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Review

Food additives and their health effects: A review on preservative sodium benzoate

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Food additives are widely used by the food industry to increase the product shelf life and/or attribute as well as enhance certain characteristics of particular foods, which are often lost during processing. With the advent of modern life, more and more additives have been employed by the food industry. Despite their wide use, they are substances that may cause adverse reactions like any other drug. This study aimed to contextualize through a review of the literature, the scientific evidence on the risks entailed by the consumption of food additives, special regards to sodium benzoate. This additive is a sodium salt, which is commonly used as a chemical preservative in foods, and it is found mainly in industrialized drinks/beverages. Sodium benzoate is considered safe by major regulatory agencies, but there is still controversy over its effects on human health.

Key words: Industrialized foods, preservatives, toxicity, sodium benzoate.

INTRODUCTION

The change in dietary habits in recent decades has caught the attention of regulators and the scientific community as a whole, since the substitution of *in natura* by industrialized foods has contributed significantly to lowering the quality of people's diet, a situation observed due to the indiscriminate use of chemical additives in processed foods (Polônio and Peres, 2009).

Just as the dietary pattern of the population has been changing in order to meet the demands of a modern and globalized world, where time is very precious, the Western people's diet has also been following this same model (Hill, 1993). In this sense, the preferred and consumed foods are those of easy and/or instant preparation, with distinguishing flavor, high energy density and usually at a low cost (Loco et al., 2015). The use of food additives has increased greatly in recent years and as a result, it is estimated that 75% of the contemporary diet is made up of industrialized foods. Estimates has shown that each person may consume 3.6 to 4.5 kg of food additives per year on average, but these values may be even greater (Zengin et al., 2011). Additives are usually used in all types of food, propagated from the minimally processed sorts to the highly-processed and modified ones (Carocho et al.,

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2015). The interaction between some food additives and the general public has not been very peaceful. In the 1980s, food additives were considered to be hazardous for consumption, which fueled widespread fears and the exclusion of some additives. Since then, the relationship between additives and consumers has improved, although some mistrust still remains (Emerton and Choi, 2008). Despite their wide use, these are substances capable of triggering adverse reactions, just as any other drug does, including allergic reactions, behavioral changes and carcinogenicity (Cardoso et al., 2017a; Aun et al., 2011).

Sodium benzoate (BS) is a sodium salt used to inhibit the growth of molds, yeasts and bacteria, found in a variety of products such as preserves, sauces, beverages and juices (Turkoglu, 2007). The Food and Drug Administration (FDA) considers the preservative sodium benzoate to be safe (FDA, 2017). However, Boris and Mendel (1994) report that the excessive intake of sodium benzoate induced hyperactivity in children, caused urticaria (Michaelsson and Juhlin, 1973), and in addition it has also been significantly harmful to the Deoxyribonucleic Acid (DNA) (Zhang and Ma, 2013). Taking into account the effects observed by the authors, the study aimed to carry out a review of the literature to contextualize the consequences caused by the chronic and high consumption of the preservative sodium benzoate.

FOOD ADDITIVES

According to Codex Alimentarius (2017), food additive means “any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether it has nutritive value or not the intentional addition to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, reasonably expected to result (directly or indirectly) in it or its by-products becoming a component or otherwise affecting the characteristics of such foods. The term does not include contaminants, or substances added to food for maintaining or improving nutritional qualities.

As these chemicals are intentionally added to food, it is essential to know their properties, so as to ensure their adequate and safe use (Cardoso et al., 2017b; (Zhang and Ma, 2013). The assessment of food additives worldwide is supported by the control system of the Acceptable Daily Intake (ADI), developed by the Joint Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (WHO) Expert Committee on Food Additives (JECFA, 2017). The importance of technological additives in food production is evident (Toledo and Tfouni, 2002). However, it is necessary to be aware of the possible toxicological risks that can be caused by frequent and/or large quantities of these substances (Turkoglu, 2007).

IDA has been developed to protect the consumer against possible adverse health effects (Loco et al., 2015). It is defined as a numerical value of the additive, measured in relation to the body weight that a person can ingest daily throughout life with no significant health risk (Varela and Fiszman, 2013; WHO, 2017). The maximum use dose is defined as the consumption of an additive in its highest concentration that Codex Alimentarius Commission (2017) has determined to be functionally effective in a food or food category, and it must be considered innocuous in relation to its consumption by humans.

In order to determine the possible harmful effects of a food additive or its derivatives, these additives must be subjected to trials to an appropriate toxicity assessment (Honorato et al., 2013). All food additives should remain under observation and evaluation whenever appropriate, considering the conditions of use and any new scientific data (Abu-Taweel, 2016).

PRESERVATIVES

Food preservatives are substances that can prevent or delay changes caused by the action of microorganisms, enzymes and/or physical agents (Tfouni and Toledo, 2002) once they are added to a given food. Its high utilization by the food industry is due to the growing demand for chemically stable, safe and durable foods (Thomas and Adegoke, 2015).

Food preservation is one of the oldest varieties of technology used by humans; different forms and means of preservation were found and perfected for this purpose (Mpountoukas et al., 2008). Preservatives may be natural (salt and sugar) or chemical, and this is the most effective type in preservation for longer periods (Martyn et al., 2013; Pongsavee, 2015). The most commonly used preservatives are: sulfur dioxide, sodium benzoate, sorbic acid, propionic acid, nitrites and sodium and potassium nitrates (Lennerz et al., 2015).

Similarly, to other classes of food additives, preservatives must meet criteria for their use, since they are chemicals and may cause adverse health effects (Akintonwa et al., 2007). Recent toxicological studies indicate that certain concentrations of synthetic preservatives and their continuous use may be potentially mutagenic and/or genotoxic (Di Sotto et al., 2014; Antunes et al., 2016; Mellado-Garcia et al., 2017). Research is being conducted to find natural compounds with antimicrobial and antioxidant activity, which may be new alternatives to replace the commonly used synthetic

preservatives or to make associations between them, thus reducing their amount in foods and their possible health risks (Ramalho and Jorge, 2006).

SODIUM BENZOATE

Sodium benzoate is a sodium salt represented by the chemical formula $C_7H_5O_2Na$, with a molecular weight of 144.1 $g.mol^{-1}$, odorless compound and soluble in water and ethanol. It is typically used as a preservative in some products from the cosmetic, pharmaceutical and food industries (Lennerz et al., 2015). In the pharmaceutical industry, it is used in the treatment of various diseases such as disorders of the urea cycle, liver diseases and multiple sclerosis (Yavav et al., 2016). In the food industry, sodium benzoate is used in foods and beverages, as it is effective to inhibit the growth of fungi and bacteria during storage, besides providing easy application (Tsay et al., 2007). It is indicated for the preservation of margarines, sauces, marmalades, gelatin, liqueurs, beers, fruit juices and soft drinks (Zhang and Ma, 2013). Despite its presence in several foods, population studies indicate that soda and juice in cartons are the main dietary sources of this preservative (Pongsavee, 2015).

Sodium benzoate has been used for many years as a preservative because of its good stability and excellent solubility in water (Ren et al., 2014). It is considered "Generally Regarded as Safe" by the FDA and may be present in foods in concentrations above 0.1% (Lennerz et al., 2015). For the FAO and WHO, the IDA for foods with sodium benzoate is 5 $mg.kg^{-1}$ body weight (Zhang and Ma, 2013; Lennerz et al., 2015). According to RDC n° 65, dated November 29, 2011 (ANVISA), the maximum limit of sodium benzoate as preservative is 0.05 g/100 mg or 0.05 g/100 mL (Zhang and Ma, 2013). According to the European Food Safety Authority (ESFA), the Lethal Dose (LD_{50}) for the preservative is 2000 $mg.kg^{-1}$ (EFSA, 2017). There are reports that oral doses of 8 to 10 g can cause nausea and vomiting, and also that small doses have little or no effect (Nair, 2001). The FDA has never set a limit for sodium benzoate and recognizes it as very safe, though it has been criticized as being negligent in its assessments in the light of publications that demonstrate contradictions (FDA, 2017). While IDA is almost unlikely to be exceeded for average consumers, large daily consumers of soft drinks and juices may exceed the ADI (Tfouni and Toledo, 2002).

As soon as it is consumed, sodium benzoate (a benzyl alcohol metabolite) is rapidly absorbed by the gastrointestinal tract, then conjugated with glycine to form pyruvate in the liver (Pongsavee, 2015), this transformation occurs by means of two steps in the mitochondria (Bridges et al., 1970). Upon entering the cell, the preservative is converted to benzoyl-CoA by means of an Adenosine Triphosphate (ATP)-dependent acid, reaction 1. Subsequently, CoA is converted to pyruvate, reaction 2, by means of glycine N-acyltransferase. In the mitochondria, SB results in the consumption of ATP, glycine and coenzyme A. The ingestion of this preservative causes an increase in the serum of benzoate and also of pyruvate (Akintonwa et al., 2007). The resulting hippuric acid is rapidly excreted in the urine within the first 6 h, and the remaining dose is completely eliminated within 2 to 3 days (Pongsave, 2015; Bridges et al., 1970).

There are several references for the same preservative, which makes its evaluation complex. In addition to that, it has been proved that certain preservatives, especially antimicrobial agents, may cause allergies, urticaria (Gören et al., 2015), and behavioral disorder such as hyperactivity and Attention-deficit/hyperactivity disorder (ADHD) (Beezhold et al., 2014; McCann et al., 2007), as well as being toxic and genotoxic when consumed above the ADI (Yavav et al., 2016). Table 1 lists some pieces of research and the results found on the consumption of the preservative.

CONCLUSION

The adverse effects caused by the consumption of food additives are well elucidated in the literature, but there is still controversy concerning results for the preservative sodium benzoate. By means of research, the present review describes the main adverse effects caused by the consumption of sodium benzoate in humans and animals, and it demonstrates that toxicity may be different, depending on the species, the dose and exposure to the preservative.

Consumption above ADI (5 mg kg^{-1}) resulted in hyperactivity, ADHD and allergic reactions. Sodium benzoate was considered genotoxic, clastogenic, neurotoxic, besides being responsible for changes in cell cycle and for having proven intercalation in the DNA structure. All the results presented were performed for a limited time, that is, cannot be considered as long-term interventions. Considering the evidence found for the consumption of the preservative, it is recommended that further studies can be conducted on the substance in order to better understand its genotoxic, immunological and behavioral effects. In addition, it is worth mentioning that drinks containing sodium benzoate are mainly those with artificial fruit flavor, carbonated drinks, teas, soft drinks and coffees, as well as drinks with lots of syrup and sugar; which already sends several warnings and precautions as to the ingestion of this substance. What is clear in this review is the need for caution and attention as to the consumption of industrialized beverages,

Table 1. Research on animals and humans with preservative sodium benzoate.

Protocol	Results	References
Doses at 7×10^{-6} mol.L^{-1} sodium benzoate concentration in splenocytes of female mice for 72 hours. Aim: In vitro evaluation of preservative on T cells and responsiveness of B cells.	Sodium benzoate suppressed functional responses of T and B lymphocytes because it altered the cell cycle in G1. It also affected the immunosuppression of expression patterns of various activation receptors, which play a role in the activation and regulation of adaptive immunity.	Yadav et al. (2016)
Evaluation of sodium benzoate interaction with DNA. It used cells from the calf thymus, acridine orange and the preservative in the concentration 45×10^{-6} mol. L^{-1}. Fluorescent spectrophotometer evaluation.	Fluorescence analysis indicated that there was a competitive interaction between the preservative and the organic acridine with the DNA, in which benzoate was an intercalating agent and was able to extinguish DNA fluorescence.	Zang and Ma (2013)
Potassium sorbate, sodium benzoate and potassium nitrate were analyzed in three cytogenetic indicators in human erythrocytes. Sample composed of two men and four women between 20 and 30 years. Doses at concentrations 8.0, 4.0, 2.0, 0.2 and 0.002 mol.L^{-1}.	When used at low concentrations (2, 0.2 and 0.02 mM), the preservatives did not show genotoxic activity, but at concentrations 4 and 8 mM, the preservatives potassium sorbate and sodium benzoate showed genotoxicity.	Mpountoukas et al. (2008)
Evaluation of the genotoxic effects of sodium benzoate and potassium benzoate in human lymphocytes – in vitro test. For 48 hours at doses 4.3×10^{-8}, 8.7×10^{-8}, 1.7×10^{-7}, 3.5×10^{-7}, and 6.9×10^{-7} mol.L^{-1}.	Chromosomal aberrations, sister chromatids and micronuclei were found in almost all treatments. There was a reduction in the mitotic index. However, the additive did not affect the replication index. Sodium benzoate significantly increased DNA damage, was clastogenic and cytotoxic.	Zengin et al. (2011)
Neurotoxic evaluation in rats resulting from consumption of sodium benzoate and citric acid. The preservatives were diluted in water and offered through gavage, 3.2×10^{-6} mol.L^{-1} of benzoate and ascorbic acid at $2.5\text{x}10^{-2}$ mol.L^{-1} for 28 days.	The structure of the cerebellum was altered after treatment with benzoate; this piece of data was found with or without the association of ascorbic acid. There were alterations and reduction in the cerebellum structure in rats treated with the preservative. Ascorbic acid alone prevented the loss of some cells in the cerebellum, but in contrast, the association between sodium benzoate and ascorbic acid did not prevent cell loss in the cerebellum.	Noorafshan et al. (2014)

because besides preservatives, there are other substances that can also be harmful to human health when consumed regularly.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Antioxidant, antimicrobial and antifeedant activity of phenolic compounds accumulated in *Hyoscyamus muticus* L.

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***Hyoscyamus muticus* is an endangered desert plant spread in the Arabian Peninsula and the Middle East deserts. The methanol extract of the aerial parts of *H. muticus* grown in the arid zones in the Northern regions of Saudi Arabia were subjected to primary phytochemical analysis which revealed the presence of phenolic compounds, flavonoids, tannins and sterols, the gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract exhibited different types of phenolic compounds, including ferulic acid, 4-hydroxy-cinamic acid-ester, methyl salicylate and methyl ferulate. The accumulation of phenolic compounds supports the antioxidative properties of the plant against oxidative stress. The antioxidant testing showed that the methanolic extract of *H. muticus* has a noticeable antioxidant activity with an IC_{50} of 8.1±0.65 mg/ml and an EC_{50} of 12.74±1.12 mg/ml. The antimicrobial investigation on 11 microbial strains revealed that the methanolic extract of *H. muticus* areal parts showed average or weak antibacterial activity against gram-positive bacteria, weak antibacterial activity against gram-negative bacteria and no antifungal activity. Moreover, the investigations exhibited the presence of an antifeedant potential on the methanol extract of *H. muticus* on the 4th instar larvae of *Spodoptera littoralis*.**

Key words: *Hyoscyamus muticus*, phytochemical analysis, antioxidant capacity, antimicrobial activity, antifeedant assay *Spodoptera littoralis.*

INTRODUCTION

All living creatures in the animal kingdom are heterotrophic and life depends directly or indirectly on plants. Through the historical development of the human race, man has always depended on plants as a source of food,

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shelter, clothing medicine, cosmetics, ceremonies and even magic, until the industrial renaissance came and introduced the manufactured and synthesized products in the life of modern man with their enormous negative impacts on the environment and health. However, In recent years, the interest in medicinal plants is growing, the demand for medicinal plant products is increasing in the developing countries as well as in the developed countries, this is because they are inexpensive, have better acceptability and compatibility and have minimal negative effects (Pal and Shukla, 2003). Herbal drugs have many features which make them preferable to modern synthetic drugs, such as the ability of the plant compounds to interact together in harmony which decreases the possible negative impact; plant compounds can support official synthetic drugs in some difficult disease treatments like cancer; and frequent consumption of some medicinal plant products could prevent the appearance of some diseases and enhance the immune system (Rasool, 2012). Accordingly, it is worthy to explore the biological activities of plants. On the other side, some medicinal plants have some toxic symptoms and others might have antifeedant effects on insects. As an example, the cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), is one of the most destructive pests in many countries in the Middle East. This insect causes critical injuries to a wide range of vegetables and crops including cotton, alfalfa, peanut, potato, pepper and tomato (Maged El - Din and El - Gengaihi, 2000; Kandil et al., 2003; Adham et al., 2009). Chemical synthetic pesticides have been used to control this pest and to reduce crop losses. This control strategy has potentially negative consequences on the environment and harmful effects on beneficial insects and natural enemies (Pavela et al., 2008). In this context, screening of botanical extracts against the target pest has been conducted by researchers in recent decades (Kebede et al., 2010; Kamaraj et al., 2010). Several studies have shown larvicidal, antifeeding and repellent activity of botanical extracts (Larocque et al., 1999; Gbolade, 2001).

