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CHEMICAL COMPOSITION AND FUNCTIONAL PROPERTIES OF *CITRULLUS VULGARIS* FERMENTED WITH MUTANT AND NON-MUTANT STRAINS OF *BACILLUS SUBTILIS* TO PRODUCE *OGIRI*

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ABSTRACT

Natural food seasoning agents are gradually gaining prominence due to purported side effects associated with artificial seasoning agents. Hence, the search for natural food seasoning with improved functional and nutritional quality. This research is aimed at investigating the amino acids composition, fatty acid contents, antioxidant and functional properties of *Citrullus vulgaris* fermented with mutant and non-mutant strains of *Bacillus subtilis* to produce *Ogiri*. *Bacillus subtilis* strains were isolated from spontaneously fermented melon seeds (*C. vulgaris*) and the *B. subtilis* isolates were exposed to two different mutagenic agents [Ultraviolet (UV) irradiations and Sodium Dodecyl Sulphate (SDS)] at varying intervals of time to obtain mutant strains. Eight (8) mutated strains of *B. subtilis* that produced high D-ribose metabolites were used for controlled starter-fermentation of *C. vulgaris* to produce *Ogiri.* The non-mutant (NMS00) and the market *Ogiri* (RTE00) were included as control samples. The properties mentioned above were determined on the *Ogiri* samples. The most abundant and limiting essential amino acids varied among the *Ogiri* samples. Mutated fermented *Ogiri* samples have improved antioxidant properties. *Ogiri* sample produced with *B. subtilis* mutant strain exposed to SDS at 110 sec (MSD51) have the highest monounsaturated fatty acid (MUFA) (15.67±0.00 mg/100 g) and polyunsaturated fatty acids (PUFA) (50.29±0.00 mg/100 g). Free fatty acids and peroxide values are higher in control samples. Modified *Ogiri* produced from the mutant strains of *B. subtilis* have improved water and oil absorption capacities high essential amino acids and antioxidant properties, hence may serve as functional condiments in food system.

Keywords: Amino acids, Antioxidant, *Citrullus vulgaris* seeds, D-ribose, Functional *Ogiri*, Mutation

INTRODUCTION

Citrullus vulgaris is one of the commonly found leguminous plants, processed into different food products such as food condiments. It is rich in protein, and high in bioactive compounds that can cause reduction of high blood pressure (Polak *et al*., 2015). Fermented food condiments are popular strong smelling fermented agro-food culinary products. They give a pleasant aroma to soups, sauces and other prepared dishes (Ogunshe *et al*., 2008). It has been revealed that condiments can serve as a substitute for protein and calorie intake for prevention of malnutrition in the diet (Nazidi *et al*., 2018).

Ogiri is one of the ethnic condiments peculiar to West African countries which occurs in different forms due to different processing techniques and substrates. *Ogiri* in present form is only applied as flavour enhancer and its value will increase if it has enhanced functionality. It is produced from different leguminous seeds (substrates) in uncontrolled solid substrate fermentation (wild) as a traditional method (Achi, 2005). It usually characterized with both pathogenic and spoilage microorganisms as contaminants and only proteolytic *B. subtilis* have been reported to be the fermenting microorganism. The mutation of *B. subtilis* produce D-ribose metabolite which is deficient in transketolase (tkt), but with several functions to include improved effects on inflammatory, pro-inflammatory cytokine and ATP synthesis increase (Li *et al*., 2021). It also served as a mediator in nucleoside antiviral agents, synthesis of vitamin B_2, nucleotide flavour enhancers, and adenine salvage rates in skeletal muscle recovery of intense contractions in both human and animal (Bayram *et al*., 2015).

The assessment of food quality has to do with its physicochemical parameters to include functional properties, nutritional level and rancidity, especially if it is oil-based. Functional properties are important parameters usually considered in food (starch, protein, fat, etc.) preparation; how the food material interact with the molecular and structural properties to reflect its tastes, appearance (looks), mouth feels and texture (Awuchi *et al*., 2019).

Amino acids and fatty acids are important factor usually considered in food. The amino acids of food is known to form the network of body protein needed for growth, body metabolic activities and functions (Samtiya *et al*., 2020) in preventing related health diseases/disorders when consumed. Likewise, the fatty acids are parts of body metabolisms. It consists of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Monounsaturated fats are of health benefits as they improve insulin sensitivity, prevent cancer, and lowers low-density lipoprotein (LDL) (bad) cholesterol level to promote high-density lipoprotein (HDL) cholesterol levels and reduce heart related problems (Katalin and Ioana-Daria, 2017). Plants are known to have natural exogenous antioxidants, which are of medicinal importance (Kasote *et al*., 2015). The plants' antioxidant potential received recognition lately due to its usage to tackle stress from oxidation associated with life-threatening diseases such as cardiovascular and neurodegenerative diseases. The antioxidant of the body serves as metabolic defense (Ighodaro and Akinloye, 2018) to combat the negative effects of oxidative stresses. Deficiency in antioxidant with malnutrition in the body have been reported to accelerate stress from oxidation quickly (Liu *et al*., 2018).

The study is aimed at determining the functional properties, amino acid, fatty acid profiles and antioxidant properties of *Ogiri* fermented with mutant and non-mutant strains of *B. subtilis* for which are currently with limited information and potential of using it as improved condiment.

MATERIALS AND METHODS

Source of raw materials

Seeds of *Citrullus vulgaris* 'bara' ('egusi') verified by Crop, Soil and Pest Management Department, Federal University of Technology Akure were bought from Owena market, Osun State, Nigeria. Already processed *Ogiri* (market sample) were purchased from Oja-Oba in Akure, Ondo State, Nigeria. They were both purchased in the month of August, 2020.
Thirty kilograms (30 kg) of *C. vulgaris* seeds was purchased as a batch and used for the production of the *Ogiri*.

Preparation of *Ogiri* extract

Each of the *Ogiri* extracts was prepared by weighing 10 g of freeze-dried *Ogiri* into 100 ml of distilled water and the mixture was shaken in automated shaker water bath machine (model SH2-82 JINOTECH instrument, China) for 4 h. It was filtered with Whatman 1.0 filter paper. The supernatant was stored for various assays.

