GSC Biological and Pharmaceutical Sciences, 2020, 13(02), 284-289

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Qualitative phytochemical and GC-MS analysis of fermented castor seed (Ogiri Igbo)

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Publication history**:** Received on 29 October 2020; revised on 16 November 2020; accepted on 18 November 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.13.2.0351

Abstract

The aim of this study was to investigate the phytochemicals present in fermented castor seed (ogiri Igbo). 1kg of castor seed was dehulled and cleaned after which seeds were wrapped in banana leaves and boiled for 6-8 h. The boiled seeds which were still wrapped in the leaves were left to ferment for 4-6 days. Later, the seeds were mixed with ash from oil palm bunch and ground into paste. Subsequently, the condiment (ogiri Igbo) formed was wrapped in leaves in small portions.Phytochemical and GC-MS analysis were performed on the condiment using standard methods. Phytochemicals investigated were tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, phlobactannins, phenols, proteins, reducing sugars and anthraquinones. While steroids, reducing sugars and anthraquinones were absent in the condiment, flavonoids, phlobactannins, and alkaloids were present in abundance (++), the least abundant being the terpenoids, cardiac glycosides and phenols (+) and the most abundant phytochemicals reportedly present in the condiment were saponins and proteins. GC-MS analysis performed on the fermented castor seed (ogiri Igbo) showed that forty eight compounds were present in the said condiment and while the most abundant compound reported was 9,12-Octadecadienoic acid (Z,Z)-methyl ester (17.43%), the least abundant was 2-t-Butyl-3,6-dimethyl pyrazine (0.35%). In conclusion, although some of the relevant phytochemicals may have been lost to fermentation, the condiment still retains some health aiding phytoconstituents in appreciable amounts.

Keywords: Ogiri; Phytochemical; Castor seed; Fermentation

1. Introduction

Castor seed (*Ricinus comminis*) an oval, shiny beanlike high nutrient profile raw material from which ogiri Igbo is made is a derivative of the castor oil plant which is a member of the family Euphorbiaceae. It grows across the tropical and sub-subtropical regions of the world [1]; [2]. The raw castor seed is a repository of a toxic protein known as ricin in addition to other toxic constituents such as ricinine and ricinoleic acids which impede its consumption [3][4].

In order to ensure that castor seed consumption is safe, fermentation, an age long traditional method of processing toxic food stuffs is extensively invoked to eliminate or reduce the toxic components, a process which births the condiment known as ogiri Igbo [5].

Ogiri Igbo like other forms of ogiri is a solid state fermentation product known for its characteristic ammoniacal flavor that impacts on the taste and flavour of the local Nigerian dishes [6]. It is a viable source of plant protein and health

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aiding phytochemicals bound to other cellular components within the food matrice and may be released during solid state fermentation [7]. Thus, it is imperative to ascertain the impact of this well thought processing approach on the phytochemicals that characterize the food matrice [8].

2. Material and methods

2.1. Seed collection and production of condiments

Exactly 1kg of castor seed procured from local market within Port Harcourt metropolis in Rivers State, Nigeria were dehulled and sorted to get rid of bad seeds, hulls and extraneous materials. Dehulled castor seeds were wrapped in banana leaves and boiled for 6-8 h. The boiled seeds which were still wrapped in the leaves were left to ferment for 4-6 days. Later the seeds were mixed with ash from oil palm bunch and ground into paste. It was wrapped in small portions and allowed to develop the characteristic flavor [9][10].

2.2. Qualitative phytochemical analysis

About 5.0 g of ogiri Igbo sample was steeped in 100 ml of distilled water in a beaker for 24 hrs, before being filtered. The resulting filtrate was used for phytochemical analysis [11].

2.3. Test for alkaloids

Mixture of 3 ml of filterate and 5 ml of 10% aqueous HCl was stirred on hot water bath. The content was filtered into the test tube into which few drops of Dragendoff's reagent were added and the appearance of orange red precipitate indicates the presence of alkaloids [12].