Hyoscyamus muticus L., is a desert plant which grows in arid areas of Egypt, known in Egypt as Egyptian henbane and belongs to the family Solanaceae, a family rich in phytochemicals of pharmaceutical properties such as tropane alkaloids and hyoscyamine that has a direct effect on the central nervous system (Elmaksood et al., 2016). Phenolic acids have two main structures, hydroxycinnamic and hydroxybenzoic acids. The derivatives of the hydroxycinnamic acid include ferulic, caffeic, p-coumaric and sinapic acids, while the derivatives of hydroxybenzoic acid consist of gallic, vanillic, syringic and protocatechuic acids. Another major class of phenolic compounds is the cell wall phenolics, which is insoluble and found in complexes with other cell wall components. The two main groups of cell wall phenolics are lignins and hydroxycinnamic acids (Callemien et al., 2008). Phenolic compounds play a critical role in the cell wall during plant growth by protecting the plant against stresses such as infection, wounding and UV radiation (Santos et al., 2004). Moreover, the presence of phenolic compounds is the reason behind the formation of the blue fluorescence (Lichtenthaler and Schweiger, 1998). The use of *H. muticus* in medicine dates back to ancient Egypt; the plant has a hallucinogenic and poisonous properties, although it is used in medicine to relieve the symptoms of Parkinson's disease, to treat some gastric disorders, to induce smooth muscle relaxation and also for treatment of motion sickness (Sevon et al., 2001). The current study aimed to evaluate some of the biological activities of the areal parts of *H. muticus* such as phytochemical, antioxidant, antimicrobial and antifeedant activity.

MATERIALS AND METHODS

Collection of plant materials

The aerial parts of *H. muticus* grown in the arid zone at Wadi Arar, Arar region, Saudi Arabia, was collected during the summer season in 2016. Collected plants have been kindly verified and authenticated in the Desert Research Center; voucher specimens were deposited in the Herbarium of Desert Research lab, dried in shade and ground to fine powder.

Plant extraction

The dried powder of the areal parts of *H. muticus* (140 g) was extracted with 80% methanol (400 ml MeOH/100 H_2O) using Soxhlet extractor at 90°C for 16 h. The polar extract was evaporated at low pressure to obtain crude methanol extract. Then, the semi-solid crude extract was kept for further analysis.

Phytochemical screening

The previously prepared methanol extract was subjected to a qualitative chemical test to detect different classes of bioactive chemical constituents present in the plant using standard methods, as previously mentioned in some reports (Yusuf et al., 2014; Mujeeb et al., 2014).

GC-MS analysis

GC-MS analysis of crude methanol extract of *H. muticus* was performed on a Perkin Elmer Clarus® 600 GC System, fitted with a Rtx-5MS capillary column (30 m × 0.25 mm inner diameter × 0.25 µm film thickness; maximum temperature 350°C), coupled to a Perkin Elmer Clarus® 600C MS. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 ml/min. The injection, transfer line and ion source temperatures were all 290°C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was programmed at 60°C (held for 2 min) to 280°C at a rate of 3°C/min. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. Then, the particle-free diluted crude extracts (1 µl) were taken in a syringe and injected into injector with a split ratio 30:1. All the resulted data were obtained by collecting the full-scan mass spectra within the scan range 40 to 550 amu. The percentage

composition of the crude extract constituents was expressed as a percentage by peak area. Finally, the identification and characterization of the chemical compounds in the crude extract of *H. muticus* was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries (Mooza et al., 2014; Admas, 1995).

Antioxidant testing

The antioxidant activity was evaluated using the ferric reducing power method (FRAP) as described by Abdallah et al. (2016), and 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging method as described by El-Sharkawy et al. (2017). For the FRAP method, 1 ml of each sample concentration was mixed with 2.5 ml of potassium hexacyanoferrate $K_3Fe(CN)_6$ solution and 2.5 ml of phosphate buffer (0.2 mol/L, pH 7.0) and incubated at 50°C for 30 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture. Then, 2.5 ml of this solution was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm and the concentration of the samples at which the absorbance of 0.5 (EC_{50}) was determined. Ascorbic acid and Quercetin were used as positive controls for comparison. For the DPPH scavenging method, 0.5 ml of each sample concentration was mixed with 0.5 ml of DPPH methanolic solution (0.04 g/l). The mixture was shaken vigorously and allowed standing for 30 min in darkness at 25°C. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer, and the percentage inhibition of activity was calculated as:

$$\% \text{ Inhibition} = \frac{(\mathbf{A}\text{blank} - \mathbf{A}\text{sample})}{\mathbf{A}\text{blank}} \times 100$$

The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against the extract concentration. Ascorbic acid and quercetin were used as positive controls for this study.

Antimicrobial investigation

The antimicrobial activity of the methanol extract of *H. muticus* was evaluated against different gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, *Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* clinical isolate), gram-negative bacteria (*Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* ATCC 27736 and clinical isolates of *Pseudomonas aeruginosa* and *Acinitobacter baumannii*) and fungi (*Aspergillus niger* ATCC 6275, *Candida albicans* ATCC 10231). Different strains from the same bacterial species were used to evaluate any potential variation in antibacterial susceptibility among them. The method used in this study was Kirby-Bauer disc diffusion test (NCCLS, 2002), with minor modification to fit with the plant extract. The crude methanol extract of *H. muticus* was reconstituted in 10% Di-methyl-sulphoxide (DMSO) to make 500 mg/ml. Microbial strains were sub-cultured in nutrient broth (for bacteria) or sabouraud dextrose broth (For fungi), samples from the broth cultures were pipette and diluted with sterile normal saline to make a suspension equivalent to the turbidity of the 0.5 McFarland standard. A sterile cotton swab was dipped in the adjusted suspension and smeared over previously prepared plates containing 20 ml Mueller Hinton agar for bacteria or sabouraud dextrose agar for fungi. Sterile paper discs, 6 mm in diameter were cut from No.1 Whatman filter paper and were immersed in the reconstituted extract (Absorb about 15 µl) and loaded over the seeded plates. Another two paper discs, one saturated with 10% DMSO while the other was erythromycin disc (15 µg) for bacteria or a disc saturated with clotrimazole (10 mg/ml) for fungi, were also used as a negative or positive control, respectively. Plates were incubated at 35 to 37°C for up to 24 h for bacteria or at 28 to 30°C for up to two days for fungi. Then, the diameter of inhibition zones (in mm) was measured and recorded.

Antifeedant activity and starvation percentage

A laboratory strain of *Spodoptera littoralis* was reared in the laboratory for more than 10 generations. Larvae were fed on fresh castor leaves, *Ricinus communius,* until pupation. Moths were fed on 10% sugar solution. Each jar was provided with branches of tafla, *Nerium oleander*, as an oviposition site. Insects were kept under controlled conditions at 26 ± 2°C and 65 ± 5 %R.H., with 8:16 L:D h photoperiod. The experiments were carried out on the 4th instar larvae. Series of ascending crude concentrations were prepared (5, 10, 20 and 40%) by dilution in 70% ethanol. Control discs were sprayed with the carrier solvent alone. 300 larvae were starved overnight, and then divided into 6 groups of 50 larvae each, four different concentrations (5, 10, 20 and 40%) of plant extract (*H. muticus)*, one group for the control and one group as starved larvae. Equal discs of fresh castor bean leaves were rinsed in each treatment and in the control, then treated and untreated leaves were shade-dried. All larvae of control and treated leaves were weighted before and after treatment for 3 days. The dried leaves were placed individually in plastic Petri-dishes. Ten larvae were transferred into each cup and allowed to feed on the treated and untreated leaves, the starved larvae were left without feeding for 24 h. Five replicates for each treatment were carried out. According to the equation of Mostafa (1969) and Abdel-Mageed et al. (1975), the starvation percentages of tested larvae were calculated as follows:

Starvation (%) = C – E/C – S × 100

Where:

C = Mean weight gain of untreated larvae after 24 h;
E = Mean weight gain of treated larvae for each concentration after 24 h; and
S = Mean weight gain of starved untreated larvae after 24 h.

The antifeedant index (AFI) was calculated according to Sadek (2003).

AFI (%) = [(C-T) / (C + T)] × 100

Where:

C: the amount of food consumed (leaves) in the control; and
T: the amount of food consumed (leaves) in the treatment.

Statistical analysis

The measurements were carried out in triplicate or in duplicate. The data obtained were presented as means ± standard error (S.E.) and the significant difference between groups was statistically analyzed using Student T-test or one-way ANOVA, as appropriate. A probability level of $P < 0.05$ was used in testing the statistical significance. The program used was SPSS-Statistical Package, version 11.

RESULTS AND DISCUSSION

The powder of dried aerial parts of *H. muticus* was

Table 1. The phytochemical analysis of 80% methanol extract of *Hyoscyamus muticus*.

Phytochemicals	Methanol extract (80% v/v)
Alkaloid	+++
Flavonoid	++
Tannin	++
Terpenoid	-
Sterol	+
Phenolic compounds	+++

+++ = Present in high amount, ++ = moderately present, + = Trace amounts, - = Absent.

extracted with 80% methanol in order to collect various non-polar and polar compounds, the obtained crude which was a brown sticky extract, was used for preliminary phytochemical investigation. Phytochemical testing revealed the presence of alkaloids, flavonoids, tannins, sterols and phenolic compounds. The major related compounds are the phenolic and alkaloid compounds, these results are shown in Table 1. The phenolic compounds of the aerial parts of *H. muticus* were analyzed by GC-MS, and the assay revealed the presence of ferulic acid, 4'-Hydroxy-3'-methylacetophenone, methyl isoferulat, methyl salicylate p-Cresol, 2,2'-methylenebis[6-tert-butyl, most of phenolic compounds found as ester–bound form, while only ferulic acid was found free as revealed in Table 2. The GC-MS assay is currently used to identify different classes of organic compounds especially phenolic compounds; these compounds were confirmed by reference sample on thin layer chromatography (TLC).

According to results represented in Tables 1 and 2, the aerial parts of *H. muticus* are rich in phenolic compounds, flavonoids, tannins and sterols, the GC-MS analysis of the methanolic extract exhibited different types of phenolic compounds, namely ferulic acid, 4-hydroxy–cinamic acid–ester, methyl silsilat and methyl ferulat. Many previous studies revealed the accumulation of phenolic compounds under environmental stress in some desert plants which grow in arid conditions under high temperature and water deficiency and exposed to different other environmental stress which affect the plant, and this may lead to the destruction of the plant cells, so plant adapt to these conditions by accumulating some antioxidant compounds to avoid these oxidative stresses. Amongst these compounds are the phenolic compounds which play an important role in protecting the plant cells from stresses. In addition, the area where *H. muticus* grows, is located in the arid zone, which is characterized by water deficiency and low levels of rainfall. Accordingly, compounds detected in the current investigations included ferulic acid, cinamic acid, benzoic acid, besides their salts; methyl ferulat, 4'-Hydroxy-3'-methylacetophenone and methyl salicylate are synthesized to support the antioxidative properties of the plant against oxidative stress. Most phenolic compounds are found in bounded ester form, the accumulation of phenolic compounds in ester form is considered as a mechanism of drought tolerance, this agrees with the report published by Stanlisla et al. (2009) as they have found the accumulation of ester bound to p-coumaric acid in *Vitis vinfiera* which grows under drought conditions in the green houses. The presence of ferulic acid also supports the role of phenolic compound in *H. muticus* as antioxidant, mainly ferulic acid belonging to biochemically active phenylpropanoids. By absorbing radiation, the phenolic compounds transform short-wave, high-energy and highly destructive radiation into the blue radiation of a longer wavelength and, therefore, it is less destructive to the cellular structures of the leaf, including the photosynthetic apparatus (Bilger et al., 2001).

Regarding the antioxidant evaluation, DPPH free radicals scavenging activity and the ferric reducing antioxidant power (FRAP) assay of methanol extract of aerial parts of *H. muticus* were carried out. The results showed that the methanolic extract of *H. muticus* has an important antioxidant activity with an IC_{50} of 8.1 ± 0.65 mg/ml and an EC_{50} of 12.74 ± 1.12 mg/ml (Table 3). The antioxidant capacity of the methanolic extract of *H. muticus*, based on the results obtained, is significantly lower than that of ascorbic acid (IC_{50}: 0.031 ± 0.001 mg/ml; EC_{50}: 0.095 ± 0.002 mg/ml) and quercetin (IC_{50}: 0.012 ± 0.002 mg/ml; EC_{50}: 0.019 ± 0.003 mg/ml) ($P < 0.05$). The antioxidant activity of this extract is due to its chemical composition, which showed the presence of different phytochemical groups that have an antioxidant activity such as alkaloids, flavonoids, tannins, sterols and phenolic compounds. Moreover, the chemical analysis using GC-MS allows identifying certain compounds which can be involved in the antioxidant mechanisms. Some studies have evaluated the efficacy of quinic acid as an antioxidant in the metabolization of tryptophan and nicotinamide (Pero et al., 2009). Also, Chuda and his group showed that quinic acid has a strong antioxidant activity (Chuda et al., 1996). Furthermore, other authors have shown the high antioxidant activity of Guaiacol (Brand-Williams et al., 1995), Cinnamic acid derivatives (Sharma, 2011), and Ferulic acid (Srinivasan et al., 2007;

Table 2. The analysis of phenolic compounds of 80% methanol extract of *Hyoscyamus muticus,* aerial parts.

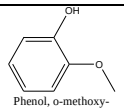
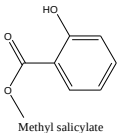
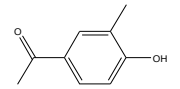
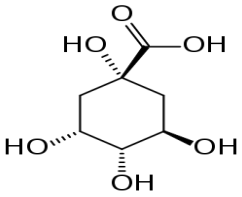
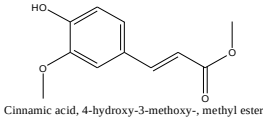
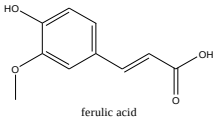
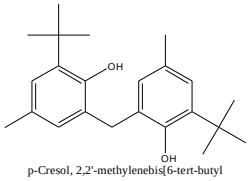
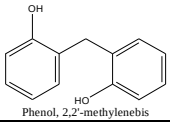
Compound	Rt	Structure	Chemical formula	Amount (%)
Phenol, o-methoxy- (Guaiacol)	1090	 Phenol, o-methoxy-	$C_7H_8O_2$	0.20
Methyl salicylate	1281	 Methyl salicylate	$C_8H_8O_3$	0.03
4'-Hydroxy-3'-methylacetophenone	1363	 4'-Hydroxy-3'-methylacetophenone	$C_9H_{10}O_2$	0.04
D-(-)-Quinic acid	1852		$C_7H_{12}O_6$	0.02
Cinnamic acid, 4-hydroxy-3-methoxy-, methyl ester (Methyl isoferulat)	1677	 Cinnamic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_{11}H_{12}O_4$	0.52
Ferulic acid	1644	 ferulic acid	$C_{10}H_{10}O_4$	0.36
p-Cresol, 2,2'-methylenebis[6-tert-butyl	2788	 p-Cresol, 2,2'-methylenebis[6-tert-butyl	$C_{23}H_{32}O_2$	0.05
Phenol, 2,2'-methylenebis	2890	 Phenol, 2,2'-methylenebis	$C_{13}H_{12}O_2$	0.03

Table 3. The antioxidant capacity of methanol extract of *Hyoscyamus muticus*.

Antioxidant capacity (mg/ml)	Methanol extract	Ascorbic acid	Quercetin
IC_{50}	8.1 ± 0.65^a	0.031 ± 0.001^b	0.012 ± 0.002^c
EC_{50}	12.74 ± 1.12^a	0.095 ± 0.002^b	0.019 ± 0.003^c

Data are averages (± S.E.). Different letters stand for statistically significant differences between the results of each test at $P<0.05$ (Student T-test).

