steam or water (Prisecaru, 2016). A mechanization machine replaces animal and manual labor from the late 18th to early 19th century. The adaption of stationary steam engines to rotary motion in food processing machines made them suitable for in food industries. Traditionally, drying was applied to both animal and vegetable products and relied primarily on the action of the sun and wind (Klein, 2008; Anderson, 2015).

Table 1. Main characteristics of industrial revolutions (adapted from Prisecaru, 2016)

Industrial revolution (IR)	Transition period	Energy resource	Main technology	Food machines	Illustration
1st IR	1780-1900	Coal	Steam engine	Textile, steel	
2nd IR	1940-1960	Oil electricity	Internal combustion engine	Extrusion line for pasta production	
3rd IR	1960-2000	Nuclear energy	Computers, Robots	Automated cooking extruders,	
4th IR	Continuing	Green energies	Internet, 3D printer, genetic engineering	Vacuum processing machine, Electronic tracing technology	

Figure 1. Workers in canning company during the 1st industrial revolution

https.//teara.govt.nz/files/p-23079-enz.jpg

In 1771 marked the first fully automated spinning mill driven by water power. Hermetic bottling techniques were invented in 1809 by a French chef, confectioner, and distiller using corked-glass containers reinforced with wire and sealing wax. The canning method used to preserve food was invented during the 1IR. A famous French investor developed a special autoclave for food canning in 1823 (Barbier, 1994). Steam sterilizing machine, metal detector, continuous freeze dryer, microwave vacuum dryers, twin-screw cooker/extruder, mixer with the electric motor, and microprocessor-controlled packaging machinery marked this period. Most food industries during 1IR developed packaging methods for food such as canning as shown in Figure 1.

The 1IR impacted food machinery by i) machines invention that replaced human labor: involving the transition from hand production methods to machines in food industries, increasing use of steam power replacing water wheels, development of machine tools, and the rise of the mechanized factory system. These machines increased mass production, the number of goods and diversity of goods produced (Malek, 2005); and ii) new energy sources to power machinery in food industries. Steam engines (Figure 2) replaced water wheels to power machines used in food factories (Malek, 2005). A good example was the Albion flour milling machine, Southwark, Surrey UK, (Salmon, 1843) powered by steam engines which replaced a water-powered grist mill.

Figure 2. Steam-powered Albion mill (1786), Water powered Grist mill (1787), and Watt steam engine (1776), respectively, Patrick and Clay (2012), Salmon (1843), Vernon (2019)

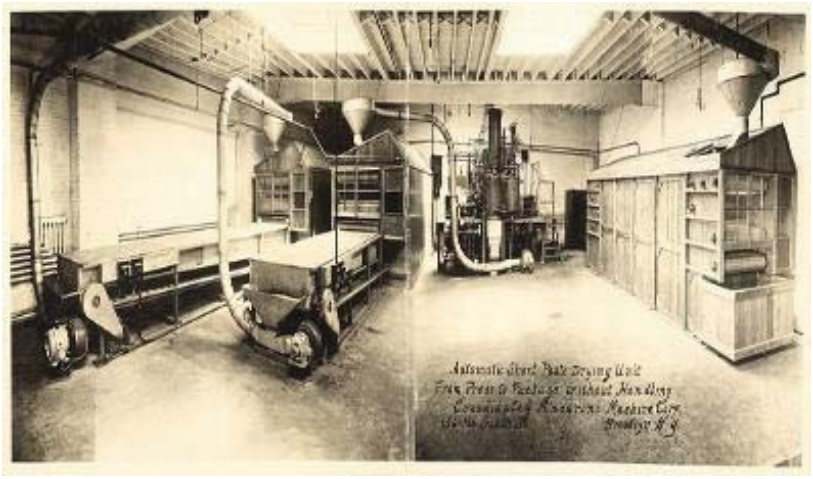

Figure 3. Dry pasta manufacturing line from the 1930s

https://en.m.wikipedia.org//wiki/food _extrusion

3. The Second Industrial Revolution and the Impact on Food Machinery

The second industrial revolution (2IR) was based on mass production achieved by division of labour concept and the use of electrical energy (Table 2), machines, and processes from the late 19th to late 20th century (Crafts, 2004). Instead of using water and steam power, most food industries used electricity-powered machines that reduce production time in bakeries, dairy industry, larger agricultural estates, and other food processing centers (Fremdling 2008). Steam engines were replaced by electricity as a primary power source to power food industries and machines (Muntone, 2013). The Bessemer process (Sheffield, England) used to make strong steel was introduced enabling better and stronger machines used in food industries to replace weak iron (Muntone, 2013). The 2IR was noticeable by the chemical industry, mechanical engineering, and the food industry (Adnan et al, 2017). The sources of energy were oil and electricity (Adnan et al., 2017). Mass production played a lead role in the revolution. The introduction of assembly lines increased automation as machines were combined and reduced the production time (Muntone, 2013). Common examples of food machinery were: i) first semi-automated extruder that uses electricity designed to manufacture packaged dry pasta and breakfast cereals around the 1930s (Figure 3), ii) refrigerators (Figure 4a) used to store and cool foods were improved and fans to cool machines during production, and iii) the oven used in oven drying (Figure 4b).

Figure 4a. Improved electric refrigerator

b. The first electric oven of 1892 (Muntone, 2013)

Table 2. Industrial revolutions and major impact on the development of food machinery

Facts on industrial revolutions (IR)	Food processing machines/machinery	Impacts (Advantages & Implications)	References
First IR (1750 - 1899): Established during the end of the 18th century. The emergence of mechanization of labor and production with industry as the foundation of the economic structure of society. Mass extraction of coal as energy resource and invention of the steam engine created a new type of energy for most processes.	• Mechanical drills for reaping or harvesting crops and threshing machines to separate the valuable grain from the stalks of cereal grain plants. • Food pasteurizer • Refrigeration machine • Weighing scale • The bulk fermentation process for bread, sun drier method	• Mechanical harvesting and threshing of grains. • Preservation of food using high-temperature steam and water to kill microorganisms. • Preservation of food by cooling and freezing. • Weighing scale for use in scaling of raw ingredients during processing and sale. • Production of equipment used in the processing of food • Environmental pollution of air high due to heat energy requirement of machines with the release of smoke	Chaloner, 1957; Chambers & Mingay, 1966; Marwala & Hurtiz, 2017; Rhoades, 2017; Min, et al 2018; Sentryo, 2018; Prisecaru, 2019
Second IR (1900 - 1960): Started in the second half of the 19th century. The era of rapid industrialization using oil and electricity as an energy resource to power production. New technological advancement initiated the emergence of electricity, gas, and oil. Chemical synthesis also developed to bring synthetic fabric, dyes, and fertilizers. Methods of communication were enhanced with the invention of telephones and so was transportation.	• Drum, spray, rapid freeze driers, brine injector technology, juice extractors, milk pasteurizer, tubular blanchers. • Improvement in agro-processing equipment like wheat harvesting tractors and separation machines. • Food canning, sterilizing/pasteurization, flash freezing machines, electric standing mixer (1908) by Herbert Johnson. Cherry Burrell corps - developed continuous pasteurization system Automated check weighing machine (1947) and vacuum pouch packaging machine (1967). • Magnetrons microwave ovens (invented by Albert W. Hull), a device to generates microwaves using electricity. One of the first electric food processes was the Star mix MX3 food processer by German company electro star in 1946. First artificial food refrigeration machine (1955) by Willem Colleen.	• Rapid industrialization using oil and electricity to power mass production. • Horsepower replaced by an internal combustion engine. • Increase in food supply due to increased crop production and improved separation processes. • Increased food preservation, sterilization by canning, and food safety. • Enhanced food production due to electricity and reduced food industry working time. • The use of equipment operated with oils or gas harmed processed food, especially with changes in color and aroma.	Chambers & Mingay, 1966; Rifkin, 2013; Marwala & Hurtiz, 2017; Min, et al 2018; Salesforce, 2019; Prisecaru, 2019.
Third IR (1961 - 2000): Computer or the digital revolution. Established during the first half of the 20th century. Advanced technology in the food industry with the emergence of nuclear energy resource whose potential surpassed its predecessors. Witnessed the rise of electronics, with the transistor and microprocessor, telecommunications, and computers. Led to the production of miniaturized material which opened doors, most notably, to space research and biotechnology.	• High-temperature short-time automatic pasteurizer, Chorleywood bread process, electrical stimulators • The Twin-rotor system combines machine to cut and separate crops or fresh produce. • Mobile software development to help farmers exchange ploughing, harvesting, and food preparation information • Use of genetic modification technology and equipment to improve crop performance and increase yields. • The invention of vacuum pouch the packaging machine (1967); high-pressure short time cooker/extruder (1964), first can-opener (1970), vacuum pressure system (1972), cook quench the chill machine (1997) and Sous vide equipment (1986).	• Electronic and IT assisted in the automation of the production/manufacturing industry, increasing food production by more than 1000% in some cases. • Computerized food processing equipment for pasteurization, sterilization, dehydration (freeze-drying), and automatic beer filtration systems. • Analytical food laboratory using computers and highly advanced machines/ equipment. • High energy consumption of machines.	Chaloner, 1957; Rifkin, 2011; Höller, 2014; Jee, 2017; Min, et al 2018; Sentryo, 2018; Prisecaru, 2019
Fourth IR (2000 - present): Typified by green energy resource. Blurring of boundaries between the physical, digital, and biological worlds. Internet of things (IoT) commonly known as industry 4.0 or 4IR.	• Novel vacuum processing machine, high-pressure processing technology, thermal and non-thermal retorting systems, electronic tracing technology, intelligence labelling, and computerised colour reflex, automatic fruit juice pasteurizer using microwaves/ultrasonic waves, hot air tray drier incorporated with computer vision;	• Ultra-centrifugal milling with extreme milling rate and competency. • Time-saving, improve productivity and reliability. • Digitalization of machine operation able to analyze different parameters in a short period,	Atzori, et al, 2010; Anderson, 2012; Al-Rodhan, 2015; Agrifood, 2017; Colombo, *et al* 2017; Jee, 2017; Marwala

A fusion of advances in artificial intelligence (AI), robotics, 3D printing, genetic engineering, quantum computing, blockchain, intelligent labelling, and other technologies. The collective force behind many products and services that are fast becoming indispensable to modern life. Very scalable industry.	Hybrid convective-infrared-rotary drum dryer. • Automated ultra-centrifugal milling • Texture analyzing and sensory evaluation machines • Automation and digitalization of processes; tracking of materials and packaging through the utilisation of radio frequency identification (RFID) tags and ERP systems. • Development of jet cook stem infusion system (2006). Food processing robots and robotic machines performing different functions for food manufacturers. • Improvement of food analysis machines. • Green extraction technologies, Pulsed electric field technology	reduction of workload, and time to complete tasks. • Predictability of outcome of a process • Replacement of manual labour by robots and machines. A new skill set will be needed to operate the robots.	& Hurtiz, 2017; Min, et al 2018; Godoi et al 2018; Salesforce, 2019; Prisecaru, 2019

The 2IR led to significant technological advancement in mobility and production. Henry Ford and others have written about the influence of the slaughterhouse practice whereby in the same factory line, one person would do a specific task, while others did a different one, resulting in meat processed easily and fast (Earle, 2010). Also, the analytical scientific equipment was not left out in these advances. A wide range of commercial driers suitable for both solid and liquid foods was developed, an example was the drum roller drying of milk, and freeze-drying involves freezing followed by sublimation of the ice under reduced pressure (Earle, 2010; Anderson, 2015).

4. The Third Industrial Revolution and the Impact on Food Machinery

New technologies, the internet, and renewable energies (Table 2) began to change history (Adnan et al., 2017) of food manufacturing during the third industrial revolution (3IR). Many modern manufacturing facilities with advanced food processing and packaging technologies came into existence. Food products were produced in a new way, using computers and new machines (Maralwa, 2019) leading to the construction of the first automated extrusion machines capable of operating all the steps from raw materials to the final product (Figure 5).

The 3IR was characterized by the implementation of electronics and information technology (IT) to automate production (Maury, 2008). It was based on the use of electronics and IT to further automate production (Fremdling, 2008) but not with much digital "disruption" as in 4IR. Examples include the adoption of i) the Chorleywood bread process, ii) vacuum packaging, as a method of food preservation, and iii) the application of digital printing on food packages (Maralwa, 2019). These developments were pointers to the innovative food packaging that now exists in the 21st-century food industry.

Automation and digitalization made possible the introduction of process lines with programmable and automated machines in food industries replacing human labor (Maury 2008). An example is an automated and programmed bakery process line (Figure 6). The technological developments that made these changes possible included innovations in farming machinery, improvements in refrigeration, the mechanization of food processing, and the invention of new packaging materials and promotional techniques (Hawken et al., 2013). The structure of the food system during 3IR raised fresh foods in large quantities far from consumers and made them available for longer periods during the year (Inikori, 2006).

Figure 5. Automated cooking extrusion machine

Source: https://en.m.wikipedia.org/ /wiki/food _extrusion

Figure 6. Automated and programmable bakery process line

5. Fourth Industrial Revolution (From 2000) and Impact on Food Machinery

The fourth industrial revolution (4IR) is based on the use of cyber-physical systems, automation, analytics, and the internet of things (IoT) from the early 21st century (Allen, 2009) in the food and agro-processing industries. This evolving food industry trend encompasses advanced technologies of digitalization as outlined in Table 2. It is characterized by making systems as well as machines intelligent and connected (Marwala, 2019). The underlying advances in technologies of the 4IR include artificial intelligence (AI), digitalization, robotics, 3D model as well as blockchain (Pirsecaru, 2019). An application of AI is machine learning (ML) which provides systems the ability to automatically learn and improve from experience without being explicitly programmed. AI enables machines to learn, adapt, evolve, and optimize; and has had a profound impact in diverse fields such as food science and technology, engineering, medical sciences, and social sciences. AI in the food industries is used to create machines that can learn, and it is also used in food sensing technologies for food safety, quality, and traceability (Maury 2008). The digitalization of manufacturing is transforming the way goods are made (Adnan et al., 2017) and AI is already responding to our needs.

The integration of robots in food machinery is now common. Robots can dispense servings of frozen yogurt, ice cream, gelato, and sorbet finished with a selection of six delicious toppings in less than 60 seconds. Robots have been invented that almost completely replace human labour (Maury, 2008, Schwab, 2015). Examples of robots in food industries are i) delta robots (Figure 7) which are used to move, pick, and place products around a production line, and ii) the dispensing robot. Such robots are faster than a human being, with the advantage of

short production time due to increased speed and accuracy. A low-level palletizer was replaced with a robotic palletizer for loading glass jars onto the sauce line which results in significant line uptime and reduced labour costs (Hawken et al., 2013). There is fear that a robotic takeover of manufacturing jobs will keep humans out of work (Adnan et al., 2017). One common example of industry 4.0 food machines is the vacuum processing machines (Figure 8) to produce food products. The vacuum processing system combines homogenizing technology, the highest reproducibility, short batch times, and high cost-effectiveness with ease to use. Automatic belt vacuum packaging machines and intelligence labelling, that are digitally operated are now in operation in food industries for packaging as well as a preservation method for various foods such as meat products and cheese (Anderson, 2015). The high-pressure processing (HPP) being a non-thermal preservation technology, innovative fluidized freezing technologies, continuous freezer control system, novel heat exchanger (IFMT 2020), and ultrasound-assisted extraction technology are all with great benefits.

Figure 7a. Delta robot (In Cookie Factory)

b. Automatic ice cream packaging machine

Figure 8. Maxx vacuum processing machine

Source: https://pfrymakoruma.com/ww-en/machinedetails/maxxd/?gclid=EAlalqobChM19r2wrLA4wlVibTtCh3ggwi4EASAYASAAEglsr_d_Bw

The 4IR now involves computer-generated product design and three-dimensional (3D) printing. It is a very scalable industry as technology is said to be a game-changer for scale (Noonan, 2019). Existing advances in this technology include designs, edible ink material, extrusion, and laser-based technologies used during the construction of computer-aided shapes (Godoi et al., 2018). The trio of robotics, 3D model, and blockchain when applied to food machinery has great potential considering that the latter is one example of using data and coupled with AI could solve the nutritional need in specific diets. This is the expectation going forward and beyond

blockchain in food industry machinery especially with rapidly evolving areas such as IIoT, operational technology cybersecurity, predictive analytics, and edge computing systems. The future holds how a combination of digitalization, robotics, AI, ML, blockchain, quantum computing can help in product development. Blockchain-enabled traceability in the food industry improved value-chain efficiency driven by improved collaboration and data visibility (Nayyar et al., 2018). Also, the advances exist in analytical scientific machines, like x-ray computed tomography, CT scanners that come in different flavors.

6. Going Forward

The different industrial revolutions, directly or indirectly, contributed and accelerated improvement and advances in processing equipment from manual operation to more advanced automation, data collection, and analysis. Technologies and processes are evolving at an exponential pace and are increasingly becoming inter-related (Marwala 2020). Although it has created jobs, it also transformed the workforce. From the present trend, and as the digital economies become knowledge-intensive, the demand for higher-order scientific and mathematical skills in food machinery is evident in the technological advancements of industry 4.0. Such skills provide a unique position to strongly contribute to a digital future and expert competence development. The future of food machinery looks exciting with the possible contributions of AI, ML, robotics, and blockchain to industry 5.0. Optimization and prediction with AI by applying ML algorithm to control the food process line, like in the air-conditioning system, is now a possibility using the modular design of the AL/ML engine for adaptive process control. These advanced techniques can help in the collection and processing of data, especially in digital format, and using this digital information to automate operations. Humans are still indispensable, hence modernization and redesign of the global education system with technology-focused subjects such as functional genomics, coding, and data analytics are necessary to prepare learners for the future food industry manufacturing operations. The tweaking of the system is much needed in the global south, which was recently highlighted in a paper in "Nature Sustainability" as an important priority for the research community (Nagendra et al., 2018). Going forward will reveal these emerging technologies in food machinery; including the prospects of food printing at home for domestic application and printing materials such as edible ink types to an innovative and economical printed reactor. Certainly, food industries will increasingly need food engineers, particularly mechatronic engineers and technicians for a good understanding of technologies in food machines of industry 4.0, those of the approaching future industrial revolutions and beyond blockchain. From 3D printing which creates an object that makes food appealing to the digital rich-future 4D printing such that a processed food requires only the addition of water to create food with desired structural and textural properties. However, 5IR in food machinery may shift attention to humans and not robots for smart science and food chain intelligence.

Conflict of Interests

The authors declare no conflict of interest.

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Microencapsulation of *Hibiscus sabdariffa* (Roselle) Extracts by Spray Drying Using Maltodextrin and Gum Arabic as Carriers

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Abstract

Microencapsulation by spray drying is one of the most common methods used to obtain food material powders. In this study, different gums (maltodextrin [MD], gum arabic [GA], and mixtures of MD:GA [60:40] at various concentrations [0–10% w/w]) were used to microencapsulate *Hibiscus sabdariffa* (Roselle) extracts by spray drying. The yield, physicochemical properties, and antioxidant characteristics (total monomeric anthocyanins [TMAs], total phenolic compounds [TPCs], and antioxidant capacity [AC]) of the microencapsulated Roselle powders (RP) were evaluated. The highest RP yield (73.3 ± 3.3%) was obtained with the 3% MD:GA blend. The red color (a^*) average for all powders (39.9 ± 2.0) decreased as the gum concentration increased. The 3% MD:GA RP showed the highest amount of TMAs (539.19 ± 13.27 mg cyaniding-3-glucoside equivalents/100 g) and TPCs (3,801.6 ± 125.9 mg of gallic acid equivalents/100 g of powder). The highest AC was observed with a 5% GA RP (1498.5 ± 44.0 mg of Trolox equivalents/100 g of powder).

Keywords: microencapsulation, spray drying, *Hibiscus sabdariffa*, Roselle, anthocyanins, phenolic compounds, antioxidant capacity

1. Introduction

Hibiscus sabdariffa is a plant in the Malvaceae family. It grows in tropical and subtropical regions and can have green or red calyces (Cissé et al., 2009). The red color of calyces reflects the high anthocyanin, mainly delphinidin-3-sambubioside (71.4%) and cyanidin-3-sambubioside (26.6%) content (Peng-Kong et al., 2002). These compounds are highly unstable and degrade easily, producing compounds with an undesirable color (browning). One of the main attributes of food quality is color and consumer acceptance depends greatly on color. Anthocyanins are colorful pigments from vegetable products; the stability of anthocyanins depends on various factors such as temperature, pH, oxygen, light, enzymes, and metallic ions (Idham et al., 2012; Ersus et al., 2007).

One technique that has been used to maintain the stability of pigments such as anthocyanins is microencapsulation. Microencapsulation of food materials is used to reduce pigment degradation due to environmental factors such as oxygen, light, temperature, and prooxidant agents, to increase stability during processing, to control their release, or as a food additive (Santos and Meireles, 2010). Encapsulating agents include natural polymers and derivatives of these or lipids. The most common of these are gum arabic (GA), carrageenin, maltodextrins (MDs), cyclodextrins, dextrins, chitosan, gelatin, sodium caseinate, pregelatinizated starch, carboximethylcellulose, methylcellulose, hydroxypropyl methylcellulose, milk proteins (caseins and whey), lactose, corn syrup (Munin and Edwards-Lévy, 2011; Selim et al., 2008; Kolanowski et al., 2006; Vega and Roos, 2006), and mesquite gum (Ochoa-Velasco et al., 2017). The ideal substances for microencapsulation are soft and unflavored and have high solubility, emulsifying properties, and characteristics that promote drying. Furthermore, their concentrated solutions should have low viscosity to facilitate drying (Vega and Roos, 2006). MDs and GA are the encapsulating agents most commonly used with spray drying. MDs solubilize rapidly and have low viscosities at high concentrations; however, their emulsifying capacity is limited. MDs in the range of

10–20 dextrose equivalents (DEs) are the most suitable for use as encapsulating agents. On the other hand, GA is a very efficient encapsulating agent; it is a polymer with 2% of its structural proteins lending excellent emulsifying properties. However, high concentrations of GA increase the viscosity of solutions (Gharsallaoui et al., 2007). Combinations of MDs with GA used with spray drying have been shown to produce good powders (Zhang, Mou, and Du, 2007; Lopez et al., 2009; Idham et al., 2012; Fazaeli et al., 2012).

Microencapsulation is a widely used process in the food, pharmaceutical, and cosmetics industries, as well as in agricultural, veterinary, medical, chemical, biotechnological, and biomedical fields. Spray drying is a widely used economical method for encapsulating food ingredients. Particle sizes of powders obtained by this method are generally in the range of 10–50 μm; however, this size may depend on the process conditions (Gharsallaoui et al., 2007). The main advantages of this process, besides its simplicity, are its suitability for use with heat-sensitive materials because the time required at high temperatures is very short (5–30 s) (Ochoa-Velasco et al., 2017), the equipment needed is readily available, options for encapsulating materials are many, the encapsulation process is efficient, the final product is stable, and there is the potential for continuous large scale production (Santos and Meireles, 2010). The parameters that have the most influence in the spray drying process are nozzle geometry, viscosity of the feeding solution, and the inlet and outlet air temperatures (Munin and Edwards-Lévy, 2011; Gharsallaoui et al., 2007). Commercially, this technique has been used to encapsulate numerous materials, including flavor agents, fats, oils, vitamins, minerals, microorganisms, enzymes, sweeteners, and colorants (Wijaya et al., 2011).

There is little research on spray drying of Roselle extracts (REs) to obtain powders. Some researchers have found that powders obtained by spray drying using encapsulating agents (mainly MDs) are more stable and have longer lasting antioxidant properties than those obtained without encapsulating agents (Langrish and Chiou, 2008a, 2008b; Farimin and Nordin, 2009; Ochoa-Velasco et al., 2017).

The aim of this work was to evaluate the use of an MD, GA, and combination of the two as encapsulating agents to obtain Roselle powders by spray drying.

2. Materials and Method

2.1 Materials

Red Creole Roselle calyces (long variety) were acquired from Chiautla de Tapia, Puebla, Mexico to produce extracts. Roselle calyces powder (RCP) was obtained using a stainless steel VeyCo MPV Mill Model 100 (Mexico). The mill has a mesh of 0.5 mm.

2.2 Methods

2.2.1 Average Particle Size

Average particle size (APS) was determined using a Microtac S3500 Particle Size Analyzer (Microtac Inc., Largo, FL, USA) with a measuring range of 0.25–2800 μm. Approximately 30 and 60 mg of spray-dried Roselle powders (RPs) and RCP were used, respectively, to assess particle size. The analysis was performed in triplicate. Particle size distributions, mean diameters of particles (d_{50}), and cumulative weight curves were obtained for the different powders (O'Hagan et al., 2005).

2.2.2 Concentrated RE

RE was obtained with a 1:10 RCP:ethanol ratio (20 g of RCP + 200 mL of 50% ethanol) at 50 ± 0.2°C for 30 min using a Riossa M80T water bath (Rios Rocha S. A., Monterrey, Nuevo Leon, Mexico), based on a method described by Salazar-González, Vergara-Balderas, Ortega-Regules, and Guerrero-Beltrán (2012). RE was filtered through Whatman paper No. 4, and the liquid was obtained in flasks that were then covered with aluminum foil (Cid-Ortega and Guerrero-Beltrán, 2014). To obtain concentrated REs, alcohol was removed using a Büchi rotary evaporator RE 111 (Brinkmann Instruments Inc., Switzerland) at 45°C and 54 cmHg for no more than 45 min (Selim et al., 2008). The concentrated RE was evaluated according to volume, weight, and physicochemical (total soluble solids [TSSs], density, viscosity) and antioxidant (total monomeric anthocyanins [TMAs], total phenolic compounds [TPCs], and antioxidant capacity [AC]) characteristics.

2.2.3 Concentrated RE-gums

A 3 × 3 factorial design with the type or blend of gum (GA, MD, and an MD:GA [60:40] blend) and their concentration (3, 5, and 10% w/w) was used (Idham et al., 2012). GA was acquired from Central de Drogas, S. A. de C.V. (Mexico State, Mexico). MD of 9–14 DEs was acquired from CP Ingredientes S. A. de C.V. (Guadalajara, Jalisco, Mexico). The gum or blend of gums was added to the RE concentrate and stirred for 15 min at room temperature (22 ± 2°C). Each RE-gum concentrate was placed in a 250-mL Erlenmeyer flask, covered with

aluminum foil, and stored a 4°C until spray drying. RE and RE-gum concentrates were analyzed for physicochemical (TSSs, density, viscosity) and antioxidant (TMAs, TPCs, and AC) characteristics.

2.2.4 Spray Drying

A mini spray dryer (Büchi B-290, Switzerland) with a two-fluid nozzle with an orifice 0.7 mm in diameter (particle diameter of 1–25 microns) was used for spray drying. The inlet and outlet air temperatures were 180.01 ± 0.25 and 105.16 ± 3.52°C, respectively. Blends of RE-gum concentrates were fed into the dryer at a flow rate of 10 mL/min (Andrade and Flores, 2004; Ochoa-Velasco et al., 2017). The aspirator power of the drying system was 100% (equivalent to an airflow of 35 m^3/h), and the spray drying airflow was maintained at 55 mm (equivalent to 670 L/h with a pressure of 1.05 bar). Calibration curves were constructed (10–100% pump power) to determine the percentage equivalent to 10 mL/min for each mixture (38–41%). RPs were weighed and placed in 100-mL amber glass bottles, and these bottles were capped and stored at room temperature (22 ± 2°C) in a desiccator containing silica. Yield, productivity, physical characteristics (moisture content, water activity [aw], average diameter, bulk and tapped densities, and color), and antioxidant characteristics (TMAs, TPCs, and AC) of RPs were determined.

2.2.5 Physical Properties of Extracts

Total soluble solids (TSSs). TSSs were measured according to the 932.14C AOAC (1995) method using a handheld refractometer (Atago Co. LTD, Tokyo, Japan). To correct values for 20°C, a standard set of tables were used (AOAC, 1995).

Density. Density was determined according to the 945.06 AOAC (1995) method and expressed in g per mL. Empty (W_1), filled with distilled water (W_2), and filled with sample (W_3) pycnometer weights were determined, and the density at 25°C was calculated according to Eq. (1):

$$\rho(g/mL) = \left[\frac{W_2 - W_1}{W_3 - W_1}\right] * \rho_{H_2O}^{25°C} \quad (1)$$

where $\rho_{H_2O}^{25°C}$ (g/mL) is the density of water at 25°C.

Absolute viscosity (μ). A Cannon-Fenske capillary viscometer (Cannon Instrument Co., State College, PA, USA) was used to determine absolute viscosity. Kinematic viscosity was calculated by multiplying the time (s) of 6.6 mL of extract at 40°C flowing through the viscometer per the constant of the apparatus (0.4754 mm^2/s^2) at the same temperature. To obtain absolute viscosity, kinematic viscosity was multiplied by the density of the extract according to Eq. (2) (Cannon Instrument Co., 2000):

$$\mu(cP) = \rho_s * \upsilon_c \quad (2)$$

where μ is the absolute viscosity (cP = mPa·s), ρ_s is the density (g/mL), and υ_c is the kinematic viscosity (mm^2/s = cSt) of an extract. The absolute viscosity at 25°C was calculated using Eq. (3) (Cannon Instrument Co., 2014):

$$C = C_o\left[1 - B\left(T_t - T_f\right)\right] \quad (3)$$

where C (0.4754 mm^2/s^2) is the constant of the apparatus at 40°C, C_o (mm^2/s^2) is the viscometer constant at the filling temperature, B (79×10^{-6}/°C) is the calibration temperature factor obtained from the calibration certificate for the viscometer, T_t is the working temperature (40°C), and T_f is the filling temperature. Using the equation above, the constant C_o was calculated and then, using the same equation, the constant C was calculated at 25°C.