Microbiological analyses

B. subtilis strains from the market *Ogiri* sample were isolated and identified based on cultural, morphological and biochemical characteristics according to Fawole and Oso (2007) and Cowan (2002) procedures. The DNA extraction (16S rRNA gene analysis) and polymerase chain reaction (PCR) of extracted DNA from isolated bacteria using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1392R (5'-GGTTACCTTGTTACGACT-3 according to Akinyemi and Oyelakin (2014) procedure. The PCR mix comprises of 1µl of 10X buffer, 0.4µl of 50mM $MgCl_2$, 0.5µl of 2.5mMdNTPs, 0.5µl 5mM Forward primer, 0.5µl of 5mM Reverse primer, 0.05µl of 5units/ul Taq with 2µl of template DNA and 5.05µl of distilled water. The PCR profile used has an initial denaturation temperature of 94 ºC for 3 min, followed by 30 cycles of 94 ºC for 60 s, 56 ºC for 60 s 72 ºC for 120 s and the final extension temperature of 72 ºC for 5 min and the 10 ºC hold forever. The amplicon was further purified before the sequencing using 2 M Sodium Acetate washing techniques. The pellet was re-suspended in 5µl sterile distilled water. The PCR mix used includes 0.5µl of BigDye Terminator Mix, 1µl of 5X sequencing buffer, 1µl of 16S Forward primer with 6.5µl Distilled water and 1µl of the PCR product making a total of 10µl. The PCR profile for Sequencing is a Rapid profile, having the initial Rapid thermal ramp to 96 ºC for 1 min followed by 25 cycles of Rapid thermal ramp to 96 ºC for 10 s, Rapid thermal ramp to 50 ºC for 5 s and Rapid thermal ramp to 60 ºC for 4 min. It was then followed by Rapid thermal ramp to 4 ºC and hold forever. The PCR sequence product was purified before the sequencing using 2 M Sodium Acetate washing techniques and the pellet was re-suspend in 5µl sterile distilled water. The combination of 9µl of Hi Di Formamide with 1µl of Purified sequence making a total of 10µl was prepared and loaded on BIO-RAY model, England and amplified fragments were visualized on ethidium bromide-stained 1.5% agarose electrophoresis gels. The Codon Code Aligner software (Codon Code Corporation, Centerville, MA) of 16S rRNA sequences of both mutant and non-mutant strains were aligned for mutation detection.
Standardization of identified bacteria to be used as starter cultures was carried out according to the procedure of Babatuyi *et al*. (2020) using McFarland standard as reference. Eighteen (18) h old broth culture of one millilitre (1 ml) (approximately 1.5×10^8cfu/ml) already harvested and washed (three times) was suspended in 10 ml 0.1 M potassium phosphate buffer (pH 7.0) and centrifuged using refrigerated centrifuge (Model Harrier 18/80, Henderson Biomedical LTD MSE, UK) at 5000 g for 10 min according to the modified procedure of Babatuyi *et al*. (2020).
The cells were aseptically re-suspended in 20 ml sterile 0.1 M potassium phosphate buffer (pH 7.0) for standardization. The result of turbidity was determined using spectrophotometer at 600 nm (model DNP-9102 721-VIS Series SEARCHTECH instruments, England).

$$0.5\ McFA = 1.5 \times 10^8 cells\ to\ be\ equal\ to\ 0.06\ (OD\ 600\ nm). \quad (1)$$

Production of mutant strains of *Bacillus subtilis*

Physical mutation
The strains of mutant *B. subtilis* were produced using the modified method of Xu *et al*. (2011). Four (4) ml of cell suspension (10^8 cells/ml) of *B. subtilis* was placed in petri dish under ultraviolet lamps (253.7nm and 366nm) at a distance of 30 cm and irradiated at different intervals between 30 s and 120 s. Each cell suspension in sterile physiological saline (0.85 %) was diluted and incubated using electro-thermal incubator (Model DNP SEARCHTECH instruments, England) in the dark at 37 ° C for 2 days on LB agar.

Chemical mutation.
The strains of mutant *B. subtilis* were produced using the modified method of Xu *et al*. (2011).The modification was that one (1) ml of SDS solution (100 mg/ml) was added to 1 ml of cell suspension of each *B. subtilis* strain (10^8 cells/ml) and placed in rotary shaker of 120 rpm at different intervals between 30 and 120 s. Each mixture was diluted several times with sterile distilled water immediately to terminate the reaction. The cells were diluted in sterile physiological saline. The plates were incubated at 37°C in dark for 3 days on LB agar.

D-ribose production

Five milliliter (5 mL) each of seeded microbial broth culture of mutant and non-mutant media containing 5 g/L xylose, 5 g/L glucose was prepared and incubated at 37 ° C for 48 h in water bath shaker (constant temperature oscillator) model SHA-C2 Labscience, England). The sugar composition of the culture broth was analyzed after incubation using HPLC-RI according to the method of Park and Seo (2004).

Production of *Ogiri*

The production of *Ogiri* sample from *C. vulgaris* seeds was determined using modified procedure of Akinyele and Oloruntoba (2013). The seeds were sorted and washed before they were boiled for 40 min for easy removal of outer coat and drained. It was boiled for another 2 h to soften the seeds, mashed and divided into 9 portions. Eight (8) out of the nine (9) portions were fermented with mutant strains of *B. subtilis*, while the last portion was fermented with non-mutant strain to serve as the control and as well as the market sample. The samples were wrapped in different low-density polyethylene packaging (LDP) materials and incubated at 28 ºC for 5 days. The fermented mashed samples were re-wrapped in low-density polyethylene packaging (LDP) materials after 5 days of fermentation, cured in a warm dry enclosure for 10 days, freeze-dried (Lab Kit FD-10-MR), milled, packaged and stored for further analysis.

Determination of functional properties of *Ogiri*

Both water and oil absorption capacities of the *Ogiri* samples were determined using the procedures of Sosulski (1962).

One gram of the *Ogiri* flour was weighed into each of already weighed clean dried centrifuge tube. Ten milliliters of distilled water or oil were poured into separate centrifuge tubes and stirred thoroughly, centrifuged at a speed of 4000/rpm for 10 min (Uniscope Model SM9023). The supernatant in each tube was discarded. Each tube and its contents were re-weighed. The increase in mass was taken as the water or oil absorbed, and calculated as follows:

$$\%\ WAC\ /OAC\ = \frac{Weight\ of\ the\ sample+\ tube\ -\ Weight\ of\ sample+tube\ after\ centrifuging}{Weight\ of\ the\ sample} x\ 100 \quad (2)$$

The foaming capacity was determined by the method of Coffman and Garcia (1977). Five grams (5 g) of the *Ogiri* sample was dispersed in 100 ml distilled water. The resulting solution was homogenized for 5 min at high speed. The volume of foam separated was noted and the result was expressed using equation 3 as given below.