2.4. Test for phenols

Exactly 2 drops of 1% ferric (III) chloride solution was added to a mixture consisting of 2 ml of filterate and 3 ml of water in a test tube. The appearance of red, blue, green (blackish or purple colour was indicative of the presence of phenols [11].

2.5. Test for phlobatannins

To test phlobactannins, 2ml of aqueous extract of ogiri Igbo was boiled with 1 % aqueous hydrochloric acid. Deposition of a red precipitate was an indication that phlobactannins was present [13].

2.6. Test for saponin

10 ml of filtrate held in a test tube was warmed with the aid of the water bath for 5 minutes before shaken to observe frothing. The persistence of froth is an indication of the presence of saponins. To the froth, 3 drops of olive oil were added. The formation of emulsion confirms the presence of saponin [14].

2.7. Test for Tannins

Mixture of the extract and distilled water was subjected to heating with the aid of a boiling water bath. The mixture was filtered and to the filtrate few drops of conc. H_2SO_4 and 5% ferric chloride were introduced, the emergence of precipitate indicates the presence of tannins [12].

2.8. Test for flavonoid

To 2 ml of extract in a test tube was added 10% of sodium hydroxide solution. Appearance of yellow colouration indicated the presence flavonoids [11].

2.9. Test for steroids

Precisely 2ml of acetic anhydride was introduced into a test tube holding 0.5 g aqueous extract of ogiri Igbo sample with 2 ml H_2S0_4. The transition of colour from violet to blue or green in some samples indicates the presence of steroids [13].

2.10. Test for terpenoids (Salkowski test)

Exactly 5 ml of aqueous extract of ogiri Igbo was mixed with 2 ml of chloroform and concentrated. 3 ml of H_2S0_4 was gently introduced to form a layer. A reddish brown colouration of the interface formed was indicates the presence of terpenoids [13].

2.11. Test for cardiac glycosides (Keller-Killani test)

Exactly5 ml of aqueous extract of ogiri Igbo was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was subsequently underlayed with 1 ml of concentrated H_2SO_4. A brownish ring at the interface is indicates the presence of deoxysugar characteristic of cardenolides [13].

2.12. GC-MS of ogiri Igbo samples

An agilent 7890B Gas Chromatography (GC) system fitted with a 30 m× 250 µm × 0.25 µm Rtx-5MS capillary column coupled to Agilent 5977A Mass Spectrometric (MS) was employed in the analysis at temperature of 325 ^oC. Ultra-high purity helium (99.99%) served as the mobile phase at constant flow rate of 1.0 cm^3/min. The injector, transfer line and ion source temperature were set at 290 ^oC. The ionizing energy was 70ev. Electron multiplier voltage was acquired from auto tune. The oven temperature was set from 60 ^oC for 2 mins, then 10 ^oC /min to 110 oC/min and then 280 ^oC at the rate of 5 ^oC/min. The sample was diluted with appropriate acetone (1/100 v/v), filtered and 1 µL was injected into the inlet. All data were collected by collecting the total ions currents (TIC). The percentage composition was determined from calibration curve (0-0.9g/cm^3). The sample and the standard were formed alike. The standard was processed separately before it was spiked into the sample and signal of the sample was obtained from the difference of the spiked sample and that of the standard. The experiment was repeated severally. As a quality control measure, percentage relative standard (%RSD) was estimated by comparing coefficient of determination (R2) values of calibration curves using both standard signal and spiked sample signal [15].