2.2.6 Antioxidant Properties

Total monomeric anthocyanins (TMAs). TMAs were determined according to the method described by Lee et al. (2005). First, 0.5 mL or 100 mg of extract or powder, respectively, were diluted with distilled water to reach 10 mL in a volumetric flask. The mixture was stirred for 5 min using a vortex at 2900–3000 rpm. One milliliter of each solution was diluted with buffer pH 1.0 or pH 4.5 to reach 5 mL in test tubes wrapped with aluminum foil. The blends were left for 30 min at room temperature (23 ± 2º C) in the dark. Then, absorbances in 4-mL glass cells were measured at 520 and 700 nm using a Cary 100 UV-Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). A blank with distilled water was used to correct these absorbances. Results were calculated as mg of cyanidin-3-glucoside equivalents per 100 mL of RE or per 100 g of powder using Eq. (4):

$$TMA = \frac{A*MW*DF}{\mathcal{E}*L} * 100 \quad (4)$$

where *TMA* is the concentration of anthocyanins (mg/100 mL or mg/100 g), $A = (A_{520nm} - A_{700nm})_{pH=1.0} - (A_{520nm} - A_{700nm})_{pH=4.5}$, *MW* is the molecular weight of cyanidin-3-glucoside (449.2 g/mole), *DF* is the dilution factor, *L* is the cell width (1 cm), ε is the coefficient of molar extinction for cyanidin-3-glucoside (26,900 L/mole-cm), and 100 is the conversion factor for obtaining mg/100 mL of RE or mg/100 g of RP.

Total phenolic compounds (TPCs). TPCs were determined using the Phenol Folin and Ciocalteu method (Singleton and Rossi, 1965) with some modifications. Three milliliters of distilled water, 150 μL of extract solution, or 100 μL of powder solution (the same solutions prepared to determine TMAs), and 250 μL of Folin and Ciocalteu reagent were placed in test tubes that were then covered with aluminum foil. Mixtures were stirred and left for a maximum of 8 min in the dark, and then 750 μL of 20% Na_2CO_3 was added and thoroughly mixed. Distilled water was added (850 or 900 μL) to reach 5 mL, mixed thoroughly, and left for 2 h at room temperature (23 ± 2°C) in the dark. Absorbances were measured at 765 nm using a Cary 100 UV-visible spectrophotometer (Varian Inc.). Various standard curves were constructed with different concentrations of gallic acid (0–0.066 mg; Sigma, St. Louis, MO, USA). The standard curve for extracts and powders is represented by the equation A = 18.810 ± 1.463 (1/mg GA) * X (mg GA) + 0.023 ± 0.007 (R^2 = 0.998 ± 0.001). The amount of TPCs was calculated as mg of gallic acid equivalents per 100 mL of RE or per 100 g of powder according to Eq. (5):

$$TPC\left(\frac{mg}{100\ mL}\right) = \left(\frac{A-b}{m}\right) * DF * 100 \quad (5)$$

where *TPC* is the total phenolic compounds content (mg/100 mL or mg/100 g), *A* is the absorbance of the sample, *b* is the intercept, *m* is the slope, and *DF* is the dilution factor for the sample.

Antioxidant capacity (AC). The DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Brand-Williams et al., 1995) was used with some modifications (Molyneux, 2004; Esmaeili et al., 2015). Two milliliters of extract solution or 100 μL of powder solution (the same solutions prepared to determine TMAs) were diluted with ethanol (99.5%) to reach 10 mL in a volumetric flask, stirred for 5 min using a vortex (2900–3000 rpm), and then filtered through Whatman paper No. 4. One milliliter of filtrate was placed in a test tube containing 1 mL of ethanol (99.5%) and 2 mL of DPPH solution (7.99 ± 0.24 mg in 200 mL of 99.5% ethanol). The solutions were thoroughly mixed and left for 45 min at room temperature (21.6 ± 3.3°C) in the dark. Absorbances were measured at 517 nm using a Cary 100 UV-Vis spectrophotometer (Varian Inc.). The AC of each solution was calculated using Eq. (6):

$$I\ (\%) = \frac{Ac-As}{Ac} * 100 \quad (6)$$

where *I* is the percent of inhibition, *As* is the absorbance of the sample and *Ac* is the absorbance of the control. Standard curves were prepared at various concentrations (0, 0.008–0.030 mg) of Trolox (6-hydroxy-2, 5, 7, 8 tetrametilcromo-2 carboxylic acid 97%). The standard curves for extracts and powders are represented by the equation I (%) = 3272.18 ± 220.83 (1/mg TE)*X (mg TE) + 0.70 ± 4.06 (R^2 = 0.978 ± 0.014), and I (%) = 3269.52 ± 251.15 (1/mg TE)*X (mg TE) + 4.78 ± 4.37 (R^2 = 0.964 ± 0.026), respectively. Results, calculated according to Eq. (7), were expressed as milligrams of TEs per 100 mL of RE or 100 g of powder.

$$AC\left(\frac{mg}{100\ mL}\right) = \left(\frac{A-b}{m}\right) * DF * 100 \quad (7)$$

where *AC* is the antioxidant capacity (mg/100 mL or mg/100 g), *A* is the absorbance of the sample, *b* is the intercept, *m* is the slope, and *DF* is the dilution factor of the sample.

2.2.7 Physicochemical Properties of Powders

Yield (Y). Yield was calculated based on the amount of TSSs in the encapsulated extract and the amount of powder obtained (Fazaeli et al., 2012) according to Eq. (8):

$$Y\ (\%) = \frac{Amount\ of\ powder}{Amount\ of\ TSS} * 100 \quad (8)$$

Moisture content. Moisture content was measured according to the 934.06 AOAC (2000) method. A Cole-Parmer (Chicago, IL, USA) vacuum oven was used to dry samples for 8 h at 70 ± 1°C and a vacuum pressure of 200–220 mmHg. Moisture content was calculated as a percentage.

Water activity (a_w). Water activity was measured using an AQUA-LAB hygrometer model 3TE (Decagon

Devices Inc., Pullman, WA, USA). The temperature at the time of measurement was 25.10 ± 0.06°C.

Bulk density. Bulk density was measured according to the method described by Jumah et al. (2000). One gram of powder was weighed in a 10-mL graduated cylinder. The cylinder was gently tapped 10 times on a polystyrene mat from a height of 15 cm. Bulk density (ρ_a) was calculated according to Eq. (9):

$$\rho_a(g / mL) = \frac{W}{V_a} \tag{9}$$

where *W* is the weight of powder (g) and *Va* is the apparent volume (mL) occupied by the powder in the cylinder after tapping.

Tapped density. Tapped density was measured according to the Mexican Official Norm number NOM-104-STPS-2001 (NOM, 2001) with some modifications. One gram of powder was weighed in a 10-mL graduated cylinder with a rubber stopper. The cylinder was subjected to a manual vibration process so that the sample were shaken from bottom to top for 8 min (estimated time at maximum volume). The tapped density (ρ_c) was calculated according to Eq. (10):

$$\rho_c\,(g/mL) = \frac{W}{Vc} \tag{10}$$

where W is the weight of powder (g) and Vc is the compacted volume (mL) occupied by the powder in the cylinder after tapping.

Color. A Colorgard system 05 colorimeter (BYK-Gardner Inc., Silver Spring, MD, USA) was used to determine the color of powders and solutions. For powders, the color parameters were obtained in reflectance mode. A plate with a light gap 1.9 cm in diameter and external diameter of 2.65 cm was used. Samples were placed in weighing bottles for color determination. For solutions, a solution of 10 mg of powder/mL of distilled water was prepared, and color parameters were determined in transmittance mode using a 3-mL quartz cell (Konica Minolta Sensing, Inc., Kyoto, Japan) (Ochoa-Velasco et al., 2017; Silva et al., 2013). Color parameters of powders and solutions were obtained using the *CIEL*a*b** scale: *L** (lightness, 0–100), *a** (green to red) and *b** (blue to yellow). From these data, purity (color saturation, $C = [a^{*2} + b^{*2}]^{1/2}$) and hue ($H = \tan^{-1}[b^*/a^*]$) were calculated.

2.2.8 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) testing using MINITAB software version 14.1. Multivariate analysis and Tukey's multiple comparison tests were used to compare differences between means. Values shown are average values. A value of 0.05 was considered significant for differences between means of treatments.

3. Results and Discussion

3.1 Antioxidant Characteristics of RE Concentrates

Table 1 shows the characteristics of RE concentrates. Roselle extracts had average initial volumes, weights, and TSSs of 73.10 ± 3.09, 7.83 ± 4.38, and 15.04 ± 0.80, respectively, and average TMAs, TPCs, and ACs of 84.10 ± 4.77, 629.97 ± 30.25, and 257.00 ± 10.68, respectively (Table 2). Statistically significant differences in TMA contents were observed ($p > 0.05$) for all extracts (70.65–91.26 mg/100 mL). Extracts with 3 and 5% GA and 3 and 5% MD added (462.43 ± 22.41, 457.66 ± 30.48, 466.53 ± 17.18, and 456.53 ± 28.07 mg/100 mL extract, respectively) showed less ($p \leq 0.05$) TPCs than those in RE alone. Extracts with 3% GA and 10% MD added (227.32 ± 5.12 and 220.99 ± 15.71 mg/100 mL extract, respectively) showed significantly lower ACs than RE alone. Significant differences ($p \leq 0.05$) in ACs for all RE-gum concentrates.

Table 1. Roselle extract concentrate and gum required for each Roselle extract-gum concentrate

Gum	Concentration (% w/w)	Gum (g)	Extract (mL)	Extract (g)	TSSs[1] (%)
RE	0	–	73.10 ± 3.09	73.83 ± 4.38	15.04 ± 0.80
GA	3	2.77 ± 0.19	88.83 ± 6.53	89.43 ± 6.29	16.78 ± 0.03
	5	4.35 ± 0.63	82.00 ± 12.49	82.67 ± 12.02	17.54 ± 0.48
	10	9.22 ± 0.64	82.17 ± 6.05	82.97 ± 5.75	17.18 ± 0.29
MD	3	2.63 ± 0.07	83.34 ± 2.36	83.84 ± 2.37	17.15 ± 0.16
	5	4.64 ± 0.28	85.21 ± 5.03	85.21 ± 5.03	16.91 ± 0.81
	10	9.60 ± 0.70	84.33 ± 6.81	85.36 ± 6.32	17.06 ± 0.46
MD:GA	3	2.18 ± 0.18	67.43 ± 6.02	69.75 ± 6.02	14.39 ± 0.37
	5	3.97 ± 0.06	72.51 ± 0.87	74.77 ± 0.93	14.34 ± 0.11
	10	8.72 ± 0.49	76.54 ± 5.24	77.53 ± 4.95	17.31 ± 0.43

[1]TSSs: total soluble solids (at 20°C) in extracts without gum. RE, Roselle extract; GA, gum arabic; MD, maltodextrin.

Table 2. Antioxidant characteristics for Roselle extract concentrates[a]

Gum	Concentration	TMA[1]	TPC[2]	AC[3]
	(% w/w)		(mg/100 mL)	
RE	0	84.10 ± 4.77abc	624.97 ± 30.25a	257.87 ± 10.68abc
GA	3	76.11 ± 3.31ac	462.43 ± 22.41c	227.32 ± 5.12bc
	5	82.07 ± 10.98abc	457.66 ± 30.48c	278.59 ± 41.77ab
	10	86.04 ± 7.69ab	491.32 ± 19.18c	235.26 ± 15.01abc
MD	3	74.11 ± 3.78bc	466.53 ± 17.18c	251.42 ± 4.42abc
	5	70.65 ± 3.37c	456.53 ± 28.07c	268.98 ± 10.47abc
	10	76.53 ± 1.91abc	499.92 ± 13.04c	220.99 ± 15.71c
MD:GA	3	90.95 ± 0.22a	617.62 ± 28.86a	281.15 ± 25.60a
	5	91.26 ± 1.50a	519.11 ± 9.12abc	263.80 ± 3.49abc
	10	83.50 ± 4.02abc	590.76 ± 43.98ab	251.72 ± 13.90abc

[a]Different letters in the same column indicate significant differences ($p \leq 0.05$) between values. [1]TMAs: total monomeric anthocyanins (cyanidin-3-glucoside equivalents). [2]TPCs: total phenolic compounds (gallic acid equivalents). [3]AC: antioxidant capacity (Trolox equivalents). RE, Roselle extract; GA, gum arabic; MD, maltodextrin.

3.2 RE-gum Concentrates

3.2.1 Physical Properties

TSSs (°Bx). Significant differences ($p \leq 0.05$) were observed between average TSS contents (Table 3) for REs with GA, MD, or MD:GA added. The TSS content in REs was 15.04 ± 0.80. An increase of gums in REs increased TSS content (Table 3).

Table 3. Physical characteristics of Roselle extract concentrates[a]

Gum	Concentration (% w/w)	TSSs (°Bx)	Density (g/mL)	Viscosity (mPa s)[1]
RE	0	15.04 ± 0.80e	1.03 ± 0.01ab	1.66 ±0.01g
GA	3	19.03 ± 0.19c	1.02 ± 0.01b	2.79 ± 0.05fd
	5	20.86 ± 0.48b	1.03 ± 0.02ab	3.68 ± 0.01c
	10	24.14 ± 0.17a	1.05 ± 0.01ab	8.44 ± 0.1a
MD	3	19.53 ± 0.22bc	1.02 ± 0.01b	2.07 ± 0.02f
	5	20.73 ± 0.39b	1.03 ± 0.01ab	2.29 ± 0.07e
	10	24.54 ± 0.08a	1.05 ± 0.01ab	2.99 ± 0.07d
MD:GA	3	17.03 ± 0.77d	1.05 ± 0.01ab	1.94 ± 0.01f
	5	18.73 ± 0.59c	1.05 ± 0.00ab	2.33 ± 0.04e
	10	24.90 ± 0.23a	1.06 ± 0.01a	4.39 ± 0.09b

[a]Different letters in the same column indicate significant differences ($p \leq 0.05$) between values. [1]Viscosity at 25° C. TSSs, total soluble solids; RE, Roselle extract; GA, gum arabic; MD, maltodextrin.

Density. Densities of RE-gum concentrates (Table 3) showed significant differences ($p \leq 0.05$) among types and concentrations of gum. Extracts with the MD:GA blend added were denser (1.06 ± 0.01 g/mL) than extracts without gum (RE) or with GA and MD (1.03 ± 0.01, 1.04 ± 0.02, and 1.03 ± 0.01 g/mL, respectively). Densities of RE-gum concentrates with the three concentrations of gums also showed significant differences ($p \leq 0.05$).

Viscosity. Viscosities of RE-gum concentrates are shown in Table 3. The viscosity of RE-gum concentrates increased as gum concentration increased (1.66 ± 0.01, 2.26 ± 0.40, 277 ± 0.69, and 5.27 ± 2.45 mPa·s, for 0, 3, 5, and 10% gums, respectively). Extracts with 10% GA showed the highest viscosity, perhaps because GA has the ability to form gels due to its protein contents (Lopez et al., 2009). Significant differences ($p \leq 0.05$) in RE-gum concentrate viscosities were observed among concentrations and types of gum. Viscosity and TSS content are important for spray drying because low viscosities along with high TSS content results in better flow during atomization and higher yields (Lopez et al., 2009). Therefore, a positive correlation between viscosity and TSS content was observed with each treatment: GA ($R^2 = 0.938$), MD ($R^2 = 0.988$), and MD:GA ($R^2 = 0.980$).

3.4 Roselle Powders (Rps)

3.4.1 Granulometry of RPs

Figure 1 shows particle size distribution and cumulative percentages of RPs. The average diameter (d_{50}) of particles was 221.03 ± 3.97 mm and the moisture content was 6.45 ± 0.43%.

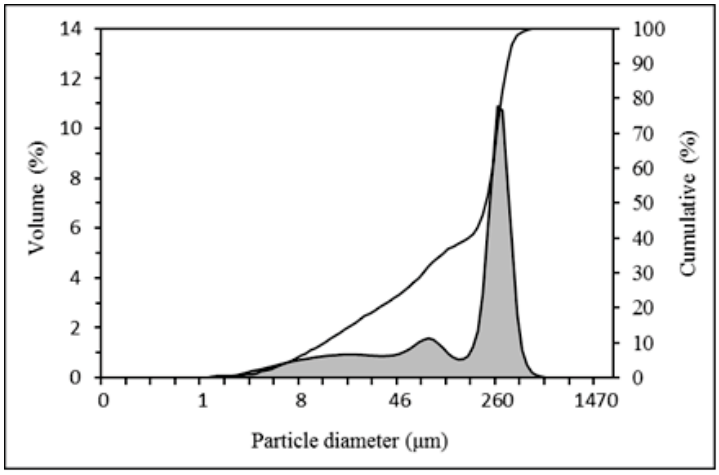

Figure 1. Particle size distribution of Roselle calyces powders

3.4.2 Yield

The yield for powder without gum was 58.19 ± 5.06cd%. The yields for powders with GA were 59.78 ± 2.47cd, 65.18 ± 5.25bc, and 68.77 ± 2.22ab% with 3, 5, and 10% gums, respectively. The yields for powders with MD were 56.46 ± 0.97d, 59.81 ± 1.49cd, 72.07 ± 2.12ab% with 3, 5, and 10% gums, respectively. The yields for powders with MD:GA were 76.38 ± 3.24a, 72.75 ± 1.16ab, and 70.79 ± 2.60ab% with 3, 5, and 10% gums, respectively. Regarding gum type, MD:GA RP showed the highest yield (73.3 ± 3.3%), and the RE and GA and MD RPs showed similar ($p > 0.05$) lower yields of 58.19 ± 5.06, 59.78 ± 2.47, and 59.81 ± 1.49%, respectively. Significant differences ($p \leq 0.05$) in yields were observed with 3, 5, and 10% gums (64.2 ± 9.5, 65.9 ± 6.3, and 70.5 ± 2.5%, respectively). The control RP (of RE) showed a low yield ($p \leq 0.05$). Ochoa-Velasco et al. 2017) obtained an average yield of 73.7 ± 1.5% for RPs microencapsulated by spray drying using mesquite gum at different concentrations (1, 2, 3, 4, and 5% w/v).

3.4.3 Physicochemical Characteristics

Moisture content. In general, RPs with the highest ($p > 0.05$) moisture contents were the following: 3.34 ± 0.30 and 3.29 ± 0.27% for 3 and 5% MD:GA, respectively; 3.29 ± 0.27 for RE; and 3.09 ± 0.24 and 2.29 ± 0.15% for 10 and 3% GA, respectively (Table 4). No significant differences ($p > 0.05$) were observed in moisture contents with gum concentrations of 0, 3, 5, and 10% (3.29 ± 0.27, 2.68 ± 0.57, 2.48 ± 0.68, and 2.60 ± 0.41%, respectively). Gonzales-Palomares et al. (2009) reported a moisture content of 4% in spray-dried powders using inlet and outlet temperatures of 180 and 80°C, respectively, for control REs, which is similar to values obtained

in this study (3.29 ± 0.27%). Likewise, Ochoa-Velasco et al. (2017) reported an average moisture content in spray-dried microencapsulated powders from REs using mesquite gum as an encapsulating agent at different concentrations (1, 2, 3, 4, and 5% w/v) of 2.29 ± 0.45%. This value is similar to the average moisture content (2.59 ± 0.55%) in RPs with different gums obtained in this study.

Table 4. Effect of gum type and concentration on the physicochemical properties of Roselle powders[a]

Gum type	GC(% w/w)	Moisture (%)	a_w (at 25.1±0.06°C)	Average diameter d_{50} (μm)	Bulk density (g/mL)	Tap density (g/mL)
RE	0	3.29±0.27a	0.183±0.012def	12.16±1.01cd	0.380±0.020a	0.483±0.021b
GA	3	2.59±0.15bc	0.167±0.012ef	9.69±0.59abe	0.220±0.010c	0.303±0.006b
	5	2.36±0.30bcd	0.210±0.010bcd	12.15±0.20cd	0.150±0.010d	0.233±0.015e
	10	3.09±0.24ab	0.247±0.006a	14.92±0.25ab	0.153±0.012d	0.250±0.017e
MD	3	2.12±0.19cd	0.153±0.015f	9.05±0.49c	0.240±0.010c	0.307±0.015b
	5	1.79±0.18fd	0.173±0.006ef	9.69±0.54de	0.163±0.012b	0.233±0.021e
	10	2.48±0.16bc	0.163±0.015f	13.93±0.51abc	0.110±0.000e	0.160±0.000f
MD:GA	3	3.34±0.30a	0.200±0.020cde	9.12±0.17e	0.353±0.021a	0.540±0.035a
	5	3.29±0.12a	0.230±0.010abc	12.42±1.53bc	0.300±0.010b	0.370±0.010c
	10	2.23±0.08cd	0.240±0.010ab	16.20±2.02a	0.097±0.012e	0.133±0.015f

[a]Different letters in the same column indicate significant differences ($p \leq 0.05$) between values. GC, gum concentration; RE, Roselle extract; GA, gum arabic; MD, maltodextrin.

Water activity (a_w). The stability of many foods depends on water activity (Fennema, 1985). High a_w indicates high free water content and thus low food stability. Table 4 shows low a_{ws} for all RPs. Average a_{ws} for RPs with MD, GA, and MD:GA were 0.163 ± 0.014, 0.208 ± 0.036, and 0.223 ± 0.022, respectively. Significant differences ($p \leq 0.05$) were observed among types and concentrations of gums. The highest values were observed with 10% GA.

Average diameter (d_{50}). Significant differences were observed between mean diameters of RE, GA, MD, and MD:GA RPs (12.16 ± 1.01, 12.25 ± 2.29, 10.89 ± 2.34, and 12.58 ± 3.32 μm, respectively (Table 4). Diameters increased significantly (0.5 ± 9.29, 11.42 ± 1.54, and 15.35 ± 2.33 μm; $R^2 = 0.990$) as gum concentrations increased from 3 to 10%. The d_{50} of RE RP (12.16±1.01 μm) was similar ($p > 0.05$) to those of 3 and 5% RPs and lower ($p \leq 0.05$) than that for 10% RPs. Particle sizes showed a unimodal distribution for RE, MD (Figure 2a), and GA RPs and a bimodal distribution for MD:GA RP (Figure 2b). Therefore, GA and MD RP particle sizes were more homogeneous and MD:GA RPs were more heterogeneous. This distribution could be attributed to agglomeration, which causes larger particles to form (Tonon et al., 2011). Figure 3 shows the TMA content for all encapsulated powders. An increase in d_{50} was observed with a decrease in anthocyanin content. This behavior was very similar to what was observed for TPCs.

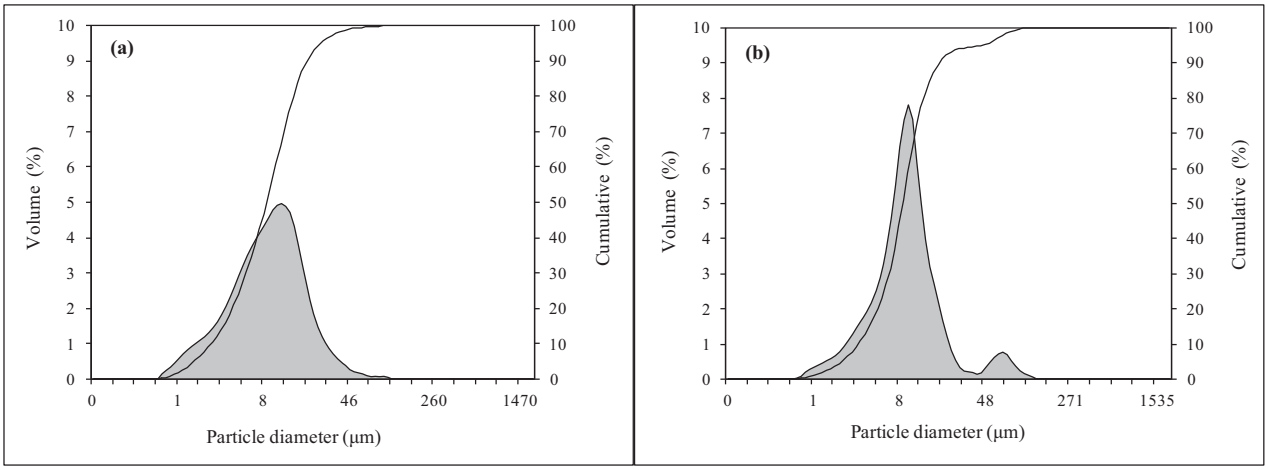

Figure 2. Particle size distribution of Roselle calyces, powders with 3% maltodextrin (MD) (a) and 3% maltodextrin:gum arabic (MD:GA) (b)

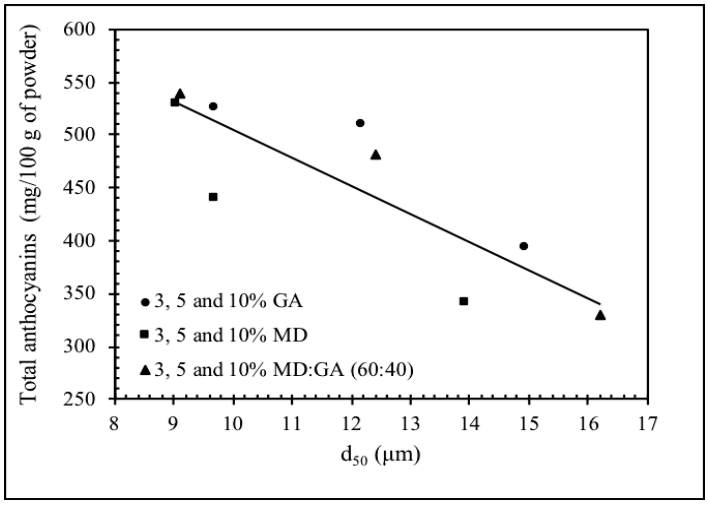

Figure 3. Correlation between total monomeric anthocyanin content and average diameter (d_{50}) of particles in microencapsulated Roselle calyces powders with gum arabic (GA) and maltodextrin (MD)

Bulk density. Significant differences ($p \leq 0.05$) were observed between bulk densities of RPs with different gum types (Table 4). RE RPs showed a higher average bulk density (0.380 ± 0.020 g/mL) than RPs with GA (0.174 ± 0.035 g/mL), MD (0.171 ± 0.057 g/mL), or MD:GA (0.250 ± 0.118 g/mL). As the concentration of gums increased, bulk densities of RPs decreased ($p \leq 0.05$): average densities of 0.271 ± 0.064, 0.204 ± 0.072, and 0.120 ± 0.027 g/mL were observed for 3, 5, and 10% gums, respectively. The highest bulk density was observed for RE (0.380 ± 0.020 g/mL) and for RPs with 3% MD:GA (0.353 ± 0.021 g/mL).

Tapped density. Table 4 shows the tapped densities of RPs. Tapped densities showed trends that were similar to those of bulk densities; however, because the RPs were tapped, all densities were higher.

3.4.4 Color of Powders

Lightness (L^*). Significant differences were observed between RPs based on type and concentration of gums (Table 5). RE RPs (41.15 ± 1.00) were darker than those with GA (55.76 ± 1.90), MD (57.14 ± 3.97), and MD:GA (52.42 ± 5.22). Lightness of RPs increased with increasing gum concentration, with lightness measures of 41.15 ± 1.00, 51.51 ± 3.18, 54.36 ± 3.03, and 59.45 ± 2.07 for 0, 3, 5, and 10% gums, respectively. Ochoa-Velasco et al. (2017) reported an average lightness value of 40.3 ± 0.71 for microencapsulated RPs obtained by spray drying using mesquite gum at different concentrations (1, 2, 3, 4, and 5% w/v); however, no significant differences were observed with the different microencapsulated powders. The authors concluded that gum concentration did not have a significant effect on all color properties. Idham et al. (2012) reported color parameters of RPs with the same gums used in this study, but they purified anthocyanins before they were mixed with the carrier for spray drying. They obtained L^*, a^*, and b^* values of 44.9, 30.3, and − 6.3 for RPs with GA; 39.3, 43.1, and −0.8 for RPs with MD; and 45.9, 34.8, and −4.3 for RPs with MD:GA. Gums were added to the extracts to reach a concentration of 20%. The mixtures were fed into the spray dryer at a flow rate of 9.5% with inlet and outlet temperatures of 150 and 110°C, respectively.

Table 5. Effect of gum type and concentration on the color properties of spray-dried powders and powders in solution[a]

Gum type	GC (% w/w)	*L**	*a**	*b**	*H* (°)	*C*
Powder						
RE	0	41.15±1.01h	42.68±0.26a	16.31±0.25a	20.92±0.40a	45.69±0.17a
GA	3	54.25±0.94de	40.66±0.33bc	11.17±0.41dcd	15.36±0.46bc	42.17±0.40cd
	5	55.59±2.33cde	39.77±0.95cd	10.31±0.64d	14.53±0.54c	41.08±1.08be
	10	57.43±0.66bc	38.72±0.86d	7.63±0.42e	11.15±0.63e	39.47±0.85fg
MD	3	52.83±0.27ef	41.11±0.09bc	12.49±0.11c	16.90±0.16b	42.97±0.08bc
	5	56.74±0.68bcd	39.16±0.20d	10.52±0.18d	15.04±0.17c	40.55±0.24ef
	10	61.86±1.00a	36.55±0.59e	8.11±0.17e	12.51±0.07de	37.43±0.60h
MD:GA	3	47.45±0.98g	41.83±0.23ab	14.76±1.06b	19.43±1.36a	44.37±0.26ab
	5	50.75±0.59f	40.97±0.22bc	12.50±0.34c	16.96±0.36b	42.83±0.30c
	10	59.05±0.82ab	37.03±0.36e	8.42±0.15e	12.81±0.10d	37.97±0.39gh
Powder in solution						
RE	0	68.32±0.24e	35.18±0.46a	14.29±0.08a	22.10±0.15a	37.97±0.46a
GA	3	71.86±0.75d	31.12±0.78b	11.65±0.26bc	20.53±0.31ab	33.24±0.80b
	5	74.71±2.29bc	29.97±1.27bc	11.13±1.00cd	20.34±0.98ab	31.97±1.52bc
	10	79.63±0.80a	23.57±0.67e	8.89±0.41e	20.66±1.06ab	25.19±0.64e
MD	3	72.11±0.71cd	30.19±0.52b	11.98±0.23bc	21.64±0.08a	32.48±0.57b
	5	76.19±0.88b	26.80±0.60d	10.10±0.17d	20.65±0.12ab	28.65±0.62d
	10	80.01±0.22a	22.22±0.62ef	7.95±0.13e	19.68±0.25b	23.60±0.63ef
MD:GA	3	72.59±0.60cd	31.38±0.41b	12.58±0.27b	21.85±0.17a	33.81±0.48b
	5	75.63±0.27b	28.14±0.41cd	11.21±0.20cd	21.73±0.47a	30.29±0.38cd
	10	80.44±0.83a	21.23±0.73f	8.49±0.25e	21.81±1.11a	22.87±0.63f

[a]Different letters in the same column indicate significant differences ($p \leq 0.05$) between values. GC, gum concentration; RE, Roselle extract; GA, gum arabic; MD, maltodextrin.