$$\%\ Foaming\ capacity\ = \frac{vol.after\ homogenisation\ -\ vol.before\ homogenisation}{vol.before\ homogenisation} x\ 100 \quad (3)$$

The method of Akpapunam and Markakis (1981) was used for determining loose and packed density of the *Ogiri* samples. The results were expressed using equations 4 and 5 as given below

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$$Loose\ density\ (g/ml)\ = \frac{Weight\ of\ the\ sample}{volume\ of\ the\ sample\ after\ pouring} x\ 100 \quad (4)$$

$$Packed\ density\ (g/ml)\ = \frac{Weight\ of\ the\ sample}{volume\ of\ the\ sample\ after\ pouring} x\ 100 \quad (5)$$

The method of Ukpabi and Ndimele (1990) was used for determining the swelling index of the *Ogiri* sample. Ten grams of the sample was transferred into a clean, dried, calibrated measuring cylinder. The *Ogiri* flour was gently leveled by tapping the cylinder and the initial volume recorded. Fifty milliliters (50 ml) of distilled water was poured into the cylinder and allowed to stand for 4 h. The value for Swelling Index (SI) was taken as the fraction increase of original volume. The results were expressed using equation. 6 as given below.

$$\%\ Swelling\ index\ (SI)\ = \frac{Final\ volume(ml)\ -\ initial\ volume\ (ml)}{Initial\ volume\ (ml)} x\ 100 \quad (6)$$

Determination of amino acid content

The amino acid profiles of the *Ogiri* samples were determined according to Spackman *et al*. (1978) using Technicon Sequential Multi-Sample Amino Acid Analyser (TSM-1) after sample hydrolysis in 6 M HCl.

The tryptophan was determined according to the procedure of Pintér-Szakács and Molnán-Perl (1990). After hydrolysis and filtration, the absorbance was measured at 380 nm and a standard tryptophan curve was prepared (0~100 µg tryptophan). Each sample concentration was determined from the standard graph, the concentration of tryptophan was calculated and expressed as g /100 g protein.

Bioactive compounds and antioxidant assays

The method of Singleton *et al*. (1999) was used for determining the total phenol content of each of the *Ogiri* extracts. The results were expressed as the Gallic Acid Equivalent (GAE) per 100 gram sample (mgGAE/100 g).

The method of Meda *et al*. (2005) was used for determining the total flavonoid content of the *Ogiri* samples. The results were expressed as the Quercetin Equivalent (QE) per 100 gram sample (mgQE/100 g).

The method of Zhang *et al*. (2008) was used for determining the ferric reducing antioxidant property of the *Ogiri* samples. The results were expressed as the milligram ascorbic acid equivalent per 100 gram sample (mgAAE/100 g).

The method of Re *et al*. (1999) was used for determining the ABTS [(2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] scavenging ability of each of the *Ogiri* samples. The results were expressed in Trolox equivalent antioxidant capacity (TEAC) using Trolox as the standard.

The method of Aluko and Monu (2003) was used for determining free radical scavenging effect of the 2, 2-Diphenyl-1-picryhydrazyl (DPPH) on each of the *Ogiri* samples. The results were expressed using equation 7 as given below.

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{517}\ of\ sample}{A_{517}\ of\ blank}\right) \text{x } 100 \quad (7)$$

The method of Girgih *et al*. (2011) used for determining the hydroxyl radical scavenging activity of each of the *Ogiri* samples. The results were expressed using equation 8 as given below.

$$\text{Hydroxyl } (\bullet OH) \text{ radical scavenging activity (\%)} = \left(\frac{(\Delta A_{536}\ /\text{min})b - ((\Delta A_{536}\ /\text{min})s}{(\Delta A_{536}\ /\text{min})b}\right) \text{x } 100 \quad (8)$$

The method of Xie *et al*. (2008) was used for determining the metal chelating activity of each of the *Ogiri* samples. The percentage chelating effect (%) of results were expressed using equation 9 as given below.

$$\text{Metal } (Fe^{2+}) \text{ chelating activity (\%)} = \left(1 - \frac{A_{517}\ of\ sample}{A_{517}\ of\ blank}\right) \text{X } 100 \quad (9)$$

Determination of fatty acid content

The fatty acid content of the *Ogiri* samples was carried out by following the standard AOAC (2015) protocols.

Determination of rancidity profile

The free fatty acid of each *Ogiri* samples was determined according to the method of Pearson and Jones (1978). The samples were converted into the methyl esters. The peak of each *Ogiri* samples was identified by comparison with standard fatty acid methyl esters. The results were expressed using equation 10 as given below.

$$\text{FFA (\% oleic acid)} = \frac{Titre\ value \times molarity\ (0.1\ M\ NaOH) \times 28.2}{Weight\ of\ sample\ x\ density\ of\ oil}\ x\ 100 \qquad (10)$$

The peroxide value (PV) of the *Ogiri* samples was determined by following the standard AOAC (2015) protocols. The results were expressed as milli-equivalents per kilogram (Meq/Kg) of the sample.

Data analysis

The results of the *Ogiri* samples analyzed were pooled and expressed as mean ± standard deviation (SD). Mean values were analyzed and compared using one-way ANOVA followed by New Duncan Multiple Range Test (NDMRT). The significance was at $p \leq 0.05$. The analyzed results for figures were expressed using GraphPad Prism version 5.00 for windows.

RESULTS AND DISCUSSION

D-ribose (%) level production by mutant strains of *Bacillus subtilis*

Twenty-four mutant strains of *B. subtilis* were obtained after exposure to UV rays and SDS. D-ribose yield of the strains from the fermentation of *C. vulgaris* ranged from 1.3 to 10 g/l. Nine (9) strains of *B. subtilis* with D-ribose production ranging from 2.2 to 10 g/l were selected for the final fermentation of *Ogiri* after molecular characterization as shown in Table 1.