Table 4. The antimicrobial activity of the methanol extract of *Hyoscyamus muticus* areal parts against different microorganisms.

Tested compound	Mean zone of inhibition (mm)*										
	Gram-positive bacteria				Gram-negative bacteria					Fungi	
	Sa1	Sa2	Se	Bc	Ec	Pa	Ab	Kp1	Kp2	As	Ca
MeOH of *H. muticus*	10.5±0.5	13.0±1.0	11.75±0.25	11.5±0. 5	8.5±0.5	12.0±0.0	11.5±0.5	7.75±0.25	6.5±0.5	6.0±0.0	6.0±0.0
Erythromycin	18.5±0.5	25.5±0.5	31.0±1.0	29.5±0.5	12.5±1.5	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	-	-
Clotrimazole	-	-	-	-	-	-	-	-	-	19.0±1.0	27.5±0.5
10% DMSO	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

*6.0 mm one of inhibition = no activity, Sa1= *Staphylococccus aureus* clinical isolate, Sa2=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 49461, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 35218, Pa=*Pseudomonas aeruginosa* clinical isolate, Ab=*Acinitobacter baumannii* clinical isolate, Kp1=*Klebsiella pneumoniae* ATCC 27736, Kp2=*Klebsiella pneumoniae* ATCC 700603, As=*Aspergillus niger* ATCC 6275, Ca=*Candida albicans* ATCC 10231

Mathew and Abraham, 2004). Our results disagree with the findings of Hajipoor et al. (2015), they reported that the antioxidant activity of *Hyoscyamus niger* collected from Iran has an EC_{50} of 377 ± 1.21 µg/ml, this differences may be related to the chemical composition, environmental conditions and/or the physiological system of this species.

The results of the antimicrobial testing are represented in Table 4 and Figures 1 to 4. Among all tested microorganisms, the gram-positive bacteria exhibited higher susceptibility towards the methanol extract of *H. muticus* areal parts which recorded 13.0 ± 1.0 mm for *S. aureus* ATCC 25923, 11.75 ± 0.25 mm for *S. epidermidis* ATCC 49461, 11.5 ± 0.5 mm for *B. cereus* ATCC 10876 and 10.5 ± 0.5 mm for *S. aureus* clinical isolate, respectively (Figure 1). However, all results of the gram-positive bacteria showed that they were most sensitive to the antibiotic (Erythromycin 15 µg/disc). 10% DMSO has no inhibitory effect on the growth of the gram-positive bacteria. The gram-negative bacteria showed average or weak antibacterial susceptibility towards the methanol extract of *H. muticus* areal parts. This recorded 12.0 ± 0.0 mm for *Pseudomonas aeruginosa* clinical isolate, 11.5 ± 0.5 mm for *Acinetobacter baumannii* clinical isolate, 8.5 ± 0.5 mm for *Escherichia coli* ATCC 35218, 7.75 ± 0.25 mm for *Klebsiella pneumonia* ATCC 27736, and 6.5 ± 0.5 mm for *Klebsiella pneumonia* ATCC 700603, respectively (Figure 2). However, the tested antibiotic (Erythromycin 15 µg/disc) showed weak or no activity against the gram-negative bacteria. Moreover, there was no statistical significance between the susceptibility of different strains from the same bacterial species (*K. pneumonia* and *S. aureus*). However, the clinical isolate of *S. aureus* was more resistant to erythromycin compared to *S. aureus* ATCC 25923 (Table 4 and Figures 1 and 2). Regarding the antifungal potential, neither *Aspergillus niger* ATCC 6275 nor *Candida albicans* ATCC 10231 revealed any susceptibility against the methanol extract of *H. muticus* areal parts, concluding that the studied plant extract has no inhibitory effect on the tested fungal strains, compared with clotrimazole 10 mg/ml (Figure 3).

Based on the above-mentioned results, the methanolic extract of *H. muticus* areal parts showed average or weak antibacterial activity against the gram-positive bacteria, weak antibacterial activity against the gram-negative bacteria and no antifungal activity. Moreover, in the current study, the gram-positive bacteria were more susceptible than the gram negative bacteria, which are attributed to the structure of the cell wall layers. In general, since the studies on antimicrobial activities of the areal parts of *H. muticus* are scanty, it would be valuable to compare our results on *H. muticus* with other available reports on different *Hyoscyamus* spp., which surprisingly showed that findings of the current study are generally in harmony with some previous studies on varied *Hyoscyamus* spp. The ethanol extract of *H. albus* showed no inhibitory effect on different bacterial strains; however, it was published that the alkaloid fraction revealed some degrees of antibacterial effects that ranged from 14.0 to 7.0 mm zone of inhibition (Kadi et al., 2013). Methanol extracts of stem, leaves and seeds of *H. niger* were investigated for antibacterial properties against some gram-positive and gram-negative bacteria, seeds showed antibacterial effects much better than leaves and stem (Snigh and Pandey, 2009). Accordingly, it is recommended to investigate the antibacterial potential of the seeds of *H. muticus*. The findings of Almalki (2017) supports our

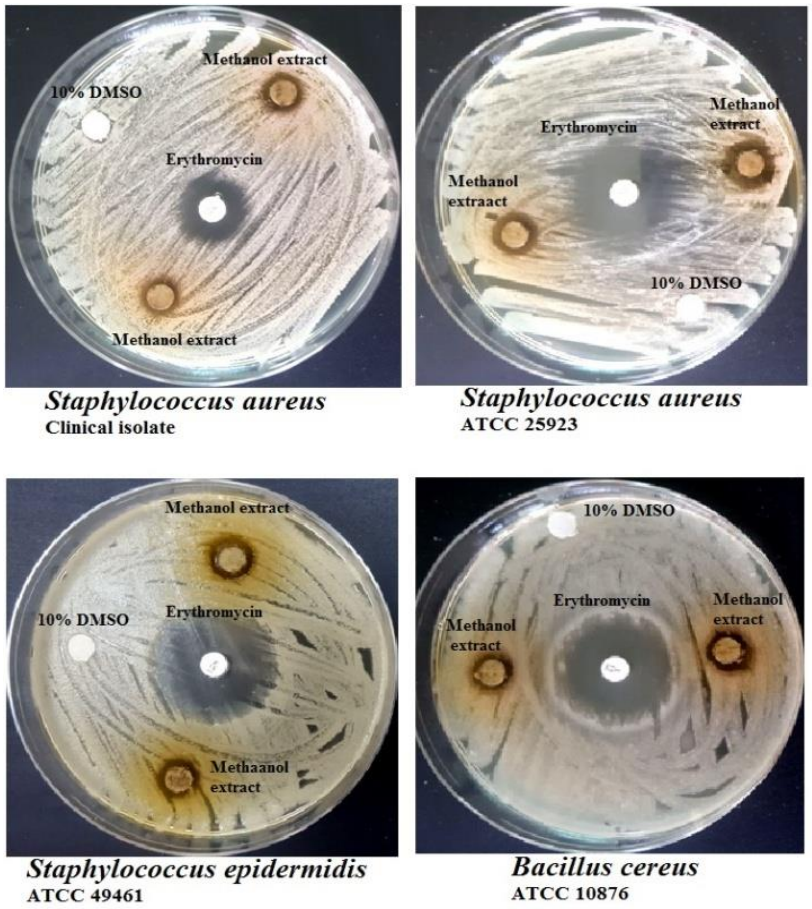


Figure 1. Susceptibility of gram-positive bacteria to methanol extract of *Hyoscyamus muticus* compared to erythromycin

recommendation, he studied the antibacterial and antifungal activity of *H. muticus* among other plants; seeds of *H. muticus* showed varying degrees of antibacterial and antifungal activities. On the other hand, in the current study, the absence of antifungal activity in H. muticus extract is a reasonable result (Figures 3 and 4). This is because; it was found that in nature, many fungal species, including endophytic fungi reside in H. muticus (El-Zayat et al., 2008). The results of the antifeedant potential of the methanol extract of *H. muticus* are tabulated in Table 5, showing that the methanolic extract of *H.* muticus exhibited antifeedant effect on the 4th instar larvae of *S. littoralis*. The antifeedant activity ranged from 86.38, 78.77, 73.81 to 73.47% at concentrations 40, 20, 10 and 5%, respectively. It was observed that the antifeedant activity increased with time in all concentrations after treatment. Data in Table 6 shows the starvation percentage of the 4th instar larvae of *S. littoralis* treated with the methanolic extract of *H. muticus*. The starvation percentage as well as the antifeedant activity increased with increasing concentration and time of exposure. Moreover, the average of the starvation percentage ranged from 98.50 to 87.18% at high concentration, whereas at lower concentration, the repellence effect ranged from 78.74 to 73.79%. This can be explained based on the phytochemical analysis of *H. muticus* which showed that this plant has special alkaloid compounds such as hyoscyamine and scopolamine that are anti-cholinergic and anti-spasmodic drugs. Moreover, this plant has anti-spasmodic, analgesic and sedative properties (Alaghemand et al., 2013). In literature, it was reported that *H. niger* was used to control the larvae of Anopheles (Mahmoodreza et al., 2017). Various botanical extracts contain a complex of chemicals with a unique biological activity (Farnsworth and Bingel, 1977). Finally, the current findings can further illustrate the perspective to control larvae of *S. littoralis* without imposing environmental damage.

Conclusion

Medicinal plants are rich sources of bioactive compounds

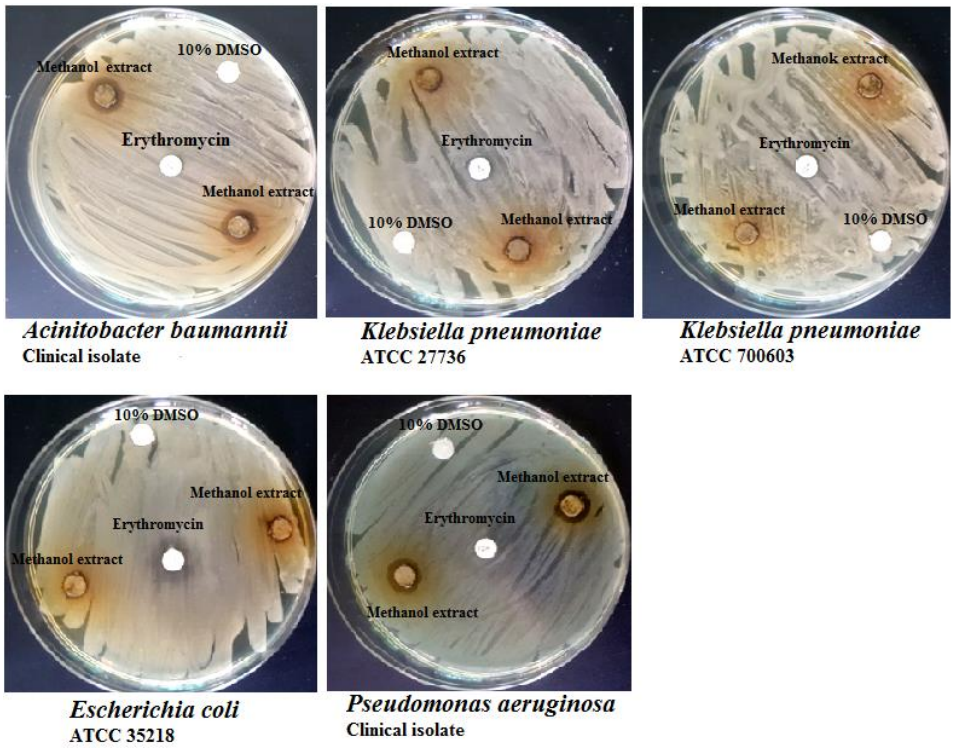
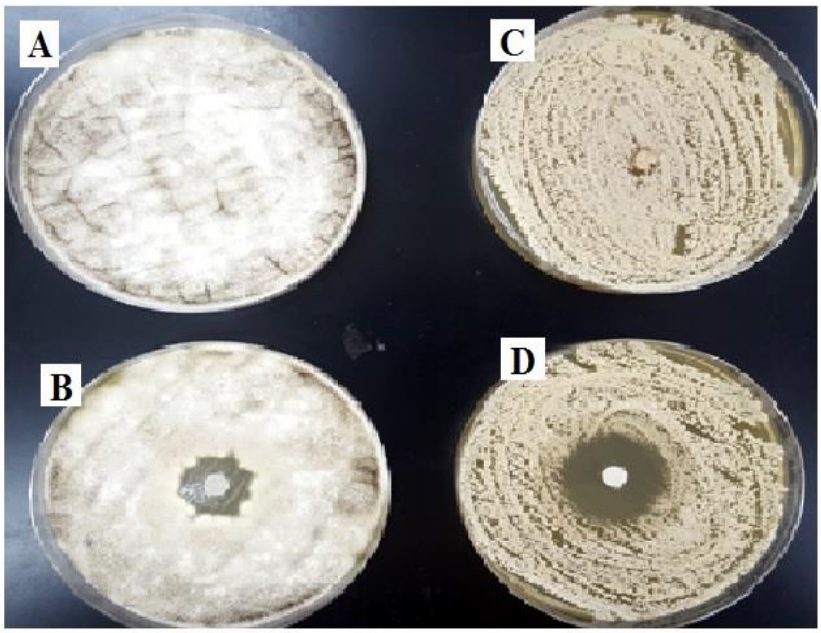


Figure 2. Susceptibility of gram-negative bacteria to methanol extract of *Hyoscyamus muticus* compared to erythromycin.



A= *Aspergillus niger* + the extract disc in the middle (No inhibition zone)
B= *Aspergillus niger* + the clotrimazole disc (Obvious inhibition zone)
C= *Candida albicans* + the extract disc in the middle (No inhibition zone)
D= *Candida albicans* + the clotrimazole disc (Obvious inhibition zone)

Figure 3. Susceptibility of some fungal strains to methanol extract of *Hyoscyamus muticus* compared to clotrimazole

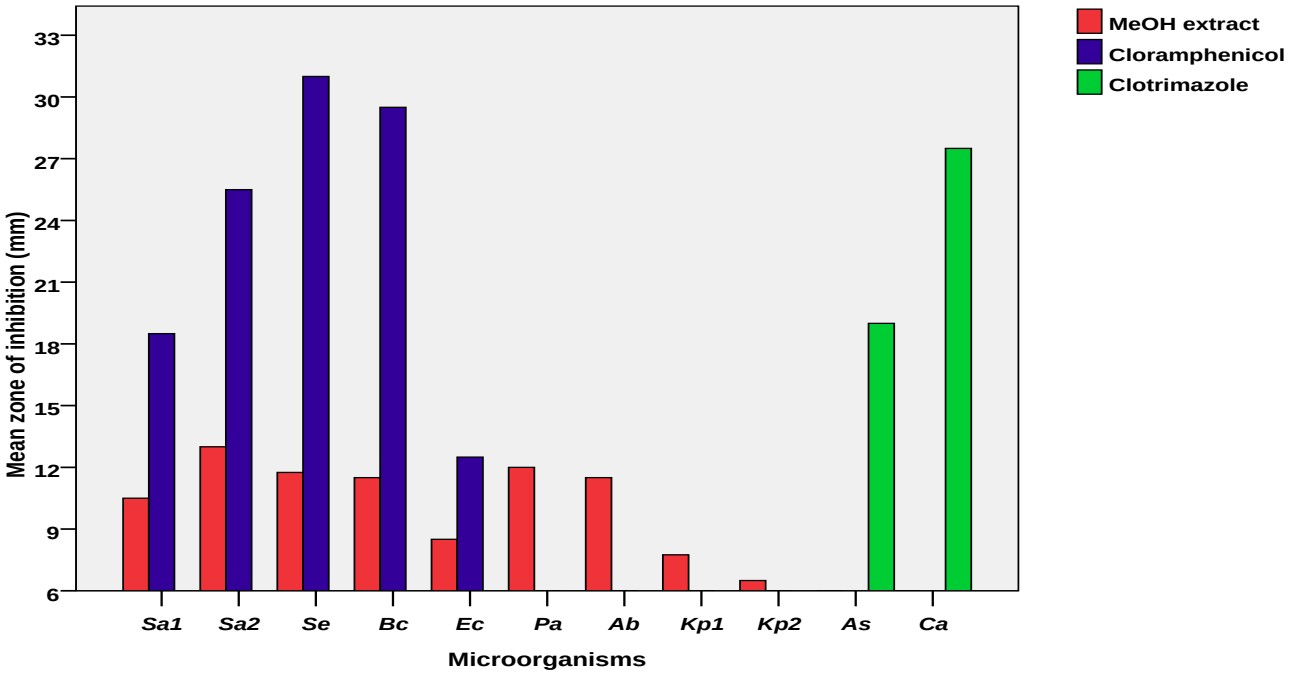


Figure 4. Mean zone of inhibitions of different microorganisms due to the effect methanol extract of *Hyoscyamus muticus* compared with antimicrobial drugs.