Green-red color (*a**). Green-red color values (*a**) decreased significantly, as gum concertation increased, with averages of 39.72 ± 1.07, 38.94 ± 2.01, 39.94 ± 2.23 for RPs with GA, MD, and MD:GA, respectively. RE RPs had the highest red color value (42.68 ± 0.26). The average green-red color values by gum concentration were 42.68 ± 0.26, 41.20 ± 0.55, 39.97 ± 0.94, and 37.43 ± 1.13 for 0, 3, 5, and 10% gums.

Yellow-blue color (*b**). Yellow-blue color values (*b**) were significantly different among different concentrations and types of gum. The overall averages were 9.70 ± 1.66, 10.38 ± 1.91, and 11.89 ± 2.84 for RPs with GA, MD, and MD:GA, respectively. RE RPs had the highest *b** value (16.31 ± 0.25). An increase in gum concertation was associated with a decrease in *b** values, with values of 16.31 ± 0.25, 12.81 ± 1.67, 11.11 ± 1.11, and 8.05 ± 0.42 for 0, 3, 5, and 10% gums, respectively.

Hue (*H*). RE RPs had a higher *H* value (20.92 ± 0.40°) than RPs with GA (13.68 ± 1.99°), MD (14.82 ± 1.91°), or MD:GA (16.40 ± 2.98°). These differences among gum types were significant. *H* values were also associated with gum concentrations: as gum concentrations decreased, *H* values increased (20.92 ± 0.40°, 17.23 ± 1.92°, 15.51 ± 1.16°, and 12.16 ± 0.83° for RPs with gum concentrations of 0, 3, 5, and 10%, respectively). Hue values are located in the red-yellow segment (0–90°) of the color space; however, these values tend to lie in the deep red or purple color areas (McLaren, 1986).

Purity (*C*). The purity (chroma) of all RPs showed trends that were similar to those of hue. Significant differences were observed based on type of gum added, with purities of RPs with GA, MD, and MD:GA of 40.91 ± 1.38, 40.32 ± 2.43, and 41.72 ± 2.90, respectively. RE RPs were purer (45.69 ± 0.17) than RPs with GA, MD, or MD:GA. The purity of powders were found to decrease as gum concentration increased, with purity values of 45.69 ± 0.17, 43.17 ± 0.99, 41.49 ± 1.18, and 38.29 ± 1.07 with gum concentrations of 0, 3, 5, and 10%, respectively. RE RPs showed the highest purity ($p \leq 0.05$). Purity values specify the position of colors between gray and a pure hue (saturation). Therefore, the purity or chroma of a color is proportional to the amount of color it has (McLaren, 1986). Figure 4 illustrates the correlation between *a** and purity values for all treatments; an increase in the *a** value was associated with an increase hue purity.

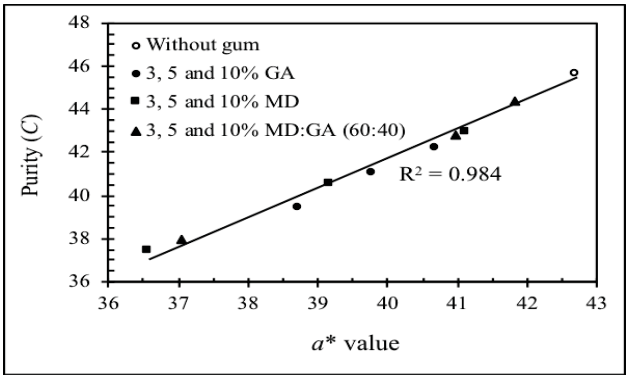

Figure 4. Correlation between purity and green-red color values for microencapsulated Roselle calyces powders with different gums

3.4.5 Color of Powders in Solution

Table 5 shows the color parameters for RPs in solution. Significant differences ($p \leq 0.05$) were observed between RPs with different types and concentrations of gums. L^*, a^*, and b^* color parameters and purity of RPs in solution were similar to those for dry RPs. The lightness (L^*) of powders in solution was higher (68–80) than that of powders because the solutions were dark red-purple but transparent. The RE RPs in solution were the darkest of all solutions. The red color (a^*) of solutions decreased as gum concentration increased. The b^* (yellow) color values for solutions were also similar to those for powders. The purity of solutions was less than those for the powders (23–38). Purities of solutions with RE (22.10 ± 0.15) and MD:GA (21.79 ± 0.61) RPs were significantly different ($p \leq 0.05$) from those of solutions with GA (20.51 ± 0.75) and MD (20.66 ± 0.86) RPs. Significant differences ($p \leq 0.05$) were also observed between RP solutions with 0, 3, 5, and 10% gum. Ochoa-Velasco et al. (2017) evaluated the color values for reconstituted RPs (100 mg/7.5 mL of distilled water) encapsulated with mesquite gum at different concentrations. The average L, a, and b color value ranges were 23.96 ± 0.66–30.54 ± 0.05, 32.83 ± 3.83–40.37 ± 0.39, and 14.56 ± 1.77–18.66 ± 0.08, respectively. These results differ from those reported in this work, possibly because of the difference in gum type, as well as solution concentration.

3.4.6 Antioxidant Characteristics

Table 6 shows TMAs, TPCs, and AC for the different RPs.

TMAs. The TMA contents in GA (476.39 ± 64.18 mg/100 g), MD (437.38 ± 79.55 mg/100 g), and MD:GA (450.10 ± 91.38 mg/100 g powder) RPs were significantly different. RE RPs had higher ($p \leq 0.05$) TMA content (665.39 ± 9.34 mg/100 g) than the other RPs. As gum concentration increased, TMA content decreased: 3% RPs showed higher TMA content than 5 and 10% RPs, with values of 531.62 ± 14.22, 477.10 ± 35.22, and 355.15 ± 30.05 mg cyanidin-3-glucoside equivalents/100 g of powder for 3, 5, and 10% gums, respectively.

TPCs. The TPC content in GA (2827.9 ± 364.8 mg/100 g), MD (499.4 ± 2956.1 mg/100 g), and MD:GA (3160.1 ± 549.4 mg/100 g of powder) RPs were significantly different. RE RPs had the highest ($p \leq 0.05$) TPC content (4929.4 ± 175.4 mg/100 g). As the gum concentration increased, TPC content decreased: RPs with 3% gum showed higher TPC content than RPs with 5 and 10% of gum, with values of 3472.9 ± 329.5, 167.3 ± 3046.2, and 2424.9 ± 138.0 mg/100 g of powder for 3, 5, and 10% gums, respectively.

AC. No significant differences ($p > 0.05$) were observed between the ACs of RE (1284.9 ± 20.8 mg/100 g), GA (1275.3 ± 185.2 mg/100 g), MD (1187.2 ± 146.4 mg/100 g), and MD:GA (1186.3 ± 102.9 mg TE/100 g of powder) RPs. Regarding gums concentration, RPs with 5% gum showed higher antioxidant capacity (1382.2 ± 106.0 mg ET/100 g powder) than RPs with 3% (1186.0 ± 94.5 mg/100 g) and 10% (1080.5 ± 45.5 mg/100 g powder) gum.

Table 6. Effect of gum type and concentration on the antioxidant properties of spray-dried powders[a]

Gum type	GC(% w/w)	TMAs[b]	TPCs[c]	AC[d]
		(mg/100 g of dry powder)		
RE	0	665.39 ± 9.34a	4929.4 ± 175.4a	1284.9 ± 20.8bc
GA	3	525.37 ± 19.34b	3090.2 ± 130.0d	1226.9 ± 115.5bcd
	5	510.33 ± 32.46bc	3036.5 ± 161.3d	1498.5 ± 44.0a
	10	393.45 ± 7.72e	2357.0 ± 92.4e	1100.6 ± 46.0d
MD	3	530.30 ± 4.46b	3527.0 ± 165.6bc	1120.7 ± 95.1cd
	5	440.20 ± 9.98b	2950.8 ± 181.0d	1368.2 ± 17.3ab
	10	341.65 ± 5.29f	2390.4 ± 111.6e	1072.6 ± 56.2b
MD:GA	3	539.19 ± 13.27b	3801.6 ± 125.9b	1210.4 ± 11.7bcd
	5	480.76 ± 9.37cd	3151.3 ± 110.1cd	1280.0 ± 83.5bc
	10	330.35 ± 16.29f	2527.5 ± 155.5e	1068.5 ± 31.7d

[a]Different letters in the same column indicate significant differences ($p \leq 0.05$) between values. [b]TMAs: total monomeric anthocyanins (cyanidin-3-glucoside equivalents). [c]TPCs: total phenolic compounds (gallic acid equivalents). [d]AC: Antioxidant activity (Trolox equivalents). GC, gum concentration; RE, Roselle extract; GA, gum arabic; MD, maltodextrin.

3. Conclusions

Based on the drying conditions used in this study, the microencapsulated RPs obtained with a mixture of MD and GA (60:40) as a carrier were the preferred powders because of its higher yields and better antioxidant and color characteristics. However, the red color (a^*) average for all powders decreased as the gum concentration increased which is due to the gum concentration. In addition, the 3% MD:GA RP showed the highest amount of TMAs (cyaniding-3-glucoside equivalents/100 g) and TPCs; however, TMAs and TPCs were well maintained in all MD:GA RPs. These results indicate that microencapsulated powders can be used successfully to produce attractive functional foods as well as imparting flavor characteristics to foods. However, a stability study should be conducted with these RPs to evaluate their carrier efficiency. A study of MD:GA mixtures at different ratios than those used in this work should also be conducted to optimize yields and physicochemical properties of RPs obtained. Therefore, more studies about stability of color, solubility, moisture sorption characteristics, and maintenance of antioxidant properties are required.

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Characterization of Pectin from Pulp and Peel of Ugandan Cooking Bananas at Different Stages of Ripening

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Abstract

East African highland cooking bananas (EA-AAA) are a staple food and major source of calories for Ugandans. Cooking bananas are considerably wasted along the postharvest chain majorly due to poor handling and ripening. Banana waste is a potential source of secondary products such as pectin, wine, beer to mention a few. The aim of this study was to extract and characterize pectin from selected cooking bananas at various stages of ripening in order to assess their potential for commercial pectin production. Pectin was extracted from the bananas at five stages of ripening i.e. stages 0 (green maturity), 1, 2, 5 and 7. Extracted pectin at stages 2, 5 & 7 was characterized.

Pectin yield from banana pulp decreased significantly with ripening ($P<0.05$) from between 18.1 to 22.65% at green maturity to between 0.65 to 1.28% at stage 7 of ripening. Pectin yield from banana peels was generally lower decreasing from between 5.34 to 6.61% at green maturity to between 1.01 to 1.38% at stage 7. The equivalent weight (1774 to 10144) of the pectin at selected stages of ripening was not significantly different ($P>0.05$) except individually. Methoxyl content was not significantly different among cultivars ($P>0.05$), however, it increased significantly through ripening stages ($P<0.05$). Anhydrouronic acid (AUA) ranged between 24.51 to 67.38% and increased with stage of ripening. AUA of pectin from pulp and peel did not differ significantly ($P>0.05$). The degree of esterification at each of the three stages was generally high (77 to 94%) implying high gelling power.

These results showed that purity of pectin increases while yield decreases with ripening and that banana pectin has a high degree of esterification implying rapid set pectin. Thus, banana peel and pulp can be good sources of industrial pectin.

Keywords: banana pulp and peel, cooking bananas, pectin extraction & characterization, ripening stages

1. Introduction

Bananas are the fourth most important food crop in the world after rice, wheat and corn (Arumugam & Manikandan, 2011). They are an important staple food particularly in tropical countries where they contribute to food security (Kabahenda, et al., 2010). The banana fruit is a good source of energy and minerals and is normally consumed fresh or processed to make products such as crisps, flour/powder, jam and wine.

Uganda is the world's largest producer and consumer of cooking bananas (Kabahenda et al., 2010) of the East African Highland (EA-AAA) genotype. Most cooking bananas (*Matooke*) are harvested and consumed at green maturity (Gafuma, Byarugaba-Bazirake, & Mugampoza, 2018). The fruits are peeled and steamed in banana leaves which are then mashed to make a thick paste that is served with sauce. Sometimes, whole fingers are boiled with beans, ground nuts or meat to make a mixture (*Katogo*). Thus, large volumes of peel are derived and either given to animals as feed or discarded on waste bins resulting in environmental pollution particularly in the urban and peri-urban areas. Moreover, *Matooke* are normally stored at ambient conditions (~25^{o}C) which accelerate ripening resulting in rejection on the market and transformed into agricultural waste. The high physical and economic losses coupled with minimum processing calls for intervention studies that could add value to waste *Matooke*. Edible by-products can be generated from banana peel and pulp as part of the waste

stream recovery (Min, et al., 2011). These materials could also be potential sources of high-quality pectin. Pectin is the second most important structural component of bananas that affects their mechanical or structural and functional properties in the living tissue where it causes firmness but also during cooking where it is involved in softening (Parre & Geitmann, 2005; Gafuma et al, 2018) particulalry in the presence of water as it undergoes solubilization.

Most of the pectin used in the food industry in Uganda is imported. Commercially, pectin is extracted from raw materials such as apple pomace or citrus peels by acid hydrolysis at high temperatures. The pH, temperature and extraction method affect pectin yield and quality (Wang, et al., 2007). Extraction and characterization of pectin have been studied for many plant materials including pumpkins, soy hull, peach pomace, sugar beet, Krueo Ma Noy (*Cissampelos pareira*; a herb traditionally used in warm regions of Asia, East Africa and South America), apple pomace, mangoes, cocoa husks, citrus peel, jackfruit waste and Saba banana waste (Castillo-Israel, Diasanta, Lizardo, Dizon, & Mejico, 2015). Therefore, ripe *matooke* could be used as alternative source of commercial pectin. Banana peels account for 40% of the total weight of the fresh banana fruit (Naggarajaiah & Prakash, 2011). Therefore, the need to examine whether or not banana peel could be a better source of high-quality pectin relative to pulp, cannot be overemphasized.

The stage of ripening at which banana peel and pulp contain the highest levels of high-quality pectin needs to be established in order to maximize pectin extraction from banana waste streams. Therefore, the aim of this study was to extract and characterize pectin from cooking banana pulp and peel at green maturity and selected stages of ripening.

2. Materials and Methods

2.1 Materials

Three Ugandan indigenous cooking banana cultivars (EA-AAA genome) were investigated basing on their abundance on the market. These include *Musakala*, *Mpologoma*, and *Nakitembe*. For each cultivar, green mature bananas were bought from Kawanda Agricultural Research Institute and directly transported to Kyambogo University Food laboratory for pectin extraction and characterization. All chemicals and reagents used were of laboratory grade and purchased from LabX Scientific Uganda Ltd.

2.2 Preparation and Ripening of Bananas

Second and third clusters were used to prepare bulk samples for each cultivar (Dadzie & Orchard, 1997). All samples were washed with portable water and ripened by covering with a tarpaulin while monitoring relative humidity (57 - 85%) and temperature (24 - 31^{o}C) every after 8 h. Samples which attained the desired level of ripeness (stages 1, 2, 5 and 7) based on the scale adopted from SH Prat's and Company (Luton UK) (Figure 1) were selected for pectin extraction and characterization. Unripe green mature bananas (stage 0) were used as the control.

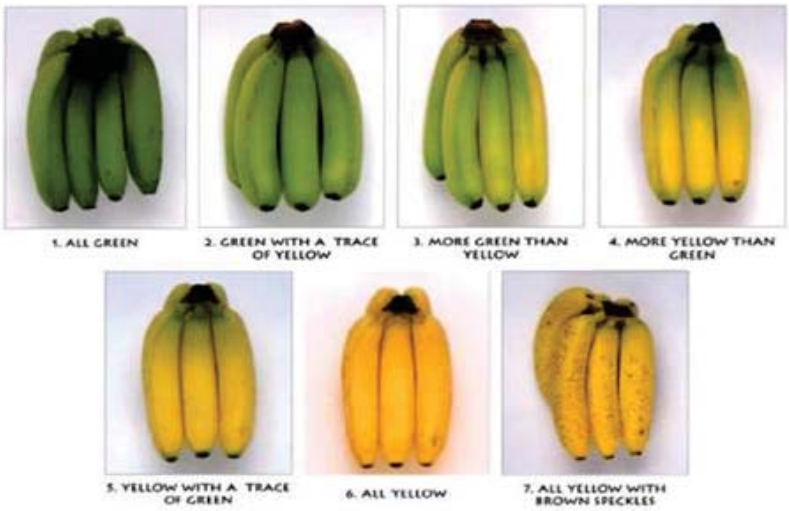

Figure 1. Colour chart used for grading of ripening bananas. Adopted from SH Pratt's & co, Luton, UK

2.3 Extraction of Pectin

Pectin was extracted using hot dilute HCl adopted from Pratik, Sheetal & Teja. (2017) with some modifications. Bananas samples at stages 0 (green maturity), 1, 2, 5 and 7 of ripening were sectioned into pulp and peel using a stainless-steel knife. Then, 50 g of each of the pulp and peel was separately weighed and crushed in a coffee grinder (Geepas, GCG289, Japan) to obtain a homogenous paste. The paste was mixed with 2.5 litres of boiling water acidified with 0.5 ml of 11.91 M HCl to give a pH of 2.2 ±0.1, followed by addition of 20 g of washed nylon threads as the filter aid. The same water was used to wash out the banana paste residue on the sides of the grinder and added to the mixture. The mixture was heated at 95 – 100^{o}C for 30 min with constant stirring. Then, the mixture was filtered and the residue was washed with 1 litre of boiling water. The filtrate was cooled before adding 2 litres of 96% ethanol containing 0.2 ml of 11.91 M HCl/litre. The mixture was slowly stirred and left to stand for 40 min to precipitate the pectin and then filtered through a fine nylon cloth. The precipitate was collected on a pre- weighed Petri dish, weighed again and dried in the air-drying oven (GenLab 12m064, UK) at 50^{o}C for 16 h. The dried pectin extract was cooled in a desiccator, weighed and pulverized in a grinder (Geepas) to a fine powder which was stored at room temperature for further use. Pectin yield was calculated as follows:

$$Pectin\ yield = \frac{EP\ x\ 100}{Bi}$$

Where Ep = extracted pectin in grams; Bi = Weight of banana pulp/peel in grams.

2.4 Characterization of the Extracted Pectin

Characterization was done for pectin extracts at stages 2, 5 and 7 of ripening since at these stages, the pectin is expected to have reducing degree of contamination from other polysaccharides such as cellulose, hemicellulose, starch and lignin. Equivalent weight, Methoxyl content, Anhydrouronic acid, and degree of esterification were determined using methods by (Owens, et al., 1952) adopted from Castillo-Israel et al. (2015).

2.4.1 Equivalent Weight

The equivalent weight (Eq. wt) of pectin extract was determined according to the formula adopted from Castillo-Israel et al. (2015). Approx. 0.1 g of pectin extract was weighed into a 250 ml conical flask and 1 ml 99% ethanol (v/v) was added. Approx. 0.5 g of sodium chloride and 60 ml of distilled water were added and heated in a water bath (Stuart SWB24D, UK) for 30 min at 45^{o}C. Finally, 2 drops of phenol red (Sigma-Aldrich, USA) were added and the solution titrated against 0.05 N NaOH. The end of the titration was indicated by development of a purple color and the titre volume recorded. Equivalent weight was calculated according to the following formula:

$$Equivalent\ weight = \frac{Weight\ of\ sample\ x\ 1000}{ml\ of\ NaOH\ x\ Normality\ of\ NaOH}$$

2.4.2 Methoxyl Content (MeO)

Methoxyl content was determined according to the formula adopted from Castillo-Israel et al. (2015). The neutral solution containing 0.5 g pectin extract from equivalent weight determination was used. To the neutral solution, 25 ml of 0.25 N sodium hydroxide was added and mixed thoroughly. The solution was kept in a flask with a stopper at room temperature for 30 min. Then, 25 ml of 0.25 N HCl was added and titrated with 0.1 N NaOH until a purple color was observed. Methoxyl content was calculated using the following formula:

$$Methoxyl\ content\ (\%) = \frac{ml\ of\ alkali\ x\ Normality\ of\ alkali\ x\ 3.1}{weight\ of\ sample\ (g)}$$

2.4.3 Anhydrouronic Acid (AUA)

Estimation of AUA was performed by making use of the titration volumes in equivalent weight and methoxyl content determinations. Hence, total AUA was determined using the following formula adopted from Castillo-Israel et al. (2015).

$$Percentage\ (\%)AUA = \frac{176 * 0.1z * 100}{w * 1000} + \frac{176 * 0.1y * 100}{w * 1000}$$

Where the molecular weight unit of AUA = 176 g; z = ml of NaOH from the equivalent weight determination; y = ml of NaOH from methoxyl content determination and w = weight of the sample.

2.4.4 Degree of Esterification (DE)

DE of pectin extract was determined on the basis of methoxyl and AUA content (Castillo-Israel et al., 2015) and calculated using the following formula:

$$\%DE = \frac{176 * Meo}{31 * \%AUA} * 100$$

2.4.5 Ash Content

The ash content of pectin extract was determined using method No. 923.3 of AOAC (2012) with modifications. One gram of pectin extract was ground to pass a 75-micron mesh screen and placed into a tared platinum crucible, then ignited in a muffle furnace (Carbolite AAF1100, Germany) at 550^{o} C for 6 h. The crucible containing ash was cooled in a desiccator and weighed. The ash content was calculated using the following formula:

$$Ash\ content(\%) = \frac{weight\ of\ ash\ x\ 100}{weight\ of\ pectin}$$

2.5 Data Analysis

Parametric statistics (means ± standard errors of means) and one-way analysis of variance (ANOVA) were computed using Predictive Analytical Software (PASW), v.23 for three independent replicates. Means were separated using Turkey test after analysis with GraphPad version 7. The P-values were set at $p<0.05$.

3. Results

3.1 Pectin Yield at different Stages of Ripening

There was a significant decrease in pectin yield ($P<0.05$) from the pulp of selected banana cultivars with increase in ripening (Figure 2). Pectin yield (wet basis) decreased from 22.65, 21.38 & 18.1% at green maturity to 1.19, 1.28 & 0.65% at stage 7 of ripening for *Nakitembe* (NKT), *Musakala* (MUS) & *Mpologoma* (MPO), respectively. Pectin yield from banana peel was generally low and decreased from 5.83, 6.61 & 5.34% to 1.01, 1.38 & 1.08% for NKT, MUS and MPO, respectively.

Pectin yield was significantly higher ($P<0.05$) in pulp than in the peel particularly at stages 0 and 1; while at stage 2, 5 and 7 the yield from both pulp and peel was not significantly different ($P>0.05$). However, pectin yield from banana peel at stage 0 was not significantly different ($P>0.05$) from that of stages 2, 5 and 7 of ripening.

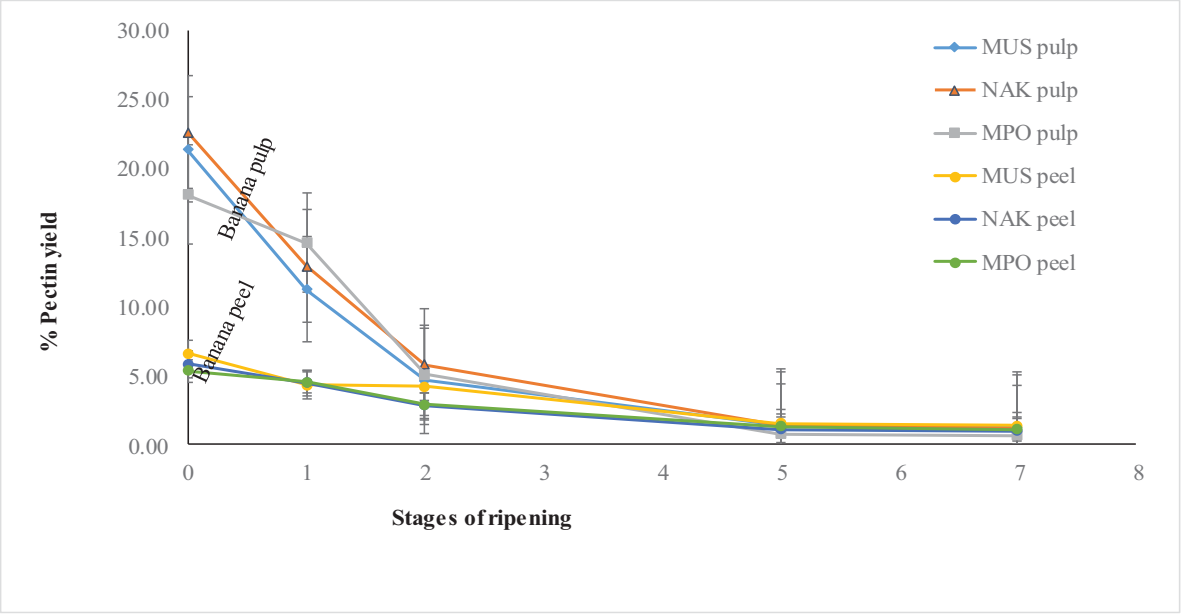

Figure 2. Changes in pectin yield from pulp and peel of selected cooking banana cultivars at different stages of ripening. Stage 0 = Green maturity

3.2 Characterization of Pectin Extract

3.2.1 Equivalent Weight (eq. wt)

Equivalent weight at each of stages 2, 5 and 7 was generally high (Figure 3). Equivalent weight of pectin from ripe banana pulp ranged from 1903 to 6421 while that of pectin from ripe banana peel ranged from 1774 to 10 144 g per mol. Pectin from *Mpologoma* peel and pulp had significantly higher equivalent weight than that from *Musakala* and *Nakitembe* ($P<0.05$). The eq. wt. of pectin from pulp and peel of *Musakala* at the three stages of ripening was not significantly different ($P>0.05$). On the other hand, equivalent weight of pectin from *Mpologoma* was initially very high and decreased significantly ($P<0.05$) with ripening while that of pectin from *Musakala* did not significantly change ($P>0.05$).

At stage 2 of ripening, the eq. wt. of pectin from *Nakitembe* pulp was 1970.9, which was the lowest followed by *Musakala* with 2041.0 and *Mpologoma* the highest with 12 657.5 g/mol. At stage 5, the equivalent weight of pectin from *Nakitembe* was 1903.8, *Musakala* was 2051.9, and *Mpologoma* was 6421.4 g/mol. At stage 7, equivalent weight of pectin from *Musakala* was 1986.4, that of pectin from *Nakitembe* was 3657.5 and that of pectin from *Mpologoma* was 4500.0 g/mol. The equivalent weight of pectin from banana peel at stage 2 was 10 144.0 g/mol (*Mpologoma*), 4878.1 (*Nakitembe*) and 1783.0 g/mol (*Musakala*). At stage 5 and 7 the equivalent weights of pectin from ripe banana peel was 1780.0 and 2556.3 for *Nakitembe*; 2085.0 and 1774.5 for *Musakala* and 5804.3 and 6384.4 g/mol for *Mpologoma* respectively.

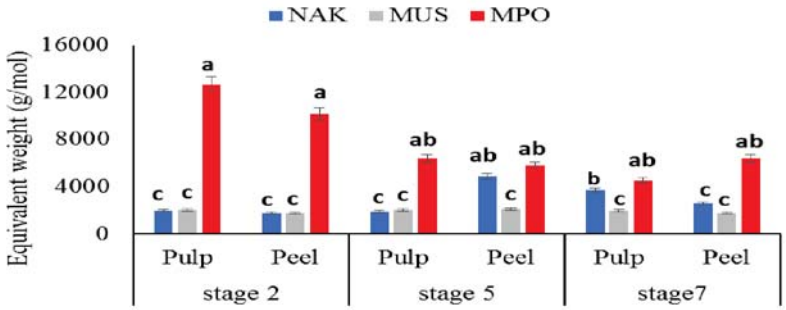

Figure 3. Equivalent weight of pectin from peel and pulp of selected cooking banana cultivars at different stages of ripening. Bars with different superscript letters are significantly different ($P<0.05$). NKT, Nakitembe; MUS, Musakala; MPO, Mpologoma

3.2.2 Methoxyl Content

The methoxyl content of pectin from ripening banana pulp and peel was not significantly different ($P>0.05$) and generally increased through the ripening stages (Figure 4). Methoxyl content of pectin from banana peel ranged from 4.07 to 11.02% while that from banana pulp ranged from 3.67 to 8.21% across the ripening stages. Methoxyl content of pectin from banana pulp at stage 2 of ripening for all the three cultivars did not differ significantly ($P>0.05$) i.e. *Nakitembe* (5.37%), *Musakala* (5.12%) and *Mpologoma* (4.07%). At stage 5, MeO was 4.73% for *Nakitembe*, 7.27% for *Musakala* and 5.73% for *Mpologoma* and was not significantly different ($P>0.05$). At stage 7 of ripening, MeO of the pectin from *Nakitembe* was 11.02%, from *Musakala* was 8.76 % and *Mpologoma* 7.88%. The pectin from banana peel at stage 2 of ripening had low methoxyl content of 4.02% for *Nakitembe*, 5.86% for *Musakala* and 4.03% for *Mpologoma*. At stages 5 and 7, the respective MeO contents of the pectin extracts were 5.87 & 3.67% for *Mpologoma*; 4.02 & 8.21% for *Nakitembe* and 7.13 & 7.75% for *Musakala*.