Table 1. D-ribose production level of mutant stains of Bacillus subtilis and their molecular identity

Bacillus subtilis **Code**	**Standard 0.2% P/A**	**D-ribose g/l**	**Molecular Identity**
Mutant Strains			
MIC67	95763.3	7.0	*B. subtilis* EBS/SRLAAH/2017
MIC64	88928.1	6.5	*B. subtilis* strain Saad27
MIC21	29681.3	2.2	*B. subtilis* 16 S KH28.1
MHL23	32893.0	2,4	*B. subtilis* strain SE10C10
MHL22	30244.0	2.2	*B. subtilis* strain Saad27
MSD99	2746.3	10.0	*B. subtilis* strain SE10C10
MSD51	70508.7	5.1	*B. subtilis* strain P2
MSD30	42443.4	3.1	*B. subtilis* strain SE10C10
Non-Mutant Strain			
NMS00	1000.1	ND	*B. subtilis* strain SE10C10

Legends:
ND: Not detected; P/A: chromatogram area, g/l: gram/litre

Functional properties of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

The functional properties of the *Ogiri* samples fermented with mutant and non-mutant strains of *B. subtilis* is presented in Table 2. The water and oil absorption capacities of the *Ogiri* samples ranged from 76 to 133 % and 38 to 60 % respectively. The water absorption capacity of *Ogiri* samples produced with *B. subtilis* strain exposed to SDS at 120 sec (MSD30), exposed to inoculating UV chamber at 90 sec (MIC67) and at 30 sec (MIC64), having values 133 %, 114 % and 114 % higher the two controls respectively. Likewise, *Ogiri* samples MSD30 (60 %), MIC67 (57 %) and MIC64 (57 %) are higher than the controls in the oil absorption capacity. High water absorption capacity will enhance its swelling power (index) and thickening property which is desirable in soup condiment. High oil absorption capacity level in some *Ogiri* samples produced with mutant *Bacillus subtilis* strains could be due to release of metabolites like protein from mutant strains of the microorganisms during fermentation to bind fat in food composition for enhancement mouth feel and flavour (Twinomuhwezi *et al*., 2020). The protein behaved in both hydrophobic and hydrophilic nature to act on the water and oil present in foods, causing both high water and oil absorption capacities (Awuchi *et al*., 2019). This is economical, as it will take more water when used during cooking, and as well enhance flavour, emulsification and mouth feel of the soup condiment (Yusufu and Ejeh, 2018).

The foaming capacity ranged from 3.20 to 9.84 %. Almost all the mutant fermented samples are higher than the controls. The increase in the foaming capacity could be due to reduction of the surface tension of both air and water interface during bubbling (Awuchi *et al*., 2019) as well as the improved protein quality and amino acid. This will make them to be better than the controls. The loose and packed densities of the *Ogiri* samples ranged from 0.93±0.00 to 1.07±0.00 g/ml and 1.66±0.00 to 3.20±0.00 g/ml respectively. Both the loose and packed densities are important in food packaging material (Ubbor *et al*., 2022) and transportation, hence economical. Loose density is about lower weight, more pores, and economical in transportation whereas, packed density is about more weight and little pore, bulky to take more quantity sample in its wrap. The swelling index of the *Ogiri* samples ranged from 94 to 240 %. Sample MSD30 (240 %) has the highest value. The high swelling index of sample MSD30 suggests that the higher the samples, the less quantity would be required during cooking, as it would take more water, hence economical and desirable thickening property of condiment (Karim *et al*., 2016).

Table 2. Functional properties of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

Sample Codes	Water absorptic capacity (%)	Oil absorption capacity (%)	Foaming capacity (%)	Loose density (g/ml)	Packed density (g/ml)	Swelling index (%)
Mutant Strains						
MIC67	114±0.00^{b}	57±0.00^{b}	6.56±0.00^{d}	0.99±0.00^{b}	2.60±0.00^{b}	236 ±0.00^{b}
MIC64	114^{b}±0.00^{b}	57 ±0.00^{b}	8.20±0.00^{c}	0.93±0.00^{c}	3.20±0.00^{a}	232±0.00^{c}
MIC21	76 ±0.00^{d}	38±0.00^{c}	3.28±0.00^{f}	0.99±0.00^{b}	2.26±0.00^{e}	223±0.00^{f}
MHL23	76±0.00^{d}	38±0.00^{c}	9.84±0.00^{b}	0.93±0.00^{c}	2.00±0.00^{f}	94±0.00^{j}
MHL22	76±0.00^{d}	38±0.00^{c}	8.20±0.00^{c}	1.07±0.00^{a}	2.53±0.00^{c}	228±0.00^{d}
MSD99	76±0.00^{d}	38±0.00^{c}	6.56±0.00^{d}	0.99±0.00^{b}	2.33±0.00^{d}	109±0.00 ^{g}
MSD51	76±0.00^{d}	38±0.00^{c}	11.48±0.00^{a}	0.93±0.00^{c}	2.26±0.00^{e}	224±0.00^{e}
MSD30	133±0.00^{a}	60±0.00^{a}	3.28±0.00^{f}	0.99±0.00^{b}	2.60±0.00^{b}	240±0.00^{a}
Non-Mutant Strain						
NMS00	76±0.00^{d}	38±0.00^{c}	4.92±0.00^{e}	0.99±0.00^{b}	1.66±0.00^{h}	103±0.00^{i}
Ready-To-Eat						
RTE00	95±0.00^{c}	38±0.00^{c}	6.56±0.00^{d}	0.80±0.00^{d}	1.86±0.00^{g}	107 ±0.00^{h}

Values represent means ± standard deviation of triplicate experiments (n = 3). Values with different letter on the same column are significantly different at P<0.05

Amino acid content of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

The amino acid content of the *Ogiri* samples fermented with mutant and non-mutant strains of *B. subtilis* is shown in Table 3. Glutamic has a taste-enhancing compound, which is formed during acid hydrolysis to serve as a major component of food seasoning (Ishiwu *et al*. 2015). *Ogiri* sample produced with *B. subtilis* strain exposed to inoculating chamber UV at 90 sec (MIC67) has the highest BV (53.32) and P-PER (2.67) in all the samples. Both biological value (BV) and predicted protein efficiency ratio (P-PER) are important parameters used in evaluating the nutritional quality of protein-rich foods. High amount of essential amino acids observed in the *Ogiri* samples; leucine (5.41 to 7.61 g/100 g), tryptophan (5.20 to 7.33 g/100 g), valine (3.55 to 6.35 g/100 g), and lysine (3.20 to 5.87 g/100 g), while threonine (3.13 to 4.30 g/100 g), isoleucine (2.04 to 3.69 g/100 g), methionine (1.19 to 2.09 g/100 g), and cysteine (0.12 to 0.65 g/100 g) are low in amount. The essential amino acids are good for promotion of health and are required in certain amount to perform protein metabolite function (cell division and growth). The high amount of tryptophan and phenylalanine (aromatic amino acid) might have been responsible for the enhanced foaming capacity (Awuchi *et al*. 2019). The amino acid score revealed that some essential amino acids are abundant which are: tryptophan (76.24 to 666.36 %), arginine (59.11 to 325 %), lysine (55.17 to 146.63 %) and phenylalanine (84.01 to 144.64 %), while methionine (54.09 to 129.05 %), lysine (55.17 to 146.63 %), phenylalanine (84 01 to 1444.64), arginine (59.11 to 325 %) and valine (61.42 to 220.29 %) are the limiting amino acids in the *Ogiri* profile. The mutant strains of *B. subtilis* fermented *Ogiri* recorded improved amino scores which suggested improved nutrition quality.