3. Results

Table 1: Result on the qualitative phytochemical analysis of fermented castor seed (ogiri Igbo)

Phytochemicals	**Results**
Tannins	+
Saponins	+++
Flavonoids	++
Steroids	-
Terpenoids	+
Cardiac glycoside	+
Phlobactanins	++
Phenolic compounds	+
Proteins	+++
Reducing sugars	-
Anthraquinnones	-
Alkaloids	++

+: abundant, ++: more abundant, -: absent

Table 2 Result of Gas Chromatography-Mass Spectrometric analysis on fermented castor seed (ogiri Igbo)

S/n	Rt	Component	Formula	Mw	%
1	3.673	Dehydromelonivlonic acid lactone	$C_6H_8O_2$	112	0.59
2	3.819	Piperidinone	C_5H_9NO	99	2.41
3	3.988	Benzofuran, 2,3-dihydro-	C_8H_8O	120	318
4	4.268	Heptanal	$C_7H_{14}O$	114	0.04
5	4.337	Benzenepropanoic acid, methyl ester	$C_{10}H_{12}O_2$	164	0.38
6	4.610	Cyclopentane, 2-ethyl-1,1-dimethyl	C_9H_{18}	126	0.38
7	4.993	1-Fluoro-2,4,6-trimethylbenzene	$C_9H_{11}F$	138	0.41
8	5.079	1,5-Heptadien-3-yne	C7H8	92	0.37
9	5.206	Phenol, 4-(2-aminoethyl)-	$C_8H_{11}NO$	137	8.64
10	5.281	Phenol, 4-(2-aminoethyl)-	$C_8H_{11}NO$	137	9.93
11	5.562	Phenol, 4-(2-aminoethyl)-	$C_8H_{11}NO$	137	0.44
12	5.701	Acetamide, n-ethyl-N-phenyl	$C_{10}H_{13}NO$	163	0.38
13	5.798	1,2,4,6-Tetrathiepane	$C_3H_6S_4$	170	0.66
14	6.203	2-t-Butyl-3,6-dimethylpyrazine	$C_{10}H_{16}N_2$	164	0.35
15	6.245	Acetamide, N-(2-phenylethyl)-	$C_{10}H_{13}NO$	163	1.04
16	6.754	Butanoic acid, 3-methyl-, 2-phenylethyl ester	$C_{13}H_{18}O_2$	206	1.04
17	6.968	N-(2-Cyanoethyl)-pyrrole	$C_7H_8N_2$	120	0.47
18	7.328	N-Acetyltyramine	$C_{10}H_{13}NO_2$	179	0.48
19	7.620	Hexadecanoioc acid, methyl ester	$C_{17}H_{34}O_2$	270	3.76
20	7.748	N-Acetyltyramine	$C_{10}H_{13}NO_2$	179	2.29
21	7.789	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.32
22	8.119	2-Butanone, 3-amino-4-phenyl	$C_{10}H_{13}NO$	163	0.40
23	8.179	2H-1-Benzopyran-4-ol,3,4-dihydro-2-(4-hydroxyphenyl)-	$C_{15}H_{14}O_3$	242	0.37
24	8.351	9, 12-Octadecadienoic acid (z, z)-, methyl ester	$C_{19}H_{34}O_2$	294	17.43
25	8.460	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	3.70
26	8.535	Dodecanamide, N-(2-hydroxyethyl)-	$C_{14}H_{29}NO_2$	243	3.77
27	8.621	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	1.16
28	8.722	DOdecanamide	$C_{12}H_{25}NO$	199	1.41
29	9.300	Eicosanoic acid, methyl ester	C21H42O2	326	1.89
30	9.480	9-Octadecyne	$C_{18}H_{34}$	250	5.38
31	9.585	Octadecanamide	$C_{18}H_{37}NO$	283	0.64
32	9.697	9, 17-Octadecadienal, (z)-	$C_{18}H_{32}O$	264	1.63
33	9.873	Undecanal, 2-methyl-	$C_{12}H_{24}O$	184	0.37
34	9.952	9, 12-Octadecadienoic acid (z,z)-	$C_{18}H_{32}O_2$	280	0.42
35	10.109	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{34}H_{68}O_3$	524	1.04
36	10.166	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284	2.62
37	10.953	Isoquinolinium, 2-methyl-, iodine	$C_{10}H_{101}N$	271	0.50
38	11.028	9, 17-Octadecadienal, (z)-	$C_{18}H_{32}O$	264	6.34
39	11.152	Cyclononanone	$C_9H_{16}O$	140	1.15
40	11.189	Heneicosanoic acid, methyl esther	$C_{22}H_{44}O_2$	340	0.66
41	11.541	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	$C_{20}H_{40}$	280	0.50
42	13.539	9-Borabicyclo[3,3,1]nonan-9-amine, N-methyl-	$C_9H_{18}BH$	151	0.59
43	14.132	9,12-Octadecadienoic acid, methyl ester (E,E)	$C_{19}H_{34}O_2$	294	0.72
44	14.237	Benzenamine, 3-methoxy-2,4,6-trimethyl-	$C_{10}H_{15}NO$	165	0.94
45	15.193	Pregna-5,16-dien-20-one, 3-hydroxy-, (3β)-	$C_{21}H_{30}O_2$	314	1.07
46	15.492	26,27-Dinorergosta-5,23-dien-3-ol -(3β)-	$C_{26}H_{42}O$	370	1.56
47	16.051	Chola-5-22-dien-3-ol, (3β, 22E)-	$C_{24}H_{38}O$	342	1.70
48	16.216	Progesterone	$C_{21}H_{30}O_2$	314	0.51