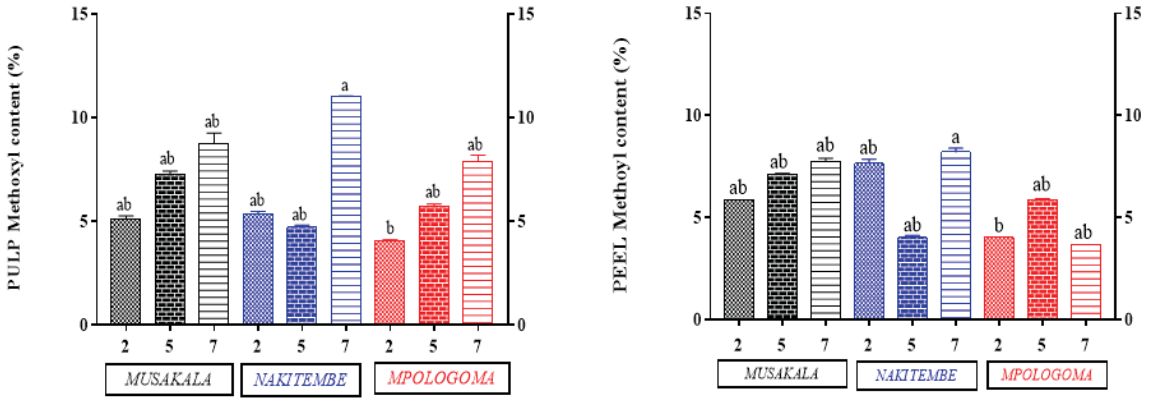

Figure 4. Methoxyl content of pectin extracted from pulp and peel of selected cooking banana cultivars at different stages of ripening

Different letters above each bar show significant difference at ($p < 0.05$).

3.2.3 Anhydrouronic Acid (AUA) Content

The AUA content of pectin from ripening bananas ranged from 24.51 to 67.38% generally increasing with the stage of ripening depending on the banana cultivar (Figure 5). There was no significant difference in the AUA ($P>0.05$) of pectin extracted from banana pulp and peel across the ripening stages. AUA of pectin from banana pulp generally increased with increase in ripening. For instance, *Nakitembe* increased from 39.41 to 67.38 %, *Musakala* increased from 37.67 to 58.57 % and *Mpologoma* increased from 24.51 to 48.64 %. AUA of pectin from the peel increased from 26.48 to 53.49% for *Nakitembe*, 43.15 to 53.94 % for *Musakala* while that for pectin from *Mpologoma* remained almost the same (24.64 to 23.60%). Generally, pectin from pulp had a higher AUA content than that from the peel.

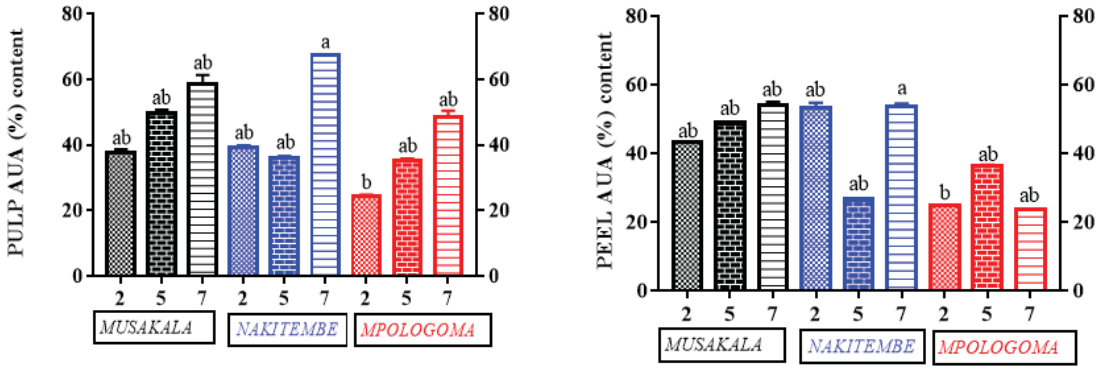

Figure 5. Anhydrouronic acid (AUA) content of pectin extracted from pulp and peel of selected cooking banana cultivars at different stages of ripening

Different letters above each bar show significant difference at ($P<0.05$).

3.2.4 Degree of Esterification (DE)

The DE of pectin extract from ripening banana pulp and peel was generally very high and ranged between 77 to 94% (Figure 6) implying high methoxyl pectin. There was no significant difference ($P>0.05$) in the DE of pectin from the peel and pulp across all the stages of ripening.

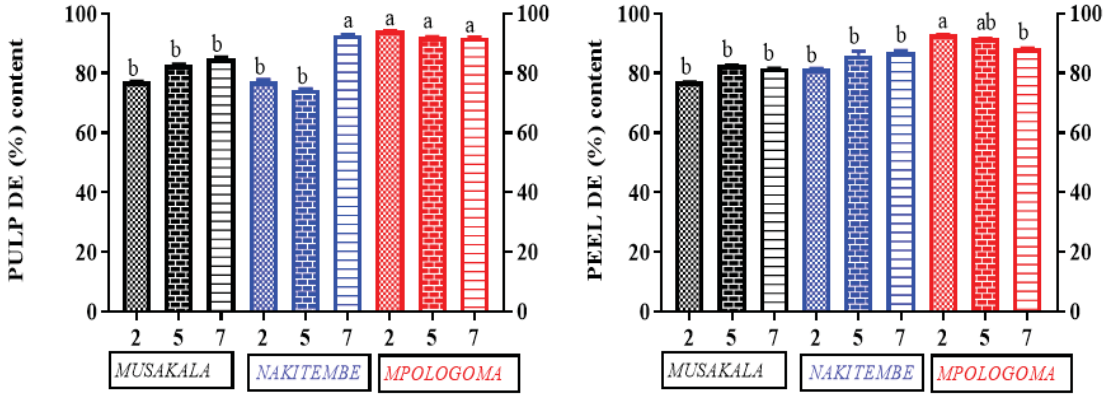

Figure 6. Degree of Esterification (DE) of pectin extracted from the pulp and peel of selected cooking banana cultivars at different stages of ripening

Different letters above each bar show significant difference at (P<0.05).

3.2.5 Ash Content

The ash content of banana pectin extracted from peel and pulp ranged between 0.3 and 5.4%. There was no significant difference (P>0.05) between the ash content of pectin extracted from the peel and pulp across the ripening stages (Figure 7).

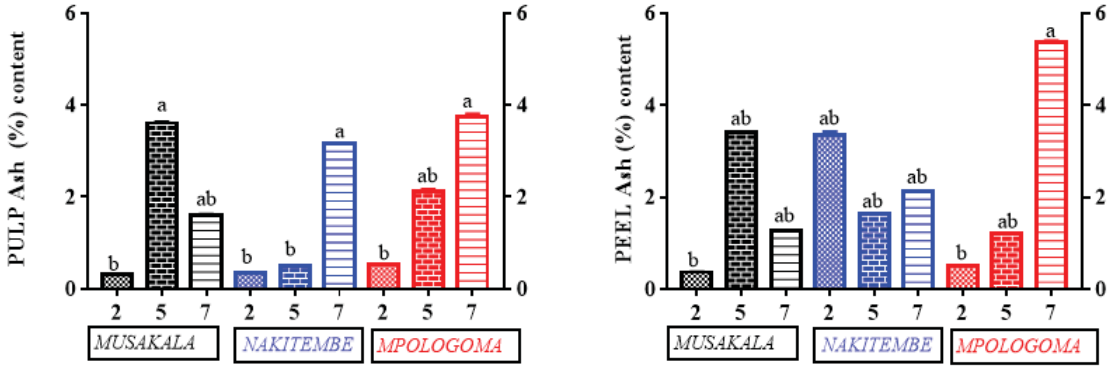

Figure 7. Ash content of pectin extracted from the pulp and peel of selected cooking banana cultivars at different stages of ripening.

Different letters above each bar show significant difference at (P<0.05).

4. Discussion

4.1 Pectin Yield

The high pectin yield at green maturity (stage 0) and at stages 1 and 2 of ripening could be due to presence of protopectin (pectin combined with hemicellulose, cellulose, lignin and starch) (Castillo-Israel et al., 2015; Conrad, 1930). According to these authors, the maturity stage of bananas affects pectin yield as the amounts of pectin, hemicellulose, cellulose and lignin vary as the fruit matures. These authors found higher pectin yield of about 16.5% from Saba banana peels at the unripe stage compared to 11.87% from the same bananas at the ripe stage. The high ionic strength of HCl (pH 2.2) also maximizes extraction and precipitation of pectin and impurities including other negatively charged ionic molecules such as cellulose and hemi-cellulose. High extraction temperatures have also been reported to increase extraction yield (Gama, De Farias Silva, Oliveira Da Silva, & Abud, 2015). Uzma, Genitha, & Farheena (2015) obtained the highest percentage of pectin (16%) from papaya peels on extraction with hydrochloric acid at pH 2.0, temperature of 80°C for 60 min of extraction, similar to the conditions used in this study. Yadav, Khan, Kunjwani, & Mular (2015) obtained pectin yield of up to 36% from orange peels using HCl at pH 2 and 85^{o}C extraction conditions. However, the yield of pectin decreased with ripening which could be explained by the increasing hydrolysis of protopectin releasing cellulose,

hemicellulose and lignins as bananas ripen. Pectin content of fully ripe bananas has been reported to range between 0.5 to 1.28% (Baker, 2006), which is in agreement with pectin yield at ripening stages 5 and 7 of this study. Pectin yield generally increases at the expense of protopectin as fruits ripen due to increasingly weaker connections between pectin and other cellular compounds thus making pectin more available for extraction. However, over ripening of bananas like at stage 7 results into a decrease in pectin yield due to degradation of pectin by native pectolytic enzymes i.e. polygalacturonase, pectin methyl esterase and pectin lyase (Emaga, Adrianaivo, Wathelet, Tchangco, & Paquot, 2007). These results imply that the stage of ripening has a significant influence on pectin yield and therefore, extraction could be optimized at stage 5 of ripening.

4.2 Characterization of the Pectin

4.2.1 Equivalent Weight

The equivalent weight (Eq. wt.) of pectin in the current study was generally high compared to literature values. Pectin produced at lower pH normally has a higher equivalent weight due to possible polymerization of the pectin molecules into longer chains (Uzma et al., 2015; Rouse, 1977). Equivalent weight represents the quantity of pectin that is reactive which can undergo cross-linking reactions through polyol functional groups and is indicative of a high degree of esterification which is in turn associated with a higher gelling power (Vaclavik & Christian, 2008; Yadav et al., 2015; Ragab, Osman, Khalil, & Gouda, 2016). The high eq. wt obtained in this study could imply strong associations between the pectin and cellulose/hemicellulose including other molecules. The high equivalent weights obtained in this study were in agreement with those reported by Castillo-Israel et al. (2015) in pectin from unripe banana peels. Therefore, the relatively high equivalent weight could be justified by the stage of maturity and ripening.

4.2.2 Methoxyl Content

Methoxyl content of banana pectin in this study increased with ripening. This means methoxyl content of pectin could be affected by the level of maturity of bananas. Castillo-Israel et al. (2015) while studying characteristics of pectin from Saba banana peels obtained methoxyl content of 6.4% and 5.25% in ripe and unripe bananas, respectively. According to Aina, et al. (2012), the methoxyl content of extracted pectins varies between 0.2 and 12% depending on the source and mode of extraction implying that the methoxyl content obatined in this study was within range at all stages of ripening. Methoxyl content of commercial pectins varies from 8-11% (Castillo-Israel et al., 2015). The methoxyl content of most pectin extracts in this study was above 6.4% meaning that they were high methoxyl pectins (Beda & Kouassi, 2014; Castillo-Israel et al., 2015) and therefore, could form strong gels with sugar especially pectins with methoxyl content above 7% (Genovese, Ye, & Singh, 2010). Pectins with methoxyl content less than 7% can form gels with lower concentrations of sugar. High methoxyl content may also imply strong adhesive and cohesive forces which could imply increased firmness of the gels. Methoxyl content is important in controlling setting time of pectin, sensitivity to polyvalent metal cations and also determines the functional properties of the pectin-gel texture (Constenla & Lozano, 2003). It also affects the dispersability of pectin in water where pectin with high methoxyl content (>7%) is readily dispersible in water (Rouse et al., 1962). Therefore, these pectins are suitable for industrial use particularly in jam and jelly production. Our results imply that banana pectin particularly that from the pulp at stage 7 could be suitable for industrial use such as in production of jam and jellies while the low methoxyl pectin at stages 2 and 5 (pulp) and that from peel could be used in production activities where weaker gels are required such as yogurt.

4.2.3 Anhydrouronic Acid (AUA)

The AUA values of pectin from green mature and ripe bananas examined in this study were in agreement with values obtained by Castillo-Israel et al. (2015) of 57.3% and 39.68% for ripe and unripe banana peels, respectively. The results were also similar to those reported by Khamsucharit, Laohaphatanalert, Gavinlertvatana, Sriroth, & Sangseethong (2018) for pectin from peels of Kluai Hin (34.56 %) and Kluai Nam Wa (66.67 %) banana cultivars from Thailand. AUA is an indicator of the purity and degree of esterification of pectin (Rangana, 1986). Pectin normally contains about 10% or more organic materials (Assifaoui, et al., 2015). Pectin with AUA of less than 65% may indicate impurities due to presence of proteins, starch and sugars (Norazelina & Nazarrudin, 2011). The recommended AUA (%) for extracted pectin for use in pharmaceuticals and as a food additive should not be less than 65% (May, 1990). The generally low AUA content in this study implies low purity of extracted pectin hence requiring further purification if the pectin is to be used commercially. According to Castillo-Israel et al. (2015), longer extraction times could be adopted for higher values of AUA. Pectin extracted at stages 2 and 5 had relatively lower AUA compared to that extracted at stage 7. Results in Figure 5 generally indicated that pectin extracted at stage 7 of ripening had a higher AUA implying increasing purity with ripening. Values of AUA for pectin from green mature bananas were also comparable with values from ripe

bananas. Pectin from the pulp seemed to be much purer relative to that from the peel.

4.2.4 Degree of Esterification

DE is important in determining the gelling and adhesive power of pectin and in case of plant tissue pectin, it has been given in the range of 60 – 90% (Shaha, Nayagi, Punichelvana, & Afandi, 2013). High DE was observed in pectin from ripe bananas and there was no significant difference in DE with respect to the stage of ripening. However, the degree of esterification differs depending on the level of maturity or ripeness, part of the fruit, botanical source and method of isolation (Bonrood, Kamonrad, & Niamsupn, 2005). During ripening, solubility of pectic substances increases mainly due to activity of pectic enzymes i.e. polygalacturonase (PG) and pectin methylesterase (PME) (Maduwanthi & Marapana, 2017). The banana pectin in this study can be categorized as rapid set pectin since it had DE greater than 72% (Shaha et al., 2013). Our DE values were within the DE range of 60 to 90% generally found in pectin from plant tissues (Shaha et al., 2013). The high DE implies high gelling power of the extracted pectin which is a good indicator for commercial application such as use in the manufacture of jams and jellies.

4.2.5 Ash Content

The ash content of all pectin extracts from the three banana cultivars from peel and pulp was less than 5.5%. This means that their purity was acceptable for commercial application (Khamsucharit et al., 2016; Shaha et al., 2013). To form gels of good quality, the maximum limit for ash content in pectin should not exceed 10% (Norazelina et al., 2011). Overall, pectin from *Mpologoma* peel at stage 7 contained the highest amount of ash indicating that it could produce the least quality pectin among the banana cultivars.

5. Conclusion

The high methoxyl content and degree of esterification of the pectin extracted from Ugandan cooking bananas implies high gelling power which makes it suitable for commercial application in industry particularly in the production of jams and jellies. If extracted at stages 2 and 5, the pectin could be used in products such as yogurt that do not require high levels of setting. Both peel and pulp could be good sources of high quality pectin due to its relatively high degree of esterification. Moreover, purity of the pectin appeared to increase with stage of ripening as observed with AUA implying higher quality pectin could be extracted from bananas at stage 5 and above.

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Bioactive Compounds, Antioxidant Activities, and Health Beneficial Effects of Selected Commercial Berry Fruits: A Review

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Abstract

Epidemiological studies have provided the evidence that regular consumption of fruits and vegetables reduce the risk of pathological condition such as cardiovascular disease, cancer, inflammation, and aging. Among fruits, berries are considered as superfruits due to their highly packed phytochemicals comprising phenolic acids, flavonoids viz. flavonols, flavanols, and anthocyanins. These bioactive compounds are associated with significant antioxidant, antidiabetic, antiinflammation, and anticancer properties. This review highlights the basic information and interesting findings of some selected commercial berries with their phytochemical composition, antioxidant properties, and potential health benefits to human.

Keywords: berries, polyphenols, antioxidant activity, antidiabetic and anticancer properties, nutrition

1. Introduction

Free radicals are reactive oxygen or nitrogen molecule that damage cellular biomolecules viz. protein, nucleic acid, lipids membranes and results in various pathological condition such as cardiovascular disease, cancer, inflammation, and aging (Sun, 1990; Valko, Leibfritz, Jan, Cronin, Mazur, & Telser, 2007; Phaniendra, Jestadi, & Periyasamy, 2015). Antioxidants are molecule that scavenge or inhibit the actions of reactions of free radical to protect the cells and tissues (Uttara, Singh, Zamboni, & Mahajan, 2009). Several epidemiologic studies demonstrated that consumption of fruits and vegetables could lower these chronic pathologies including obesity, inflammation, cardiovascular diseases, and cancer due to their strong antioxidant activities (Kristo, Klimis-Zacas, & Sikalidis, 2016; Kalemba-Drożdż, Cierniak, & Cichoń, 2020). Among fruits, berries are important part of the human diet for many centuries and are receiving considerable attention continuously all over the world due to their beneficial effects to the human health and nutrition (Bravo, 1998; Nile& Park, 2014, Cianciosi, Simal-Gándara, & Forbes-Hernández, 2019).

Berries are considered as superfruits due to their high packed phytochemicals, dietary fibers, vitamins, and minerals. Berries polyphenolic compounds composed of diverse group of compounds which include phenolic acids, flavonoids viz., flavonols, flavanols, and anthocyanins. Phenolic acids are the derivatives of benzoic acid and cinnamic acid and consist of an aromatic ring structure with hydroxyl group. However, hydroxy derivatives of cinnamic acid are much more abundant than hydroxybenzoic acid among berries. Among flavonoids berries phenolic compounds include anthocyanins, flavanols, flavonols, and proanthocyanidins. These groups differ each other in the spatial positions and numbers of hydroxyl and alkyl groups on the basic chemical structure. Anthocyanins are the most abundant among flavonoids and are responsible for the color of the fruits. In their structure anthocyanins are glycosylated with glucose, galactose, rhamnose, xylose, or arabinose are attached to the aglycone called anthocyanidins mainly cyanidin, pelarogonidin, malvidin, petunidin, delphinidin, and peonidin. Usually the sugar components of anthocyanins are connected to the anthocyanidin skeleton via the C3 hydroxyl group in ring C of the anthocyanin.

Due to the presence of these polyphenolic compounds berries and their extracts exhibit several health benefits such as retarding inflammation, lowering cardiovascular diseases, or protect to lower the risk of various cancers,

and antioxidant activities (Heinonen, Meyer, & Frankel, 1998; Seeram, Adams, Zhang, Lee, Sand, Scheuller, & Heber, 2006; Pan, Skaer, Yu, Hui, Zhao, Ren, Oshima & Wang, 2017; Reboredo-Rodr´ıguez, 2018; Pan et al., 2018). However, the contents of polyphenols and nutrients of berries are highly dependent on genotypes, environments, and the cultivation techniques. In addition to these, various agronomic factors such as pre and postharvest practices, maturity at harvest, storage, and processing operations plays crucial role in the quality and levels of phytochemicals in berries. Interestingly among these factors the genotype plays most significant role which regulates the content of nutrients and phytochemicals and influence health beneficial activities. In an important study Halvorsen et al., (2006) evaluated 1120 food samples listed in USDA for antioxidant content and found that blueberries, strawberries, cranberries and their juice product occupied top position in the first 50 antioxidant rich foods (Halvorsen et al., 2006). Large body of literature is available with studies of berries' health benefits to humans. Recently Yeung, et al., (2019) concluded that berries which were mentioned at the 100 top cited research articles dealing with anticancer and antioxidant activities were strawberry, blueberry, cranberry, raspberry, blackberry, billberry, and grape berry. However, at the current time in addition to these commonly cultivated berries some other native berry fruits viz, sea buckthorn, acai, maqui, viburnum, and elder berries are gaining remarkable attention worldwide due to their rich source of antioxidants. Due to the increasing demand of antioxidant rich berries, continuous research on the identification of phytochemicals and their health benefits of these berries have been carried out. However, a very few review and research articles are seen covering most of the top commercial berries. In this review article we provide an overview of polyphenolic composition of selected top commercial berries and their health beneficial properties.

2. Commercial Berries

Berries are available with distinctive skin and flesh colors such as red, blue, or purple. They are highly perishable fruits. Some of the top commercial varieties of berries include members of *genera*: *Fragaria* (strawberry), *Vaccinium* (blueberry, cranberry, bilberry), *Prunus* (cherries), *Hippophae* (sea buckthorn), *Rubus* (raspberries), *Euterepe* (açaí berry), *Aristotelia* (Maqui berry), and *Sambucus* (elderberry, red elderberry).

2.1 Strawberries

2.1.1 Source

Strawberry fruits belong to the family of *Rosaceae* and genus *Fragaria* are globally cultivated for their popularity due to distinctive aroma, bright red color, and juicy texture. The plant is widely cultivated worldwide, intensively in Europe, USA, and China. The USA is the world leading producer of strawberries after Turkey and Spain. The US strawberry industry has significantly rising as per person consumption increasing because of the high consumer acceptance for its sensory attributes.

2.1.2 Composition

Vitamin C is one of the major nutrients available in strawberries. Other vitamins such as thiamine, riboflavin, niacin, and vitamins A, E, K, and B6 including carotenoids are also available in strawberries (Rothwell et al., 2013, Fierascu, Temocico, Fierascu, Ortan, & Babeanu, 2020). However, depending on the varieties, geographic, and agronomic condition these levels vary among them (Aaby, Mazur, Nes, & Skrede, 2012; Nowicka, Kucharska, Sokół-Ł¸etowska, & Fecka, 2019, Akimov, et al., 2019). The major polyphenolic compounds in strawberries are flavonol, flavanol, anthocyanins, and phenolic acids (Kähkönen, Hopia, & Heinonen, 2001; Aaby, Skrede, & Wrolstad, 2005; Giampieri, et al., 2020), (Table 1). Moreover, the major polyphenolic compounds in strawberries are anthocyanins and they are responsible for the color of the fruits. The major anthocyanins in strawberries are derivatives of pelargonidin and cyanidin with glycosides or acylated forms such as pelargonidin 3-glucoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-galactoside, pelargonidin 3-rutinoside, pelargonidin 3-arabinoside, pelargonidin 3 malylglucoside etc (Lopes-da-Silva, de Pascual-Teresa, Rivas-Gonzalo, & Santuos-Buelga, 2002; Giampieri, et al., 2020). Quercetin, kaempferol, fisetin, and their glycoside were found to be as major flavonols in strawberries. Among flavanols catechin, proanthocyanidin B1, proanthocyanidin trimer, proanthocyanidin B3 were reported to be major ones. Phenolic acids such as 4-coumaric acid, p-hydroxybenzoic acid, ferulic acid, vanillic acid, sinapic acid were found to be in significant level in strawberries. Storage and processing greatly influence the quality and levels of phenolic and anthocyanin compounds of strawberries. In the processed products such as jams, jellies, puree, and juices the levels of phenolic compounds decrease relative to the fresh strawberries (Álvarez-Fernández, Hornedo-Ortega, Cerezo, Troncoso, García-Parrilla, 2014; Méndez-Lagunas, Rodríguez-Ramírez, Cruz-Gracida, Sandoval-Torres, & Barriada-Bernal, 2017). The color and composition of anthocyanins are affected by pH. During storage lower pH (2.5) were found to be better to preserve its polyphenols.

Table 1. Major phenolic compounds in selected commercial berries

Berries	Flavonol	Phenolic acid	Anthocyanin	Flavanol
Strawberries	Kaempferol 3-O-glucoside	4-Hydroxybenzoic acid 4-O-glucoside	Cyanidin	(+)-Catechin
	Kaempferol 3-O-glucuronide	5-O-Galloylquinic acid	Cyanidin 3-O-(6"-succinyl-glucoside)	(+)-Gallocatechin
	Quercetin 3-O-glucuronide	Ellagic acid glucoside	Pelargonidin	(-)-Epicatechin 3-O-gallate
	Kaempferol	5-Caffeoylquinic acid	Pelargonidin 3-O-(6"-malonyl-glucoside)	(-)-Epigallocatechin
	Myricetin	Caffeoyl glucose	Pelargonidin 3-O-arabinoside	Procyanidin dimer B1
	Quercetin	Cinnamic acid	Pelargonidin 3-O-glucoside	Procyanidin dimer B2
		Feruloyl glucose	Pelargonidin 3-O-rutinoside	Procyanidin dimer B3
		p-Coumaric acid	pelargonidin 3 malylglucoside	Procyanidin dimer B4
		p-Coumaric acid 4-O-glucoside		Procyanidin trimer
		p-Coumaroyl glucose		
		Vanillic acid		
		Protocatechuic acid		
		Sinapic acid		
Blueberries	Kaempferol	4-Hydroxybenzoic acid	Cyanidin 3-O-(6"-acetyl-galactoside)	
	Myricetin	Ellagic acid	Cyanidin 3-O-(6"-acetyl-glucoside)	
	Quercetin	Gallic acid	Cyanidin 3-O-arabinoside	
	Kaempferol 3-O-glucoside	Caffeic acid	Cyanidin 3-O-galactoside	
	Myricetin 3-O-arabinoside	Ferulic acid	Cyanidin 3-O-glucoside	
	Myricetin 3-O-rhamnoside	p-Coumaric acid	Delphinidin 3-O-(6"-acetyl-galactoside)	
	Quercetin 3-O-acetyl-rhamnoside	5-Caffeoylquinic acid	Delphinidin 3-O-(6"-acetyl-glucoside)	
	Quercetin 3-O-arabinoside		Delphinidin 3-O-arabinoside	
	Quercetin 3-O-galactoside	Syringic acid	Delphinidin 3-O-galactoside	
	Quercetin 3-O-glucoside	Vanillic acid	Delphinidin 3-O-glucoside	
	Quercetin 3-O-xyloside	4-Hydroxybenzoic acid 4-O-glucoside	Malvidin 3-O-(6"-acetyl-galactoside)	
		Gallic acid 4-O-glucoside	Malvidin 3-O-(6"-acetyl-glucoside)	
		Protocatechuic acid 4-O-glucoside	Malvidin 3-O-arabinoside	
		3-Caffeoylquinic acid	Malvidin 3-O-galactoside	
		4-Caffeoylquinic acid	Malvidin 3-O-glucoside	
		5-Caffeoylquinic acid	Peonidin 3-O-(6"-acetyl-galactoside)	
		5-Feruloylquinic acid	Peonidin 3-O-(6"-acetyl-glucoside)	
		5-p-Coumaroylquinic acid	Peonidin 3-O-galactoside	
		Caffeic acid 4-O-glucoside	Peonidin 3-O-glucoside	
		Ferulic acid 4-O-glucoside	Petunidin 3-O-(6"-acetyl-galactoside)	
		p-Coumaric acid 4-O-glucoside	Petunidin 3-O-(6"-acetyl-glucoside)	
			Petunidin 3-O-arabinoside	
			Petunidin 3-O-galactoside	
			Petunidin 3-O-glucoside	
			Cyanidin 3-O-(6"-acetyl-galactoside)	
			Cyanidin 3-O-(6"-acetyl-glucoside)	
			Cyanidin 3-O-arabinoside	
			Cyanidin 3-O-galactoside	
			Cyanidin 3-O-glucoside	
			Delphinidin 3-O-(6"-acetyl-galactoside)	
			Delphinidin	(-)-Epicatechin

			3-O-(6"-acetyl-glucoside) Delphinidin 3-O-arabinoside Delphinidin 3-O-galactoside Delphinidin 3-O-glucoside Malvidin 3-O-(6"-acetyl-galactoside) Malvidin 3-O-(6"-acetyl-glucoside) Malvidin 3-O-arabinoside Malvidin 3-O-galactoside Malvidin 3-O-glucoside Peonidin 3-O-(6"-acetyl-galactoside) Peonidin 3-O-(6"-acetyl-glucoside) Peonidin 3-O-arabinoside Peonidin 3-O-galactoside Peonidin 3-O-glucoside Petunidin 3-O-(6"-acetyl-galactoside) Petunidin 3-O-(6"-acetyl-glucoside) Petunidin 3-O-arabinoside Petunidin 3-O-galactoside Petunidin 3-O-glucoside	
Bilberries	Myricetin Quercetin	Caffeic acid p-Coumaric acid Chlorogenic acid Chlorogenic acid Gallic acid 4-O-glucoside Cinanamic acid	Cyanidin 3-O-glucosides Delphinidin-3-O-glucosides Peonidin 3-O-glucosides Petunidin3-O-glucosides Malvidin 3-O-glucosides Cyanidin 3-O-galactosides Cyanidin 3-O-arabinoside Delphinidin 3-O-arabinoside Peonidin 3-O-arabinoside Petunidin3-O-arabinoside Malvidin s3-O-arabinoside	
Cranberries	Kaempferol 3-O-glucoside Myricetin 3-O-arabinoside Quercetin 3-O-arabinoside Quercetin 3-O-galactoside Quercetin 3-O-rhamnoside Myrecetin Quercetin	2,4-Dihydroxybenzoic acid 3-Hydroxybenzoic acid 4-Hydroxybenzoic acid Benzoic acid Vanillic acid Caffeic acid Cinnamic acid Ferulic acid p-Coumaric acid Sinapic acid 4-Hydroxybenzoic acid 5-Caffeoylquinic acid Caffeic acid Ferulic acid p-Coumaric acid	Cyanidin 3-O-arabinoside Cyanidin 3-O-galactoside Cyanidin 3-O-glucoside Peonidin 3-O-arabinoside Peonidin 3-O-galactoside Peonidin 3-O-glucoside Cyanidin 3-sophoroside Pelargonidin 3-glucoside pelargonidin 3-rutinoside	
Cherries	Quercetin Quercetin 3-glucoside Quercetin 3-rutinoside Kaempferol 3-rutinoside	3-Caffeoylquinic acid 3-Feruloylquinic acid 3-p-Coumaroylquinic acid 4-Caffeoylquinic acid 4-p-Coumaroylquinic acid	Cyanidin 3-O-glucoside Cyanidin 3-O-rutinoside Pelargonidin 3-O-rutinoside Peonidin 3-O-glucoside Peonidin 3-O-rutinoside	(+)-Catechin (-)-Epicatechin (-)-Epicatechin 3-O-gallate (-)-Epigallocatechin Procyanidin dimer B1