Table 5. Antifeedant activity of the methanolic extract of *Hyoscyamus muticus* against 4th instar larvae of *S. littoralis*.

Conc. (%)	Antifeedant index (%) post-treatment			Mean*
	1st day	2nd day	3rd day	
5	62.16	69.68	88.57	73.47
10	64.58	70.87	85.99	73.81
20	76.20	74.32	85.79	78.77
40	83.65	89.04	86.45	86.38

Data are expressed as mean ± SE (n=5), * total mean of each treatment at different time intervals, values were analyzed by one-way ANOVA, where means within each column followed by different letters are significantly different (P< 0.05 by LSD).

Table 6. Starvation percentage (%) of the 4th instar larvae of *S. littoralis* treated with the methanolic extract of *Hyoscyamus muticus.*

Treatment	Time	Average weight (mg/larva)	Difference* (mg/larva)	Starvation (%)	Average
5%	0 min	56.50	-	-	73.79%
	24 h	63.40	+6.90	65.20	
	48 h	65.60	+9.10	71.91	
	72 h	67.90	+11.4	84.28	
10%	0 min	56.01	-	-	78.74%
	24 h	59.60	+3.60	74.62	
	48 h	62.30	+6.30	75.90	
	72 h	65.01	+9.00	85.70	

Table 6, Contd.

20%	0 min	60.20	-	-	87.18%
	24 h	61.40	+1.20	81.45	
	48 h	59.01	-1.20	86.58	
	72 h	56.00	-1.40	93.52	
40%	0 min	59.02	-	-	98.50%
	24 h	54.01	-5.01	99.14	
	48 h	50.02	-9.01	97.69	
	72 h	46.12	-12.9	98.67	
Control	0 min	61.10	-	-	-
	24 h	90.09	+29.8	-	
	48 h	120.7	+59.6	-	
	72 h	214.8	+153.7	-	
Starved larvae	0 min	67.90	-	-	-
	24 h	65.60	-5.310	-	
	48 h	63.40	-10.62	-	
	72 h	56.50	-15.14	-	

with varied effects on human, animals, plants, insects and microorganism. Plants that survive under environmental stresses could produce important unique phytochemicals. *H. muticus* revealed the presence of phenolic compounds, flavonoids, tannins and sterols. The methanolic extract was found to be rich in phenolic compounds, ferulic acid, 4-hydroxy–cinamic acid–ester, methyl salicylate and methyl ferulate; these compounds support the antioxidative properties of the plant againstoxidative stress, as seen in the antioxidant evaluation. The antimicrobial investigation exhibited moderate or weak antibacterial effects, perhaps these moderate efficacy particularly against the gram-positive bacteria, which are enough to control these bacteria prevalent in soil, or may have some sort of associations with this plant. The absence of antifungal compounds may allow the endophytic fungi to grow in/on the tissues of *H. muticus*. Moreover, this plant protects itself from insects by means of some antifeedant compounds as revealed from the findings of present study. Accordingly, the results generated from the current study provides the bases for further future investigations to isolate some important compounds of bioactive properties which could be useful for biocontrol and pharmaceutical industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Production of rice cereal-based Kefir beverage

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The aim of the present research was to evaluate the use of the sugary Kefir grains as a starter culture for new rice cereal-based Kefir beverage. Fermentation was performed by inoculating Kefir grains in rice extract. Flasks containing Kefir grains and substrates were statically incubated at 28°C for 24 h. The microbiota of sugary Kefir grains and rice cereal-based Kefir beverage were genera *Lactobacillus, Lactococcus* and *Acetobacter* as well as yeasts, such as *Saccharomyces, Kluyveromyces, Lachancea* and *Kazachstania*. The sugary Kefir grains were able to ferment the rice extract and produced Kefir beverage that are functional and healthy that satisfy nutrition-related conditions such as allergies and malabsorption, food intolerances, and lifestyle choices, for example vegetarianism and low salt. The use of starter cultures as Kefir grains offers a promising tool for innovation and diversification of cereal-based beverages. This study was the first to report the rice cereal-based Kefir beverage production. This result opens up perspectives for this innovative application of sugary Kefir grains for developing cereal-based beverages.

Key words: Probiotic, fermentation, food intolerances, vegetarianism.

INTRODUCTION

The use of cereals grains around 10,000 B.C. led to cereals becoming source of nutrients for population throughout the world. Cereals grains are a source of dietary fibers and carbohydrate and provide nutrients such as vitamins and minerals and low-gluten or gluten-free. Soy-based food and beverages, cereals, fruits, and vegetables have been considered as ingredients for functional food/beverages that satisfy dietary lifestyles such as allergen-free and veganism (Peyer et al., 2016; Fiorda et al., 2017). On a worldwide basis, rice is the prevailing crops in terms of area reserved for cereal cultivation and total cereal production. In Segundo, the world rice production in 2017 was 756.7 million tonnes. However, cereals, such as quinoa and buckwheat, have

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generated interest in western countries, because of their higher content in dietary fiber, starch, vitamins and minerals (Peyer et al., 2016).

Kefir is a traditional Middle Eastern beverage. It originated in the Caucasus in Asia in thousands of years (Magalhães et al., 2010a; Nalbantoglu et al., 2014; Fiorda et al., 2017). Kefir is a symbiosis between yeast and bacteria. A vast variety of different species of microorganisms formed the Kefir grains (Miguel et al., 2011; Nalbantoglu et al., 2014; Viana et al., 2017; Magalhães-Guedes et al., 2017; Fiorda et al., 2017; Roos and Vuyst, 2018). *Lactobacillus* genera are the most frequent in kefir. Other lactic bacteria including *Lactococcus* and *Leuconostoc* genera are also common in Kefir (Nalbantoglu et al., 2014; Magalhães-Guedes et al., 2017; Fiorda et al., 2017; Roos and Vuyst, 2018). *Acetobacter* genera represent acetic bacteria and the yeast isolates are *Kluyveromyces, Candida* and *Saccharomyces* genera (Leite et al., 2013; Marsh et al., 2013; Viana et al., 2017).

The microbial species from Kefir grains carried out three types of fermentation during the process: lactic, alcoholic and acetic. Kefir can easily adapt to different substrates and lead to production of new probiotic products (Nalbantoglu et al., 2014; Fiorda et al., 2016; Fiorda et al., 2017). “Traditional” way of Kefir beverage production is using pasteurized or Ultra-high-temperature (UHT) processing treated milk. Kefir beverage is mainly considered a probiotic resource (Leite et al., 2013). Kefir may help bridge the gap between the health benefits and consumption of non-dairy foods and provide the benefits of probiotic without milk or diary product consumption (Leite et al., 2013; Garofalo et al., 2015; Fiorda et al., 2016).

Due to the numerous positive effects of Kefir on the human health, alternative substrates others may be used for kefir grains fermentation. Names of the resulting beverages are changed in case additional fruit, molasses or vegetable are used as medium of fermentation (Magalhães et al., 2010a,b; Miguel et al., 2011; Nalbantoglu et al., 2014; Fiorda et al., 2016, 2017). The adaptation of Kefir grains into different substrates has shown potential for production of Kefir beverages with distinct sensory characteristics and functional proprieties. For all the aforementioned, the objective of this study was the use of Kefir grains as starter culture for fermented rice cereal-based beverage.

MATERIALS AND METHODS

The raw materials used were polished rice type 1 (*Oryza sativa*) and brown sugar, from the city of Salvador, Bahia. The sugary Kefir grains were obtained from the Probiotics Laboratory of the Federal University of Bahia, UFBA.

Preparation of rice extract

The rice extract was elaborated based on the methodology proposed by Peyer et al. (2016). The Kefir grains were initially washed in water to reduce or eliminate soil contamination. In a stainless, the beans were cooked in the ratio of 1 part grain to 2 part water (1: 2 w/w), for 30 min. The product was filtered, drained and homogenized.

Experimentation and chemical analysis

Samples (in triplicate), control and rice extract, were inoculated with sugary Kefir grains following the traditional method (Figure 1). The control (C) and test (T) samples were 1000 mL of filtered 5% sugary water and rice extract, respectively. The proportion of 10% sugary Kefir grains was added in sterile glass containers (5% sugary water and rice extract). The fermentation time of the samples corresponded to 24 h/28°C. pH, acidity, and Kefir grains weight mass, were performed in 0 and 24 h. pH was determined using a Neomed model pH meter. Titratable acidity (in lactic acid) and mass weight (Shimadzu) were done according to the rules of the Adolfo Lutz Institute (2008).

DNA extraction and PCR-DGGE analysis

The sugary Kefir (grains and rice cereal-based Kefir beverage) microbiological analysis was carried out at the Molecular Biology Laboratory of the Federal University of Lavras, UFLA, Brazil. For analysis, 1 g of sugary Kefir grains and 1 mL of rice cereal-based Kefir beverage sample were transferred into a plastic tube and was subjected to DNA extraction using a Nucle-oSpin Tissue kit (Macherey-Nagel, Du ¨ren, Germany). DNA extraction was performed according to the manufacturing instructions. The bacterial community DNA was amplified with primers 338fgc and 518r spanning the V3 region of the 16S rDNA gene (Puerari et al., 2015). The yeast community DNA was amplified using the primers NS3 and YM951r (Magalhães et al., 2010b). The amplification was carried according to the method of Magalhães et al. (2010b). Polymerase chain reaction (PCR) products were analysed by denaturing gradient gel electrophoresis (DGGE) using a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad, Richmond, CA, USA). Samples were applied to 8% (w/v) polyacrylamide gels in 0.5% TAE. Optimal separation was achieved with a 15 to 55% urea-formamide denaturing gradient for the bacterial community and a 12 to 50% gradient for the yeast community, where 100% is defined as 7 M urea and 40% (v/v) formamide. Electrophoresis was carried out for 3 h at 200 V at 60°C, and the gels were stained with SYBR-Green I (Molecular Probes, Eugene, OR, USA) (1:10,000 v/v) for 30 min. The gels were visualised via U. V. transilluminator, and images were captured using a Polaroid camera (Concord, USA). The bands were excised with a sterile surgical blade and stored at -20°C until further analysis.

DGGE bands were excised from the acrylamide gels and the fragments were purified using the QIAEX II gel extraction kit (Qiagen, Chatsworth, CA, USA). DNA recovered from each DGGE band was reamplified using the primers 338f (without GC clamp) and 518r for bacteria and NS3 (without GC clamp) and YM951r for yeast. The PCR amplicons were then sequenced (Applied Biosystems, Foster City, CA, USA). GenBank searches (http://www.ncbi.nlm.nih.gov/BLAST/) were performed to determine the closest known relatives of the partial ribosomal DNA sequences obtained.

Statistical analysis

In order to obtain the means of the control and test samples, Statistical package for Social Science (SPSS) statistical software, version 20.0 was used. Student's t test, using the 95% confidence level, was also used.

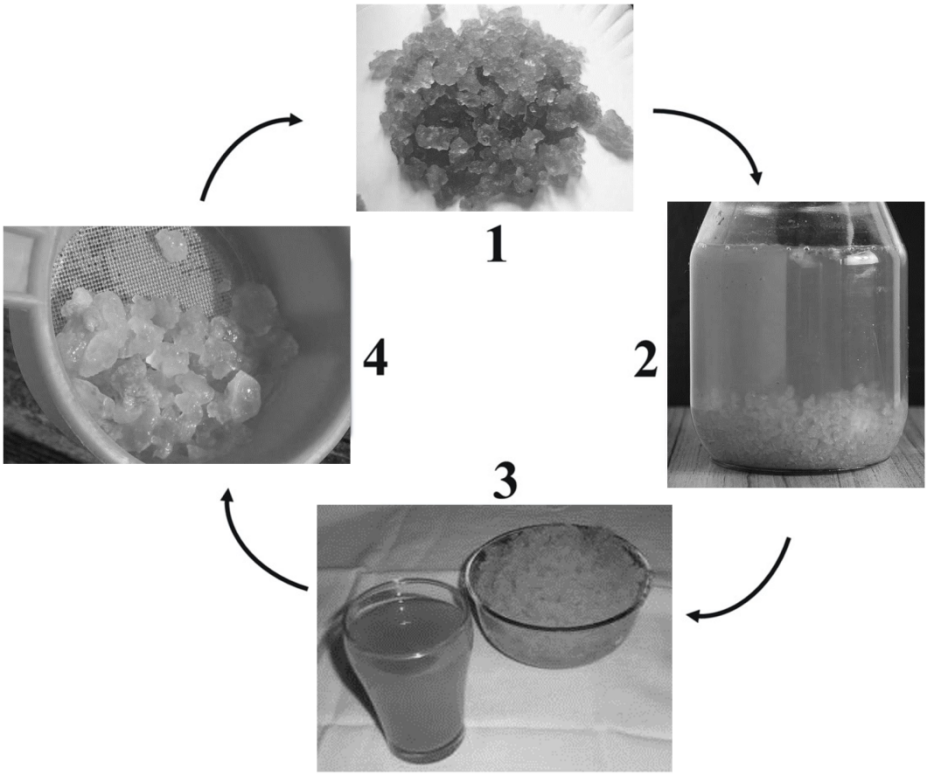


Figure 1. Kefir beverage production. Kefir grains (1) are added to substrate and are left to stand at room temperature for fermentation 24 h; (2) substrate is then fermented forming the Kefir beverage; (3) after which they are filtered, and (4) ready to start another cycle. The fermented beverage that results from step 3 is appropriate for consumption.

Table 1. Kefir grains weight in 24 h fermentation period.

Kefir grains weight (g)	Time (h)	
	0	24
C	$5.00^{a} \pm 0.01$	$10.2^{b} \pm 0.20$
T	$5.00^{a} \pm 0.01$	$15.1^{c} \pm 0.15$

C, Control; T, test. Values represented by samples means ± standard deviation in triplicates. Different letters in same column indicate differences between ($p<0.05$) values in the group, according to the Student's t-test.

RESULTS AND DISCUSSION

After 24 h fermentation period, a significant difference ($p<0.05$) was observed between the Kefir grains weight of the control (C) and test (T) samples (Table 1). The cell mass (T) growth was higher in relation to the (C) cultures (Table 1). According to Magalhães et al. (2010a, b), the amount of Kefiran (polysaccharide) released during the fermentation depends on the microorganisms involved, the culture medium composition, the temperature and the fermentation time. Probably, the growth of the (T) samples is related to the greater availability of nutrients present in the rice extract.

The decreased pH value (~4.3 to ~3.5) and the increased acidity (~0.2 to ~0.3) during 24 h of fermentation are shown in Table 2. These observations indicated that the fermentation process was followed by the production of acids. This also demonstrates that the sugary Kefir grains were able to ferment the rice extract. The low pH value (~3.5) and high acidity value (~0.3) present at the end of fermentation appears to be responsible for the presence of lactic acid bacteria as the major bacterial species at 24 h (Table 2).