Table 3. Amino acid content of *Ogiri* samples fermented with mutant and non-mutant strains *Bacillus subtilis*

Parameter	**Sample codes**									**(g/100 g protein)**
	Mutant Strains								**Non-Mutant Strain**	**Ready-To-Eat**
Amino acids	**MIC 67**	**MIC 64**	**MIC 21**	**MHL 23**	**MHL 22**	**MSD 99**	**MSD 51**	**MSD 30**	**NMS 00**	**RTE 00**
Non-Essential Amino Acids (NEAAs)										
GLY	4.84±0.02^{a}	3.81±0.02^{g}	3.91±0.02^{e}	3.99±0.03^{e}	.67±0.02^{i}	3.51±0.03^{j}	3.89±0.02^{f}	4.09±0.02^{d}	4.21±0.04^{c}	4.42±0.03^{b}
ALA	5.01±0.03^{a}	2.74±0.02^{f}	4.00±0.03^{d}	4.05±0.02^{d}	.62±0.03^{g}	2.40±0.02^{h}	2.85±0.02^{e}	4.24±0.02^{c}	4.11±0.02^{c}	4.77±0.01^{b}
SER	4.71±0.02^{a}	3.45±0.03^{h}	3.77±0.02^{e}	3.63±0.02^{f}	.39±0.03^{i}	3.28±0.02^{j}	3.54±0.0^{g}	3.92±0.01^{d}	4.12±0.01^{c}	4.24±0.02^{b}
PRO	2.71±0.02^{a}	1.94±0.02^{f}	2.03±0.02^{e}	2.04±0.02^{e}	.87±0.01^{g}	1.66±0.01^{h}	1.95±0.02^{f}	2.20±0.03^{d}	2.31±0.02^{c}	2.44±0.03^{b}
ASP	9.04±0.01^{a}	7.20±0.03^{g}	7.91±0.03^{f}	8.19±0.02^{e}	.03±0.02^{h}	6.74±0.02^{i}	7.31±0.0^{g}	8.54±0.02^{d}	8.59±0.02^{c}	8.70±0.03^{b}
GLU	12.03±0.01^{a}	8.97±0.02^{h}	10.17±0.02^{f}	10.77±0.01^{e}	.86±0.02^{i}	8.49±0.02^{j}	9.29±0.02^{g}	11.02±0.01^{d}	1 11.93±0.02^{b}	1 11.82±0.0 2^{c}
TYR	2.99±0.03^{a}	.21±0.04^{e}	2.24±0.02^{e}	.21±0.03^{e}	.07±0.02^{f}	1.94±0.02^{g}	2.19±0.02^{e}	2.40±0.03 ^{d}	2.64±0.02^{c}	2.79±0.02^{b}
ARG	6.50±0.02^{a}	.90±0.02^{f}	4.87±0.02^{e}	5.16±0.02^{d}	3.73±0.02^{h}	3.60±0.03^{i}	3.81±0.0^{g}	5.99±0.03 ^{c}	5.94±0.02^{c}	6.31±0.02^{b}
ORN	0.44±0.02^{a}	.14±0.01^{e}	0.14±0.01^{e}	0.16±0.01^{f}	.09±0.01^{g}	0.03±0.01^{h}	0.13±0.01^{e}	0.21±0.03 ^{d}	0.24±0.02^{c}	0.34±0.01^{b}
ΣNEAs	48.27±0.00^{a}	.36±0.00^{e}	39.04±0.00^{d}	40.20±0.00^{c}	33.33±0.00^{e}	31.65±0.00^{e}	34.96±0.00^{e}	42.16±0.00^{c}	44.09±0.00^{c}	45.83±0.00^{b}
Essential Amino Acids (EAAs) + Histidine										*****
PHE	4.05±0.01^{a}	3.42±0.03^{g}	3.51±0.03^{f}	3.58±0.02^{e}	3.31±0.02^{h}	3.21±0.03^{i}	3.42±0.01^{g}	3.71±0.03^{d}	3.80±0.02^{c}	3.94±0.02^{b}	3.8
HIS	3.53±0.02^{a}	2.85±0.01^{e}	2.81±0.03^{e}	2.90±0.02^{d}	2.63±0.02^{f}	2.46±0.02^{i}	2.81±0.03^{g}	3.05±0.02^{d}	3.13±0.02^{c}	3.23±0.02^{b}	1.5
MET	2.09±0.02^{a}	1.48±0.02^{f}	1.54±0.02^{e}	1.65±0.02^{d}	1.35±0.02^{g}	1.19±0.03^{h}	1.56±0.02^{e}	1.77±0.02^{c}	1.77±0.01^{c}	1.91±0.03^{b}	1.6
VAL	6.35±0.02^{a}	3.71±0.02^{f}	4.84±0.02^{e}	5.41±0.02^{d}	3.71±0.02^{g}	3.55±0.02^{j}	3.95±0.01^{g}	5.80±0.02^{d}	5.91±0.02^{c}	6.04±0.02^{b}	3.9
TRP	7.33±0.01^{a}	5.64±0.02^{e}	5.67±0.01^{e}	6.37±0.02^{d}	5.39±0.02^{f}	5.20±0.03^{i}	5.61±0.03^{g}	6.92±0.01^{c}	6.86±0.02^{c}	7.07±0.02^{b}	0.6
THR	4.30±0.03^{a}	3.33±0.01^{f}	3.61±0.03^{d}	3.57±0.02^{e}	3.19±0.02^{g}	3.13±0.01^{j}	3.36±0.02^{g}	3.82±0.03^{d}	3.92±0.01^{c}	4.05±0.03^{b}	2.3
CYS	0.65±0.02^{a}	0.23±0.02^{e}	0.23±0.02^{e}	0.29±0.02^{d}	0.16±0.01^{f}	0.12±0.01^{g}	0.24±0.02^{e}	0.46±0.01^{c}	0.47±0.01^{c}	0.54±0.02^{b}	0.6
ISO	3.69±0.02^{a}	2.25±0.01^{f}	2.73±0.02^{e}	2.97±0.01^{e}	2.15±0.02^{i}	2.04±0.02^{j}	2.37±0.02^{g}	3.06±0.01^{d}	3.22±0.01^{c}	3.47±0.01^{b}	3.0
LEU	7.61±0.02^{a}	5.83±0.02^{f}	6.09±0.02^{e}	6.24±0.01^{e}	5.67±0.01^{i}	5.41±0.02^{j}	5.73±0.02^{h}	6.91±0.02^{c}	6.81±0.03^{c}	7.10±0.02^{b}	5.9
LYS	5.87±0.01^{a}	3.71±0.03^{f}	4.74±0.02^{e}	5.07±0.02^{d}	3.50±0.03^{i}	3.20±0.02^{j}	3.80±0.02^{g}	5.12±0.02^{c}	4.92±0.02^{d}	5.44±0.02^{b}	4.5
ΣEAAs	45.47±0.00^{a}	32.4±0.005^{f}	35.77±0.00^{e}	38.05±0.00^{d}	31.06±0.00^{g}	29.51±0.00^{h}	32.85±0.00^{f}	40.62±0.00^{c}	40.81±0.00^{c}	42.79±0.00^{b}	
Predicted Nutritional Qualities											**** REF (Egg)**
TEAA	45.47^{a}	32.45^{f}	35.77^{e}	38.05^{d}	31.06^{g}	29.51^{h}	32.85^{f}	40.62^{c}	40.81^{c}	42.79^{b}	57.30
BV	53.32^{a}	37.70^{e}	37.50^{f}	38.51^{d}	32.44^{g}	34.60^{g}	34.22^{h}	40.42^{d}	42.09^{c}	43.67^{b}	100
P-PER	2.673^{a}	1.947^{e}	2.067^{d}	2.133^{c}	1.889^{f}	1.784^{i}	1.903^{g}	2.417^{c}	1.677^{g}	2.462^{b}	3.90
LAA	MET	MET	MET	ARG	ARG	MET	LYS	PHE	MET	VAL	TRY
AAA	TRP	TRP	ARG	LYS	PHE	TRP	TRP	ARG	TRY	ARG	LEU