		5-Caffeoylquinic acid	Cyanidin 3-sophoroside	Procyanidin dimer B2
		p-Coumaroylquinic acid	Pelargonidin 3-glucoside	Procyanidin dimer B3
		4-p-Coumaroylquinic acid	Cyanidin 3-O-glucosyl-rutinoside	Procyanidin dimer B4
		5-Caffeoylquinic acid		Procyanidin dimer B5
		5-Feruloylquinic acid		Procyanidin dimer B7
		5-p-Coumaroylquinic acid		Procyanidin trimer C1
Sea Buckthorn	Isorhamnetin	Gallic acid		(epi)gallocatechin
	Kaempferol	p-Coumaric acid		Catechin
	Quercetin	Ferulic acid;		Proanthocyanidin(dimer, trimer, tetramers)
		Ellagic acid		
Viburnum berries	Quercetin	Chlorogenic acid	Cyanidin-3-glucoside	Epicatechin
	Isorhamnetin		Cyanidin-3-rutinoside	Catechin
			Cyanidin 3-sambubioside	
Raspberries	Quercetin-3-glucuronide	Gallic acid	Cyanidin 3-sophoroside	(+)-Catechin)
	Kaempferol-3-glucuronide,	Salicylic acid	Cyanidin 3-glucosylrutinoside	
	Quercetin-3- rutinoside	Caffeic acid	Cyanidin 3-glucoside	
	Quercetin-3-rhamnoside	p-Hydroxybenzoic	Cyanidin 3-rutinoside	
	Apigenin	ferulic acid	Cyanidin 3-sambubioside	
	Naringenin	p-Coumaric acid	Cyanidin 3-rutinoside	
		Cinnamic acid	Cyanidin 3-xylosylrutinoside	
		Vanillic acids acid		
Acai berries	Quercetin	Gallic acid	Cyanidin-3-glucoside	
	Kaempferol	3,4-Dihydroxybenzoic acid	Delphinidin-glucoside	
	Dihydrokaempferol	Chlorogenic acid	Malvidin-glucoside	
		Caffeic acid	Pelargonidin-3-glucoside	
		Syringic acid	Peonidin glucoside	
		Ferulic acid	Cyanidin-3-sambubioside	
		Trans-cinnamic acid	Peonidin 3-rutinoside	
		Vanillic acid		
Maqui berries	Quercetin	Caffeic acid	Cyanidin and delphinidin	
	Dimethoxy-quercetin	Ferulic acid	Cyanidin 3-glucosides	
	Quercetin-3-rutinoside	Gallic acid	Cyanidin 3,5-diglucosides	
	Quercetin-3-galactoside	p-Coumeric acid	Cyanidin 3-sambubiosides	
	Myrecetin	Chlorogenic acid	Cyanidin 3-sambubioside-5-glucosides	
			Delphinidin 3-sambubioside-5-glucosides	
	Kaempferol			
Elderberries	Kaempferol	Chlorogenic	Cyanidin-3-glucoside	+)-Catechin
	Quercetin	neo-chlorogenic acid	Cyanidin 3-sambubioside-5-glucoside	(-)-Epicatechin
	Isorhamnetin	crypto-chlorogenic acid	Cyanidin 3-rutinoside	Proanthocyanidin monomer
	Quercetin 3-O-rutinoside	Caffeic acid	Pelargonidin 3-glucoside	Proanthocyanidin dimer
	Quercetin 3-O-glucoside	p-Coumaric acid	Delfinidine-3-rutinoside	Proanthocyanidin trimer
	Kaempferol 3-O-rutinoside		Cyanidin-3-sambubioside	Proanthocyanidin tetramer
	Isorhamnetin 3-O-glucoside		Cyanidin 3,5-diglucoside	
	Myricetin 3-O-rutinoside		Cyanidin 3-rutinoside	
			Pelargonidin 3-sambubioside	
			Delfinidine-3-rutinoside	
			Petunidin 3-rutinoside	

2.1.3 Health Benefits

Strawberries shows anti-inflammatory, anti-oxidative, anticancer properties, and other health benefits because of the presence of high levels of flavonoids, anthocyanins, and vitamin C. Large number of studies have been investigated to screen their antioxidant activities adopting DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS

(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), peroxyl, and superoxide free radical scavenging assays (Wang & Lin, 2000; Kähkönen, Hopia, & Heinonen, 2001, Giampieri, et al., 2012). It has been observed that total phenolic compounds were highly correlated with the radical scavenging activities. Prymont Przyminska et al., (2014) found that daily consumption of strawberries increased the plasma antioxidant activity measured by DPPH assay in healthy subjects (Prymont Przyminska et al., 2014). Strawberries containing anthocyanin crude extracts have been reported to show i*n vitro* antioxidant and anti-proliferative activities in human tumor cell. Ellagic acid and quercetin from strawberries have been shown to promote anti-cancer activity by suppressing the growth of human oral, colon, breast, and prostate cancer cells (Zhang, Seeram, Lee, Feng, & Heber, 2008; Casto, Knobloch, Galioto,Yu, Accurso, & Warner, 2013). *In vivo* and *in vitro* studies demonstrated that strawberries bioactive compounds reduces intracellular reactive oxygen species concentration, increases the activity of antioxidant enzymes, reduces DNA (Deoxyribonucleic Acid) damage; reduces inflammation, reduces oxidative stress, slows down the aging process, treats stomach ulcers, improves plasma lipid profile, and reduces oxidation of low-density lipoproteins (Giampieri, et al., 2012, Basu, Morris, Nguyen, Betts, Fu, & Lyons, 2016)). Agarawal et al., (2019) has reported that consumption of strawberries reduces the risk of Alzheimer's dementia (Agarwal, Holland, Wang, Bennett, & Morris, 2019). Schell et al. (2017) reported that dietary strawberries supplemented to obese adults suffering from osteoarthrities have resulted analgesic and anti-inflammatory effects (Schell et al., 2017). Basu et al., (2016) reported that dietary strawberry selectively increased plasma antioxidant biomarkers in obese adults with elevated lipids (Basu, Morris, Nguyen, Betts, Fu, & Lyons, 2016).

2.2 Blueberries

2.2.1 Source

Historically, blueberries have been a popular fruit due to their well-known health and nutritional benefits. Blueberries belongs to family *Ericacea* and genus *Vaccinum*. Numerous species (approx. 450) of blueberries are grown wildly or cultivated worldwide. Major commercially available blue berries that are grown across the worldwide are rabbit eye blueberries (*Vaccinium ashei*), lowbush blueberry (*Vaccinium angustifolium* A.) and highbush blueberry (*Vaccinium corymbosum* L.). USA is the largest producer of blue berries in world after Australia and Canada. However various part of the Europe and other countries produce blueberries commercially. Blueberries are highly perishable and therefore blueberries are processed after harvesting. Various postharvest techniques for storage and processing are applied to prolong their shelf lives and preserve quality properties of blueberry.

2.2.2 Composition

Blueberries are packed with various nutrients and bioactive active compounds. They are listed top of the superfoods. Blueberries are the richest source of polyphenolic compounds. It also contains several vitamins including vitamin C. Numerous studies have been carried out to screen the level polyphenolic compounds including analysis by chromatographic and mass spectrophotometric analysis. Analysis in the content of total phenolic compounds and total anthocyanins demonstrated that it has several folds differences among the different varieties depending on the geographic location and agricultural practices. Major polyphenolic compounds of blueberries are flavonols (mainly quercetin derivatives), anthocyanins, flavan-3-ols, proanthocyanidins, and phenolic acids (Table 1). Among the phenolic acids of blueberries hydroxycinnamic and hydroxybenzoic acids and their derivatives such as chlorogenic, caffeic, gallic, p-coumaric, ferulic, ellagic, syringic, vanillic acids are common (Kalt & McDonald, 1996;Kalt, Forney, Martin, & Prior, 1999, Gu et al., 2004; Rodriguez-Mateos, Cifuentes-Gomez, Tabatabaee, Lecras, Spencer, 2012,;). Anthocyanins are the major polyphenolic compounds comprising 60% of total phenolic compounds in blue berries. In a study of 215 phenotypes of blueberries cyanidin-3-glucoside was reported to be the major again among the anthocyanin compounds (Kalt, et al., 2001). In some other studies it was found that malvidin, delphinidin, petunidin, and peonidin are the major components comprising 75% of all identified anthocyanins (Scibisz & Mitek, 2007; Routray & Orsat, 2011).The composition and levels of anthocyanins in blueberries vary with cultivars and varieties. The color of blueberries also varies with the composition of anthocyanins (Routray & Orsat, 2011). The level of polyphenolic compounds differs with maturity stages of blueberries where ripening stages showed higher level of anthocyanins than the other phenolic compounds. Postharvest condition such as oxygen level, temperature, and light of blueberries impacts the nutritional quality and phenolic contents (Kalt, Forney, Martin, & Prior, 1999).

2.2.3 Health Benefits

Blueberries have tremendous pharmacological properties. Consumption of blueberries help in controlling

diabetes and its complication such as lowering blood pressure and blood cholesterol (Martineau, et al., 2006; Basu, et al.,2010; Stull, Cash, Johnson, Champagne, & Cefalu, 2010, McAnulty, Collier, Pike, Thompson, & McAnulty, 2019). It possesses anti-diabetic properties and help to protect pancreatic β-cells from glucose-induced oxidative stress (Al-Awwadi, et al., 2005; Martineau, et al., 2006). It has been reported that consumption of blueberry significantly reduced H_2O_2-induced DNA damage (Del Bo′, et al., 2013). Phytochemicals present in blueberry could inhibit the growth and metastatic potential of breast and colon cancer cells (Adams, Phung, Yee, Seeram, Li, & Chen, 2010; Schantz, 2010). It was found that pure anthocyanins, such as cyanidin, delphinidin, as well as peonidin 3-glucoside, suppressed growth of human tumor cells and apoptosis of colon and breast cell line. In a recent clinical study comprising 52 US adult veterans reported that consumption of 22 g freeze-dried blueberries for 8 weeks could beneficially affect cardiometabolic health parameters in men with type 2 diabetes (Stote, et al., 2020). Recently Rodriguez-Daza et al., (2020) revealed the key role of blueberry extract with proanthocynaidins in modulating the gut microbiota and restoring colonic epithelial mucus layer triggering health effects of blueberry polyphenols (Rodriguez-Daza et al., 2020). Moreover Türck, et al., (2020) evaluated the effect of blueberry extract on functional parameters and oxidative stress levels in rat lungs with pulmonary arterial hypertension (PAH) and reported that intervention with blueberry extract mitigated functional PAH outcomes through improvement of the pulmonary redox state (Türck et al., 2020). Tian et al., (2019) reported that cyanidin-3-arabinoside extracted from blueberry as a selective Protein Tyrosine Phosphatase 1B Inhibitor (PTP1B) which is an important target for type 2 diabetes (Tian et al., 2019). PTP1B inhibitors can reduce blood glucose levels by increasing insulin sensitivity. Jielong et al., (2019) reported that extracts of blueberry reduces obesity complications through the regulation of gut microbiota and bile acids via pathways involving FXR(Farensoid X Receptor) and TGR5 (Jielong, Xue, Hongyu, Weidong, Yilin, & Jicheng, 2019).

2.3 Bilberries

2.3.1 Source

Bilberry (*Vaccinium myrtillus* L.) a small dark blue berry belongs to *Ericacea* family and is native to Europe and North America. Bilberry is also known as European blueberry differs from blueberry relative to *Vaccinium corymbosum* and *Vaccinium angustifolium* with their morphology and flesh color. The blue coloration is due to its high content in anthocyanin (Prior et al., 1998). Cultivation of bilberries have been increasing continuously during several years.

2.3.2 Composition

Bilberry contains several polyphenolic compounds such as lignin, flavonoids, tannins, and phenolic acids (Bravo, 1998) (Table1). Among flavonoids of bilberries anthocyanins are the major compounds while simple phenolics constitute phenolic acids such as cinnamic acid, gallic acid, caffeic acid, and chlorogenic acid (Puupponen-Pimiä et al., 2001; Taiz & Zeiger, 2006). The bilberries contain five different anthocyanidins comprising cyanidin, delphinidin, peonidin, petunidin, and malvidin with three sugar moieties viz., 3-O-arabinoside, 3-O-glucosides and 3-O-galactosides (Martinelli, Baj, Bombardelli, 1986).

2.3.3 Health Benefits

Due to the presence of anthocyanins as major bioactive compounds in bilberry fruit it exhibits several health-promoting properties (Park, Shin, Seo, Kim, 2007; Schantz, Mohn, Baum, & Richling, 2010, Pieberger et al., 2011; Kolehmainen et al., 2012). Recently, Arevstrom et al., (2019) in a study involving 50 patients found that bilberry powder supplementation (40 g/day) over eight weeks significantly reduced both total and LDL (low-density lipoprotein) cholesterol compared to baseline (Arevstrom et al., 2019). Karlsen et al., (2010) investigated the effect of bilberry juice on serum and plasma biomarkers of inflammation and antioxidant status in subjects with elevated levels risk factor for cardiovascular disease (CVD) and found that supplementation with bilberry polyphenols modulated the inflammation processes (Karlsen et al., 2010)). Triebel et al., (2012) investigated the influence of bilberry (*Vaccinium myrtillus* L.) extract containing anthocyanins on pro-inflammatory genes in IFN-γ/IL-1β/TNF-α stimulated human colon epithelial cells (T84) and demonstrated that anti-inflammatory activity mostly depends on the aglycon structure and the sugar moiety of the billberry anthocyanin (Triebel, Trieu, & Richling, 2012). Roth et al., (2014) reported that bilberry-derived anthocyanins reduced IFN-γ-induced pro-inflammatory gene expression and cytokine secretion in human THP-1 monocytic cells (Roth, Spalinger, Müller, Lang, Rogler, & Scharl, 2014). These findings suggested a crucial role for anthocyanins in modulating inflammatory responses. Billberry extract showed antihypoglycemic effect by inhibition the action of intestinal α-glucosidase activity (Martineau et al., 2006). Takkikawa et al., (2010) investigated antidiabetic activities of billberry extract and found that dietary anthocyanin-rich bilberry extracts

ameliorated hyperglycemia and insulin sensitivity in diabetic mice (Takikawa, Inoue, Horio, & Tsuda, 2010). In another study Cignarella, et al., (1996) found that bilberry extract decreased levels of plasma glucose and triglycerides in streptozotocin (STZ)-induced diabetic mice (Cignarella, Nastasi, Cavalli, & Puglisi, 1996).

2.4 Cranberries

2.4.1 Source

Cranberries (*Vaccinium macrocarpon* Ait.) also known as lowbush cranberries are native to USA belongs to *Ericaceae* family. USA is the world leader of cranberry producer with 90% of world production. Cranberries are consumed as fresh fruits, dried, jams, and juices. The US per capita consumption of cranberries is raising continuously mostly in the form of juices and remains at the top of healthy drinks.

2.4.2 Composition

Cranberries are rich source of various phytochemical compounds viz., flavan-3-ols, A type procyanidins (PACs), anthocyanins, benzoic acid, ursolic acid, and vitamin C (Table1). Among the PAC's comprising catechin, epicatchin, epigallocatechin cranberries have been known to have epicatechin as the major one. Although many fruits have proanthocyanidins but only cranberry have significant level of A type PAC. Recently Wang et al., (2020) investigated the analysis of cranberry proanthocyanidins using UPLC (Ultra Performance-ion mobility-high-resolution mass spectrometry and identified total of 304 individual A-type and B-type proanthocyanidins, including 40 trimers, 68 tetramers, 53 pentamers, 54 hexamers, 49 heptamers, 28 octamers, and 12 nonamers (Wang, Harrington, Chang, Wu, & Chen, 2020). Anthocyanins in cranberries are composed of glycosides of the 6 aglycones with cyanidin, peonidin, malvidin, pelargonidin, delphinidin, and petunidin (Wu, & Prior, 2005). The major phenolic acid including hydroxycinnamic acids in cranberry are p-coumaric, sinapic, caffeic, and ferulic acids. Quercetin is the major flavonol compound present in cranberries. Ellagic acid with and without glucosides represent more than 50% of total phenolic compounds. However, level of phenolics and anthocyanins depends on the maturation stage of the cranberries.

2.4.3 Health Benefits

Cranberries have been used for several decades to prevent urinary tract infection. This health benefit is attributed to cranberries because proanthocyanidin can prevent adhering of *Escherichia coli* to uroepithelial cells in the urinary tract (Ermel, Georgeault, Inisan, Besnard, 2012). After consumption juice and various products of cranberries it was also believed to enhance the plasma antioxidant activities (Pedersen et al., 2000). In vitro study confirmed that cranberry extracts inhibited activities of angiotensin converting enzyme and thus it showed the potential in lowering blood pressure (Apostolidis, Kwon, & Shetty, 2006). Cranberry also helped to reduce the cardiovascular disease risks and to protect against lipoprotein oxidation. Several studies confirmed that cranberries bioactive compounds have anti-cancer and antimutagenic activities (Prasain, Grubbs, & Barnes, 2020; Howell, 2020). Recently Hsia et al., (2020) investigated that whether consumption of cranberry beverage would improve insulin sensitivity and other cardiovascular complications and reported that daily consumption for 8 weeks may not impact insulin sensitivity but could be helpful in lowering triglycerides and alters some oxidative stress biomarkers in obese individuals with a proinflammatory state (Hsia, Zhang, Beyl, Greenway, & Khoo, 2020). Chew et al., (2019) reported the health benefits of cranberry beverage consumption on gluco regulation, oxidative damage, inflammation, and lipid metabolism in healthy overweight humans (Chew et al., 2019). Consumption of significant amount of cranberry beverage improved antioxidant status and reduced cardiovascular disease risk factors by improving glucoregulation, downregulating inflammatory biomarkers, and increasing HDL cholesterol.

2.5 Viburnum Berries

2.5.1 Source

Viburnum opulus L. (Adoxaceae), commonly known as European guelder, is also called as European cranberry bush, guelder rose, cherry-wood, and snowball bush. It grows in Europe, North and Central Asia, and North Africa. Viburnum is commonly used both in conventional and folk medicine in Russia. The State Pharmacopoeia of the Russian Federation (XI edition, issue 2) contains a monograph on the preparation of Viburnum fruits, and two medicinal drugs with Viburnum fruits are entered in the State Register of Medicinal Remedies of the Russian Federation. While parts of viburnum such as bark, flowers, and fruits are widely used in traditional medicine some fruits are used as cooking ingredients. In Russia, Ukraine, and among many Siberian nations the *viburnum opulus* (VO) fruits are used in traditional cuisine such as marmalades, jams, and "Kalinnikov" pies, and herbal teas.

2.5.2 Composition

Viburnum opulus contain high level phenolic compounds such as hydroxybenzoic acids, tannins, flavonoids, anthocyanins, chlorogenic acid, catechin, epicatechin, cyanidin-3-glucoside, cyanidin-3-rutinoside and quercetin (Velioglu, Ekici, & Poyrazoglu, 2006; Perova, I. B., Zhogova,Cherkashin,Éller, &Ramenskaya, 2014).) (Table 1). In an investigation to profile phenolic compounds in viburnum berries using high-performance liquid chromatography Velioglu et al., (2006) identified chlorogenic acid (upto 2037 mg/kg), catechin, epicatechin, cyanidin-3-glucoside, cyanidin-3-rutinoside and different glucosides of quercetin and cyanidin derivatives (Velioglu, Ekici, & Poyrazoglu, 2006). Zakłos-Szyda (2019) has identified and quantified the major phenolic compounds where they reported chlorogenic acid as main component. Catechin was found to second most abundant phenolic compounds and Cyanidin 3-sambubioside was found to be major anthocyanin (Zakłos-Szyda, Majewska, Redzynia, & Koziołkiewicz, 2019). In addition to these flavonol compounds quercetin-pentose, quercetin-hexose, quercetin-deoxyhexose and isorhamnetin glycosides, rutin and isorhamnetin were obtained. Çam et al. (2007) have showed that *Viburnum Opulus* seeds are also good source of total phenolics and flavonoids (Çam, Hisil, & Kuscu, 2007).

2.5.3 Health Benefits

Viburnum opulum phenolic compounds impart various health beneficial properties. Number of in vitro studies reported high antioxidant activities by viburnum berries. Zakłos-Szyda et al, (2019) investigated the antioxidant activities of *viburnum opulus* and reported the strong correlation between total phenolics and radical scavenging activities such as ABTS and ORAC with high Pearson's correlation coefficients, r = 0.993 and 0.991 respectively(Zakłos-Szyda, Majewska, Redzynia, & Koziołkiewicz, 2019). Similar linear relationships between phenolics and antioxidants activities were observed by Karaçelik et al., (2015) in their study of identification of bioactive compounds of *Viburnum opulus L.* using on-line HPLC-UV-ABTS (High Performance Liquid Chromatography-Ultra violet- 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay and LC-UV-ESI-MS (Liquid Chromatogrpahy-Ultra Violet Electro Spray Ionization Mass Spectrometry) (Karaçelik et al., 2015). *Viburnum opulus* extract also showed anticancer activities. *In vitro* studies using Caco-2 cell culture indicated that *Viburnum opulus* phenolic rich fraction prompted to decrease the uptake of free fatty acids and lower the accumulation of glucose and lipids by Caco-2 cells without affecting their viabilities (Zakłos-Szyda, Majewska, Redzynia, & Koziołkiewicz, 2019). Moreover i*n vivo* antitumoral activity of *Viburnum opulus* were confirmed by Ceylan et al., (2018) in Ehrlich ascites tumor model. Phenolic extract of *Viburnum. opulus* fruit also reported to be strong inhibitor of α-amylase, α-glucosidase, and/or PTP-1B phosphatase enzymes involved in lipid and carbohydrate metabolism (Zakłos-Szyda, Majewska, Redzynia, & Koziołkiewicz, 2015).

2.6 Cherries

2.6.1 Source

Cherry is one of the major small fruits with bigger benefits belongs to family: *Rosaceae* and genus: *Prunus.* It is one of the major berries native to United States and is the second-largest producer in the world. The two major types of cherries are sweet cherries (*Prunus avium*) and tart or sour cherries (*Prunus cerasus*).

2.6.2 Composition

Sweet and sour cherries are distinguished from each other by their ratios of sugars (e.g., glucose, fructose, and others) to organic acids (mainly maleic acid). Cherries are rich source vitamins A, B, C, E, K, carotenoids, minerals, and phenolic compounds. Sour cherries have higher contents of vitamin A and betacarotene. Tart cherries contain significant levels of melatonin (13.46 ± 1.10 ng/g and 2.06 ± 0.17 ng/g in Balaton and Montmorency, respectively) (Burkhardt, Tan, Manchester, Hardeland, & Reiter, 2001). Chemical profiling of tart cherries indicated presence of cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-sophoroside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside, peonidin 3-glucoside, and peonidin 3-rutinoside as important flavonoids (Kirakosyan, Seymour, Llanes, Daniel, Kaufman, & Bolling, 2008) (Table 1). Among phenolic acids, hydroxycinnamates (neochlorogenic acid and p-coumaroylquinic acid) are reported to present in significant levels. Cherries also contain flavonols and flavan-3-ols such as catechin, epicatechin, quercetin 3-glucoside, quercetin 3-rutinoside, and kaempferol 3-rutinoside. Different cultivars of sweet and sour cherries have different levels of phenolics and flavonoids. For example, in a study Kim et al., (2005) studied different cultivars it was found that total anthocyanins of sweet cherries were 30 - 79 mg cyanidin-3-glucoside equivalents (CGE)/100 g, whereas in sour cherries these were 45 - 109 mg CGE/100g (Kim, Heo, Kim, Yang, & Lee, 2005).

2.6.3 Health Benefits

Various studies demonstrated that tart cherries extract, and its compounds showed strong antioxidant activities

(Blando, Gerardi, & Nicoletti, 2004). Kirakosyan et al., (2008) reported the high TEAC (trolox equivalent antioxidant capacity) of two tart cherries viz Balaton and Montmorency and cyanidin and its derivatives were found to be the important antioxidants in the assays (Kirakosyan, Seymour, Llanes, Daniel, Kaufman, & Bolling, 2008). Numerous studies indicated that cherry consumption inhibited inflammatory pathways. Consumption of cherries also helped to lower blood pressure, control blood glucose, protect against oxidative stress, and reduce inflammation (Martin, Burrel, & Bopp, 2018; Martin, & Coles, 2019). Kelley *et al.* (2006) showed that intake of sweet cherries decreased levels of C-reactive protein (CRP), a biomarker for inflammation and cardiovascular disease in healthy subjects (Kelley, Rasooly, Jacob, Kader, Mackey, 2006). *In vitro* and *in vivo* studies suggested that anti-inflammatory properties of polyphenolic compounds of cherries evidenced by the inhibition of activity of the cyclooxygenase II (COX II), another biomarkers for inflammation, carcinogenesis, cell proliferation, and angiogenesis (Wang, Nair, & Strasburg,1999). Consumption of Cherry also showed to lower serum urate levels and inflammation (Martin, & Coles, 2019). Zhang et al., (2012). reported that cherry consumption affected the risk of recurrent gout attacks (Zhang, Neogi, Chen, Chaisson, Hunter, & Choi, 2012). Recently Lamb et al., (2020) also demonstrated the effect of tart cherry juice to reduce risk of recurrent gout flare (Lamb, Lynn, Russell, & Barker, 2020). Di Bonaventura et al., (2020) indicated that tract cherry has potential role to prevent obesity-related risk factors, especially neuroinflammation based on a rat model study (Di Bonaventura et al., 2020). In a mice model study Smith et al., (2019) found that cherry supplementation (5% and 10%) improved bone mineral density (BMD) and some indices of trabecular and cortical bone microarchitecture and they proposed that these effects were likely attributed to increased bone mineralization (Smith et al., 2019).

2.7 Sea Buckthorn Berries

2.7.1 Source

Sea buckthorn, known as seaberry, (*Elaeagnus rhamnoides L.*) belongs to the family *Elaeagnaceae*. Even though sebuckthorn is cultivated mostly in Russia and China, now a days it is cultivated around other countries like Finland, Germany, and Estonia.

2.7.2 Composition

Sea buckthorn have been found to have a range of bioactive compounds including vitamin A, C, E, carotenoids, minerals, and polyphenols (Olas, 2016; Gradt, Kuhn, Morsel, & Zvaigzne, 2017). A recent intensive analysis on composition of seabuckthorn berries indicated the presence of 21 phytochemicals such as isorhamnetin, quercetin, kaempferol glycosides and catechin. Phenolic compounds also include primarily proanthocyanidins, gallocatechins and flavonol glycosides (Dienaitė, Pukalskas, Pukalskienė, Pereira, Matias, & Venskutonis, 2020) (Table 1). Criste et al., (2020) also reported that seabuckthorn berries are great source of phenolic compounds such as derivatives of quercetin and hydrocinnamic acid. (Criste et al., 2020).

2.7.3 Health Benefits

Sea buckthorn exhibits a wide spectrum of pharmacological activities such as anti-inflammatory, anticancer, antioxidant, and anti-atherosclerotic activities (Zeb, 2006; Basu, Prasad, Jayamurthy, Pal, Arumughan, & Sawhney, 2007; Olas, 2016). They also induce apoptosis and strengthen the immune system. In a study on the content and antioxidant activities of phenolic compounds of seabucthorn Gao et al., (2000) reported that antioxidant activities were strongly correlated with the content of total phenolic compounds and ascorbic acid (Gao, Ohlander, Jeppsson, Bjork, & Trajkovski, 2000). It was also found that antioxidant activity of the lipophilic extract correlated with the total carotenoids content. A strong correlation existed between flavonoid content in seabuckthorn and their antioxidant activities ($r = 0.96$) (Criste et al., 2020). To investigate other health benefits recently Guo et al., (2020) reported that administration of freeze-dried seabuckthorn powder lowered body weight, Lee's index, adipose tissue weight, liver weight, and serum lipid levels induced by obesity (Guo, Han, Li, & Yu, 2020). Tkacz et al., (2019) reported high *in-vitro* anti-oxidant and anti-enzymatic activities related to digestion system due to the presence of phytochemicals such as phenolic acids, flavonols, xanthophylls, carotenes, tocopherols, and tocotrienols(Tkacz, Wojdyło, Turkiewicz, Bobak, & Nowicka, 2019). Number of studies reported that seabuckthorn oil exhibits anti-tumor properties due to the presence flavonoid compounds kaempferol, quercetin, and isorhamnetin (Christaki, 2012). Hao et al., (2019) found that seabuckthorn seed oil extracts were effective in reducing blood cholesterol in hypercholesterolemia hamsters (Hao et al., 2019).