Traditionally, many plating procedures are only partially

Table 2. pH and acidity analysis in Kefir beverages.

pH	Time (h)	
	0	24
C	$4.30^{a} \pm 0.01$	$3.58^{b} \pm 0.10$
T	$4.35^{a} \pm 0.01$	$3.88^{b} \pm 0.15$
Acidity		
C	$0.20^{c} \pm 0.01$	$029.2^{d} \pm 0.20$
T	$0.20^{c} \pm 0.01$	$0.38^{d} \pm 0.15$

C, Control; T, test. Values represented by samples means ± Standard Deviation in triplicates. Different letters in same column indicate differences between ($p<0.05$) values in the group, according to the Student's t-test.

selective and exclude members of the microbial community (Miguel et al., 2011; Nalbantoglu et al., 2014; Corona et al., 2016; Fiorda et al., 2017; Cho et al., 2018; Roos and Vuyst, 2018). Thus, to determinate the total composition of microbiota in the sugary Kefir grains and rice cereal-based Kefir beverage, PCR-DGGE analysis was used. The V3 region of the 16S rDNA gene of the bacteria and NS3 region of the 18S rDNA gene of the yeast were amplified and representative DGGE fingerprints are as shown in Figure 2. To determine the composition of microbiota, individual bands observed in the PCR-DGGE profiles were excised from the acrylamide gel and re-amplified to provide a template for sequencing. After BLAST analysis, sequence results showed between 99 and 100% identity with the sequences retrieved from GenBank accession numbers. DGGE bands a and d were clearly identified as *Lactobacillus paracasei, Lactobacillus parabuchneri, Lactobacillus kefiri, Lactococcus lactis, Lactobacillus casei, L. paracasei* subsp. *paracasei, Leuconostoc citreum, L. paracasei* subsp. *tolerans, Lactobacillus buchneri, Acetobacter lovaniensis, Saccharomyces cerevisiae, Kluyveromyces lactis, Lachancea meyersii* and *Kazachstania aerobia.*

Descriptions of the different types of yeast and bacteria present in different Kefir grains have been provided by different authors (Jianzhong et al., 2009; Magalhães et al., 2010a; Miguel et al., 2011; Nalbantoglu et al., 2014; Viana et al., 2017; Corona et al., 2016; Fiorda et al., 2017; Cho et al., 2018). Previous results have shown that three groups of microorganisms co-exist in Kefir grains: lactic acid bacteria, acetic acid bacteria and yeast (Jianzhong et al., 2009; Miguel et al., 2011; Nalbantoglu et al., 2014; Viana et al., 2017; Corona et al., 2016; Fiorda et al., 2017; Cho et al., 2018; Roos and Vuyst, 2018).

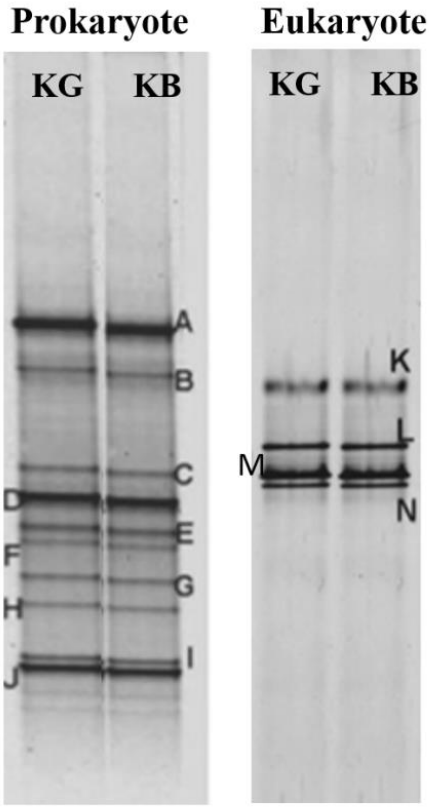
This data indicated that the sugary Kefir grains contained a diverse spectrum lactic acid bacteria group including *Lactobacillus, Lactococcus* and *Leuconostoc*. Another important bacterium found in sugary Kefir grains is *L. kefiri*. There are reports on the presence of *L. kefiri* as a member of the lactic acid microbiota in Kefir grains (Garrote et al., 2001; Jianzhong et al., 2009; Corona et al., 2016; Fiorda et al., 2017; Cho et al., 2018; Roos and Vuyst, 2018). The following lactic acid bacteria were also found: *L. parabuchneri, L. lactis* and *L. casei*, besides the species of *L. citreum*. Previous studies showed that a variety of lactic acid bacteria's different species have been isolated and identified in Kefir grains from around the world (Jianzhong et al., 2009; Magalhães et al., 2010a; Miguel et al., 2011; Nalbantoglu et al., 2014, Corona et al., 2016; Fiorda et al., 2017; Cho et al., 2018; Roos and Vuyst, 2018). The acetic acid species, *A. lovaniensis*, was also identified. The species *Acetobacter pasteurianus* has been also described in fermented Kefir beverages (Corona et al., 2016; Viana et al., 2017; Fiorda et al., 2017; Cho et al., 2018).

The lactose-fermenting yeast, *K. lactis* was identified in the sugary Kefir grains together with non-lactose-fermenting yeast *S. cerevisiae, K. aerobia* and *L. meyersii* (Figure 2). Of these yeasts, *S. cerevisiae* represented the most commonly identified yeast isolates in Kefir grains. The presence of *S. cerevisiae* contributes to the organoleptic quality enhancement of the Kefir beverages, promoting a refreshing and pungent taste (Cho et al., 2018).

The results demonstrated that rice extract could be an ideal alternative substrate for the production of functional cultured Kefir beverage, especially for vegans and lactose intolerant consumers, because the microorganisms of the Kefir grains successfully fermented the rice extract.

Conclusion

The results indicate that genera of bacteria, such as *Lactobacillus, Lactococcus* and *Acetobacter*, as well as yeast, such as *Saccharomyces, Kluyveromyces, Lachancea* and *Kazachstania* were the microorganisms



	Closest relative	Identity (%)	Accession number
	Bacteria		
A	*Lactobacillus paracasei*	98	AB368902.1
B	*Lactobacillus parabuchneri*	99	AB368914.1
C	*Lactobacillus kefiri*	99	AB362680.1
D	*Lactococcus lactis*	99	EU194346.1
E	*Lactobacillus casei*	99	EU626005.1
F	*Lactobacillus paracasei* subsp. *paracasei*	99	NR025880.1
G	*Leuconostoc citreum*	99	FJ378896.1
H	*Lactobacillus paracasei* subsp. *tolerans*	98	AB181950.1
I	*Lactobacillus buchneri*	99	FJ867641.1
J	*Acetobacter lovaniensis*	98	AB308060.1
	Total		
	Yeast		
K	*Saccharomyces cerevisiae*	100	EU649673.1
L	*Kluyveromyces lactis*	99	AJ229069.1
M	*Lachancea meyersii*	99	AY645661.1
N	*Kazachstania aerobia*	99	AY582126.1
	Total		

Figure 2. Profiles of microbial communities from Kefir grains and cereal-based Kefir beverage.

present in sugary Kefir grains and rice cereal-based Kefir beverage. The sugary Kefir grains were able to ferment the rice extract and produce Kefir beverage functional and healthy that satisfy nutrition-related conditions such as allergies and malabsorption, food intolerances, and lifestyle choices, for example vegetarianism and low salt. This study is the first to report the rice cereal-based Kefir beverage production. The use of starter cultures as Kefir grains offers a promising tool for innovation and diversification of cereal-based beverages.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Agronomic evaluation and web blight resilience of common bean genotypes in the littoral region of Ecuador

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Common bean is very important in the daily diet of Ecuadorians. Web blight (*Rhizoctonia solani* (Kuhn) is the major constraint faced by common bean farmers in Ecuador and everywhere in the world, causing high losses of yield grain every year. A constant selection of genotypes with excellent productive and resilience to disease features in each region is presented as a potential solution to these problems. The present study aimed to evaluate the phenology, web blight resilience and grain yield production of 17 common bean genotypes in field conditions, in the Guayas River Basin, Ecuador. Three commercial varieties (two belonging to determined feature and one to undetermined feature) and 14 more promising genotypes were evaluated. Day period for phenological stage R_6 (full bloom) and R_9 (flowering maturity), web blight resilience, number of harvested pods, number of grains per pod per plant, 1000-seed weight, grain yield and later yield per hectare were measured. Scott Knott test ($p \leq 0.05$) was executed for mean comparisons and principal component analysis (PCA) statistical test was performed using the productive and agronomical variables, successfully distinguishing two group of genotypes according to their types of growth (determined and undetermined), highlighting the genotypes EVG-6-103, EVG-6, CAL-96, INIAP-473, AFR-619, INIAP-474, AFR-298, SER-29, SER-35, EVG-16-08 which showed the higher agronomic, sanitary, and productively averages in approximately all the studied variables.

Key words: *Phaseolus vulgaris* L., *Rhizoctonia solani* (Kuhn), yield.

INTRODUCTION

Factors such as increase of crop yield, lessening production costs, preserving the health of farmers by avoiding the use of chemicals on fields for pest and diseases control have been considered by the agronomists nowadays as parts of the programs for plant protection and breeding (Muñoz-Rengifo et al., 2014; Villamar-Torres et al., 2016; Martinez et al., 2017).. Common bean *(Phaseolus vulgaris* L.) in Ecuador is consumed owing to its nutritional value and accessibility, contributing as a main constituent of the primary diet of the Ecuadorian population. Many advantages are known about the consumption of common beans. Among them, one is providing important nutrients for a correct alimentation for humans as well as for animals, because it contains high proportion of proteins, vitamins, and minerals (Petry et al., 2015). On the other hand, common bean plants are also known to fix the atmospheric nitrogen and improve the soil fertility, hence contributing to the output of other crops (Rondon et al., 2007). An overview of five years' data from 2011 to 2015 on the production of common bean in Ecuador indicated that 311,147 ha in all parts of the Ecuadorian territory were harvested approximately, from which 174,964 ha were of dry bean and 136,183 ha of fresh bean, representing 56.23 and 43.77% of national production, respectively. Likewise, 59,139 ton of dry bean and 87,487 ton of fresh bean have been reported for this period. The average nationwide harvested area in 2012 was 63,487 ha, corresponding to 52.7% of dry bean and 47.3% to fresh bean, with a yield of 0.28 t ha^{-1} for dry bean and 0.53 t ha^{-1} for fresh bean respectively. A decline of 36% (~35,258 ha) in the sowing area respecting the year 2011 was observed. However, a higher yield percentage (15 and 10%, respectively) in 2012 has been noted.

Geographical distribution is given around the central area of the Ecuadorian coast, which is one of the most suitable zones for productive agricultural development of this leguminous plant, highlighting the cantons of: Milagro, Naranjito and Pedro Carbo in the province of Guayas; Babahoyo, Vinces and Quevedo in the province of Los Rios (Godoy-Montiel et al., 2011). This makes it one of the most representative areas for planting this valuable legume, although there is also an important representation in the meso-thermal high mountain valleys of the country (Falconí-Castillo, 2005; Torres-Navarrete et al., 2013). In the province of Los Rios and its influence area (neighboring cantons of the provinces of Guayas, Manabi, Bolivar and Azuay), mostly recommended materials for other areas of the country (Guayas, Manabi or Imbabura), recycled seeds usually from the informal market are used especially by small farmers. Habitually, the producer does not obtain a germination rate above 80%, reflecting in a low crop production and less profitability for the producer. This crop is also suffering from a pathogen, namely necrotic fungus *Rhizoctonia solani* (Kuhn), causing the leaf disease named web blight. Web blight disease, impacting yield (Costa-Coelho et al., 2014; Rodríguez et al., 2015) and many plant features (number of nodes, number of pods and number of seeds per plant), is consequently considered as the main fungal disease, representing big losses for the small farmers in Latin America (Godoy-Lutz et al., 2008; Rodríguez et al., 2015), Ecuador and around of the Province of Los Rios (Garcés-Fiallos et al., 2013). The direction of Research, Science, and Technology (DICYT) of the Technical State University of Quevedo (UTEQ) has been working to obtain common bean varieties with ideal productive and sanitary features for the central coastal zone of Ecuador. Nowadays, it has led to obtaining the first and promising genotypes with determined and undetermined growth habit, that some of them were studied in this work. Consequently, based on the need to continue this study of common bean genotypes, the aim of this work was to evaluate the phenology, web blight resilience and grain yield production of common bean genotypes in Ecuador field conditions.

MATERIALS AND METHODS

Trial management

This experiment was carried out during the dry season (summer) of 2012 at the Experimental Farm of “La María”, UTEQ, Quevedo - Ecuador whose geographical coordinates are Western longitude 79° 27´ 42´´ and Southern latitude 01° 06´ 0´´. The conditions of the study site are as follow: moist forest – tropical climate zone, average temperature of 24.2°C, and relative humidity of 77.4%, total sunlight of 823 h/light/year, and annual precipitation of 1537 mm. The ground topography is flat with a clay-loamy soil texture and pH of 5.7. Climatic conditions during the experimental time when the crop was established maintained normal and suitable for agronomic and productive development of common beans. The temperature throughout the experiment had an average of 26.04°C, with accumulative sunlight and rainfall of 86.4 h and 56.22 mm respectively (Table 1).

With respect to the soil preparation, an only-pass plow and two dredges were performed three days before planting in order to prepare the experimental site. Before sowing, the seeds were previously disinfected by a dose of 1 g per 1 kg seed of a mixture of Carboxin and Captan (active ingredient). Subsequently, the seeds were planted manually using a handspike, placing two seeds per hole and after 12 days proceeded with the thinning. The spacing used between rows was 0.50 m, while the separation distance between plants was 0.20 m (5 plants per meter in row or 100,000 plants per hectare). The experimental area contained 68 plots, each with 7.5 m^2 areas; constituted of four rows and totaling 510 m^2.

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Table 1. Temperature (°C), sunlight (hours) and water precipitation (mm) during crop cycle.

Experimental months	Average temperature (°C)	Sunlight (h)	Precipitation (mm)
May	27.00	123.4	409.30
June	26.80	87.3	30.60
July	25.80	70.8	1.80
August	24.80	68.6	1.30
September	25.80	81.9	0.90
Average	26.04	86.4	56.22

Fertilization was done in two fractions, at phenological status V3 (3rd trifoliolate leaf unfolded at node 5), using a NPK source of: 80-40-40 kg ha^{-1}. To control weeds in a pre-emergence manner, herbicide application corresponding to Paraquat and Pendimethalin was done at doses of 2 and 2.5 L ha^{-1} respectively, and completed by four manual weeding during crop development. Insect pests were controlled by applying Imidacloprid (0.4 L ha^{-1}) and Carbaryl (0.6 kg ha^{-1}). Three sprinkler irrigations were made to compensate for the crop water requirement; with the first at 15 days after sowing (DAS), the second before flowering and the third after grain filling. The harvest was performed manually during the phenological status R_9 (115 DAS). The promising common bean genotypes as genetic material were used in this study, of which 6 were genotypes usually offered in the market: EVG-6, EVG-6-103, INIAP-473 and INIAP-474 with feature determined whilst EVG-16 and EVG-16-08 of characteristic undetermined, respectively. The genotypes CAL-96, AFR-298, AFR-619, EVG-6 (determined) SER-03, SER-08, SER-20, SER-29, SER-31, SER-35, SEQ-1033 and SEQ-1039 (undetermined) were introduced from the International Center for Tropical Agriculture (CIAT, as its Spanish acronym), Cali, Colombia; through a scientific collaboration to evaluate genotypes that may adapt to this part of the coastal zone of Ecuador. The 17 genotypes were evaluated using Randomized Complete Block Design (RCBD) with four replications.

Plant phenological, sanitary and grain yield measurements

Day period for the phenological stage R_6 (full bloom) and R_9 (flowering maturity) were considered (Hall, 1994). The number of days was recorded from planting to when 50% of plants had one or more open flowers as well as to when 90% plants were completely dry. Web blight resilience was evaluated at reproductive growth stages R_6 (full bloom) and R_8 (pod filling) on leaflets, according to CIAT scale (van Schoonhoven and Pastor-Corrales, 1987), where 1 (considered the fewer value into the scale), means there is no diseased leaf and 9 (the higher value into the scale), means that it exists in the 100% of foliar infected area. Furthermore, the variables related to grain yield and its components were estimated after harvesting of plants. Number of pods per plant and number of grains per pod per plant were variables evaluated in ten plants taken at random in each plot as post-harvest; thereafter, the number of pods and grains were quantified as well as their averages. For the variable 1000-seed weight (g), 1000 grains were weighed and expressed in grams, considering the healthiest ones (without presence of insect or disease damage) obtained from each plot. For grain yield (kg ha^{-1}), all grains of each useful plot (two central rows) were weighed and their value recorded in grams, being homogenous to water content. Finally, these values were transformed to unit kg ha^{-1} obtaining yield per ha.