Values represent means ± standard deviation of triplicate experiments (n = 3). Values with different letter on the same row are significantly different at P<0.05

Bioactive compounds and antioxidant assays

The bioactive compounds of the *Ogiri* samples are presented in Table 4. The *Ogiri* samples of the total phenol and total flavonoid ranged from 95.45±6.43 to 284.77±15.75 and 25.39±1.45 to 168.87±5.62 respectively. Sample MIC67 exhibited the highest total phenol (284.77 mgGAE /100 g) and flavonoid (168.7 mg QE/100 g) contents. Total phenol and flavonoid compounds do play important roles in promoting human's health by preventing oxidative damage that can lead to related disease. High exhibition of total phenol and total flavonoid contents was peculiar to *Ogiri* sample produced with *B. subtilis* strain exposed to inoculating chamber UV at 90 sec (MIC67) among other *Ogiri* samples determined. This is consistent with the findings in previous studies (Ighodaro and Akinloye, 2018; Amic *et al*., 2003) and this could suggest that the *Ogiri* sample have potential capacity to promote anti-inflammatory functional. The scavenging ability of ABTS in the *Ogiri* samples ranged from 4.23 to 6.70 µ/mol TEAC/ g. Sample MIC67 recorded the highest (6.70 µ/mol TEAC/ g) ABTS results. Sample MIC67 recorded significant ferric reducing activity with 74.35 mgAAE/100 g which is higher than other samples. Ferric reducing antioxidant property (FRAP) is usually used to measure the capacity of an antioxidant reaction with ferric tripyridyltriazine (Fe^{3}+-TPTZ) to give a colored ferrous tripyridyltriazine (Fe^{2}+-TPTZ) by donating an electron/ hydrogen atom (Rahman *et al*., 2015). This free radical reaction breaks when donating hydrogen atom, after which, it stabilizes and terminates the radical chain. It is observed that the phenolic contents correlate well with FRAP and ABTS assays confirming that phenolic compounds are likely to contribute to radical scavenging activity of *Ogiri* samples, which can modulate and prevent any oxidative stress related diseases such as cardiovascular disease, diabetes, cancer, and cognitive diseases like Alzheimer's and dementia (Rahman *et al*., 2015).

Table 4. Bioactive compounds of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

Sample Codes	Total phenol Content (mgGAE/100 g)	Total flavonoid content (mgQE/100 g)	FRAP (mgAAE/100 g)	ABTS radical scaven ability (µ/mol TEAC
Mutant Strains				
MIC67	284.77±15.75^{a}	168.87±5.62^{a}	74.35±0.39^{a}	6.70±0.02^{a}
MIC64	102.05±4.18^{g}	31.46±6.09^{h}	7.82±0.39^{j}	5.36 ±0.17^{f}
MIC21	109.77±0.96^{f}	64.68±0.94^{c}	10.01±0.78^{h}	4.23±0.03^{j}
MHL23	98.41±8.68^{h}	34.44±3.12^{g}	18.31±029^{d}	5.29±0.28^{g}
MHL22	168.86±4.82^{c}	42.49±4.84^{f}	36.90±2.33^{c}	5.89±0.02^{d}
MSD99	123.86±7.24^{d}	25.39±1.45^{j}	12.69±3.75^{g}	5.28±0.00^{h}
MSD51	123.18±7.71^{e}	47.46±0.94^{e}	8.57±0.29^{i}	5.99±0.02^{c}
MSD30	97.50±2.25^{i}	26.05±5.30^{i}	13.99±0.97^{f}	5.04±0.00^{i}
Non-Mutant Strain				
NMS00	95.45±6.43^{j}	61.15±1.56^{d}	15.29±2.81^{e}	6.23±0.12^{b}
Ready –To-Eat				
RTE00	206.36±10.93^{b}	142.49±2.97^{b}	54.32±5.63^{b}	5.69±0.13^{e}

Values represent means ± standard deviation of triplicate experiments (n = 3). Values with different letter on the same column are significantly different at P<0.05

The Antioxidant properties of *Ogiri* samples produced with mutant and non-mutant strains of *Bacillus subtilis* results are presented in Fig. 1a to 1c. The DPPH radical scavenging ability (Fig. 1a) is observed to scavenge in a dose-dependent manner. DPPH is a free, simple stable radical widely used method usually used to evaluate the activities of antioxidants within a short period in food samples. The percentage scavenging capacity of the *Ogiri* samples at lower concentration (8.33 mg/ml) ranged from 60.00 to 76.89 % and at higher concentration (16.67 mg/ml), it ranged from 66.09 to 83.11 %. Sample MIC67 showed the highest ability to scavenge DPPH radical (76.89 and 83.11 % inhibition) at both concentrations. The values obtained in this study is higher than the findings (Arueya *et al*., 2017) in soybean-*ogiri* (70.06 % inhibition). Likewise, samples MIC64, the one exposed to inoculating UV chamber at 120 sec (MIC21) and the one exposed to hand UV lamp at 100 sec (MHL23) also exhibited higher scavenging radical activities (73.99 %, 73.35 % and 72.31 % respectively) than soybean-*ogiri*, but little lower (76.06 % inhibition) than one of the control samples (RTE00). Sample MIC67 was higher than the glutathione (79.03 %) used as standard. The result suggests that free hydrogen atoms can easily be donated, making DPPH to be deficient in the electron and thereby terminate the reaction of the radical chain (Olagunju *et al*., 2018).