2.8 Raspberries

2.8.1 Source

Raspberries, a popular soft fruit grown in Eastern Europe belongs to the family *Rosaceae* and genus *Rubus*. It is cultivated all over the world mainly in Europe (European red raspberry), North America (American variety), and

Asia. In the early of 19th century raspberries were grown in the United State of America. Now it is the third highest producer of raspberries. Black raspberries are also grown commercially in America. Purple raspberries are the hybrid of red and black raspberries. There are approximately 250 species of *Rubus* genera fruits however red raspberry (*Rubus idaeus L.*), the North American red raspberry (*R. idaeus*), and the black raspberry (*Rubus occidentalis L.*) are the most important commercial varieties (Wu, et al., 2019).

2.8.2 Composition

Raspberries are considered as healthy superfruits due to their rich source of vitamins C, A, B, B1, B2, E, folic acid, polyphenols, anthocyanins, and minerals. Raspberry as fruits are rich sources of polyphenols such as flavonoids, phenolic acids, ellagitannins, and ellagic acid. Among anthocyanins the major components in red raspberries (*R. idaeus*) are cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside and cyanidin 3-rutinoside (Table 1). Black raspberries (*Rubus occidentalis*) have cyanidin 3-sambubioside, cyanidin 3-rutinoside, and cyanidin 3-xylosylrutinoside. Ellagitannins and their derivative ellagic acid are other important hydrolysable tannins bioactive compounds that are available in fruit pulp and seeds of raspberries. Other biologically active phenolic compounds are quercetin-3- glucuronide and kaempferol-3-glucuronide, flavan-3-ols (catechin), and phenolic and hydroxy acids (gallic, salicyl, caffeic, p-hydroxybenoic, ferulic, p-cumaric, cinnamic and vanillic acids (Määttä-Riihinen, Kamal-Eldin, & Törrönen, 2004; Tian, Giusti, Stoner, & Schwartz, 2006; Mazur, Nes, Wold, Remberg,& Aaby, 2014)).

2.8.3 Health Benefits

Raspberries confers significant antioxidant activities because of their polyphenolic compounds. (Lee, Dossett, & Finn, 2012; Chen, Xin, Zhang, & Yuan, 2013). Raspberries have been known to use traditional drug such as antipyretic and diaphoretic drug. It has been used in managing diabetes and hypertension, and inflammation (Liu,Schwimer, Liu, Greenway,Anthony, & Woltering, 2005; Cheplick, Kwon, Bhowmik, & Shetty, 2007; Medda et al., 2015). Polyphenol compounds of raspberries exerted antiproliferative activities against cervical and colon cancer cells (McDougall, Ross, Ikeji, & Stewart, 2008). The raspberries extract also showed anti-proliferative activities against colon, prostate, breast, and oral cancer cells. (Wedge et al., 2001; Seeram et al., 2006; Ross, McDougall, & Steward, 2007; Peiffer, 2018). Raspberry phenolics exhibited antimicrobial and antiviral activities. A growing evidence was found that berries could modify the composition of the gut microbiota (May, McDermott, Marchesi & Perry, 2020). Recently Tu et al., (2020) investigated that administration of a diet rich in black raspberry changed the composition and diverse functional pathways in the mouse gut microbiome which suggested important role of the gut microbiome in the health effects of black raspberry extract (Tu et al., 2020).

2.9 Acai Berries

2.9.1 Source

Açaí a palm fruit, belongs to family *Arecaceae* and genus *Euterpe.* They are native to South America and grows significantly in the Amazon River delta in Brazil. Two primary species of açaí fruit that are popular are *Euterpe precatoria* (EP) and *Euterpe oleracea* (EO).They are highly consumed by the native people in that region but it has gained international reputation because of their potential nutrition and health benefits. The use of açaí berries by native people to treat malaria related symptoms such as fever, pain, inflammation, and anemia has been seen long time. In the US marketplace commercial products containing açai fruit have been increasing rapidly during recent years (Lee, 2019).

2.9.2 Composition

Açaí fruit is a great source of polyphenolic compounds such as anthocyanins and phenolic acids (Yamaguchi, Pereira, Lamarão, Lima, & Da Veiga-Junior, 2015) (Table 1). However, there were significant differences in the levels of these phytochemicals between the species such as *Euterpe precatoria* (EP) and *Euterpe oleracea* (EO). EP reported to have higher level of polyphenolic compounds compared to EO (Xie et al, 012). The major derivatives of anthocyanins in these berries are cyanidin-3-glucoside and cyanidin-3-rutinoside. In a study by Poulose et al., (2014) reported the level of anthocyanin content in the EP and EO extracts were very significant such as 2035 -66 ng/mg; cyanidin 3-glucoside, 18434 - 575 ng/mg ; cyanidin 3-rutinoside, 113 - 220 ng/mg; delphinidin-glucoside, 538 - 27 ng/mg, for malvidin-glucoside, 84 -8 ng/mg; pelargonidin-glucoside, and 371 - 65 ng/mg for peonidin glucoside. Other phenolic compounds such as catechins, ferulic acid, quercetin, resveratrol, and vanillic acid were also greatly varied between the two species.

2.9.3 Health Benefits

Due to the presence of various polyphenolic composition, acaí berries exhibit important health benefits. Various

cell and animal model studies indicated that açaí extracts showed antioxidant, anti-inflammatory, anti-atherosclerotic, anti-aging, analgesic, and neuromodulatory properties.

Through antioxidant and anti-inflammatory activities acai berry extracts reduced the risk of atherosclerosis (Mertens-Talcott et al., 2008). Moreover, Xie et al. (2012) proposed that anti-inflammatory activities were attributed to the flavone velutin (Xie, et al., 2012). *In-vivo* and *in-vitro* cell and animal model study confirmed that extracts of acai fruits reduce oxidative stress and neuroinflammation via inhibition of activities and expression of nitrous oxide synthase (iNOS), cyclooxygenase-2 (COX-2), p38 mitogen-activated protein kinase (p38- MAPK), tumor necrosis factor-α (TNF-α), and nuclear factor κB (NF-κB) (Poulose et al., 2012). Extract of açaí fruit pulp specially from EO protected from neurotoxicity induced by lipopolysaccharide in mouse brain (Noratto, Angel-morales, Talcott, & Mertenstalcott, 2011; Poulose et al., 2012). In addition to the *in vivo* and *in vitro* antioxidant and anticancer activities it was reported that Açaí juice from EO exhibited neuroprotective, anticonvulsant, and anti-seizure properties (Souza-Monteiro et al., 2015). Ferriera, et al., (2019) investigated potential use of acaí polyphenols as novel antimalarial compounds *in vitro* and *in vivo* and indicated its potential effects of proteostasis as major molecular target (Ferriera, et al., 2019). Magalhães et al., (2020) demonstrated the protective effect of açaí pulp components on intestinal damage in 5-fluorouracil-induced Mucositis, as well as the ability to control the response to oxidative stress, in order to mobilize defense pathways and promote tissue repair (Magalhães et al., 2020). Recently de Liz et al., (2020) evaluated the effects of moderate-term açaí juice intake on fasting glucose, lipid profile, and oxidative stress biomarkers in healthy subject by assigning 200 mL/day for four weeks and collected blood before and after consumption. They found that there were increased the concentrations of HDLC (high- density lipoprotein cholesterol) by 7.7%, TAC (total antioxidant capacity) by 66.7%, antioxidant enzyme activities catalase by 275.1%, and glutatathone peroxidase activity by 15.3% (de Liz et al., 2020).

2.10 Maqui Berries

2.10.1 Source

Maqui berry (*Aristotelia chilensis*), belongs to the family *Elaeocarpaceae*. This purple berry is native to Chile (Aristotelia chilensis) is one of the emerging Chilean superfruit with high nutraceutical value. It is consumed as fresh and dried fruits or also used to make tea, jam, cakes, drink, juice, alcoholic beverages.

2.10.2 Composition

Maqui berry are one of the richest sources of polyphenol compounds. The total phenol content of maqui berry is reported to be much higher than even superfruits blue berries (97 μmol GAE g^{-1} FW and 17 μmol GAE g^{-1} FW respectively) (Ruiz et al., 2010). Major phenolic compounds in Maqui berries are phenolic acids and flavonoids that includes flavonols, flavanols, and anthocyanins (Table 1). Among polyphenols maqui berries have highest level of anthocyanins. The major anthocyanins are 3-glucosides, 3,5-diglucosides, 3-sambubiosides and 3-sambubioside-5-glucosides of cyanidin and delphinidin (delphinidin 3-sambubioside-5-glucoside). Other flavonoids compounds are quercetin and its derivatives such as dimethoxy-quercetin, quercetin-3-rutinoside, quercetin-3-galactoside, dimethoxy-quercetinand ellagic acid.

2.10.3 Health Benefits

Maqui berry is reported to exhibit high antioxidant activities. The ORAC values of maqui was found to be 37,174 μmol Trolox per 100 g of dry weight which was much higher than in commercial berries such as raspberries, blueberries and blackberries cultivated in Chile (Speisky, López Alarcón, Gómez, Fuentes, & Sandoval Acuña, 2012). Bastías-Montes (2020) et al., also recently showed that seed oil from Maqui berry and their tocols (α, β, γ, δ-tocopherols, tocotrienols, and β-sitosterol) promoted for clinical investigation due to their high antioxidative and antiobesity potential against DPPH, HORAC (Hydroxyl Radical Antioxidant Capacity), ORAC (Oxygen Radical Absorbance Capacity), FRAP (ferric reducing antioxidant power), Lipid-peroxidation (TBARS), α-amylase, α-glucosidase, and pancreatic lipase (Bastías-Montes et al., 2020). The purified delphinidin extract maqui berry helped in the generation of nitrogen oxide (NO) in endothelial cells, decreased platelet adhesion, and possessed anti-inflammatory effects. Miranda-Rottmann, et al., (2002) reported that maqui berry extracts could prevent the oxidation of low-density lipoproteins and protected the cultures of human endothelial cells (Miranda-Rottmann, Aspillaga, Pérez, Vasquez, Martinez, & Leighton, 2002). Maqui berries are used as dietary management in patients with respiratory disorders as anthocyanin maqui extract could normalize H_2O_2 and IL-6 concentrations in exhaled breath condensates (EBC) by asymptomatic smokers (Vergara, Ávila, Escobar, Carrasco-Pozo,, Sánchez, & Gotteland,2015). Recently Zhou et al., (2019) reported that ethyl acetate fraction from maqui berry crude extract was rich in phenols and exhibited strong antioxidant and anti-inflammatory activities. They suggested that there was a possible prevention of cognitive damage due to the

antioxidant activity of the maqui berry (Zhou et al., 2019). In a study with male rat brain exposed to ozone and treatment with extract of Maqui berry it was found that maqui berry extracts improved memory and decreased oxidative stress (Bribiesca-Cruz, Moreno, García-Viguera, Gallardo, Segura-Uribe, Pinto-Almazán, & Guerra-Araiza, 2019).

2.11 Elderberries

2.11.1 Source

Elderberry (*Sambucus nigra)* is one of the richest sources of anthocyanins and are used as great source for production of antioxidants, colorants, and bioactive compounds industrially. Traditionally they have been used as medicinal components and food ingredients in fruits, jams, and juices. They are also more frequently used in the manufacture of various types of liqueurs.

2.11.2 Composition

Nutritional composition analysis reported that elderberry is a good source of nutrients like protein, amino acids, dietary fibres, vitamin B, A, and C phytochemicals. Some elderberries have higher level of organic acid and lower level of sugars which is important to industrially processing foods. More significantly elderberry is one of the richest sources of bioactive compounds like flavonols, flavanols, phenolic acids, proanthocyanidins, and anthocyanins (Table 1). Elderberries have been reported to have high level of anthocyanins containing total anthocyanin levels upto 1816 mg/100g FW. Major anthocyanins in elder berries are cyanidin-3-O-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-O-sambubioside, yanidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-sambubioside, pelargonidin 3-glucoside, pelargonidin 3-sambubioside, and delfinidine-3-rutinoside. However, their levels vary with different cultivars. Some other anthocyanins are also present in trace amounts. Major flavonols in elderberries were derivatives of quercetin, kaempferol and isorhamnetin. In the quercetin group quercetin 3-rutinoside and quercetin 3-glucoside were found to be in significant level. Among phenolic acids chlorogenic, crypto-chlorogenic and neochlorogenic acids were identified as major while small amounts of ellagic acids were also available in elderberry fruits. Proanthocyaninidins with monomers, dimers, and trimers, and tetramars have been found in elderberries (Veberic, Jakopic, Stampar, & Schmitzer, 2009; Mikulic-Petkovsek, et al., 2014; Sidor, & Gramza-Michałowska, 2015; Młynarczyk, Walkowiak-Tomczak, & Łysiak, 2018;).

2.11.3 Health benefits

Elderberry has been used as folk medicine for the treatment of common cold, fevers, allergies, and ailments. Several reports demonstrated that elderberries are associated with antioxidant, anti-inflammatory, antibacterial, antiviral, and inflammation properties and various health beneficial properties (Sidor & Gramza-Michałowska, 2015; Porter & Bode, 2017; Olejnik, et al., 2015). Antioxidant activities of elderberries and its extracts were confirmed by *in vitro* antiradical activity assays viz., DPPH, ABTS, hydroxyl, and peroxyl. However, the potency of antioxidant activities depended on the assay, method of extraction bioactive compounds as well as type of elderberry cultivars. In some studies, it showed a less activities than choke berries and black berries and whereas in some other studies it showed higher than other berries (Viskelis, Rubinskiene˙, Bobinaite˙, & Dambrauskiene, 2010; Wu et al. (2004)). Wu et al. (2004) investigated ability of elderberry extract to scavenge the peroxyl radical (ROO•) in the ORAC assay and reported upto 5783 μmol TE/g extract which was higher than the activity of other extract of berries in the respective assay (Wu, Gu, Prior, & McKay,2004). *In vivo* studies showed that an enhanced plasma and serum antioxidant activity was observed after consumption of elderberry (Netzel et al. (2005).

Several studies indicated the antidiabetic properties of elderberry extract. Administration of elderberry extract to diabetic rats helped to maintain glycemic index and reduced the increase in glycemia (Badescu, Badulescu, Badescu, & Ciocoiu, 2012). Bhattacharya et al., (2013) reported the possible role of elderberry in the prevention and treatment of diabetes via the increasing in the secretion of insulin (Bhattacharya et al., 2013). Ho et al., (2017 a, b) reported that elderberry extracts showed high stimulation of glucose uptake in human liver cells and human skeletal muscle cells and inhibitory effect towards carbohydrate hydrolyzing enzymes after treatment with elderberry extracts (Ho, Nguyen, Kase,Tadesse, Barsett, & Wangensteen, 2017). *In vivo* studies with STZ-induced diabetic rat fed with high fat diet Salvador et al., (2017) found that polar extract of elderberry modulated glucose metabolism by correcting hyperglycemia and in other way the lipophilic extract lowered insulin secretion (Salvador et al., 2017). Elderberry extract reported to boost immune system (Badescu, Badulescu, Badescu, Ciocoiu, 2015). Anti-inflammatory properties by elderberry extracts were evident from the findings that elderberry stimulated the production of proinflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α (tumour necrosis factor) as well as anti-inflammatory cytokine IL-10 (Barak, Birkenfeld, Halperin, & Kalickman,

2002). Several studies indicated elderberry extract for antimicrobial and antiviral activity against human pathogenic bacteria as well as influenza viruses (Krawitz, Mraheil, Stein, Imirzalioglu, Domann, Pleschka, &Hain, 2011). Elderberry flower extract inhibited the influenza A virus (H1N1)-induced Madin–Darby canine kidney (MDCK) cell infection (Roschek, Fink, McMichael, Li, & Alberte, 2009). Recently it was reported that *Sambucus Formosana* Nakai stem ethanol extract displayed strong anti-HCoV-NL63 related to respiratory tract illnesses including runny nose, cough, bronchiolitis, and pneumonia (Weng et al. 2019). A significant study demonstrated the anticancer properties of elderberries including European and American elderberry fruits which demonstrated chemopreventive potential through strong induction of quinone reductase and inhibition of cyclooxygenase-2 (Thole et al, 2007).

3. Conclusion

A wide spectrum of *in vitro* and *in vivo*, and human studies has proven the berries antioxidant status and potential health benefits including cardiovascular, neuroprotective, anticarcinogenic potential, and antidiabetic properties. However, the bioavailability of polyphenolic compounds appears to be different with their structure, composition, and diet sources. Abundancy of polyphenols may not correlate strongly with the bioavailability. A thorough knowledge of the bioavailability of the series of polyphenolic compounds will help in promoting healthy choices for maximum health benefits. Further studies in profiling bioavailability and medicinal value are needed for potential application.

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The Potential Effect of Aqueous Extract of *Detarium microcarpum* Bark on Certain Metabolic Disorders Associated with an Atherogenic Diet in Rats

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Abstract

Atherosclerosis is the leading cause of the cardiovascular disease (CVD). This study aimed to evaluate the effect of the aqueous extract of *Detarium microcarpum* on metabolic disorders in rats fed with an atherogenic diet. The extract at two doses (200 mg/kg and 400 mg/kg) was co-administered in rats with an atherogenic diet. After 28 days, rats were sacrificed, blood collected in ethylene diamine tetraacetic acid (EDTA) tubes for plasma preparation, and the heart used for homogenate preparation. These plasma and heart homogenates were used to assess lipid profile, cardiac function (NO, ASAT), and hepatic function (ALAT, ASAT, and totals proteins). The results obtained showed that treatment (200 mg/kg and 400 mg/kg extract) led to a significant decrease in TG, VLDL-c, LDL-c, HDL-c, and non-HDL-c compared to untreated rats (positive control) ($p < 0.001$). Similarly, the cardiovascular risk index (IR, CRR, AC and AIP), were significantly low in the treated groups compared to untreated groups ($p < 0.001$). Meanwhile, the CPI was higher in threated groups ($p < 0.001$). The percentage of vascular protection in rats receiving the extract was higher compared to rats treated with atorvastatin. The evaluation of cardiac function showed high levels of NO and ASAT in the heart of rats treated with the extract ($p < 0.05$). The plasma activity of ASAT and ALAT was low in the groups treated with the extract ($p < 0.05$). A low plasma level of total protein was also observed in the same groups ($p < 0.001$). Therefore, the aqueous bark extract of *D. microcarpum* administered orally showed anti-atherogenic, cardioprotective, and hepatoprotective potential.

Keywords: Anti atherogenic, atherogenic diet, cardioprotective, cardiovascular risk, *Detarium microcarpum*, and hepatoprotective

1. Introduction

Cardiovascular disease (CVD) is a category of diseases that involves the heart or blood vessels such as coronary heart disease, myocardial infarction, and angina pectoris, causing deaths in both developed and developing countries (Shafiee-Nick *et al.,* 2017; WHO, 2018). The development and progression of CVD are associated with several risk factors such as dyslipidemia or dyslipoproteinemia, hypertension, hyperglycemia, insulin resistance, obesity, and atherosclerosis (Brunzell *et al.,* 2008). Most cases of CVD complications result from atherosclerosis (Habauzit & Morand, 2012).

Atherosclerosis, which develops mainly in the arteries, is strongly associated with dyslipidemia characterized by high plasma levels of VLDL-c, LDL-c, total cholesterol (TC), non-HDL-c, triglycerides (TG), and low levels of HDL-c. Non-HDL-c represents the major lipoprotein group linked with a higher risk of cardiovascular diseases. Indeed, the increase in plasma cholesterol levels leads to a change in the impermeability of arterial endothelial cells, thus allowing lipids, particularly LDL-c particles, to migrate into the arterial wall (Al-Qahtany *et al.,* 2018). Once in the subendothelial space, these small LDL-c particles will be oxidized causing the endothelial cells to express the adhesion molecules ICAM-1, VCAM-1, E, and P-selectin (Prasad *et al.,* 2014). These adhesion molecules expressed on the endothelial surface will recruit circulating monocytes that will differentiate into macrophages. These macrophages, by expression of the receptors A, B1, CD36, CD68 will massively accumulate cholesterol (Bergheanu, Bodde & Jukema, 2017) and transform into foamy cells. The atherogenic process will continue with a set of vascular modifications. These changes include fat streaks, chronic

inflammation, pathological thickening of the intima, the appearance of fibrous plaques vulnerable to rupture, thrombosis, or stenosis (Aziz & Yadav, 2016). The initiation and evolution of the atherosclerotic process are strongly associated with oxidative stress which is characterized by an increase in the production of reactive oxygen species (ROS). These ROS cause a narrowing of the arterial wall (vasoconstriction) at the vascular level by decreasing the bioavailability of nitric oxide (NO) and the loss of intracellular tissue components (liver, hearts, kidneys, brain) as well as their function by altering their membrane architecture (lipoperoxidation). This damage caused by ROS is due to their toxicity on lipids, proteins, and nucleic acids which are major components of cells (Valko, Rhodes, Moncol, Izakovic & Mazur, 2006). Given the complexity of the mechanisms involved in atherogenesis and the associated complications, the search for new compounds with multiple pathways of action is becoming a necessity.

Polyphenols, especially flavonoids, are compounds derived from plants with variable biological properties. They have beneficial effects on vascular health (inhibition of LDL-c oxidation), dyslipidemia (decrease in LDL-c, TC, TG and increase in HDL-c), inflammation (decrease in pro-inflammatory cytokine production), diabetes (hypoglycemic and insulin sensitizer effect), antioxidant, endothelial dysfunction (inhibits the expression of adhesion molecules, promotes vasodilatation) (Morand & Milenkovic, 2014; Amiot, Riva & Vinet, 2016). For several decades, low-income populations have been using medicinal plants for the management of several pathologies. *Detarium microcarpum* or small sweet detar, a woody plant of the Fabaceae family (Sani, Agunu, Danmalam & Ibrahim, 2014), is one of these plants. In traditional medicine, the decoction or maceration of the leaves, roots, fruits, and bark of this plant is used to treat constipation, meningitis, arthritis, tuberculosis, hypertension, and rheumatism (Sani, Agunu, Danmalam & Ibrahim, 2014; Oibiokpa, Godwin, Abubakar & Kudirat, 2014). The decoction of the stem bark is traditionally used in the treatment of hemorrhoids, blennorrhoea, simple and sanguinolent diarrhea (Adama, 1997). The studies carried out by Hama, Ouedraogo & Adama in 2019, on the methanolic extract of various organs of this plant revealed that the stem bark contained more polyphenols and had a better antioxidant activity *in vitro*. View its antioxidant activity correlated with the polyphenols they contain; this study was carried out to evaluate some biological activities of *Detarium microcarpum* stem bark extract in rats fed with an atherogenic a diet. The effect on lipid profile, atherogenic index, markers of cardiac and hepatic function was evaluated.

2. Methodology

2.1 Reagents

Analytical grade reagents used were purchased from Sigma Aldrich (USA). They were: Folin-ciocalteu reagent, catechin, ethanol, aluminum chloride, potassium acetate, quercetin, sodium chloride, sulfanilamide, naphthyl ethylene diamine dichloride, bovine serum albumin, orthophosphoric acid, L-alanine, L-aspartate, alpha-ketoglutarate, mono, and disodium phosphate.

2.2 Plant Material

The stem bark of *D. microcarpum* was collected in Kousseri (Far North region of Cameroon) and identified at Cameroon national herbarium as No. 49834. These barks were then dried at ambient temperature in a dark room, ground to a powder, and used to prepare the aqueous extract.

2.3 Extraction

The aqueous extract was prepared by decoction. Briefly, 500 g of powder was dissolved in 4 L of distilled water. The extraction ratio was 1/8 (w/v). The mixture was homogenized and then brought to a boil for 45 minutes. The decoction from this boiling was cooled and filtered using Whatman paper No. 1 (Whatman Int. Ltd., Maidstone, U.K). The filtrate obtained was evaporated in the oven at 60°C for 72 hours. The extract obtained was stored at 25°C in the laboratory.

2.4 Quantitative Phytochemical Analysis of the Extract

2.4.1 Determination of Total Polyphenol Content

The amount of total polyphenols in the extract was determined using the Singleton and Rossi method (1965). Briefly, 30 µL of extract (1 mg/mL) was added to 1 mL of the 0.2 N Folin Ciocalteu reagent. A Spectrophotometer (Genesys 20) was used to measure the absorbance at 750 nm of the complex formed between Folin and polyphenols after 30 minutes of incubation at room temperature against the blank. Curcumin (0- 1000 mg/mL) was used as standard and the result obtained was expressed as equivalent mg of curcumin per g of dry matter.

2.4.2 Determination of Total Flavonoid Content

The presence of flavonoids in the extract was assessed according to the procedure described by Aiyegoro & Okoh (2010). For a 1 mL extract (5 mg/mL), 1 mL 10 % aluminum chloride, 1 mL potassium acetate (1 M) and 5.6 mL distilled H_2O was added and mixed. The mixture was then incubated at room temperature for 30 minutes. A spectrophotometer (Genesys 20) was used to measure the absorbance at 420 nm against the blank. The standard was catechin (0 - 40 µg/mL) and the flavonoid concentration was expressed in µg catechin per g of dry material.

2.5 Experimentation

2.5.1 Food Composition

Table 1 shows the composition of the diets used in this study.

Table 1. Dietary composition of the normal and atherogenic diet (Ble-Castillo et al., 2012, with some changes)

Ingredient	Composition in g per 100 g of food	
	Normal diet	**Atherogenic diet (DESF)**
Milk	10	10
Fish meal	10	10
Refined palm oil	10	12.5
Margarine	/	12.5
Sucrose	/	18
Depulped cornflour	35	18
Wheat flour	20	15
Salt	1.5	1.5
Bone meal	2.5	2.5
Polyvitamins	1	1
Fibers (cellulose)	1	1
Distilled water	10	/

DESF: a diet enriched with sucrose and fat

The changes made on the composition of the atherogenic diet were a reduction in the proportion of saturated fats, the addition of unsaturated fats, and an increase in the amount of sucrose.

2.5.2 Animals

Animals were handled according to the European Union Animal Care (CEE Council 86/609) guideline adopted by the Cameroon National Ethics Committee. Adult male *Wistar* albino rats of the age range 12 - 16 weeks and 200-340 g body weight were maintained at their housing conditions with relative humidity (50%) at 25°C temperature. They were obtained from the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the University of Yaounde I and were acclimated for 7 days before used. The animals had free access to water, a normal diet, and were maintained in a 12-hour light/dark cycle.

2.5.3 Experimental Design

A total of Twenty-five rats were used. Five of them (210-240 g) constituted the normal group (normal control) and received a normal diet (ND). Twenty (330-340 g) received an atherogenic diet supplemented with fructose at 10 % (g/v). They were divided into four groups of 5 rats as follows: untreated group (positive control): DESG + distilled water; treated group 1: atherogenic diet (DESF) + 200 mg/kg extract; treated group 2: atherogenic diet (DESF) + 400 mg/kg extract and standard group: atherogenic diet (DESF) + atorvastatin at 5 mg/kg. The administration of the extract and atorvastatin was done daily by gavage using an esophageal probe. The experiment lasted 28 days during which the animals had access to water and the diet *ad libitum*. Their weight was taken each week, which allowed the calculation of the weight variation according to the following formula:

$$\text{Weight variation (\%)} = \frac{(\text{Final weight} - \text{Initial weight})\ \text{g}}{\text{Final weight (g)}} \text{ X } 100$$

2.5.4 Preparation of Samples

At the end of the experiment, the rats were sacrificed by cervical dislocation after slight ether anesthesia. Blood was immediately collected in the EDTA tubes and centrifuged at 3000 rpm for 10 minutes to obtain the plasma. The heart was then collected for the preparation of the 10% (w/v) homogenate in NaCl (0.9%). Succinctly, the

organ after sampling was rinsed in sodium chloride (0.9%) and then wrung out on filter paper and crushed. The corresponding volume of NaCl was added and centrifuged at 900 g for 10 minutes and the supernatant was recovered. Both samples (supernatant and plasma) were aliquot stored at -20°C for biochemical analyses.

2.6 Determination of Lipid Profile Parameters

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were assessed in plasma using standard chronolab brand assay kits. Low-density lipoprotein cholesterol (LDL-c) and very-low-density lipoprotein cholesterol (VLDL-c) were determined using the formula of Friedewald, Levy & Friedrickson (1972).

$$\mathrm{LDL-c\ (mg/dL)} = \mathrm{TC} - \left(\mathrm{HDL-c} + \frac{\mathrm{TG}}{5}\right)$$

$$\mathrm{VLDL-c\ (mg/dL)} = \frac{\mathrm{TG}}{5}$$

The concentration of non-HDL cholesterol was calculated using the formula established by Brunzell *et al.,* 2008):

$$\text{non-HDL-c (mg/dL)} = [\mathrm{TC}] - [\mathrm{HDL-c}]$$

2.7 Calculation of Insulin Resistance and Atherogenic Indices

Lipid profile parameters were used to calculate insulin resistance, atherogenic indices cardioprotective index, and vascular protection. Insulin resistance (IR) was assessed using the following formula:

$$\mathrm{IR} = \frac{[\mathrm{TG}]}{[\mathrm{HDL-c}]}$$

The atherogenic index of plasma (AIP), atherogenic coefficient (AC), cardiac risk ratio (CRR), and cardioprotective index (CPI) were calculated as follows.

$$\mathrm{AIP} = \mathrm{Log}\left(\frac{[\mathrm{TG}]}{[HDL-c]}\right) \qquad \text{(Althunibat } \textit{et al.;}\text{ 2019) ;}$$

$$\mathrm{AC} = \frac{([\mathrm{TC}]-[\mathrm{HDL-c}])}{[\mathrm{HDL-c}]} \qquad \text{(Althunibat } \textit{et al.;}\text{ 2019) ;}$$

$$\mathrm{CRR1} = \frac{[\mathrm{TC}]}{[\mathrm{HDL-c}]} \quad ; \quad \mathrm{CRR2} = \frac{[\mathrm{LDL-c}]}{[\mathrm{HDL-c}]} \qquad \text{(Ikewuchi \& Ikewuchi, 2009a, b);}$$

$$\mathrm{CPI} = \frac{[\mathrm{HDL-c}]}{[\mathrm{LDL-c}]} \qquad \text{(Oršoli´}\textit{et al.,}\text{ 2014).}$$

The formula below was used to calculate vascular protection.

$$\text{Vascular protection (\%)} = \frac{\text{AIP positive control} - \text{AIP treated group}}{\text{AIP positive control}} \text{ x } 100$$

2.8 Determination of Cardiac Function Parameters

Plasma and cardiac nitric oxide (NO) was evaluated based on the diazotization reaction (Griess, 1879; Chaea, Lee, Kim & Bae, 2004) and the activity of cardiac aspartate aminotransferase (ASAT) by that of Reitman & Frankel (1957).

