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Effect of Wood Charcoal Powder on Rate of Microbial Production of Lactic Acid in Dehulled and Undehulled *Vigna unguiculata* Pastes

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Abstract

Effect of Wood Charcoal Powder (WCP) on rate of microbial production of Lactic Acid (LA) in ground dehulled and an undehulled *Vigna unguiculata* (cowpea) paste was studied. The pastes were analysed for proximate compositions, energy values, tannin contents and microbial loads. Later, 4.0 g pastes were treated with incremental concentrations (0.03-0.05 g) of WCP diluted with 50 mL distilled water and fermented for 2 h at ambient temperature (29.5 ± 2.0°C). Results showed that decortication of cowpea significantly reduced ($p<0.05$) the crude protein, crude fibre, crude fat and tannin contents as well as energy value and microbial loads. Fermentation produced ethanol and LA. The WCP inhibited LA production in dehulled cowpea paste, whereas LA fermentation continued unhindered in Undehulled Cowpea Paste (UCP). A WCP-tannic acid-cellulose interaction study suggested that WCP interacted more with the fibre and tannin components of UCP thereby reducing the amount that would have bound the fermentative microorganisms. In conclusion, wood charcoal inhibited microbial production of lactic acid in ground dehulled cowpea paste.

Keywords: Cowpea; Decortication; Lactic acid; Microbial fermentation; Wood charcoal

Introduction

Vigna unguiculata (cowpea) is commonly consumed in developing countries, like Nigeria, as a source of plant protein [1]. The consumption of cowpea cuts across societal economic strata. It simply can be cooked whole with pepper, salt and palm oil. The paste of its dehulled form can be fried in hot edible oil into bean balls (*Akara* in Igbo and Yoruba) or wrapped in nylon, *Pandenus candelabrum* leaf, plastic container, aluminum foil or container and cooked into bean cake (*Moi-moi* in Igbo and Yoruba).

Microorganisms are ubiquitous. They degrade food materials producing organic acids, alcohols, toxins and other chemicals which impact off-flavour [2]. Food that is attractive and appealing can be transformed by microorganisms into a sour, fungus-covered or foul-smelling mass [3]. In order for food to serve its purpose, it must be protected from deterioration while being processed from its raw form to a consumable product. Food prepared from degraded raw materials is unappealing to the consumer and can lead to abandonment of the food and food wastage, downstream. Neta et al. [4] reported that sour taste results from low pH and the presence of organic acid(s); with the intensity of sourness being directly related to proton concentration and the number of molecules with at least one protonated carboxyl group.

Charcoal is an allotrope of carbon and forms covalent bonds with sulphur, oxygen and nitrogen. It is got by burning firewood in a limited amount of oxygen. Activated charcoal has been used in the management of chronic wounds [5], adsorption of verotoxin and verotoxin-producing *Escherichia coli* [6]. Wood charcoal adsorbs dyes [7] and gases [8]. Wood charcoal powder is traditionally used to clean dirt-coated teeth, glasses and mirrors because of its ability to adsorb dirt. A lump of wood charcoal is traditionally placed on pastes made from ground dehulled beans to prevent souring preparatory to processing into *Akara* or *Moi-moi*. This type of fermentation leads to food deterioration and/or spoilage because the taste of the final products is unacceptable to consumers. This study reports the effect of wood charcoal on the rate of microbial production of lactic acid in dehulled and undehulled cowpea pastes.

Materials and Methods

Collection of cowpea and wood charcoal

Cowpeas and dry wood charcoal used were purchased from Eke Ukwu market in Owerri Municipal Local Government Area, Imo State, Nigeria. The cowpeas were authenticated by Dr. F.N. Mbagwu, a taxonomist in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. Cowpea samples were deposited in the institution's herbarium with voucher number IMSUH 248.

Treatment of samples

A quantity (25.0 g) of apparently healthy cowpea seeds were put in each of two 500 mL capacity plastic bowels containing 150 mL of distilled water. The samples were soaked for 1 h and drained of water. One set was manually decocted, whereas the other set was left undehulled. The two sets of soaked bean samples were separately ground into pastes using the Thomas-Willey milling machine (ASTM D-3182; India) in 50.0 mL of distilled water. The dry wood charcoal was ground into powder using ceramic mortar and pestle.