The Hydroxyl ion (•OH) scavenging ability of *Ogiri* samples (Fig. 1b) are dose-dependent. The percentage scavenging capacity of the *Ogiri* samples at lower concentration (2.17 mg/ml) ranged from 23.11 to 38.51 % and at higher concentration (4.34 mg/ml), it ranged from 29.50 to 49.41 %. The Hydroxyl ion (•OH) scavenging ability to neutralize or reduce oxidative stress is expressed in some food samples. Samples MIC67 and another sample exposed to hand UV lamp at 110 sec (MHL22) exhibited higher (•OH) radical scavenging ability (38.51 and 37.51 %; 49.41 and 47.13 %) at both concentrations better than samples RTE00 (32.87 and 28.71 %) and the one fermented with non-mutant *B. subtilis* (NMS00) strain (41.98 and 34.06 %) as control samples, as well as glutathione standard (30.93 and 39.03 %) respectively. The ability of the bioactive compounds in these samples (MIC67 and MHL22) to scavenge hydroxyl radicals could cause the hindering of lipid peroxidation, hence might be used as anticancer agents by restraining hydroxyl radicals having interaction with DNA (Rahman *et al*., 2015).

The Fe^{2+}chelation abilities of the *Ogiri* samples (Fig. 1c) are also concentration-dependent. The percentage iron chelation at lower concentration ranged from 50.80 to 76.65 %, while at higher concentration, it ranged from 64.29 to 86.72 %. All *Ogiri* samples fermented with mutant strains of *B. subtilis* have higher iron chelation ability at both low and high concentrations than the control samples and the glutathione

standard. Samples MSD30 recorded the highest abilities (76.65 and 86.72 %) at both concentrations. Samples MIC67 (80.68 %), MHL22 (78.77 %), MSD99: *Ogiri* exposed to SDS at 100 sec (78.37 %), MSD51 (77.56 %), and MSD30 (86.74 %) are able to reduce Fe^{3+} to Fe^{2+} better than the control samples and glutathione standard: RTE00 (72.73%), NMS00 (64.29%) and glutathione (69.07 %) respectively. The connection of Fe^{2+} with its involvement in peroxyl and alkoxyl radical formation to initiate lipid oxidation through the Fenton reaction has the ability to bypass the blood-brain barrier in a case of extracellular Fe^{2+} overload (Crichton *et al*., 2011). Therefore, these *Ogiri* samples revealed their potential role as iron chelators that could be employed in the management of oxidative stress-related diseases.

Fatty acid profile of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

The fatty acid profile of the *Ogiri* samples fermented with mutant and non-mutant strains of *B. subtilis* is shown in Table 5. Both monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of *Ogiri* samples ranged from to 3.71 to 15.67 mg/100 g and 23.49 to 50.29 mg/100 g respectively. Monounsaturated and polyunsaturated are the essential healthy fatty acids needed by body for metabolic activities; however, saturated fatty acids in foods such 16-carbon palmitic acid, 14-carbon myristic acid, 12-carbon lauric acid, etc. are needed in little concentrations (less than 10%) of our daily calories as recommendations by Snetselaar *et al*. (2021). Sample MSD51 had the highest values of MUFA (15.67 mg/100 g) and PUFA (50.29 mg/100 g). This suggest that these *Ogiri* samples are rich in both omega-3 and omega-6, which can help in prevention of chronic inflammation that could lead into diseases such as rheumatoid arthritis, cancer, cardiovascular, neuropsychiatric and neurological diseases as well as to help to maintain both the structural integrity and the critical functioning of cellular membranes throughout the body (Katalin and Ionana, 2017).

Rancidity profile of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

Table 6 shows the rancidity profile (free fatty acid and peroxide value) of the *Ogiri* samples fermented with mutant and non-mutant strains of *B. subtilis*. The free fatty acid of the *Ogiri* samples ranged from 1.07 to 1.58 %. The peroxide value (PV) of the *Ogiri* samples fermented with mutant and non-mutant strains of *B. subtilis* ranged from 2.11 to 5.36 meq/kg. The market sample (RTE00) had the highest (5.36 meq/kg) peroxide value.

Free fatty acid (FFA) in food indicates the level of hydrolytic rancidity, and a higher level of FFA makes food go rancid (Mahesar *et al*., 2014). All samples fermented with mutant strains of *B. subtilis* have lower free fatty acids than the controls. This suggests that the *Ogiri* samples will not easily be prone to rancidity hence, may have long shelf life as FFA is predisposed by heating due to decrease in water content of the food products and/or by alteration in the enzyme activity. PV is known to decompose to a very high unbalanced minor oxidation product under heat. The high level of sample RTE00 may be due to the oxidization of double bonds of unsaturated fats to release peroxide as one of the oxidization products. However, the freshness of the *C. vulgaris* (melon seeds) used and mutant strains of *B. subtilis* used to ferment some *Ogiri* samples could have contributed to the low level of peroxide value (PV) recorded as it measures the oxidation during storage and freshness of the lipid matrix at the early stages of rancidity under mild condition (Hosseini *et al*., 2017).