2.9 Determination of Hepatic Function Parameters

Plasma activity of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) was evaluated by the colorimetric method described by Reitman & Frankel (1957). The determination of total plasma protein levels was carried out using the method of Lowry, Rosebrough, Farr & Randall (1951).

3. Statistical Analysis

The results obtained were expressed as mean ± standard error on the mean. The values were analyzed with the statistical package for social science (SPSS) software version 20.0. The ANOVA test was used for the descriptive analysis and the comparison between the groups was performed by the post hoc LSD test. The difference in significance was noted at $p < 0.05$ and $p < 0.001$. The excel spreadsheet was been used to plot the graph.

4. Results

4.1 The Polyphenol Content of the Extract

The total polyphenol and flavonoid content of aqueous bark extract of *D. microcarpum* is shown in table 2.

Table 2. Total polyphenol and flavonoid content of the aqueous bark extract of *D. microcarpum*

Detarium microcarpum	**Aqueous bark extract**
Total polyphenols (mg ECu/g of DM)	777.50 ± 9.14
Total flavonoids (µg d'EC/g of DM)	53.62 ± 1.63

ECu: Curcumin equivalent, EC: Catechin equivalent; DM: dry material.

4.2 Effect of Oral Administration of Extract on the Body Weight of Rats

An increase in weight was observed in both control groups (Figure 1). However, administration of aqueous bark extract at doses of 200 mg/kg/day and 400 mg/kg/day and atorvastatin at 5 mg/kg/day respectively limited weight gain in the different treated groups. This effect was more pronounced in the group treated with the 200 mg/kg/day extract.

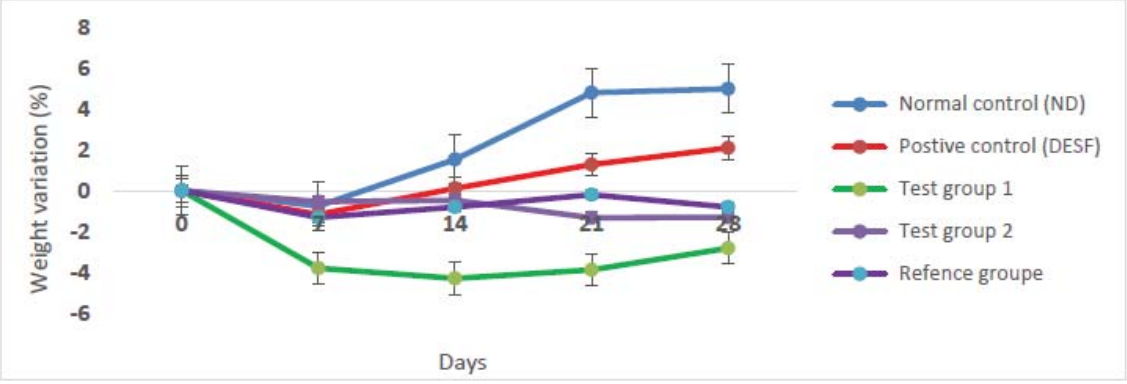

Figure 1. Influence of administration of the aqueous extract of *Detarium microcarpum* bark on the weight variation of experimental rats

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin

4.3 Effect of Detarium microcarpum Extract on Plasma Lipid Profile

An increase was observed in TG, TC, VLDL-c, non-HDL-c and a decrease in HDL-c in the untreated group (positive control) compared to the normal control group ($p < 0.05$) (table 3). Oral administration of the extract at doses of 200 mg/kg body weight and 400 mg/kg body weight limited the increase in TG, TC, VLDL-c, LDL-c, and non-HDL-c compared to the untreated group ($p < 0.001$). The extract at both doses resulted in an increase in HDL-c ($p < 0.001$).

Table 3. Influence of extract on plasma levels of triglyceride, total cholesterol, very-low-density lipoproteins cholesterol, low-density lipoproteins-cholesterol, high-density lipoproteins-cholesterol (HDL) and non-HDL-c

	Normal control (ND)	Positive control (DESF)	Treated group 1	Treated group 2	Standard group
TG	31.13 ± 1.40^{a}	129.90 ± 0.40^{b}	$77.61\pm0.60^{c*}$	$85.65\pm0.04^{d*}$	$62.69\pm4.48^{e*}$
TC	65.07 ± 5.30^{a}	92.07 ± 0.25^{b}	91.92 ± 0.36^{b}	92.07 ± 0.60^{b}	65.43 ± 4.48^{da}
VLDL-c	6.23 ± 0.28^{a}	25.98 ± 0.08^{b}	$15.52\pm0.12^{c*}$	$17.13\pm0.01^{cd*}$	$12.53\pm0.90^{e*}$
LDL-c	30.31 ± 4.60^{a}	49.55 ± 0.41^{b}	$31.02\pm0.99^{a*}$	$21.17\pm0.94^{c*}$	$17.53\pm1.31^{d*}$
HDL-c	28.53 ± 0.60^{a}	16.54 ± 0.41^{b}	$45.38\pm0.99^{c*}$	$53.77\pm0.94^{d*}$	$35.37\pm0.91^{e*}$
non-HDL-c	36.75 ± 4.70^{a}	75.53 ± 0.16^{b}	$46.54\pm0.63^{c*}$	$38.30\pm0.34^{d*}$	$30.06\pm3.56^{a*}$

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; TG ; triglyceride ; TC : total cholesterol ; VLDL : very low density lipoproteins-cholesterol ; LDL : low density

lipoproteins-cholesterol ; HDL : high density lipoproteins-cholesterol ; c: choleterol ; the assigned values of the alphabetic letters are significantly different ($p < 0.05$); * represents the difference in significance ($p < 0.001$) between the positive control and the treated groups.

4.4 Effect of the Extract on Insulin Resistance and Atherogenic Risk

Table 4 presents data on the effects of the extract on IR, CRR, AC, AIP, CPI, and vascular protection (%). IR, CRR, AC and AIP were higher in rats of the positive control compared to the normal control groups ($p < 0.001$). CPI was low in positive control than normal control groups ($p < 0.001$). On the other hand, the administration of the extract at two doses and atorvastatin resulted a significant decrease in IR, CRR, AC and AIP, in the treated compared to positive control ($p < 0.001$). While, the CPI was higher in threated groups ($p < 0.001$). The percentage of vascular protection obtained was higher in the group treated with 400 mg/kg extract body weight compared to the group treated with 200 mg/kg extract and 5 mg/kg atorvastatin.

Table 4. Effect of the extract on insulin resistance, atherogenic indices, and vascular protection

	Normal control (ND)	**Positive control (DESF)**	**Treated group 1**	**Treated group 2**	**Standard group**
IR	1.09 ± 0.03^{a}	7.88 ± 0.17^{b}	$1.71\pm0.02^{c*}$	$1.59\pm0.03d^{c*}$	$1.76\pm0.08^{ad*}$
CRR_1	2.27 ± 0.14^{a}	5.58 ± 0.12^{b}	$2.03\pm0.04^{c*}$	$1.70\pm0.03^{a*}$	$1.84\pm0.08^{c*}$
CRR_2	1.06 ± 0.14^{a}	2.99 ± 0.03^{b}	$0.68\pm0.01^{c*}$	0.39 ± 0.01^{dc}	$0.49\pm0.02^{ec*}$
AC	1.27 ± 0.14^{a}	4.58 ± 0.12^{b}	$1.02\pm0.04^{c*}$	0.71 ± 0.03^{d}*	$0.84\pm0.08^{e*}$
AIP	0.04 ± 0.01^{a}	0.90 ± 0.01^{b}	$0.23\pm0.01^{a*}$	$0.20\pm0.01^{a*}$	$0.24\pm0.02^{c*}$
CPI	1.04 ± 0.14^{a}	0.45 ± 0.01^{b}	$1.47\pm0.01^{c*}$	$2.56\pm0.07^{d*}$	$2.06\pm0.10^{e*}$
% protection	-	-	74.44	77.78	73.33

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; CRR : cardiac risk ratio ; AC : atherogenic coefficient; AIP : atherogenic index of plasma ; IR : insulin resistance ; CPI : cardiprotective index ; The assigned values of the alphabetic letters are significantly different ($p < 0.05$) * represents the difference in significance ($p < 0.001$) between the positive control and the treated groups.

4.5 The Ability of the Extract to Improve Cardiac Function

Plasma nitric oxide (NO) in rats in the groups receiving aqueous bark extract of *D. microcarpum* (treated group) at both doses was significantly lower compared to positive control ($p < 0.05$) (Table 5). However, in the heart, NO was higher in the treated groups than in the untreated group. The same observation was made after the evaluation of the activity of plasma aspartate aminotransferase (ASAT). In treated groups, there was a significant increase ($p < 0.05$; $p < 0.001$) in cardiac NO, ASAT and significant decrease ($p < 0.05$; $p < 0.001$) in plasma NO.

Table 5. Effect of the extract on cardiac, plasma nitric oxide levels and cardiac activity of aspartate aminotransferase

	Normal control (ND)	**Positive control (DESF)**	**Treated group 1**	**Treated group 2**	**Standard group**
NO (Cardiac)	42.72 ± 0.93^{a}	33.67 ± 0.20^{b}	$36.15\pm0.64^{c*}$	$38.24\pm0.44^{d*}$	$37.54\pm0.62^{e*}$
NO (Plasma)	32.69 ± 1.94^{a}	83.56 ± 2.68^{b}	45.75 ± 0.70^{c}	$36.94\pm0.46^{ad*}$	$23.95\pm0.34^{e*}$
ASAT (Cardiac)	49.05 ± 0.39^{a}	41.66 ± 0.51^{b}	44.17 ± 0.46^{c}	43.33 ± 0.47^{d}	44.05 ± 0.58^{e}

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; NO : nitric oxide; ASAT: aspartate aminotransferase; the assigned values of the alphabetic letters are significantly different ($p < 0.05$) ; * represents the difference in significance ($p < 0.001$) between the positive control and the treated groups.

4.6 The Ability of Extracts to Improve Liver Function

The results of the plasma assessment of transaminase activity (AST and ALT) and total protein levels are presented in Table 6. In the groups treated with the extract, there was a decrease in total proteins compared to the untreated group ($p < 0.001$). The activity of ALAT and ASAT in the group receiving the extract at 200 mg/kg

body weight was low compared to the untreated group. While the activity of both transaminases was higher in the group treated with 400 mg/kg body weight. In the standard group, these activities were lower compared to the untreated group ($p < 0.001$).

Table 6. Effect of the extract on plasma activity of alanine aminotransferase, aspartate aminotransferase, and total protein levels

	Normal control (ND)	Positive control (DESF)	Treated group 1	Treated group 2	Standard group
ALAT	96.25 ± 2.98^{a}	275.73 ± 17.84^{b}	263.00 ± 7.72^{b}	300.19 ± 40.17^{db}	$54.13\pm5.48^{c*}$
ASAT	80.53 ± 1.91^{a}	139.05 ± 16.05^{b}	133.31 ± 5.21^{cb}	148.70 ± 9.41^{db}	$75.95\pm13.50^{a*}$
Total protein	25.80 ± 2.23^{a}	31.44 ± 0.25^{b}	$21.06\pm0.28^{c*}$	$22.17\pm0.39^{d*}$	29.33 ± 1.30^{ae}

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; ALAT : alanine aminotransferase; ASAT : aspartate aminotransferase ; the assigned values of the alphabetic letters are significantly different ($p < 0.05$) * represents the difference in significance ($p < 0.001$) between the positive control and the treated groups.

5. Discussion

Insulin resistance is at the center of several metabolic disorders. These include obesity, type 2 diabetes, dyslipidemia, and non-alcoholic fatty liver disease, all important risk factors for cardiovascular disease (Kato *et al.,* 2015; Oseini & Sanyal, 2016). The objective of this study was to evaluate the effect of the aqueous extract of *Detarium microcarpum* (*D. microcarpum*) bark on the lipid profile, atherogenicity index, markers of cardiac and hepatic function in rats fed on an atherogenic diet (DESF). Preliminary results on the phenolic compound composition of the extract revealed the presence of total polyphenols and flavonoids (Table 2). The values obtained in this work are different from those obtained by Hama, Ouedraogo & Adama (2019) who worked on the antioxidant activity of méthanolic extracts from various organs of *D. microcarpum*. The difference in solubility of polyphenols in the solvent system (Ghedadba, Bousselsela, Hambaba, Benbia & Mouloud, 2014); the environmental conditions of the plant (Falleh *et al.,* 2008), and the extraction method used to justify the variability observed. The existence of these phenolic compounds would suggest that the extract has hypolipidemia, antioxidant, anti-atherogenic, anti-inflammatory potential and will have the ability to improve endothelial function and limit cardiovascular risk. Thus, the preventive effect of *D. microcarpum extract* on certain metabolic and physiological disorders was investigated for 28 days in Wistar rats. The rats fed on a normal diet had a greater weight variation than those receiving DESF alone (figure 1). It was 5.46% and 2.15% for the normal and untreated groups, respectively. This result shows a low dietary intake of untreated rats that would be justified by a decrease in appetite following a change in diet type. However, a high-calorie diet, although poorly consumed, leads to the development of metabolic disorders associated with chronic diseases. Jin, Yi & Mei (2013), showed that rodents fed a high-fat diet develop insulin resistance, dyslipidemia, and hepatic steatosis. Concomitant administration of DESF supplemented with fructose and extract at doses of 200 mg/kg (treated group 1) and 400 mg/kg (treated group 2) and atorvastatin at 5 mg/kg (reference) limited the weight gain of animals in these groups with respective percentage changes of - 2.81%; - 0.97% and -0.80% compared to the untreated group. The polyphenols content in the extract limit rat weight gain by inhibiting lipid digestion, and/or the absorption of their digestion products leading to an increase in their fecal excretion. Polyphenols such as catechin reduce weight gain by stimulating energy expenditure at the cellular level by increasing lipolytic protein expression or by inhibiting lipogenesis through the suppression of fatty acid synthase expression (Yamashita *et al.,* 2007; Kyung, Myoung, Keunae, & Hwang, 2011).

Dyslipoproteinemias are strongly associated with the development of atherosclerosis and its complications (Mulvihill, Burke & Huff, 2016, Bergheanu, Bodde & Jukema, 2017). Thus, all compounds with lipid-lowering and anti-obesogenic properties limit the occurrence of atherosclerosis and its clinical complications like cardiopathy and hepatopathy. In general, the effect of the extract on plasma lipid levels showed a decreased of TG, TC, LDL-c, non-HDL-c, VLDL-c and increased HDL-c compared to the positive control ($p < 0.001$) (table 3). This lipid-lowering potential (especially low levels of LDL-c and non-HDL-c) of the extract reflects its ability to prevent atherogenic risk in rats and associated vascular and tissue complications. The evaluation of the atherogenic index and the IR was therefore correlated with this ability of the extract to lower plasma lipid concentrations. Significant low ($p < 0.001$) IR, CRR, AC, and AIP values were observed in the groups receiving the extract (Table 4), showing its anti-atherogenic potential. This protective power of extract against

atherogenesis induced by DESF is justified by the achievement of high vascular protection and CPI. The indices (IR, CRR, AC, AIP) are useful for assessing the risk of developing CVD, the more accurate the increase, the higher the risk of CVD (Oršoli *et al.,* 2019). On the other hand, the CPI reflects the cardioprotective potential of a compound. The compound is more effective when the index is higher. The presence of polyphenols/flavonoids in *D. microcarpum* extract gives it the ability to normalize plasma lipids, reduce the onset of coronary heart disease, and the accumulation of liver fat as observed. Middleton, Kandaswami & Theoharides (2000), showed that *citrus* flavonoids lowered LDL and TG cholesterol levels in normolipidemic and hyperlipidemic rats. These phenolic compounds used for the prevention/treatment of cardiovascular diseases and their related complications act at various levels. Notably, the inhibition of lipid-digesting enzymes, stimulation of lecithin acyl cholesterol transferase expression (Okafor, Ezeanyika, Nkwonta & Okonkwo, 2015). Flavonoids inhibit the expression of cholesteryl ester transport protein (CETP) which reduces plasma LDL-c levels (Kuivenhoven *et al.,* 1997). This decrease in plasma LDL-c is associated with increased expression of LDL receptors (LDLR) under the control of sterol regulatory element-binding protein-2 (SREBP-2) (Soutar & Naoumova, 2007; You, Su & Zhou, 2008). LDLR is involved in the lysosomal degradation of LDL-c. Flavonoids also lower plasma cholesterol levels by inhibition of acyl CoA cholesterol acyltransferases (ACATs) resulting in inhibition of intestinal cholesterol absorption and hepatic production of VLDL (Keti, Ketan, Randolph, Arroo, Roberta & Matteo, 2017). Studies of Masao *et al*. (2009); Saranan *et al*. (2009) and Nagendra, Rajasekhar & Raghn, (2017) on pine, *Terminalia arjuna*, *Cinnamomum zeylanicum* barks respectively, have also shown beneficial effects on lowering weight gain, preventing dyslipidemia and reducing atherogenicity. Besides, all these barks have shown a protective effect of the aorta against atherosclerotic lesions.

Endothelial dysfunction associated with the overproduction of reactive oxygen species (ROS) is responsible for the onset of atherosclerosis and tissue complications. The key event is vascular production of ROS such as superoxide anion (O^{2-}), catalyzed by NADPH oxidase (NOX) (Griendling, Sorescu, & Ushio-Fukai, 2000). Our results reveal the beneficial effects of *D. microcarpum* on the improvement of endothelial and hepatic function (Table 5 and 6). A decrease in the bioavailability of cardiac NO is indicative of possible vasoconstriction, while an increase in cardiac NO is beneficial for vascular health. Thus, the high level of NO observed in this study is thought to result from the activation of the protein kinase B (Akt) pathway, which by phosphorylation of the endothelial nitric oxide synthase (eNOS) leads to NO production. Rocha *et al* (2019) showed that the leptin/Akt/eNOS signaling pathway is associated with this NO production. Also, polyphenols by inhibition of NOX limit the production of the O^{2-}, which, by action with NO, forms the highly reactive peroxynitrite ion (ONOO-) (Tong, Du-Ok, Bo-Sup, Kyung-Sik & Seong-Gook, 2015). This compound with a high oxidative power leads to an alteration of the tissue membranes. In plasma, the significant low activity of ALAT, ASAT, low level of NO, and total proteins obtained thus reflects the capacity of the extract to protect the liver and heart from oxidative changes associated with a hyperproduction of ROS. Hama, Ouedraogo & Adama (2019) have shown that *D. microcarpum* stem bark can trap free radicals and complex metals. This activity would, therefore, explain the plant's protective power against tissue damage caused by excessive ROS production, as observed in this work.

6. Conclusion

The results of this study revealed the ability of the stem bark aqueous extract of *D. microcarpum* due to the presence of polyphenols to regulate plasma lipid/lipoprotein levels (TC, TG, LDL-c, VLDL-c, HDL-c, and non-HDL-c) and to limit weight gain in rats. *D. microcarpum* significantly reduced atherogenic risk (AIP, CRR, AC), insulin resistance index (IR), and increased cardioprotection index (CPI). In general, this plant improved cardiac and liver function, hence its cardioprotective and hepatoprotective potential. In addition to this work, further studies need to be carried out on transgenic models of cardiovascular disease and monocytes in culture to elucidate the precise mechanism of action of the extract.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Nutritional Value of *Raphia hookeri* Fruit, Hematological Properties of Its Powder and Aqueous Extract in A Model of Aluminum Chloride Inducing Neurotoxicity by Using Rats

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Abstract

This study was aimed at evaluating the nutritional value of the mesocarp of *Raphia hookeri* (Rh) fruit and the effect of powder and aqueous extract of the fruit on hematological parameters in rats which have undergone neurotoxicity by aluminum chloride. The nutritional content was evaluated with the standard method. Seven groups of six *Wistar* rats were used, neurotoxicity was induced by 4.2 mg/kg of body weight of aluminum chloride 3 times a week intraperitonealy with treatment. Rat treatment was as follow: aqueous extract at 200 and 400 mg/kg body weight, 5 and 10% of formulation, negative, positive and normal groups. The experiment lasted 28 days. The data obtained from the nutritive value showed that Rh mesocarp is a good source of lipids (48.97%), fibers (25.82%), calcium (3183.3mg/100g of dry matter), potassium (1218.3 mg/100g of DM), zinc (0.88 mg/100g of DM) and selenium (8.6 mg/100g of DM). Nevertheless, it contains a little amount of phytic acid and hydrocyanic acid which is acceptable for human consumption. It can then be used in many formulations as a source of these nutrients. Aluminum administration indicates the reduction in food intake, a low weight gain and Hematological alteration in the Positive control group. However, consumption of *Rh* mesocarp indicated an increase in food intake, weight gain and a restoration of hematological parameters to the normal level with the best values in groups that were administered Rh powders (*Rh*5% and *Rh*10%).

Raphia hookeri mesocarp is a rich source of nutrients involved in the prevention of hematological disorder.

Keywords: *Raphia hookeri*, nutritional value, hematological parameters, aluminum chloride

1. Introduction

Aluminum (Al) is a metal which is widely distributed in the environment. It is found in the soil, water and air. This metal is extensively used in daily life as medicine, food additive, vaccines adjuvants, phosphate binders, dialysis, total parenteral nutrition solutions and foods, hence increasing exposure of human being to it (Newairy et al., 2009). The increasing use in preparation and storage of food in aluminum utensils may increase one's exposure to aluminum, particularly when used with salty, acidic or alkaline foods (Lukyanenko et al., 2013). Furthermore, excessive consumption of food baked with aluminum foil may bring a serious health risk (Sahin et al., 1994). Biological effects of aluminum (Al) are linked to the development of many diseases. Many studies have shown that $AlCl_3$ induces toxic effects on the brain, bone, immune and hematopoietic system (Gu et al., 2009). A chronic exposure to aluminum diminishes affinity of transferrin for aluminum due to the fact that the binding sites of transferring available for aluminum are mostly occupied by iron. (Azadeh & Mohammad, 2011).

However, there is certainly growing evidence that several fruits possess interesting pharmacological effects. This is probably the case of *Rh* fruit. It is the largest palm tree in Africa and is commonly found in the tropical

rainforest (Ndon, 2003). Rh is one of the most economically useful plants in Africa. Leaves are used for shelter and stem produces palm sap which is drunk as beverage. The fruits are oblong to ovoid and covered with glossy golden brown scales. They can be eaten once boiled, or used in traditional medicine for its laxative and stomachal properties in the treatment of dysentery and hemorrhage (Leung et al., 1968; Ukwubile et al., 2013). The phytochemical evaluation of the mesocarp and peel of *Raphia* palm fruits showed that the mesocarp is rich in bioactive compounds such as vitamin E, niacin, alkaloid, saponins, flavonoid and phenols (Edem et al., 1984; Ogbuagu, 2008). The effects of this fruit have been shown on exogenous testosterone and eostradiol induced benign prostatic hyperplasia (Mbaka et al., 2013) and it leaf to modulate carbohydrate hydrolyzing enzymes linked to type-2 diabetes (Dada et al., 2017). However, the effect of Rh fruit on hematological disorders is not well known. Bioactive compounds contained in Rh fruit may also have some benefit effects on hematological parameters. This leads us to evaluate nutritional value of *Raphia hookeri* fruit and hematological properties of its powder and aqueous extract in a model of Aluminum chloride inducing neurotoxicity by using rats.

2. Materials and Methods

2.1 Plant Material

Fresh mature *Raphia hookeri* fruits were harvested from swampy field of the West region of Cameroon from April to June 2018.

2.2 Methods

2.2.1 Preparation of Powder, Different Formulation and Extraction of Aqueous Extract of Mesocarp

The fresh fruits were cleaned and peeled. The mesocarp was cut into small pieces using a rustproof knife and dried in an electric air-dried oven at 45°C for 48 hours. The dried mesocarp was grinded in a blender machine (*Moulinex*) and sieved (Diameter of pore: 1 mm) for further analysis. The formulation (Rh5% et Rh10%) was done as follows: Rh 5% was prepared using 95g of food staple + 5g of *Rh* powder and *Rh* 10% was prepared using 90g of food staple + 10g of *Rh* powder. The food staple was composed as follows: corn flour (77.8%), fish flour (20%), bone flour (0.1%), palm olein (1%), vitamins (0.1%), and salt (1%). 20g of Rh powder was extracted into 200ml of water and the mixture was regularly shaken during the extraction. After 48 hours of maceration, the mixture was filtered with a Wathman N°1 filter paper and the filtrates were subjected to evaporation at 45°C under reduced pressure for the removal of the solvent. Dry extracts were stored at 4°C for further analysis.

2.2.2 Evaluation of the Nutritive Value of *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

- **Determination of macronutrient content in *Raphia hookeri* mesocarp, food staple and different formulation**

Determination of moisture content, carbohydrate, protein according to the Association of Official Analitical Chemists (AOAC., 1980), fat content with soxhlet method according to the International Union of Pure and Applied Chemistry (UIPAC) and the determination of fiber content was done according to (Pauwels et al., 2007).

- **Determination of micronutrient content of *Raphia hookeri* mesocarp, food staple and different formulation**

Determination of ash content (AOAC., 1980), phosphorus, Iron, Calcium, Magnesium, Sodium, Potassium, zinc, Manganese and selenium (Pauwels E., Erba P. and Kostkiewicz M. 2007).

- **Determination of anti-nutritional content (hydrocyanic acid and phytic acid) of *Raphia hookeri* mesocarp, food staple and different formulation**

Hydrocyanic acid was determined according to (Makkar et al., 2007) and the phytic acid according to (Edem et al., 1984)

2.3 Experimental Animals: Induction of Neurotoxicity with Aluminum Chloride and Administration of Aqueous Extract and Different Formulation of Rh Mesocarp

Seven groups of six animals each weighing between 200 and 230g were obtained from the Animal Centre Department and allowed to be accustomed to their new environment for 1 week according to the guidelines of the Organization for Economic Cooperation and Development (OECD, 2008). Animals were individually housed under room temperature (25 °C), (12-h light/12-h dark cycle) and had free access to water and diet. $AlCl_3$ (4.2mg/kg/intraperitonial) was administered three time a week to all groups except the vehicle group for 28 successive days (Bhatia et al., 2018). *Rh aqueous extract* (200 and 400 mg/kg body weight) and powder (5% and 10%) were administered daily to different groups of rats orally for 28 consecutive days. Otherwise the negative

control group received 200 mg vitamin C per Kg body weight and positive control group was given only aluminum chloride without any treatment. All experiments were carried out according to the regulations and ethical approval of Experimental Animal Welfare and Ethics Committee of the Institution (No. 2017/056).

2.4 Evaluation of the Amount of Food Intake by the Animals During the Treatment with Aluminum Chloride, Aqueous Extract and Different Formulation of Rh Mesocarp

The amount of food intake was determined everyday by calculating the difference between the amount of food given the previous day and the food left the next day.

2.5 Evaluation of the Weight Gain by Animals during Treatment with Aluminum Chloride, Aqueous Extract of Rh Mesocarp and Different Formulation

The weight gain was evaluated every day and the percentage was determined as follows:

$$\boldsymbol{p}(\%) = \frac{\mathbf{wf-w0}}{\boldsymbol{wf}} * 100$$

P (%) = percentage of weight gain on day x

wf = body weight of animal on day x

wo= initial body weight of animal (after the beginning of treatment)

2.6 Evaluation of the Hematological Parameters of Rats during Treatment with Aluminum Chloride, Aqueous Extract and Different Formulation of Rh Mesocarp

Blood samples collected in Ethylene DiamineTetraacetic Acid (EDTA) tubes were used to perform a blood count using an impedance hematology automated system. The counting principle is based on impedance variation and flow cytometry to determine the size and type of blood cells. Flow cytometry measures on a suspension of particles (cells, bacteria, parasites, beads). Individual characteristic of each particle such as the size, shape and complexity of any component or function that can be detected by a fluorescent compound. The cells in suspension passed one by one in front of one or more laser beams (X) and detectors pick up signals emitted by each cell. Each passage of a cell through the opening then causes an increase in electrical resistance. This increase is translated into electrical pulses whose height is directly proportional to the cell volume. The cells emit naturally or after treatment signals which are analyzed by the computer linked to the cytometer making it possible to establish for the leucocytes the leucocyte formula giving the percentages of the different types of leucocytes. The number of red blood cells is determined by the total number of pulses recorded. The hematocrit level is then deduced by the formula:

Hematocrit = red blood cell × average cell volume / 10

Hemoglobin is assayed by the spectrophotometric method (525 to 550 nm) after lysis of red blood cells (Driscoll et al., 1980).

2.7 Statistical Analysis

Results obtained in the present study were subjected to one-way analysis of variance (ANOVA) with turkey test using Minitab version 17.0 to evaluate the statistical significance of the data. A probability value at $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Results

3.1.1 Nutritive Value of *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

3.1.1.1 Chemical Composition of Macronutrients of *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

Table 1 presents the approximate chemical composition of *Raphia hookeri* mesocarp fruit, the staple food and the different formulations (Rh5% and Rh10%). It shows that there is a significant difference ($P< 0.05$) between the water content of the different samples. The highest content was noted in the staple food (19.52 ± 0.32%), followed by formulations of Rh5% (18.27 ± 1.37%) and Rh10% (17.61 ± 0.71%) respectively. The staple food also had the highest protein content (12.83 ± 0.63%) followed by the different formulations Rh10% (10.27 ± 1.37%) and Rh5% (8.61 ± 0.71%). In terms of lipid content, the highest content was found in *Rh* fruit mesocarp powder (48.97 ± 1.02%), followed by the different formulations: Rh10% (22.98 ± 0.08%) and Rh5% (21.55 ±

2.01%). There is also a significant difference between the carbohydrate content of the different samples with the highest content in the staple food (42.78± 1.20%). The highest fiber content was noted in *Rh* fruit mesocarp powder (25.82 ± 0.27%), followed by the formulation of Rh5% (23.21 ± 1.36%) and then Rh10% (22.41 ± 0.61%). For the ash content, no significant difference (P <0.05) was observed between the different samples. *Rh* fruit mesocarp powder had the highest energy value (585.85 ± 54.80%) followed by the formulation Rh10% (426.02 ± 40.10%).