Statistical analysis

Bartlett and Shapiro-Wilk tests were conducted to verify the homoscedasticity (variances) and normality (residues). Two-way ANOVA was used for data analysis, considering two factors: genotypes (17), and replicates (four) correspondingly. For measure comparison between treatments, Scott Knott test ($p \leq 0.05$) (Scott and Knott, 1974) was employed. Statistical program ASSISTAT 7.6 beta (Silva and Azevedo, 2016) was used for the first analysis. Moreover, Principal Component (PCA) and Cluster Analyses were carried out individually. XLSTAT (Statistical software and data analysis add-on for Excel) package free version 2015.1 was executed for PCA analysis and graphic representation, whilst, RStudio software free version 3.2.2 was used for the elaboration of graphical representation of the dendrograms by WARD2 method.

RESULTS AND DISCUSSION

Phenology cultivars

Statistical differences for the phenological states R_6 and R_9 were found. The genotype EVG-6 after 29 days reached the phenological stage R_6 (full bloom) in less time as early plant, however, the genotypes EVG-6-103, AFR-619 and INIAP-473 obtained averages numerically close to 31, 33 and 31 days respectively, while the tardy lines were SER-03, SER-35, SEQ-1039 and EVG-16-08 completing 43 days correspondingly for each one. The SER-35 and SEQ-1039 genotypes, both with 73 days were considered as the tardiest to reach the stage R_9 (physiological maturation), compared to the others (Figure 1). The results obtained for the phenological behavior of the genotypes fluctuated around the values described (Garcés-Fiallos et al., 2011) for the Ecuadorian areas; as these might be related to similar genetic materials used by both studies, and to the behavior of cultivars under similar environmental conditions (Table 1).

Evaluation of web blight resilience

No significant differences at the stage R_6 was found for the first evaluation of web blight resilience. Conversely, at the phenological stage, highly significant R_8 differences were observed between the genotypes. The genotypes CAL-96, SER-03, SER-08, SER-31, SER-35 and SEQ-1039 had lower severity of disease compared to the other genotypes under study, reaching the follow values: 2.8, 2.8, 2.5, 2.8, and 3.3 respectively, highlighting as those with most resilience to web blight. These results are

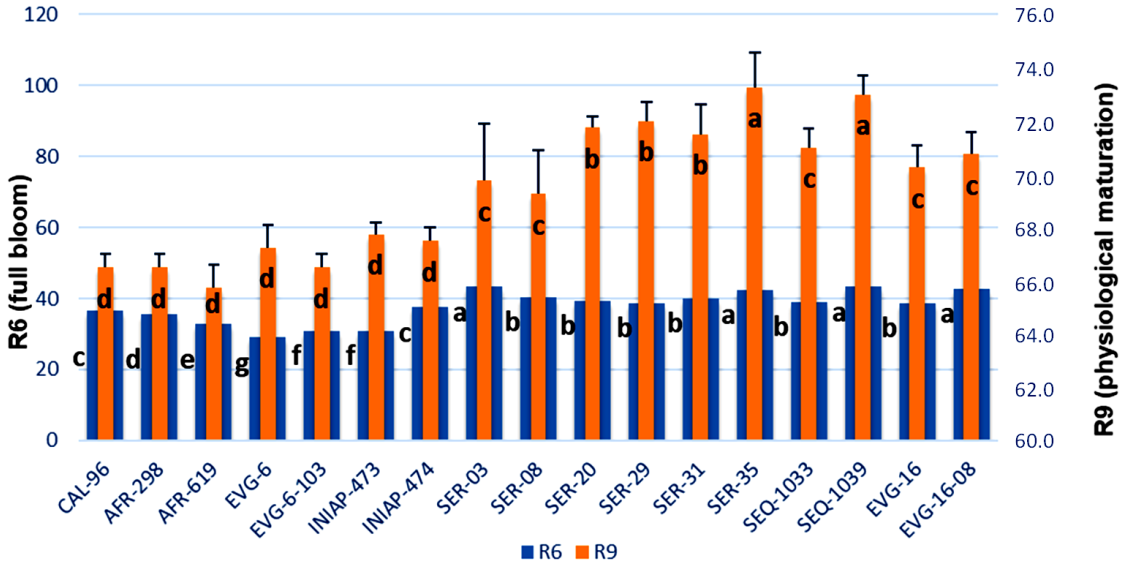

Figure 1. Days from sowing to reproductive phenological stages R_6 (full bloom) and R_9 (physiological maturation) in 17 common bean genotypes. Lowercase letters indicate significant differences between genotypes in each phenological stage (Scott Knott test ($p \leq 0.05$)), n = 150 for each genotype.

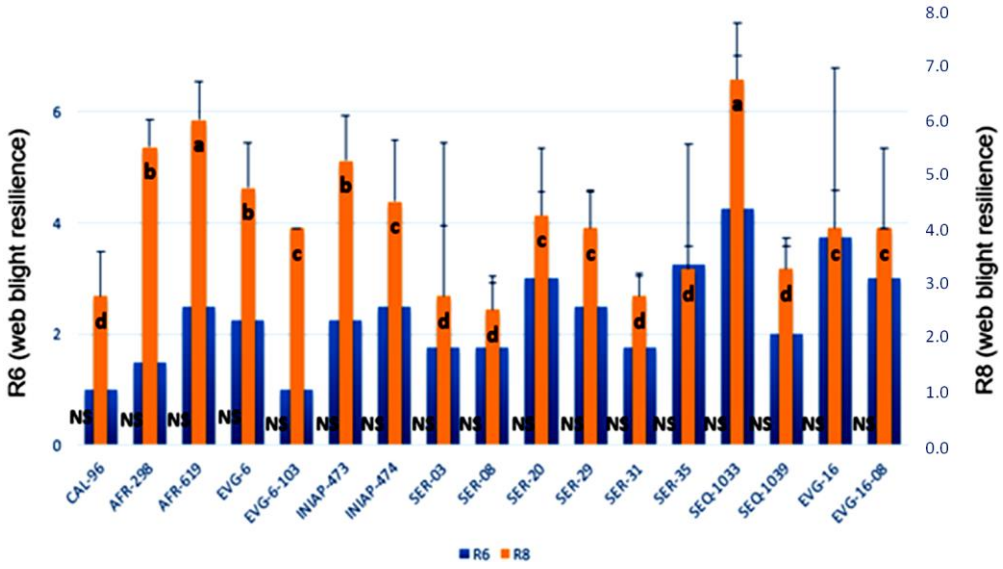

Figure 2. Web blight resilience obtained for the genotypes studied. Evaluations done at phenological stages R_6 (full bloom) and R_8 (pod filling) in 17 common bean geno-types. Lowercase letters indicate significant differences between genotypes at phenological stage R_8. Not significance differences were found at phenological stage R_6 (Scott Knott test ($p \leq 0.05$)), n = 150 for each genotype.

opposed to the previous study (Garcés-Fiallos et al., 2013), who found a highest severity of the disease for the genotypes SER-03, SER-08, SER-31. These results might be related to the low fungal incidences during crop establishment time, as sowing was performed on leaving the rainy season, with an average 56.22% of water precipitations (mm) during crop cycle, thus, climatic conditions were not optimal for a high severity of web blight disease (Figure 2). By contrast, the genotypes SEQ-1033 and AFR-619 had the highest web blight severity with an average of 6.8 and 6.0 each one (Figure 2). Genotypes can have different levels of resilience to the disease, nevertheless, tolerance and resistance to web blight disease will also depend on the agro-

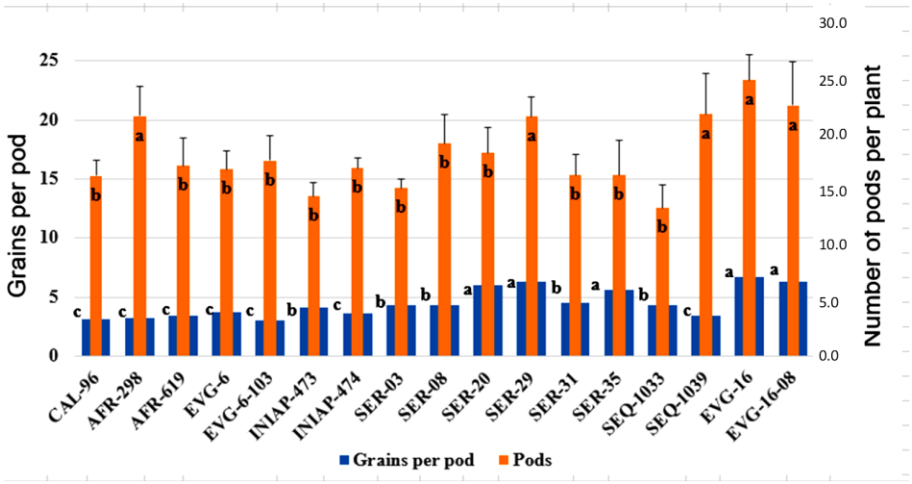

Figure 3. Grains per pod and number of pods per plant in 17 common bean genotypes. Lowercase letters indicate significant differences between genotypes (Scott Knott test ($p \leq 0.05$)), n = 150 for each genotype.

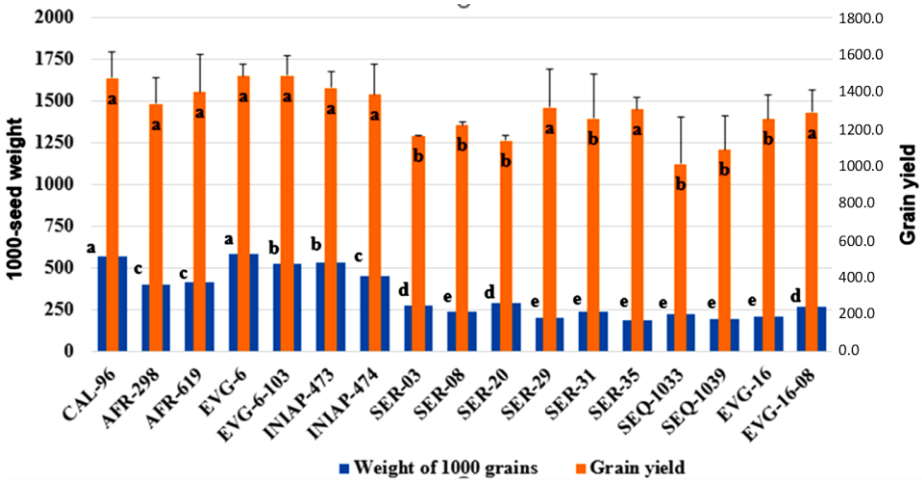

Figure 4. 1000-seed weight (g) and grain yield of seventeen common bean genotypes. Lowercase letters indicate significant differences between genotypes in each production (Scott Knott test ($p \leq 0.05$)). Error bars represent the mean standard deviation, n = 150 for each genotype.

ecological adaptation of each material to the environmental conditions where they are growing (Alves et al., 2009).

Yield and its components

The genotypes including AFR-298, SER-29, SEQ-1039, EVG-16 and EVG-16-08 achieved highest number of pods per plant, in comparison with the other genotypes, obtaining 21.8, 21.7, 22, 25.1 and 23 pods per plant respectively. Equally, the uppermost number of grains per pod was reported for the genotypes SER-20, SER-29, SER-35, EVG-16, EVG-16-18 compared with the others, and with averages 6.0, 6.0, 6.0, 7.0 and 6.0 grains per pod correspondingly. Both variables showed statistical differences (Figure 3).

Concerning the variable 1000-seed weight (g), significant differences were reported, highlighting the genotypes EVG-6, CAL-96, INIAP-473 and EVG-6-103 with the averages 571.3, 580.8, 521.3 and 533.3 (g), respectively (Figure 4). Likewise, for grain yield, statistical significant differences were found, sticking out the genotypes CAL-96, AFR-298, AFR-619, EVG-6, EVG-6-103, INIAP-473, INIAP-474, SER-29, SER-35, EVG-16-08 which gained the higher yields with the (individual)

Table 2. Yield per ha of the 17 genotypes.

Genotypes	kg ha $^{-1}$	(48) p < 0.05
EVG-6-103	1484.3 a	
EVG-6	1480.8 a	
CAL-96	1473.2 a	
INIAP-473	1419.7	a
AFR-619	1394.4	a
INIAP-474	1381.0	a
AFR-298	1333.5	a
SER-29	1314.2	a
SER-35	1304.5	a
EVG-16-08	1288.1	a
SER-31	1254.0	b
EVG-16	1249.6	b
SER-08	1216.8	b
SER-03	1156.8	b
SER-20	1133.7	b
SEQ-1039	1084.3	b
SEQ-1033	1008.6	b
Average	1292.79	
C.V. (%)	12.52	

Different letters in column shows significant difference at 5% probability, n = 150 for each genotype. According to the analysis for 48 freedom degree F = 3.038, with a likelihood to 5% = 1.859.

averages of 1473.2, 1335.5, 1394.4, 1480.8, 1484.3, 1419.7, 1381.0, 1314.2, 1304.5, and 1288.1 kg ha^{-1} respectively and compared to the other genotypes (Figure 4).

Several differences were obtained in other investigations for each one of the productive variables under study in the current research. Regarding the number of pods harvested per plant, our results were opposed to the findings reported by other authors. Infante et al. (2003) obtained averages from 26.67 to 38.38 and Santos et al. (2009) from 7.56 to 16.40 pods per plant correspondingly. The same happened with the variable grain per pod per plant, where the averages reported with significant differences, as shown by Infante et al. (2003) who reported averages from 8.83 to 9.95 and Barrios-Gómez et al. (2010) presented the values fluctuating from 3.1 and 4.4 grains per pod per plant.

Finally, relating to yield per hectare, significant differences were found, for the genotypes EVG-6-103, EVG-6, CAL-96, INIAP-473, AFR-619, INIAP-474, AFR-298, SER-29, SER-35, EVG-16-08 which obtained a higher yield average (Table 2).

Results reported in this research for the variable yield ha^{-1} differ from that reported by other authors as González Torres et al. (2008) who found highest yield with averages between 1138 to 2550 kg ha^{-1} during irrigation season, whilst in optimal temporal season obtained an increasing of the 17% in the yield for almost all the genotypes studied, compared with the irrigation season. These results also contrasted what was described by Santos et al. (2009) with averages from 393 to 1230 kg ha^{-1}; and Godoy-Montiel et al. (2011) from 435.3 to 462 kg ha^{-1}; both reporting fewer yields in their studies. Alteration probably could have been caused by the genetic and adaptive differences of the germplasm used in each one of the studies.

Set of genotypes grouped by productive and phenological variables

Principal components analysis (PCA)

Six productive and phenological variables (R_6, R_9, Pods per plant, Grains per pod, 1000 seed-weight and yield ha^{-1}), were selected to discriminate similarity among the genotypes. According to PCA, 100% of the variance total was distributed in five principal components. However, two firsts' components shown in Table 3 present the values that explained 82.94% total of the existing variation jointly, with a contribution of 64.83% for the first component, where the features were grouped as to the majority of PCA. It was followed by the second component explaining the 18.11% of the variation (Table 3). In the first component, the most significant variables were R_6 (−0.88), R_9 (−0.91), grains per pod (−0.73),

Table 3. Principal component analysis of six productive and phenological variables for the 17 common bean genotypes.