Analyses of cowpea pastes

The proximate compositions and tannin contents of the ground bean pastes were determined using the methods of AOAC [9], energy content was determined as described by Codex Alimentarius [10], and FAO [11], and microbial analyses were carried out using the methods of UK SMI [12].

Fermentation study of cowpea pastes

A quantity (4.0 g) of each ground cowpea paste was weighed into four 50.0 mL capacity beakers. Ground charcoal was not put into the first beaker, but 0.03, 0.04 and 0.05 g of ground charcoal were added to the second, third and fourth beakers, respectively. The content of each beaker was diluted with 50.0 mL of distilled water and the beakers were allowed to ferment for 2 h at room temperature (29.5 ± 2.0°C). At the end of the fermentation period, each broth was mixed and filtered through a Whatman No. 24 filter paper. The presence of lactic acid and ethanol in the filtrates was detected as described by Mathotra [7], and Ibegbulem [13], respectively. The total acidity (as lactic acid) of the filtrate was determined using the method of Haddad et al. [14]. The rate of formation of lactic acid in the diluted paste for the 2 h study period was calculated as the ratio of lactic acid formed to time in minutes.

Tannic acid–charcoal–cellulose interaction study

Aliquots (10.0 mL each) of a 0.12% tannic acid solution were dispensed into three test tubes. To the first test tube, 0.04 g of charcoal was added. To the second test tube, 0.04 g of cellulose was added. To the third test tube, 0.04 g each of charcoal and cellulose were added. The content of each test tube was mixed thoroughly, allowed to stand at room temperature (29.5 ± 2.0°C) for 2 h and filtered using Whatman No. 24 filter paper. The tannic acid contents of the filtrates were determined using the methods of AOAC [9].

Statistical analysis

Data were analysed using percentage coefficient of variation and one-way analysis of variance (ANOVA) at 95% confidence limit where appropriate.

Results and Discussion

Dehulling or decortication of cowpea reduced its protein, mineral, crude fibre, energy and tannin contents (Table 1).

Parameter	Sample		Mean	SD	CV%
	Dehulled	Undehulled			
Crude protein (%)	5.81	7.58	6.69	0.88	13.23
Moisture (%)	72.55	69.94	71.25	1.3	1.82
Ash (%)	0.94	0.86	0.89	0.07	7.96
Crude fibre (%)	0.27	0.35	0.31	0.04	11.99
Crude fat (%)	0.36	0.8	0.58	0.22	37.65
Digestible carbohydrates (%)	20.07	20.53	20.3	0.23	1.13
Energy content (kcal/100 g)	107.84	121.04	114.44	6.6	5.77
Tannin (mg/100 g)	0.86	1.53	1.2	0.33	27.5
*wet-weight basis					

Table 1: Proximate composition, energy and tannin contents of dehulled and undehulled cowpea pastes*.

It suggests that when bean seeds are processed by soaking in water and decortication for the preparation of local delicacies like *Moi-moi* or *Akara*, some of their nutrients, fibre and polyphenols are lost. The loss of polyphenols was in agreement with the report of Ibegbulem et al. [15]. Tannins have been reported to reduce absorption of iron from the gastrointestinal tract [2], reduce digestibility because they bind and precipitate digestive enzymes, act as antioxidants because they scavenge free radical and have styptic and astringent properties [16] and inhibit microbial growth [3]. The crude protein, moisture, ash, crude fibre, crude fat and digestible carbohydrates contents of the raw, unsoaked cowpea seeds were found to be 29.48%, 7.81%, 3.27%, 1.72%, 4.68% and 53.04%, respectively; suggesting that is a carbohydrate-based food. Soaking the seeds in water markedly increased their moisture contents and decreased the other nutrients.

The concentrations of *Micrococcus* spp., *Escherichia coli*, *Staphylococcus* spp., *Bacillus* spp., *Streptococcus* spp. and *Lactobacillus* spp. isolated from the ground dehulled cowpea paste were significantly lower than those isolated from the ground undehulled cowpea paste (Table 2).

The seed coat seemed to have harboured much of the microorganisms. It suggests that decortications of beans prior to preparation of local delicacies like *Moi-moi* or *Akara*, causes loss of some of their naturally occurring microorganisms. Microbes found associated with cowpea include lactic acid bacteria such as *Lactobacillus casein*, *Lactobacillus leichmanni*, *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *P. acidilactici* [17].