4. Discussion

Phytochemicals are biologically active compounds which are widely acknowledged principally for their medicinal [16]. Castor seed although known for its high nutrient profile is a repository of certain toxins such recinine and recinoleic acid among others which when consumed are extremely deleterious to health [4]. Fermentation is an age long traditional food processing approach to eliminating toxins from foods and has been widely employed especially by the local Nigerian people to translate the inedible castor seed into the edible fermented product (ogiri Igbo). Microbial fermentation has impact on phytochemical profiles of food matrices [8]. Table 1.0 shows the result on the qualitative phytochemical analysis performed on fermented castor seed (ogiri Igbo). Phytochemicals screened for included tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, phlobactannins, phenols, proteins, reducing sugars and anthraquinones. Steroids, reducing sugars and anthraquinones were reportedly absent in the condiment. This may be as a result of the absence of the corresponding hydrolytic enzymes for their conjugated forms. However, flavonoids, phlobactannins, and alkaloids were abundant (++), the least abundant being the terpenoids, cardiac glycosides and phenols (+) and the most abundant phytochemicals reportedly present in the condiment were saponins and proteins. This may be attributed to the presence of different carbohydrate cleaving enzymes. These results are in tandem with the work of Bhanja et al [17] which affirmed that different carbohydrate enzymes are responsible for the release phenolics during solid state fermentation of wheat by *A. oryzae* and *A. awamori*. GC-MS analysis was carried out with the aid of the National Institute Standard and Technology (NIST) database which has over 62,000 patterns. Spectrum obtained on unknown compounds was compared with that of known compounds deposited in the NIST library. The name, molecular weight and structure of the compounds were determined. GC-MS analysis performed on fermented castor seed (ogiri Igbo) revealed the presence of forty eight compounds in the said condiment as shown in Table 2.0 while the most abundant compound reported was 9,12-Octadecadienoic acid (Z,Z)-methyl ester (17.43%), the least abundant was 2-t-Butyl-3,6-dimethyl pyrazine (0.35%).

5. Conclusion

In addition to the detoxification potential of fermentation, the outcome of this study has further consolidated its viability as a tool for engineering bioavailability of essential phytochemicals in food stuffs such as fermented castor seed (ogiri Igbo).

Compliance with ethical standards

Acknowledgments

Authors wish to acknowledge the technical staff of the Department of Medical Biochemistry, Rivers State University. Nkpolu Oroworukwo. Port Harcourt Nigeria

Disclosure of conflict of interest

Authors hereby declare that no conflict of interest exists.

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