Variable	PC1	PC2
R_6	− 0.88	− 0.17
R_9	− 0.91	− 0.12
Pods per plant	− 0.40	0.83
Grains per pod	− 0.73	0.42
1000 Seed-weight	0.96	0.04
Yield/ ha	0.81	0.42
Variability explained (%)	64.83	18.11

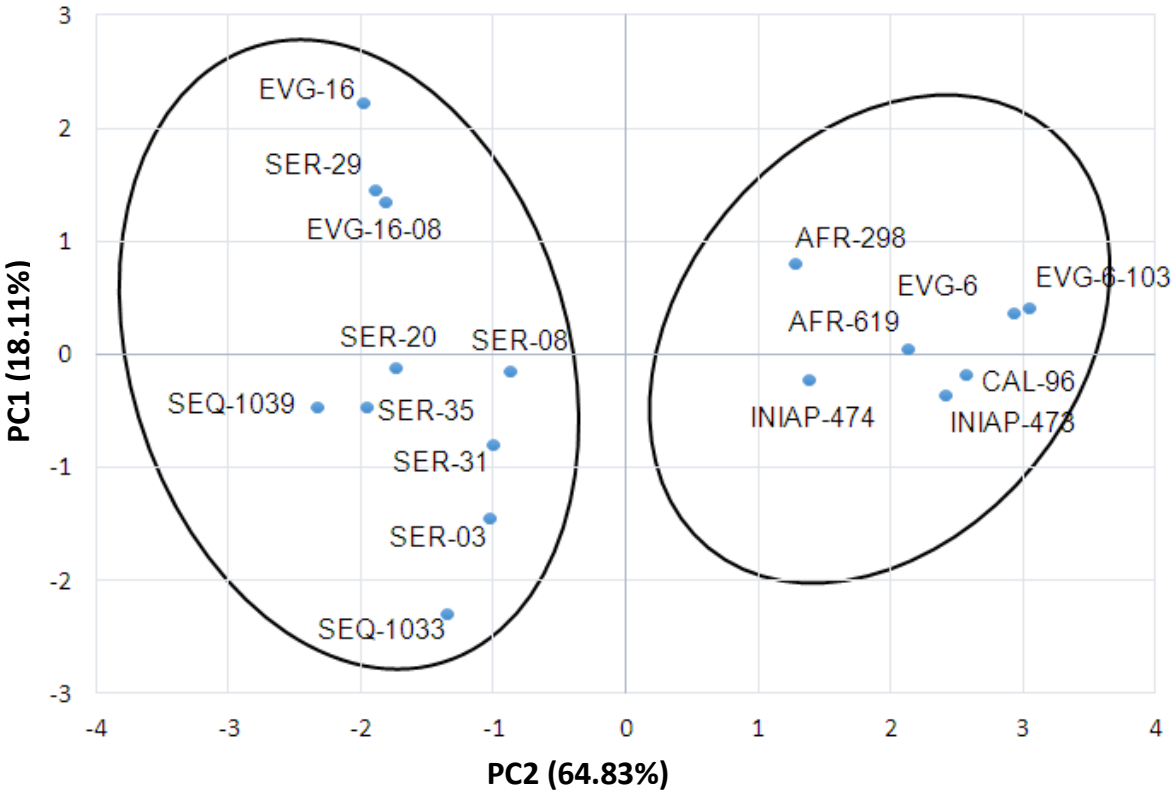

Figure 5. Two set of genotypes grouped by six productive and phenological variables for the 17 common bean genotypes.

1000-seed weight (0.96) and yield per hectare (0.81), correspondingly. Therefore, according to these outcomes, it seems that the genotypes which precociously reached the phenological stage R_6 arrived in less time to the stage R_9 and obtaining a smaller number of grains per pod, nonetheless, these obtained a greater weight of 1000 seeds and yield ha^{-1}. Subsequently, for the second component, the most outstanding variable was pod per plant; despite that grain per pod and yield ha^{-1} obtained a medium representation (Table 3). In addition, Figure 5 shows the distribution of the points, corresponding to the 17 genotypes. The graph allows to observe a wide distribution of the genotypes in the plane and demarcated by the axes assigned to the components 1 and 2, highlighting two groups well-defined and probably segregated by a specific genetic character, related to the type of growing feature (determined and undetermined). To the left of the plane, the first group is observed including the genotypes EVG-16, SER-29, EVG-16-08, SER-20, SER-08, SEQ-1039, SER-35, SER-31, SER-03 and SEQ-1033 and the other one (second) is located to the right of the plane including AFR-298, EVG-6, EVG-6-103, AFR-619, CAL-96, INIAP-474 and INIAP-473. All these differences might be explained by a genetic basis.

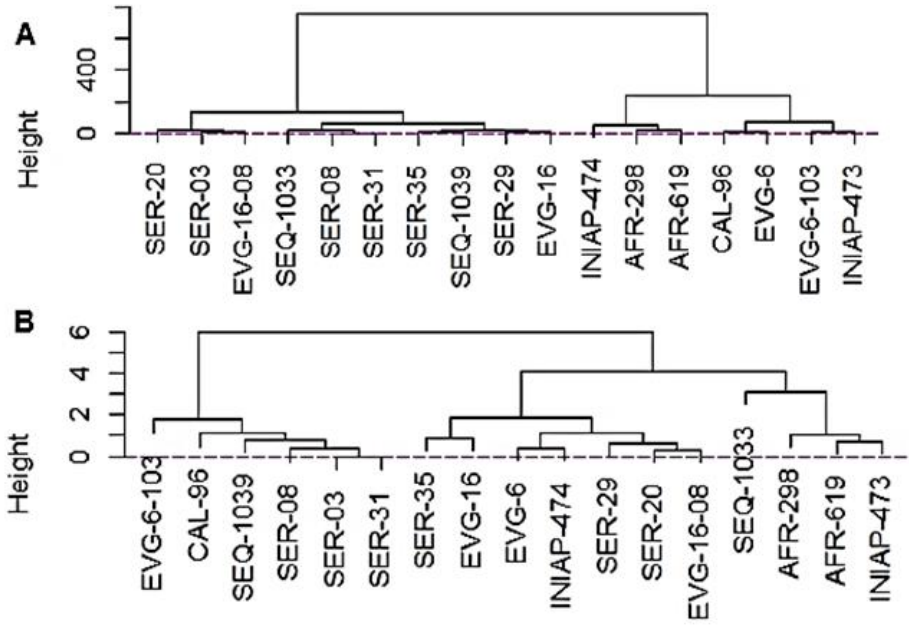

Figure 6. Dendrogram of similitude of 17 common bean genotypes. **(A)** Productive and phenological variables. **(B)** Web blight resistance were subject to analyses.

The authors have demonstrated, that a high correlation between phenotypic and genotypic variability (Machado et al., 2017) exists. Ultimately, the relative regularity of the genotypes in the plane allows us to accept that for this experiment, we used an important and representative sample amount of the available genetic resources of common beans in Ecuador, and the others introduced from Colombia.

Cluster analysis

Dendrogram were obtained using the matrix of Euclidean distances by means of the method distance average of WARD2. The noted variability shown between the genotypes (Figure 5) and the variables used for dividing them separate them in two subsets in each cluster (Figure 6A and B). All the genotypes belonging to the first subset joined according to the common features, which were different for the genotypes of the other subset (second).

Regarding cluster A the variables used to shape the two subsets were the productive and phenological variables. It implies that the subset number one (from left to right) was shaped by 10 genotypes including SER-20, SER-03, EVG-16-08, SEQ-1033, SER-08, SER-31, SER-35, SEQ-1039, SER-29 and EVG-16. These genotypes showed the lower averages for phenological and reproductive variables, whilst the second subset (from right to left) including the 7 genotypes INIAP-474, AFR-298, AFR-619, CAL-96, EVG-6, EVG-6-103, INIAP-473 presented higher averages in almost all phenological and productive variables (Figure 6A).

On the other hand, by the same procedure, for the elaboration of the dendrogram B, the variables related to the resilience against web blight R_6 (full bloom) and R_8 (pod filling) were considered. Two subsets were created. For the first subset (from left to right), presented by 6 genotypes EVG-6-103, CAL-96, SEQ-1039, SER-08, SER-03 and SER-31, the less attacked plants by the disease during the two resilience evaluations (R_6 and R_8) against web blight were seen. The remaining 11 genotypes including SER-35, EVG-16, EVG-6, INIAP-474, SER-29, SER-20, EVG-16-08, SEQ-1033, AFR-298, AFR-619 and INIAP-473 were more susceptible (Figure 6B).

Thus, PCA and dendrogram analysis enabled distinguishing of marked architectural differences among the germplasms under study, separated principally by their growth features in determined and undetermined genotypes respectively. These results agree with those obtained by Garcés-Fiallos et al. (2015), who in a study of 18 lines of common bean in the same zone of Quevedo reported similar outcomes identifying two clear set of genotypes grouped by variables agronomic, productive, and sanitary. It seems that this result might be related to the fact that the genetic material used in the present research saved certain similitude with the genetic material used by Garcés-Fiallos et al. (2015).

Conclusions

Significant differences among the 17 genotypes under

study for all the evaluated variables, phenological, web blight resilience as well as the productive variables were seen. Results evidenced that high-quality of plant architecture is a useful strategy for web blight management. A genetic material with certain double features as tolerance to disease and high yield ha^{-1} was found (three genotypes undetermined) apart from the seven determined genotypes which showed the higher yield characteristics. Our results allowed identifying the materials, which becomes an excellent option for sowing, and can be recommended to the farmers in this region of Ecuador. Nevertheless, it would be imperative to continue testing the genotypes, but in other conditions more auspicious for the disease (rainy season).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Impact of clarification process on the nutritional, mineral and vitamin composition of cashew (*Anacardium occidentale*) apple juice

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This study investigates the impact of clarification process on the nutritional, mineral and vitamin composition of the cashew apple juice. The proximate composition in percentage for moisture, dry matter, ash, crude fibre, ether extract, crude protein and carbohydrate were determined in line with the recommended methods of Association of Official Analytical Chemist (AOAC), and vitamin profile was analysed using Gas Chromatography coupled with the Pulsed Flame Photometric Detector (GC-FPD). The mineral analysis also followed the recommendation of AOAC. The clarified cashew apple juice had values in mg/100 of 10.1, 6.1, 2.2, 28.8, 10.2 and 0.22 for calcium, magnesium, sodium, potassium, phosphorus and iron, respectively. The study showed that cashew apple juice is rich in nutrient and contains a good level of trace elements necessary for healthy living. Rice gruel as a natural sourced clarifying agent can better replace industrial clarification agents for a safer drink production.

Key words: Clarification process, nutritional composition, mineral composition, vitamin composition, cashew apple juice.

INTRODUCTION

Nutrients are chemical constituents' in food that are required by living organisms to keep them alive and active. Nutrients are present in food as large molecules which have to be broken down into tiny particles before it can be used by the living organisms. Good nutrition according to S´anchez-Moreno et al. (2006) means getting adequate amount of nutrients from healthy foods such as fruits, vegetables among others in appropriate combination. Fruits have been found to contain essential nutrient such as vitamins, minerals, fibres and phytochemicals which make them essential for a balance diet (Anu and Rajinder, 2006). Consumption of fruits and vegetables contributes to good health and well being of man. Many people prefer fruits due to the fact that they believed it is a source of diet with low fats and sodium content. Consumption of fruit everyday have been reported to reduce by halve the risk of developing cancer and also the risk of cardiovascular diseases, diabetes,

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stroke, obesity, birth defects, cataract, osteoporosis among others (Anu and Rajinder, 2006). Due to high cost and/or unavailability of food substance containing the essential classes of food and other needed nutrients, there is need to search for other alternative sources of nutrients using fruits juices. Fruits are highly perishable and seasonal in nature and during glut season, a lot of fruits are wasted due to lack of the knowhow by farmers in the processing of the fruit to make the product available during off season. Cashew apple is one of such fruits discovered to have rich essential vitamins, minerals, fibre and other phytochemicals that can be processed into juice (Ashok and Upadhyaya, 2012). However, the juice from cashew apples contain tannins which give the raw juice an astringent taste and dry mouth feeling when consumed (Odurole et al., 2001; Ashok and Upadhyaya, 2012). To make the juice attractive, the tannins which give it the undesirable taste need to be removed through clarification. Clarification is a process by which the semi-stable emulsion of colloidal plant carbohydrates that support the insoluble cloud material of a freshly pressed juice is “broken” such that the viscosity is dropped and the opacity of the cloudy juice is changed to an open splotchy look (Sharma et al., 2015). Clarification is an important step in the processing of fruit juice mainly in order to remove pectin and other carbohydrates which are present in the juice. Rice gruel can be used for clarification on the assumption that fruit juice on the addition of clarifying agents such as rice gruel or gelatin, the colloids present in the juice coagulate and form a flocculent precipitate which settles down. The precipitation is due to the electrostatic attraction between the positively charged rice gruel particles and the negatively charged colloids in the juice. Generally, clarifying procedures can be achieved by centrifugation, enzymatic treatment or non-enzymatically by applying heat and use of clarifying agents such as gelatine, bentonite, silica sol, and polyvinyl pyrrolidone (Chatterjee et al., 2004). Clarification processes of fruit juice may have significant influence on the nutritional, mineral and vitamin composition of the juice. At times, essential nutrients in fruits and vegetables are often damaged or transformed during clarification. Tiwari and Cummins (2013) and Tiwari et al. (2009) reported that conventional thermal, non-thermal, domestic and industrial processing widely degrade the levels of phytochemicals in processed food. Food processing according to Tiwari et al. (2013) changes the nutritional properties of some foods for instance, partial hydrogenation of vegetable oil results in the formation of trans-fatty acid and heat treatment of protein solutions in an alkali environment results in the formation of lysinoalanine. On the other hand, some nutrients and bioactive compounds that are naturally present in fruits may undergo transformations during food processing that neither decrease their nutritional value nor bioactive value but may increase it by favouring their absorption and metabolism in the human body (S´anchez-Moreno et al., 2006). Available literature revealed that most of the industrial clarification of fruit juice is carried out using gelatin of which there is some concern about the safety of gelatin because it comes from animal sources, some of these animals such as cow may be suffering from mad cow disease (bovine spongiform encephalopathy) and as a result may go along to pose health challenges on the consumers. Therefore, safer and cost effective bio-process clarification of fruit juice using natural products of plant origin such as rice gruel is necessary. This study therefore focused on determining the impact of clarification process on the nutritional, mineral and vitamin composition of cashew (*Anacardium occidentale*) apple juice using rice gruel. Cashew apple is that part of the fruits which is eaten fresh. The apple constitutes the major parts of the fruit representing 90% of its weight. It is consumed in fresh state and has rich flavor, aroma and vitamin C content higher than that of orange, mango and guava juices (Soares et al., 2007). The juice has to be processed and preserved to keep it available all year round.

MATERIALS AND METHODS

The plant materials used for the experiment were fresh ripped cashew apples obtained from Ochaja cashew plantation farm, Kogi State, Nigeria and polished rice also obtained from Kogi State (7.49°N and 6.45°E), a locality of high cashew tree plantation in Nigeria. The fresh ripped cashew apples were transported under refrigeration conditions to agricultural laboratory of the department of agricultural and bio-resources education, University of Nigeria, Nsukka. The study adopted experimental design and spanned from November, 2016 to March, 2017. It covered clarification of cashew juice using rice gruel as clarifying agent, nutritional, mineral and vitamin analysis were also carried out.

Preparation of clarifying agent (rice gruel)

Six glass of water was measured into a pot and brought to boil. One cup of parboiled rice was measured, washed and poured into the boiled water, mixed well and the pot covered and kept on a low heat. The rice was cooked on a low heat between 20 and 25 min. The pot was removed from heat and the rice with the water poured into a sieve to get the water referred to as rice gruel. The rice gruel was then allowed to cool down and was filtered using a muslin cloth. The filtrate was kept in a container for clarification.

Juice extraction

The collected cashew apples transported to the laboratory had their nuts detached. The apples were washed thoroughly using distilled water. The apples were then cut into slices and the juice obtained by pressing the mash through a muslin cloth. The obtained juices were then separated into two different containers for the clarification assay and sample analysis.

Experimental clarification of the cashew apple juice

The clarification process of the cashew apple juice using rice gruel was carried out in line with the recommendation of Cormier (2008).