Lactic acid and ethanol were detected in the fermentation filtrates. This appears to suggest that the microbial modes of fermentation of the bean pastes may have included homolactic, heterolactic, and alcoholic fermentations. Majority of the microorganisms isolated from the pastes such as *Lactobacillus* spp., some *Bacillus* spp. and *Staphylococcus* spp. are lactic acid bacteria that can ferment glucose to lactic acid [3,17]. *E. coli* can ferment dextrose to acetic, formic and lactic acids [18] whereby pyruvate is first converted to format, then to carbon (IV) oxide and molecular hydrogen via formic acid

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fermentation, whereas Streptococcus can carry out mixed acid fermentation to produce ethanol, succinate, lactate and CO_2 [3]. *Micrococcus* spp. cannot ferment glucose [19,20]. However, of all the microorganisms isolated in this study, only the Bacillus spp. can hydrolyse starch [21].

Microorganism isolated	Microbial load of sample (cfu/mL)		Mean	SD	CV%
	Dehulled	Undehulled			
Micrococcus spp.	1.20×10^2	3.00×10^2	2.10×10^2	0.90×10^2	40.86
Escherichia coli	1.30×10^2	2.00×10^2	1.65×10^2	0.35×10^2	21.21
Staphylococcus spp.	1.00×10^2	2.00×10^2	1.50×10^2	0.50×10^2	33.33
Bacillus spp.	1.50×10^2	2.50×10^2	2.00×10^2	0.50×10^2	25
Streptococcus spp.	1.60×10^2	2.00×10^2	1.80×10^2	0.20×10^2	11.11
Lactobacillus spp.	1.60×10^2	2.50×10^2	2.05×10^2	0.45×10^2	21.95

Table 2: Microbial load of dehulled and undehulled cowpea pastes.

The rate of microbial production of lactic acid in the dehulled bean sample, devoid of wood charcoal powder, was significantly higher than that in its undehulled equivalent (Table 3).

Condition	Sample		Mean	SD	CV%
	Dehulled	Undehulled			
Without charcoal	4.17×10^{-3}	3.75×10^{-3}	3.96×10^{-3}	0.21×10^{-3}	5.3
With 0.03 g charcoal	4.50×10^{-3}	4.10×10^{-3}	4.30×10^{-3}	0.20×10^{-3}	4.65
With 0.04 g charcoal	5.25×10^{-3}	3.45×10^{-3}	4.35×10^{-3}	0.90×10^{-3}	20.69
With 0.05 g charcoal	3.85×10^{-3}	6.75×10^{-3}	5.30×10^{-3}	1.45×10^{-3}	27.36

Table 3: Rate of microbial production (g/100 mL/min) of lactic acid in dehulled and undehulled cowpea paste ferments.

Lactic acid production in the dehulled bean paste fermentation mixture increased but later decreased with increase in the concentration of wood charcoal powder unlike the undehulled bean paste fermentation mixture where lactic acid production increased consecutively with increase in the concentration of wood charcoal powder. Whereas rate of production of lactic acid in the undehulled bean paste fermentation mixture containing 0.04 g charcoal was not significantly higher than that of its dehulled equivalent, production of lactic acid in the undehulled bean paste fermentation mixture containing 0.05 g charcoal was significantly higher than that in its dehulled equivalent. Accordingly, the present study showed that at a critical wood charcoal concentration of 0.04 g, inhibition of fermentation of ground undehulled bean paste was lost but inhibition of fermentation was achieved in ground dehulled cowpea paste at 0.05 g wood charcoal powder.

The free tannic acid contents of the tannic acid–charcoal, tannic acid–cellulose and tannic acid–charcoal–cellulose solutions were significantly lower ($p<0.05$) than that of the benchmark tannic acid solution (Table 4). This seems to suggest that wood fibre can interact with both charcoal and plant pigments such as tannins.

The present study showed that increasing concentrations of wood charcoal powder did not inhibit the rate of fermentation of the undehulled bean pastes (Table 3). This may have been due to greater fibre–charcoal–phytochemical interactions in the undehulled bean, rather than charcoal–microorganism interaction. The undehulled bean paste contained more protein, fibre and oil than its dehulled equivalent (Table 1). Fibre bound wood charcoal and tannins (Table 4) thereby reducing the level of available charcoal that was supposed to adsorb the fermentative microorganisms. Adsorption of fermentative microorganisms by wood charcoal removes and makes them unavailable for fermentation [6]. This may explain its usage in medicine.