Table 1. Evaluation of macronutrients present in *Raphia hookeri* mesocarp, food staple and different formulation

Paramètres	*Rh*	Al	*Rh*5%	*Rh*10%
Moisture(%)	9.54 ±3.54^{c}	19.52 ± 0.32^{a}	18.87 ±1.37^{a}	18.06± 0.71^{ab}
Protein(%DM)	1.93 ± 0.51^{b}	12.83 ± 0.63^{a}	12.18 ± 0.423^{a}	11.54 ± 1.27^{a}
Lipid (%DM)	48.97 ± 1.02^{a}	18.75 ± 1.77^{c}	21.55 ±2.01^{bc}	22.98± 0.08^{b}
Carbohydrate(%DM)	32.66 ± 4.74^{b}	42.78 ± 1.20^{a}	40.76 ± 3.15^{a}	40.92± 1.12^{a}
Fiber (%DM)	25.82 ± 0.27^{a}	21.04± 0.11^{b}	22.41± 1.36^{ab}	23.21± 0.61^{a}
Ash(%DM)	6.90 ± 0.26^{a}	6.12 ± 0.26^{a}	6.64 ± 0.36^{a}	6.50± 0.61^{a}
Energy(Kcal/100g)	585.31±54.80^{a}	401.2± 21.40^{b}	415.24 ± 45.4^{b}	426.1± 40.10^{b}

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05; Al : food staple; Rh: powder of *Raphia hookeri mesocarp*; Rh 5%: formulation food with 5% of *Rh mesocarp* powder; Rh 10%: formulation food with 10% of *Rh mesocarp* powder; DM: dry matter

3.1.1.2 Composition of Micronutrients Present in *Raphia hookeri* Mesocarp, Food Staple and Different Formulation

Table 2 shows the mineral composition of the different samples. Calcium content differed significantly in the different samples and ranges between 2433.79 ± 47.62 mg and 3183.30 ± 199.80 to / 100g of DM. The highest value is found in Rh mesocarp powder fruit and the lowest in the staple food. The magnesium content varied significantly between 1102.30 ± 19.80 and 1220.00 ± 60.80 mg / 100g of DM. The highest content was observed in the staple food and the lowest in the Rh mesocarp. The potassium content was also evaluated and the values differed significantly between the different samples ranging between 689.03 ± 4.10and 1218.3 ± 182.70 mg / 100g of DM. The highest value was observed in the fruit powder. The sodium and phosphorus contents varied significantly between 122.00 ± 18.70 and 291.3 ± 7.9 mg/100g of DM and 198.00 ± 8.66 and 226.87 ± 10.79 mg / 100g of DM respectively. Zinc content ranged from 0.88 ± 0.03 to 0.41 ± 0.03mg/100g of DM, the highest value being found in *Rh* fruit and the lowest in the staple food. Iron content ranged from 22.12 ± 2.50to 32.76 ± 2.06 mg / 100g of DM, with the highest value being found in the staple and the lower in fruit powder. Selenium content also varied significantly and ranged from 0.88 ± 0.03 to 2.04 ± 0,11µg/100g.

Table 2. Composition of micronutrients present in Raphia hookeri mesocarp, food staple and different formulation

Component	*Rh*	Al	*Rh*5%	*Rh*10%
Ca (mg/100g)	3183.30± 199.80^{a}	2433.79 ± 47.62^{d}	2630.00 ± 60.80^{c}	2865.43 ±242.50^{b}
Mg (mg/100g)	1102.30± 19.80^{b}	1220.00 ± 60.80^{a}	1197.33 ± 4.62^{a}	1145.33 ± 12.50^{ab}
k (mg/100g)	1218.3 ± 182.70^{a}	689.03 ± 4.10^{b}	695.13 ± 85.28^{b}	701.21± 37.9^{b}
Na (mg/100g)	122.00 ± 18.70^{c}	291.3 ± 37.9^{a}	230.33 ± 15.28^{b}	247.33 ± 4.10^{b}
P (mg/100g)	198.00 ± 8.66^{b}	226.87 ± 10.79^{a}	225.90 ± 10.02^{a}	210.90 ±23.29^{ab}
Zn (mg/100g)	0.88±0.03^{a}	0.41± 0.03^{b}	0.45±0.13^{b}	0.57±0.08^{b}
Fe (mg/100g)	22.12 ± 2.50^{c}	32.76 ± 2.06^{a}	26.40± 5.01^{b}	29.84±1.2^{a}
Se (µg/100g)	8.600 ± 0.27^{a}	2.04±0.11^{b}	2.93± 0.30^{b}	3.51 ± 0.31^{b}

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05. Al : food staple; Rh: powder of *Raphia hookeri mesocarp*; Rh 5%: formulation food with 5% of *Rh mesocarp* powder; Rh 10%: formulation food with 10% of *Rh mesocarp* powder

3.1.1.3 Anti-nutrient Composition of Mesocarp Powder of *Raphia hookeri* Fruit, Different Formulations and Food Staple of Animals

The table 3 below shows the amount of phytic acid and hydrocyanic acid present in mesocarp powder of *Raphia*

hookeri fruit, the different formulations and the food staple. We observed that the amount of phytic acid differ significantly between the mesocarp powder of *Rh* fruit and food staple. The highest value which is 91.81 ± 8.76mg/100g of DM is found in food staple and the lowest value which is 40.16 ± 3.31mg/100g of DM is in mesocarp powder of *Raphia hookeri* fruit. However there is no significant value between the different formulation and the staple food. The amount of hydrocyanic acid was also evaluated and significantly differs between the mesocarp powder of *Rh* fruit, different formulation and food staple. The *Rh mesocarp* fruit present the highest value which is 0.36 ± 0.06 mg/100g of DM and the lowest value which is 0.18 ± 0.03mg/100g of DM was found in staple food.

Table 3. Phytic acid and hydrocyanic acid composition of mesocarp powder of *Raphia hookeri* fruit, the different formulations and the food staple of animals

Sample	%Phytic acid(mg/100g of DM)	% HCN (mg/100g of DM)
Al	91.81 ± 8.76^{a}	0.18 ± 0.03^{c}
Rh	40.16 ± 3.31^{b}	0.36 ± 0.06^{a}
Rh5%	86.24 ± 5.73^{a}	0.22 ± 0.06^{b}
Rh10%	63.12 ± 8.76^{a}	0.26 ± 0.08^{b}

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05; Al : food staple; Rh: powder of *Raphia hookeri mesocarp*; Rh 5%: formulation food with 5% of *Rh mesocarp* powder; Rh 10%: formulation food with 10% of *Rh mesocarp* powder; HCN: hydrocyanic acid

3.1.2 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Amount of Food Intake and the Weight of Animals

3.1.2.1 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Amount of Food Intake

The following figure shows the effect of the aqueous extract of the mesocarp of *Raphia hookeri* fruit and the different formulations on the amount of food intake.

It appears from this figure that the food intake of animals varies during treatment and is between 20 and 32g. During the first two weeks, except for the VC200 and the Rh5% groups, food intake decreased in the other groups. However, during the third week of treatment, there was an increase in food intake in all animals except the VC200 group which remained constant. The highest amount of food intake is observed in ARh200 group follows by Rh5% and Rh10%.

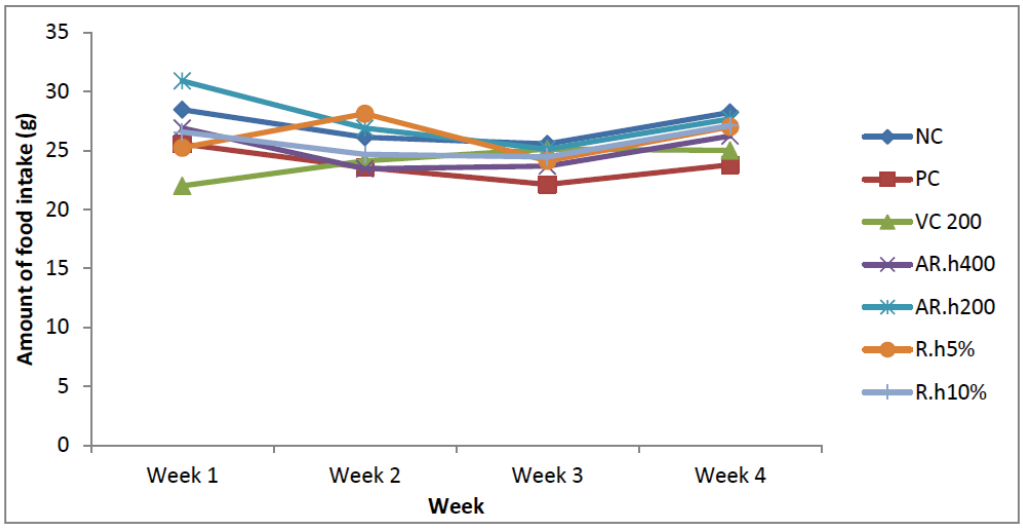

Figure 1. Effect of the aqueous extract and the different formulations of *Raphia hookeri mesocarp* on the amount of food intake

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05; NC: vehicle group received water; PC: induced rats (positive control rats) received water; VC200: induced rats received 200 mg/kg bw of vitamin C(negative control

rats) ; A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder.

3.1.2.2 Effect of the Aqueous Extract and the Different Formulations of the Mesocarp of *Raphia hookeri* on the Weight of Animals

Figure 2 shows the effect of the aqueous extract of *Raphia hookeri* fruit mesocarp and the different formulations on the weight gained of animals. A weight increase between 5 and 10% was observed during the first week except for the ARh200 group, which showed a weight gain less than 5%. This weight gain increased to the end of treatment except in the Rh5% group who presented a constant weight gain from the second week to the fourth week. The highest weight gain (between 20 and 25%) is observed in the ARh400 group and the lowest in the PC group with a weight gain between 10 and 15% throughout the treatment.

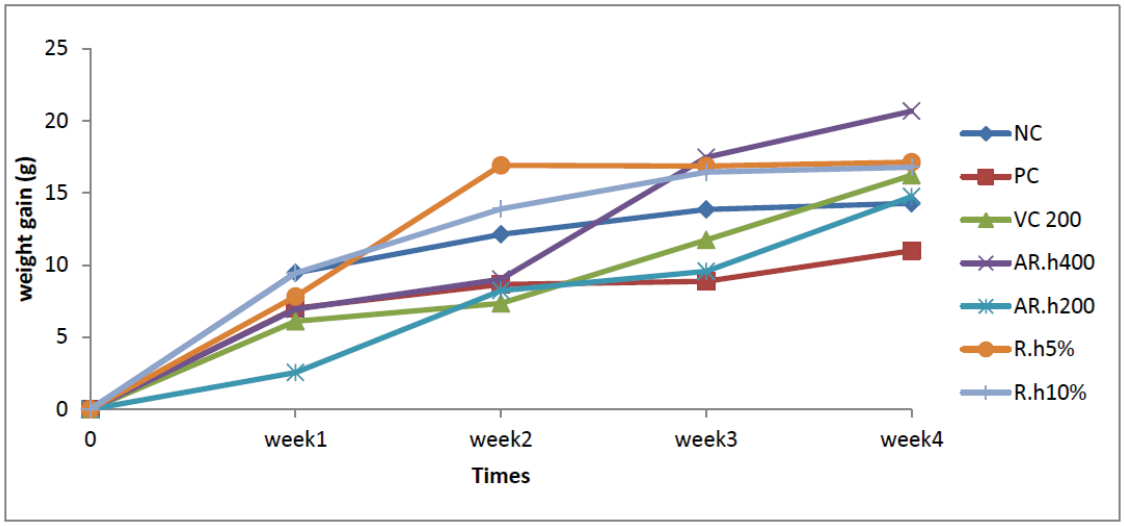

Figure 2. Evolution of weight gain of different group during the treatment

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05; NC: vehicle group received water; PC: induced rats (positive control rats) received water; VC200: induced rats received 200 mg/kg bw of vitamin C (negative control rats); A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder.

3.1.3 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Hematological Parameters

3.1.3.1 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on Red Blood Cell Counts

The following table 4 shows the effect of the different treatments on the count of red blood cells and the figured elements of the blood. It is noted that the induction of stress has led to a significant decrease in red blood cell count and hematocrit in induced group with the value of 5.4210^{6} /µl and 11.65% respectively. On the other hand, the administration of the aqueous extract increased significantly in the red blood cell count and the hematocrit level in the latter. It is also observed that the intake of the various formulations leads to an increase in the red blood cell count in the animals. The high level of RBC, HGB and HCT is observed in groups Rh5% with the value of 8.86 10^{6} /µl, 17.2g/dl and 13.21% respectively.

Table 4. Effect of the aqueous extract and the different formulations of *Raphia hookeri* on the count of red blood cells and the figurate elements of blood

Groups	RBC (10^6 /µl)	HGB (g/ dl)	HCT (%)	MCV (fl)	MCHC (g/dl)
NC	6.96± 0.96^{b}	14.36±0.96^{b}	13.25±4.85^{a}	53.13±2.99^{a}	22.5±0.96^{a}
PC	5.42± 0.99^{c}	14.43±0.99^{b}	11.65±4.59^{b}	54.16±3.5^{a}	22.5±1.22^{a}
VC 200	6.81±0.37^{b}	14.53±0.31^{b}	13.87±1.44^{a}	54.23±0.92^{a}	21.26±0.5^{a}
ARh200	6.46±1.54^{b}	14.5±1.54^{b}	13.43±8.91^{a}	54.63±1.7^{a}	22.3±0.55^{a}
ARh400	6.82±1.55^{b}	14.73±1.55^{b}	12.78±5.72$^{a\,b}$	5 3.7±4.34^{a}	21.7±1.03^{a}
Rh5%	8.86±0.76^{a}	17.2±3.93^{a}	13.21±5.01^{a}	53.6±1.34^{a}	21.8±0.72^{a}
Rh10%	6.39±0.19^{b}	13.6±0.19^{b}	10.92±2.67^{b}	52.96±2.97^{a}	21.23±0.2^{a}

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05 ; NC: vehicle group received water; PC: induced rats (positive control rats) received water; A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder; VC200: induced rats received 200 mg/kg bw of vitamin C (negative control rats); RBC: red blood cell count; HGB:hemoglobin; HCT: hematocrit; MCV: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

3.1.3.2 Effect of the Aqueous Extract and the Different Formulations of *Raphia hookeri* Mesocarp on the Leucocyte Formula

The following table 5 shows the effect of the different treatments on the leucocyte formula. It shows that the induction of stress has led to a significant increase in white blood cell, monocyte and granulocyte levels with the value of 18.0610^{3}/µl, 8.23% and 16.56% respectively. However, the administration of the aqueous extract and the various formulations has led to a significant decrease in these parameters in the animals. The best reduction of WBC is observed with the formulation in group Rh10% with the value of 10.66 10^{3}/µl and the best reduction of monocyte and granulocyte in the formulation 5% with the values of 4.5% and 8.76% respectively.

Table 5. Effect of the aqueous extract and the different formulations of *Raphia hookeri* mesocarp on the leucocyte formula

Groups	WBC(10^3/µl)	LYM (%)	MID(%)	GRAN (%)	PLT (10^3/µl)	MPV (fl)
NC	9.63±1.1^{c}	79.83±6.56^{ab}	6.6±0.86^{ab}	13.16±0.28^{b}	333±23.6^{b}	8.4±0.52^{a}
PC	18.06±1.2^{a}	76.03±8.52^{b}	8.23±2.54^{a}	16.56±0.8^{a}	290.33±8.54^{c}	8.86±0.15^{a}
VC 200	13.62±2.03^{b}	77.53±1.1^{b}	7.66±2.63^{a}	14.8±17.6^{ab}	353.33±69.24^{b}	8.86±1.09^{a}
ARh200	21.8±0.0^{a}	79.9±0.95^{b}	7.03±0.57^{a}	13.16±1.48^{b}	293.66±15.01^{c}	8±0.2^{a}
ARh400	11.8±1.82^{b}	82.53±1^{a}	5.1±0.81^{b}	13.03±0.92^{b}	286.33±78.51^{c}	8.46±0.85^{a}
Rh5%	11.16±0.28^{b}	85.86±1.3^{a}	4.5±0.6^{b}	8.76±1.41^{b}	439±20.78^{a}	9.3±0.97^{a}
Rh10%	10.66±2.02^{bc}	80.96±1.46^{a}	6.56±1.45^{ab}	14.13±0.63^{ab}	412.33±73.32^{a}	8.7±0.34^{a}

The values of this table represent the means ± standard deviation of 6 repetitions and the value which have different letters are signitificaly different at P< 0.05 ; NC: vehicle group received water; PC: induced rats (positive control rats) received water; A.Rh 200: induced rats received 200 mg/kg bw aqueous extract of *Rh* mesocarp; A.Rh 400: induced rats received 400 mg/kg bw aqueous extract of *Rh* mesocarp; Rh 5%: induced rats received formulation food with 5% of *Rh mesocarp* powder; Rh 10%: induced rats received formulation food with 10% of *Rh mesocarp* powder; VC200: induced rats received 200 mg/kg bw of vitamin C (negative control rats);WBC: white blood cell count ;LYM: lymphocyte; MID: monocyte; GRAN: granulocyte; PLT: platelet count

3.2 Discussion

The characterization of different samples was done. Water content is a parameter used in the treatment and organoleptic properties of foods. The water contents of our samples were all low and the lowest water content noted in *Rh mesocarp* (9.54 ± 3.54). This would justify the fact that this product can be preserved for a long time without enzymatic browning process being initiated. These results are in agreement with those of (Esiegbuya et

al., 2013) which showed that the mesocarp of the *Rh* had a water content of 9.87%.

Proteins play a major role in the body as they build up all the structures of an organism. They are mostly contained in foods from animal sources but also in vegetables. The highest protein content was observed in the staple food. This is surely due to the fact that the staple food contained the highest quantity of fish, meat and soya beans compared to other food samples. The mesocarp of *Rh* showed the lowest protein content (1.93 ± 0.51%). This value is lower than that obtained by (Leung et al., 1968), who found that the core of *Rh* fruit had a protein content of 8% and slightly lower than that of (Akpabio et al., 2012) who found a protein content of 2.5% on the exudates of *Rh*. This difference can be attributed to several factors such as the genetic constitution of the plant, the climatic conditions, the stage of maturity of the fruits and the part of the plant studied (Morris et al., 2004).

Lipids are one of the essential components of our diet. This study revealed that the *Rh* fruit had a lipid content of 48.97%.This result does not corroborate with that of (Esiegbuya et al., 2013), who found a lipid content of 8% in fresh *Rh* mesocarp and of 20.88% in the dry one. The difference observed may be due to fruit maturity, weather conditions under which the plant grew up and the fruit moisture content (dry or fresh) (Morris et al., 2004).

Carbohydrates content was also determined as they represent the main macronutrient in human diet with an energy requirement ranging between 50 and 55% a day. The highest carbohydrates content was observed in the food staple. This may be explained by the fact that the food staple highly contained corn flour which is known as a very good source of carbohydrate. As the results show, the *Rh* mesocarp presented the lowest carbohydrate content. It can therefore be used for this beneficial property in the treatment of metabolic disorders due to high carbohydrate intake including diabetes. The studies of (Dada et al., 2017) have shown that phenolic compounds of *Rh* leaf extract have a modulatory effect on starch hydrolysis by activating enzymes like α-amylase and α-glucosidase. *Rh* fruit presented the highest energy value (485.74Kcal/100g). It depends on the macronutrient content with the highest energy intake in lipids. The difference observed is due to a difference in the proportion of macronutrient present in different samples. Our results are showed higher value than those of (Leung et al., 1968) and (Bhatia et al., 2018), which obtained an energy value of 326 and 375Kcal/100g respectively. The higher fiber content of *Rh* mesocarp (25.82%) makes it a food of choice in the prevention of certain diseases such as: cancer of the colon, diabetes, cardiovascular diseases and others (Igboh et al., 2009). The results obtained in this study are lower than those of (Esiegbuya et al., 2013), which had a fiber content of 56.15%, which can be attributed to the degree of maturity of the fruit, the geographic conditions and the condition of the fruit. Indeed, fresh fruits have a higher fiber content compared to dried fruits (Esiegbuya et al., 2013).

Ash is an inorganic residue remaining after water and organic matter are removed by calcination. It indicates the amount of minerals contained in a food (Mcclements, 2003). Minerals are important components of diet because of their physiological role and metabolic function in the body. In this study, the main minerals present in the different samples were: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and phosphorus (P). *Rh mesocarp* powder sample had the highest calcium content. Indeed, humans need a significant amount of calcium for the construction and maintenance of bones, blood clotting, transmission of nerve impulses, formation of teeth and bones. It is also an important factor in enzymatic metabolic processes (Senga et al., 2013). The amount of calcium obtained was 3183.30 mg /100g of dry matter. This value is highly above the one recommended daily by WHO (800 mg). This result showed that *Rh* fruit is an important source of calcium that can be used as a supplement in calcium deficient people (Murray et al., 2000). This result obtained is higher than that of (Esiegbuya et al., 2013) who showed that *Rh* fresh mesocarp contained 789.1mg / 100g of fresh matter. This difference is probably due to the fact that in this study, a dry matter was used to determine the calcium content contrary to other studies where a fresh matter was used.

Phosphorus is jointly absorbed with calcium. It is essential for the fortification of bones and teeth as well as children and nursing mothers. Phosphorus is vital for the flow of metabolic reactions especially those involving buffers in body fluids (Andzouana & Mombouli, 2012). Energy production within living cells involves the formation or breakdown of high-energy bonds that bind oxygen from phosphorus to carbon or carbon-nitrogen compounds. Phosphorus is also an essential element for plants (Abugre, 2011). The daily dose recommended in adults and children is 800mg / day (Pillai & Nair, 2013). The highest phosphorus content noted in the staple food may be due to the fact that phosphorus is present in large quantities in foods like meat and fish because these ingredients are mostly found in the staple food than the other samples.

The magnesium content was also evaluated and the highest content was observed in the staple food. In fact, magnesium plays an important role in the body by activating enzymes involved in protein synthesis. The recommended daily dose is 420mg for men and 320mg for women. However, magnesium deficiency leads to

growth retardation, behavioral disorders, body weakness and muscle contractions (Murray et al., 2000).

Potassium is also an important mineral for the body because it maintains the acid-base balance, the osmotic pressure and the conduction of nerve impulses. The recommended quantity is 2.5mg / day and its deficit leads to muscle weakness and paralexia. It is found in foods such as milk, meat and fruits. The results showed that the *Rh* fruit had the highest content. The amount obtained was 1218.3mg/100g of dry matter. This fruit represents an important source of exploitable potassium for food formulations. These results do not line with those of (Akpabio et al., 2012) who found very lower potassium content from *Rh* exudates (20.95mg/100g). This difference could be due to the plant part used which is different in the two studies.

Sodium content was determined and the highest amount was observed in the staple food (291.3mg/100g). It has numerous functions in the body including the maintenance of acid-base balance and osmotic pressure between cells and interstitial fluid. The recommended daily dose is 115-75000mg/kg for adolescents, 324-975mg/kg for children and 1100-3300mg/kg for adults (Lawal & Fagbohun, 2012).

Zinc plays a vital role in human growth and development (Divrikli et al., 2006). Highest zinc content was observed in *Rh* fruit (0.88 mg/100 g DM). The recommended daily dose is between 0.3 and 1 mg/kg in adults (FAO / WHO, 2014). This result is not similar to that of (Esiegbuya et al., 2013) who obtained 10.45 Ppm/100g fresh matter from the *Rh* fresh mesocarp. The difference is certainly linked to the maturity of the fruit.

The present study showed that the highest iron content was observed in the staple food. Nevertheless, the amount of iron present in mesocarp of *Rh* fruit was also higher 22.12mg/100g dry matter than the recommended daily dose which is 10mg/100g dry matter(FAO / WHO, 2014). Iron is a component involved in numerous metabolic pathways including proteins (Andzouana & Mombouli, 2012). It is important for a normal functioning of the central nervous system and proteins transportation. Iron is also essential in the diets of pregnant women, nursing mothers, infants and elderly so as to prevent anemia and other related diseases (Alinor & Oze, 2011). So, this fruit can be recommended to people constantly exposed to anemia in order to prevent hemolysis caused by oxidative stress, inflammation, erythrocyte deformability and mechanical rupture from foot strike (FAO / WHO, 2014).

However, food usually contains anti-nutritional substances that prevent the absorption of some nutrients. This is why some anti-nutrients such as hydrocyanic acid and phytic acid were evaluated in this study. Hydrocyanic acid content was evaluated and the highest value (0.36mg/100 dry matter) was obtained in the *Rh* powder. This value is much lower than the lethal dose (35mg/100 g of weight body). This result is very lower than that of (Akpabio et al., 2012) who obtained a value of 23.76mg/100 of fresh matter with Rh exudates. This difference could be due to the plant matrix that was used and also the fruit moisture content (dry or fresh fruit). Storage and drying may significantly reduce hydrocyanic acid content.

Phytic acid content was also determined. The highest value (91.81mg/100g dry matter) was observed in the staple food and the smallest value in *Rh* mesocarp (40.16 mg/100g dry matter). This value is extremely higher than that of (Akpabio et al., 2012) who obtained 7.82mg/100g DM. Low anti-nutrients contents obtained in Rh mesocarp fruit could be exploited to formulate some foods (Nwokolo & Bragg, 1977).

Induction of stress by aluminum chloride resulted in a low percentage of weight growth and a decrease in food intake in untreated induced rats. This is due to the fact that aluminum has a direct effect on the appetitive pathways and /or malabsorption of nutrients induced by $AlCl_3$ effects on the gastro-intestinal tract and/or inhibition of protein synthesis. And therefore a low food intake would be the cause of low weight gain (Lukyanenko et al., 2013). Concomitant food intake with increased weight gain would be due to the fact that the mesocarp powders and aqueous extract of *Rh* fruit would stimulate appetite, as the work of (Mann & Wendl, 1864), reveal that the mesocarp powder of this fruit and used in certain food formulations to enhance the flavor.

Induction of stress with aluminum chloride resulted in a significant decrease in red blood cell count and hematocrit levels in animals. It can be explain by the fact that the oxidative stress caused by $AlCl_3$ increase production of free radicals, decrease catalase activity and the erythrocyte ATP concentration (Newairy et al., 2009; Rim, 2007; Al-Hashem, 2009). All or some of these deleterious effects of $AlCl_3$ on RBCs membrane caused increased membrane fragility, increased RBCs destructions. The decrease of hematocrit would be due to an increase in the rate of destruction or a reduction in the rate of formation of red blood cells. Indeed, aluminum can disrupt erythropoiesis through its combined effect on mature erythrocytes and delayed cellular metabolism of progenitor erythroids (Bouasla et al., 2014). Aluminum can be accumulating in the liver and decrease or inhibing the erythropoese activitie (Ojiako et al., 2018). However, administration of aqueous extract and differents formulations of the mesocarp of *Rh* least to an increase of theses parameters. It is due to the present of some metabolites which stimulate hematopoietic activity in the bone marrow. (Bouasla et al., 2014) showed the

antioxidant effect of alpha lipoic acid which stimulate hematopoietic activity in the bone marrow of the hepatotoxicity rat induced by aluminum chloride. In the same line, (Domingo et al., 2011) showed that malic acid can act as chelating agent, reduce the concentration of $AlCl_3$ levels in the brain by fecal and urinary excretion. Malic acid act as powerful antioxidant by decreasing the oxidative stress in comparison with group that was received $AlCl_3$ alone Sadhana, 2011). The different formulation also contain high amount of iron which in addition to vitamin B12, vitamin A, folate, riboflavin, and copper are required for the proper production of hemoglobin (De Benoist et al., 2008).

Induction of stress with aluminum chloride also led to a significant increase in the level of white blood cells, monocytes and granulocytes in the plasma of positive control group of rats. Oxidative stress usually leads to activation of the white blood cells that are lymphocytes and neutrophils (Vignais, 2002) which indicated the activation of defence and immune system (Mahdy & Farrag, 2009). These results are similar to those of (Turkman et al., 2005), which following induction of fibrosis by carbon tetrachloride showed a significant increase in plasma white blood cell level of rats. Also, to those of (Bouasla et al., 2014) which observe the decrease of theses parameters in rats which has induced hepatotoxicity with aluminum chloride. Moreover, the administration of the aqueous extract of the *Rh mesocarp* and the different formulations resulted in a significant decrease in the rate of these parameters. This drop is due to the presence of metabolites (minerals and phenolic compound) present in our extract and formulations. In addition, the work of (De Benoist et al., 2008) showed that antioxidants in plants reduce white blood cell levels by inhibiting pro-inflammatory pathways associated with acute or chronic toxicity in rats. These results are similar to those of (Ojiako et al., 2018) who observed a decrease in white blood cell count after inducing hepatic stress with carbon tetrachloride and administering crude and infused herbs in *wistar* rat.

4. Conclusion

The evaluation of the nutritional value of the different samples showed that Rh mesocarp fruit is rich in macronutrients namely lipids and fibers and micronutrients including calcium, potassium, zinc, iron and selenium. However anti-nutrients such as phytic acid and hydrocyanic acid were also present but under the toxic concentration prescribed by WHO. The evaluation of hematological parameters showed that the formulation made with Rh mesocarp powders presented the best result by increasing the concentration of red blood cells, hemoglobin and hematocrit and reducing white blood cell concentration.

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