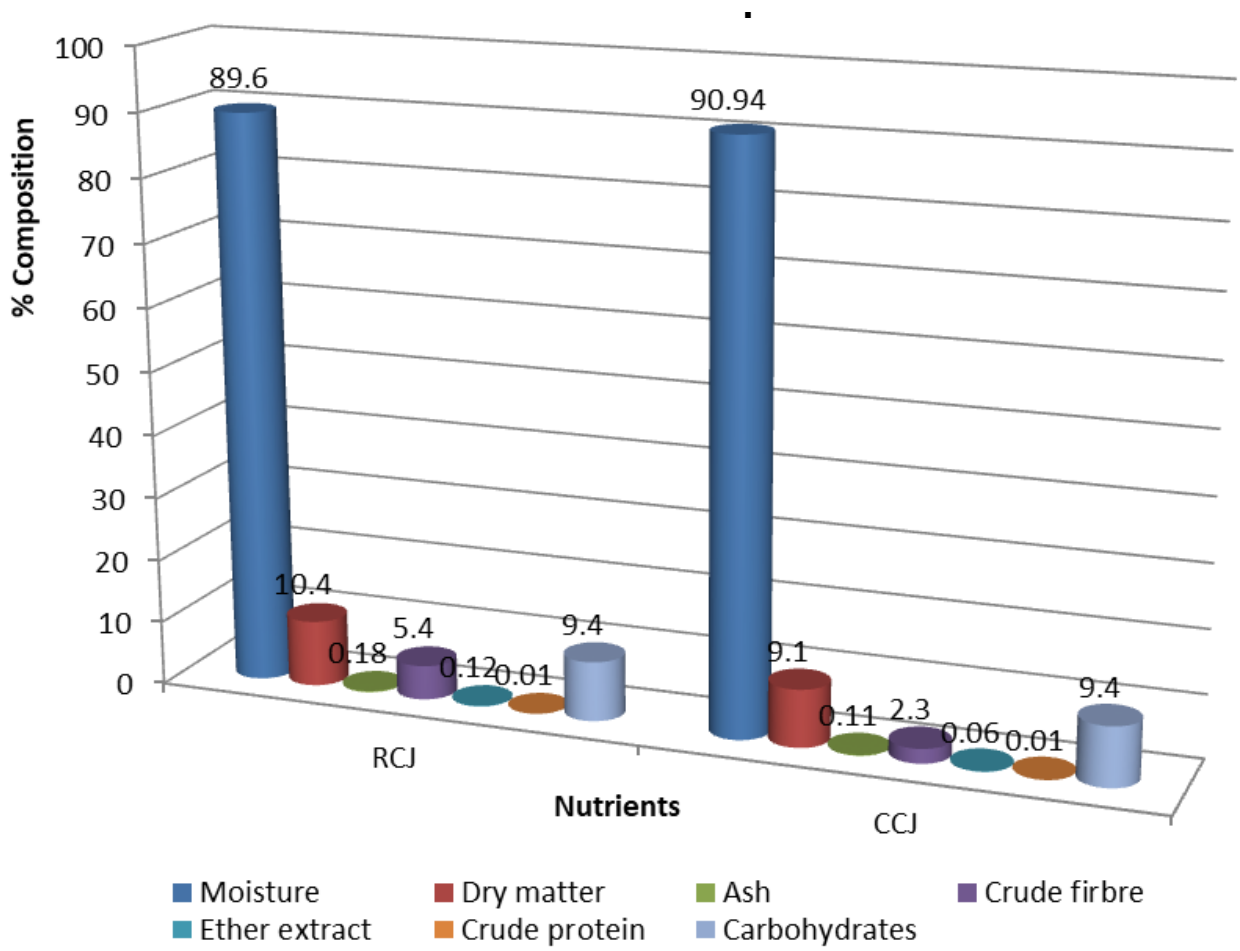

Figure 1. Histographic representation of the result of the proximate analysis carried out on cashew apple juices. RCJ, Raw cashew juice; CCJ, clarified cashew juice.

With 1 L of the raw juice in a stainless steel vessel, the juice was stirred in a circular motion and 125 mL of rice gruel was slowly poured into the juice and was constantly stirred for 5 min to ensured thorough blend of juice and solution. The juice was allowed to rest until the tannins settled at the bottom of the vessel. At this point, the vessel was covered. The cleared juice was siphoned using rubber turbines into a cleaned plastic container and labelled sample B. The raw juice in the other plastic container was labelled sample A.

Data collection

The data for the study was obtained through laboratory analysis of cashew juice samples. Both samples labelled as Raw Cashew Juice (RCJ) sample A and Clarified Cashew Juice (CCJ) sample B were analyzed for physical and chemical properties in line with the recommended methods of Association of Official Analytical Chemist (AOAC, 1984).

Analysis of sample

Analysis of moisture, ash, protein and crude fat were carried out using the recommended methods of the AOAC (1984). Vitamin profiles were analyzed using Gas Chromatography coupled with the Pulsed Flame Photometric Detector (GC-FPD) methods. The mineral analysis also followed the recommendation of AOAC (1984).

RESULTS

The result in Figure 1 reveals the percentage composition of raw cashew juice as follows in (%): moisture 89.60, dry matter 10.40, ash 0.18, crude fibre 5.40, ether extract 0.12, crude protein <0.01 and carbohydrates 9.40. The clarified cashew juice had 90.94, 9.10, 0.11, 2.30, 0.06, <0.01 and 9.40 for moisture, dry matter, ash, crude fibre, ether extract, crude protein and carbohydrate, respectively

The result in Figure 2 shows mineral analysis of raw and clarified juice in mg/100. The raw juice in mg/100 revealed 10.2 for calcium, 6.2 for magnesium, 10.8 for sodium, 28.7 for potassium, 5.1 for phosphorus and 0.22 for iron. The clarified juice indicated 10.1 for calcium, 6.1 for magnesium, 2.2 for sodium, 28.8 for potassium, 10.2 for phosphorus and 0.22 for iron, respectively.

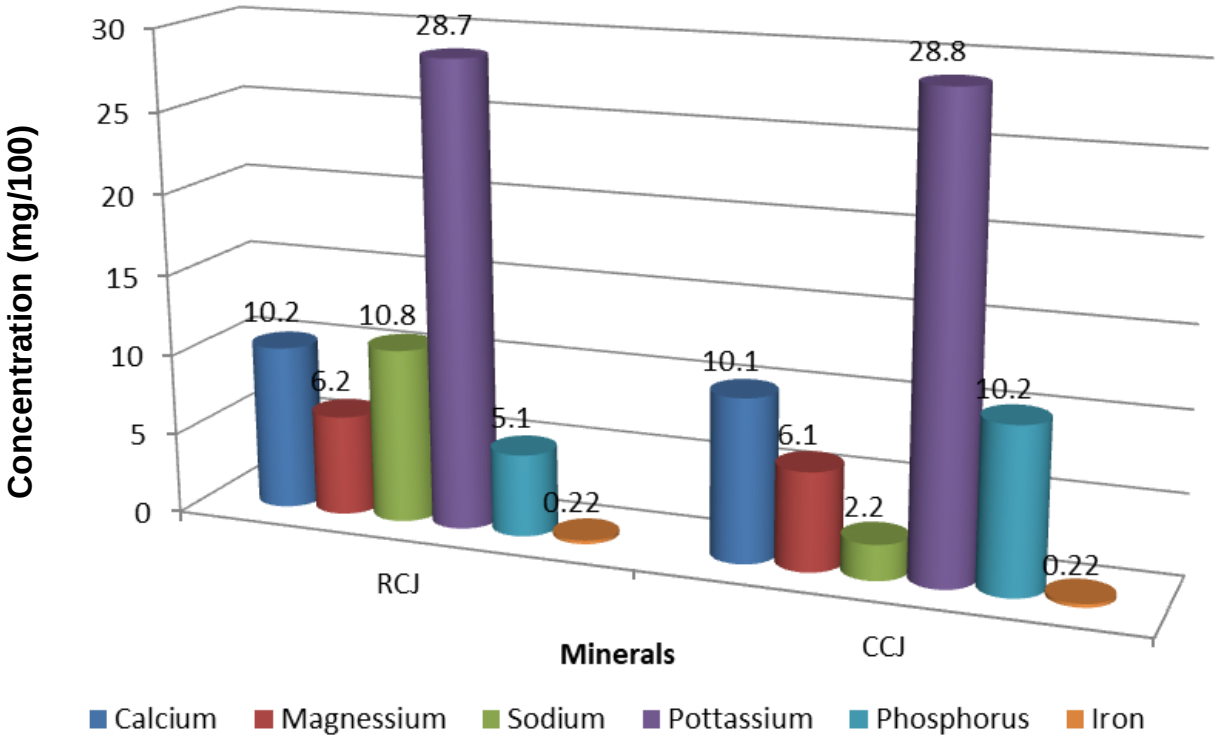

Figure 2. Histographic representations of results of the mineral composition carried out on the cashew apple juices. RCJ, Raw cashew juice; CCJ, clarified cashew juice.

Table 1. Vitamin composition of the raw and clarified cashew juices.

Vitamin	Retention time (min)	RCJ [pA.s]	Retention time (min)	CCJ [pA.s]
A	17.10	189.10	17.10	110.93
B1	18.10	314.10	18.10	230.07
B2	18.84	179.81	18.84	130.38
B3	12.27	16.70	12.27	12.15
B5	22.61	277.61	22.61	217.29
B6	13.74	112.70	13.74	78.23
B9	20.54	94.95	20.54	70.31
C	16.04	153.24	16.04	109.41
D	19.24	105.71	19.24	77.35
E	19.52	297.94	19.52	217.37
K	21.82	156.73	21.82	165.42

RCJ, Raw cashew juice; CCJ, clarified cashew juice; pA.s, picoAmpseconds

The result in Table 1 reveals the vitamin profiles per area and the retention time of both raw and clarified cashew apples juice. Vitamin composition for the raw cashew apple revealed the following results in (pA.s): vitamin B_3=16.70, vitamin B_6= 12.70, vitamin C=153.24, vitamin A=189.10, and vitamin B_1= 314.10, respectively, whereas the clarified cashew apple juice had the values of 12.15, 78.23, 109.41, 110.93, and 230.10 pA.s for vitamin B_3, B_6, C, A and B_1, respectively.

DISCUSSION

The result of proximate analysis revealed that there was a ($P<0.05$) significant difference in the moisture contents between the raw and clarified forms of cashew juice. A

significant (P<0.05) decrease in dry matter was observed when clarified. This indicated that processing decreases the dry matter of cashew juice. This is however not a good development since the presence of moisture or high range of moisture in food makes it spoil faster indicating decrease in the shelf life of the food. A non-significant (P>0.05) decrease in ash content was observed when cashew juice was clarified. Ash is a non-organic compound containing mineral compound of food and nutritionally it aids in the metabolism of other organic compounds such as fat and carbohydrate (Okonkwo and Ozoude, 2015). The observed decrease in ash content on processing was not significant (P>0.05) and therefore of no major concern. It was also observed that there was no significant (P<0.05) change in crude protein when the cashew juice was clarified. Protein is very important for the repair of worn out tissues in the body, promotes growth and organ development. The carbohydrate content of the cashew juice was not altered, an indication that clarification of cashew juice using rice gruel neither decreased nor increased the carbohydrates. Cashew juice have moderate concentration of carbohydrate and are therefore an energy food implying that the consumption of cashew juice by young and old gives enough energy to carry out specific metabolic functions (Giwa and Abiodun, 2010).

The result of the proximate analysis carried out on cashew juices in the raw and clarified form also showed that there was a significant change in some parameters tested for except for carbohydrates, crude protein, and dry matter which showed no significant (P>0.05) difference. Crude fiber is known to aid digestion in humans. Ihekoronye and Ngoddy (1985) indicated that food or diet low in fiber content is undesirable and can cause constipation and such diets have been associated with diseases of colon like piles, appendicitis and cancer (Okonkwo and Ozoude, 2015). There was significant decrease in ether extract composition on clarification of the cashew juice. This decrease is a good development since rancidity that promotes the development of unpleasant and odorous compounds is low. Diets too high in fats predispose consumers to different illness such as obesity, coronary heart disease (Okpala and Chinyelu, 2011) and are therefore not desirable.

There was a significant (P<0.05) difference in mineral composition between the raw and the clarified cashew juices. The results show that clarification increased some of the minerals tested for in cashew juices significantly (P<0.05), while there was no significant change for some minerals such as calcium and magnesium except for sodium where there was a significant (P<0.05) decrease after clarification. It may therefore be advised that cashew juice be taken in its clarified form. Calcium is essential for the normal development of the body. It is an important constituent of bones and teeth. It is also essential for many metabolic processes including nerve function, muscle contraction and blood clothing. A deficiency of calcium in the body leads to conditions such as rickets, osteomalacia, and osteoporosis. A deficiency of calcium in the blood may lead to tetany. The presence of calcium in trace amount is very necessary and can alleviate nutritional impairments such as rickets (Okonkwo and Ozoude, 2015). Magnesium just like calcium is important for proper functioning of muscle and nervous tissues. It is required as a cofactor for many enzymes in the body. Phosphorus just like calcium is important in bone formation, metabolism and in energy conversion and storage in the body. Sodium and potassium are important for nerve transmission and osmolarity while iron is an essential component in the transport of oxygen in the body. A deficiency of iron will lead to anaemia. Therefore, adequate intake of potassium and iron is necessary for a healthy life (Okonkwo and Ozoude, 2015).

Table 1 shows that there was a significant (P>0.05) decrease in all the vitamins present in cashew juice after clarification except for vitamin K which increase significantly (P<0.05) from 156.73 to 165.42 pA.s on clarification. The result revealed that clarification has impact on the vitamin content of cashew juice. However, it is noted that clarified juice contained considerable amount of vitamin which makes it suitable for consumption. The importance of vitamin in diet cannot be neglected. Vitamin B_1, B_2, B_3 and B_6 (thiamin, riboflavin, niacin and pyridoxine) serve as coenzymes in all part of the body, they take part in protein, carbohydrates and fat metabolism, they are also important in the structure and function of the nervous system (IM, 1998; ASNS, 2004; Lukaski, 2004). Vitamin C is essential in maintaining healthy connective tissues, cell wall integrity, collagen synthesis and prevention of scurvy (S´anchez-Moreno et al., 2003). Vitamin A like other vitamins is essential for growth, vision and maintenance of soft mucous tissues (Okonkwo and Ozoude, 2015). Vitamin E is useful in reducing the risk of developing degenerative disease (Bramley et al., 2000; S´anchez-Moreno et al., 2003). The appreciable increase of vitamin K as shown in Table 1 is a welcome development; this is due to the importance of vitamin K in blood clothing as well as its function in the inhibition of the growth of certain types of cancer including those of liver and gut tumour (Martins and Paul, 2008).

Cashew juices have appreciable amounts of vitamins, minerals and nutrients. The ascorbic acid content also indicates that cashew juices in clarified form can be used to prevent or at least minimize the formation of carcinogenic substances from dietary materials (Okonkwo and Ozoude, 2015). The presence of carbohydrates, fat and protein also make it a good source of energy. The present research with rice gruel for cashew juice clarification shows that clarification has impact on the proximate composition of the clarified juice. The study revealed that some nutrients such as crude fiber, ether extract and ash have been decreased as a

result of clarifications. The study showed that carbohydrates and crude protein remain unchanged while moisture content increased after clarification. The study also revealed that no major changes occurred in the mineral composition of the clarified juice when compared with the raw juice except for sodium which decreased upon clarification. Clarification increased the phosphorus composition from 5.1 to 10.2 mg upon clarification. The study further showed a negligible decrease in all vitamin composition of the clarified juice except for vitamin K which increased from 156.73 to 65.42 pA.s. Cashew juice has good amount of vitamins, minerals and nutrient. And its high ascorbic acid (Vitamin C) indicates its usefulness as anti-carcinogenic substances.

CONCLUSION AND RECOMMENDATION

The study conducted confirms the effectiveness of rice gruel as a clarifying agent for the removal of tannins content of cashew juice which makes it more desirable for consumption. The clarified juice was not significantly affected in terms of the proximate, mineral and vitamin composition. The rice gruel used in this study showed that it is an efficient and economic natural product for the clarification of cashew apple juice. Information on the utilization of rice gruel for cashew apple juice clarification should be made available for farmers in order to help them have access to a cheaper and natural agent of clarification.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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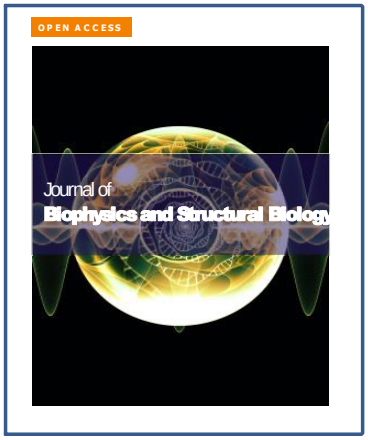

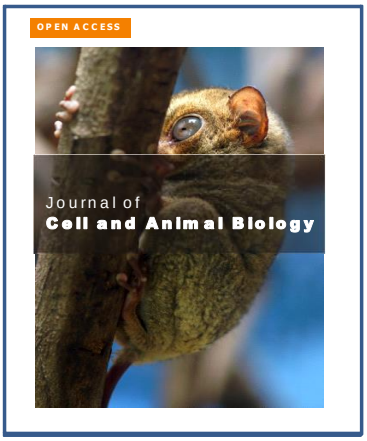

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