Solution	Tannic acid content (mg/100 mL)
Tannic acid	12.00 ± 0.00^a
Charcoal+tannic acid	9.40 ± 0.05^c
Cellulose+tannic acid	9.60 ± 0.49^c
Charcoal+cellulose+tannic acid	11.00 ± 0.37^b

[*]Values are mean ± SD of duplicate determinations.

Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

Table 4: Free tannic acid content of solution.

Conclusion

The inhibition of fermentation in ground dehulled bean paste by wood charcoal may explain its traditional use to prevent the spoilage of ground bean pastes prior to processing them into bean balls or bean cake.

References

1. Phillips RD, McWatters KH, Chinnan MS, Hung YC, Beuchat LR, et al. (2003) Utilization of cowpea for human food. Field Crops Res 82: 193-213.
2. Wardlaw GM, Kessel MW (2002) Food safety (5th edn.). In: Perspective in Nutrition. Wardlaw GM, Kessel MW (eds.) McGraw-Hill, Boston pp: 745-788.
3. Prescott LM, Harley JP, Klein DA (2002) Microbiology (5th edn.). McGraw-Hill, Boston.
4. Neta ERC, Johanningsmeier SD, Drake MA, McFeeters RF (2007) A chemical basis for sour taste perception of acid solutions and fresh-pack dill pickles. J Food Sci 72: 352-359.
5. Kerihuel JC (2010) Effect of activated charcoal dressings on healing outcomes of chronic wounds. J Wound Care 19: 208-215.
6. Naka K, Watarai S, Inoue K, Kodama Y, Oguma K, et al. (2001) Adsorption effect of activated charcoal on enterohemorrhagic *Escherichia coli*. J Vet Med Sci 63: 281-285.
7. Mathotra VK (1989) Practical Biochemistry for Students (3rd edn.). Jaypee Brothers Medical, New Delhi, India.
8. Zakaria Z, George T (2011) The performance of commercial activated carbon absorbent for adsorbed natural gas storage. Int J Res Rev Appl Sci 9: 225-230.
9. Association of Official Analytical (AOAC) (2006) Official Methods of Analysis. (18th edition) Association of Official Analytical Chemists, Washington DC, USA.
10. Codex Alimentarius (2001) Codex Guidelines on Nutrition Labelling CAC/GL 2-1985. Codex Alimentarius Commission. Joint FAO/WHO Food Standards Programme. Food and Agricultural Organization of the United Nations/World Health Organization, Rome.
11. Food and Agricultural Organization (FAO) (2003) Food Energy-Methods of Analysis and Conversion Factors. Food and Nutrition Paper 77. Food and Agricultural Organization of the United Nations, Rome.
12. UK SMI (2015a) UK Standards for Microbiology Investigations. Public Health England.
13. Ibegbulem CO (2012) Nutritional effects of drinking terminalia iittoralis seem decoction. Int J Biochem Res Rev 2: 126-136.
14. Haddad PR, Sterns M, Warclaw J (1978) Analysis of wine–an undergraduate project. Edu Chem 15: 87-88.
15. Ibegbulem CO, Nwaogu LA, Iworie IL (2006) Effect of dehulling bean on volume of water drunk after its meal. J Agric Food Sci 4: 61-66.
16. Evans WC (2002) Trease and Evans Pharmacognosy (15th edn.). W.B. Saunders, Edinburgh.
17. Hedley CL (2001) Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics. CABI Publishing, NY, USA.
18. Frampton EW, Restaino L (1993) Methods for E. coli identification in food, water and clinical samples based on beta-glucuronidase detection. J Appl Bacteriol 74: 223-233.
19. Hugh R, Leifson E (1953) The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J Bacteriol 66: 24-26.
20. UK Standards for Microbiology Investigations (2015b) Standard Units, Microbiology Services. UK Standards for Microbiology Investigations. Public Health England. Bacteriology Test Procedures Oxidation/ Fermentation of Glucose Test pp: 1-13.
21. Ibrahim SE, El Amin HB, Hassan EN, Sulieman AME (2012) Amylase production on solid state fermentation by *Bacillus* spp. Food Public Health 2: 30-35.