Table 5. Fatty acid profile of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

Fatty acids	**Sample codes**						**(mg/100 g)**			
	Mutant Strains								**Non Mutant**	**Ready-To-Eat**
Monounsaturated (MUFA)	**MIC67**	**MIC64**	**MIC21**	**MHL23**	**MHL22**	**MSD99**	**MSD51**	**MSD30**	**NMS00**	**RTE00**
Palmitoleic acid (C16:1)	0.36±0.01^{a}	0.05±0.01^{e}	0.09±0.01^{d}	0.02±0.01^{f}	0.00±0.00^{g}	0.00±0.00^{g}	0.15±0.01^{c}	0.00±0.00^{g}	0.23±0.01^{b}	0.00±0.00^{g}
Oleic acid (C18:1)	3.01±0.03^{b}	9.88±0.02^{e}	11.94±0.02^{d}	7.94±0.02^{f}	3.88±0.01^{i}	5.88±0.01^{g}	15.24±0.01^{a}	3.71±0.01^{j}	12.87±0.03^{c}	4.07±0.02^{h}
Erucic acid (C22:1)	0.28±0.01^{a}	0.11±0.01^{d}	0.16±0.01^{c}	0.07±0.01^{e}	0.00±0.00^{g}	0.02±0.00^{f}	0.28±0.01^{a}	0.00±0.00^{g}	0.23±0.02^{b}	0.00±0.00^{g}
∑MUFA	13.65±0.00^{b}	10.04±0.00^{e}	12.19±0.00^{d}	8.03±0.00^{f}	3.88±0.00^{i}	5.90±0.0^{g}	15.67±0.0^{a}	3.71±0.00^{j}	13.33±0.00^{c}	4.07±0.00^{h}
Polyunsaturated (PUFA)										
Linoleic acid (C18:2)	21.59±0.03^{i}	31.94±0.02^{c}	35.88±0.02^{b}	28.85±0.02^{d}	23.11±0.02^{g}	25.49±0.03^{e}	46.04±0.01^{a}	21.90±0.02^{h}	21.40±0.03^{j}	23.36±0.0 ^{f}
Linolenic acid (C18:3)	5.92±0.03^{a}	1.15±0.02^{e}	1.26±0.03^{d}	1.08±0.01^{f}	0.89±0.02^{h}	1.02±0.01^{g}	2.56±0.02^{c}	0.77±0.02^{i}	5.73±0.01^{b}	0.99±0.04^{g}
Arachidonic acid (C20:4)	2.00±0.03^{a}	1.10±0.01^{e}	1.16 ±0.0 ^{d}	1.04±0.01^{f}	0.87±0.01^{h}	0.94±0.02^{g}	1.69±0.02^{c}	0.82±0.01^{i}	1.91±0.02^{b}	0.93±0.02^{g}
∑PUFA	29.51±0.00^{e}	34.19±0.00^{c}	38.30±0.00^{b}	30.97±0.00^{d}	24.87±0.00^{i}	27.45±0.00^{g}	50.29±0.00^{a}	23.49±0.00^{j}	29.04±0.00^{f}	25.28±0.00^{h}
MUFA/PUFA	0.46±0.00^{a}	0.29±0.00^{c}	0.32±0.00^{b}	0.26±0.00^{d}	0.16±0.00^{f}	0.22±0.00^{e}	0.31±0.00^{b}	0.16±0.00^{f}	0.46±0.00^{a}	0.16±0.00^{f}

Values represent means ± standard deviation of triplicate experiments (n = 3). Values with different letter on the same row are significantly different at P<0.05

Table 6. Rancidity profile of *Ogiri* samples fermented with mutant and non-mutant strains of *Bacillus subtilis*

	Rancidity profile	
Samples codes	**Free fatty acid (FFA) (%)**	**Peroxide value (PV) (meq/Kg)**
Mutant Strains		
MIC67	1.07±0.01^{g}	2.11±0.01^{f}
MIC64	1.09±0.01^{e}	2.24±0.02^{d}
MIC21	1.09±0.01^{e}	2.11±0.02^{f}
MHL23	1.13±0.01^{c}	3.17±0.02^{c}
MHL22	1.07±0.01^{g}	2.22±0.01^{e}
MSD99	1.11±0.01^{d}	2.22±0.01^{e}
MSD51	1.08±0.01^{f}	2.03±0.01^{g}
MSD30	1.07±0.01^{g}	2.11±0.01^{f}
Non-Mutant Stain		
NMS00	1.45±0.02^{b}	4.00±0.01^{b}
Ready –To- Eat		
RTE00	1.58±0.01^{a}	5.36±0.01^{a}

Values represent means ± standard deviation of triplicate experiments (n = 3). Values with different letter on the same column are significantly different at P<0.05

CONCLUSION

Ogiri produced by the fermentation of *C. vulgaris* using mutant strains of *B. subtilis* possess improved water and oil absorption capacities, improved swelling index and high in essential amino acids. The mutant *Ogiri* samples also have improved phenolic, flavonoid, and high antioxidant properties. Therefore, the mutant *Ogiri* could serve as functional food that can modulate oxidative stress with improved nutrient quality, especially sample MIC67.

List of abbreviations

FFA: Free Fatty Acid
PV: Peroxide Value
SI: Swelling index
UV: Ultraviolet ray/light
SDS: Sodium Dodecyl Sulphate
C. vulgaris: *Citrullus vulgaris*
B. subtilis: *Bacillus subtilis*
*: FAO/WHO/UNU/ (2007
**REF: USDEC, (1999)
BV: Biological value
P-PER: Predicted Protein Efficient Ratio
LAA: Limiting amino acid
AAA: Abundant amino acid
PHE: Phenylalanine
HIS: Histidine
MET: Methionine
VAL: Valine
TRP: Tryptophan
THR: Threonine
ISO: Isoleucine
LEU: Leucine
LYS: Lysine
GLY: Glycine
ALA: Alanine
SER: Serine
PRO: Proline
ASP: Aspartic
CYS: Cysteine
GLU: Glutamine
TYR: Tyrosine
ARG: Arginine
ORN: Ornithine
TNEAA: Total non-essential amino acid
TEAA: Total essential amino acid ratio
TAA: Total amino acids
MIC67: *Ogiri* produced with *B. subtilis* mutant strain exposed to inoculating chamber UV at 90 sec
MIC64: *Ogiri* produced with *B. subtilis* mutant strain exposed to inoculating chamber UV at 30 sec
MIC21: *Ogiri* produced with *B. subtilis* mutant strain exposed to inoculating chamber UV at 120 sec
MHL23: *Ogiri* produced with *B. subtilis* mutant strain exposed to hand UV lamp at 100 sec
MHL22: *Ogiri* produced with *B. subtilis* mutant strain exposed to hand UV lamp at 110 sec
MSD99: *Ogiri* produced with *B. subtilis* mutant strain exposed to SDS at 100 sec
MSD51: *Ogiri* produced with *B. subtilis* mutant strain exposed to inoculating chamber UV at 110 sec
MSD30: *Ogiri* produced with *B. subtilis* mutant strain exposed to inoculating chamber UV at 120 sec
NMS00: *Ogiri* produced with Non-mutant *B. subtilis* strain without D-ribose yield,
RTE00: Ready-to-eat *ogiri* market sample

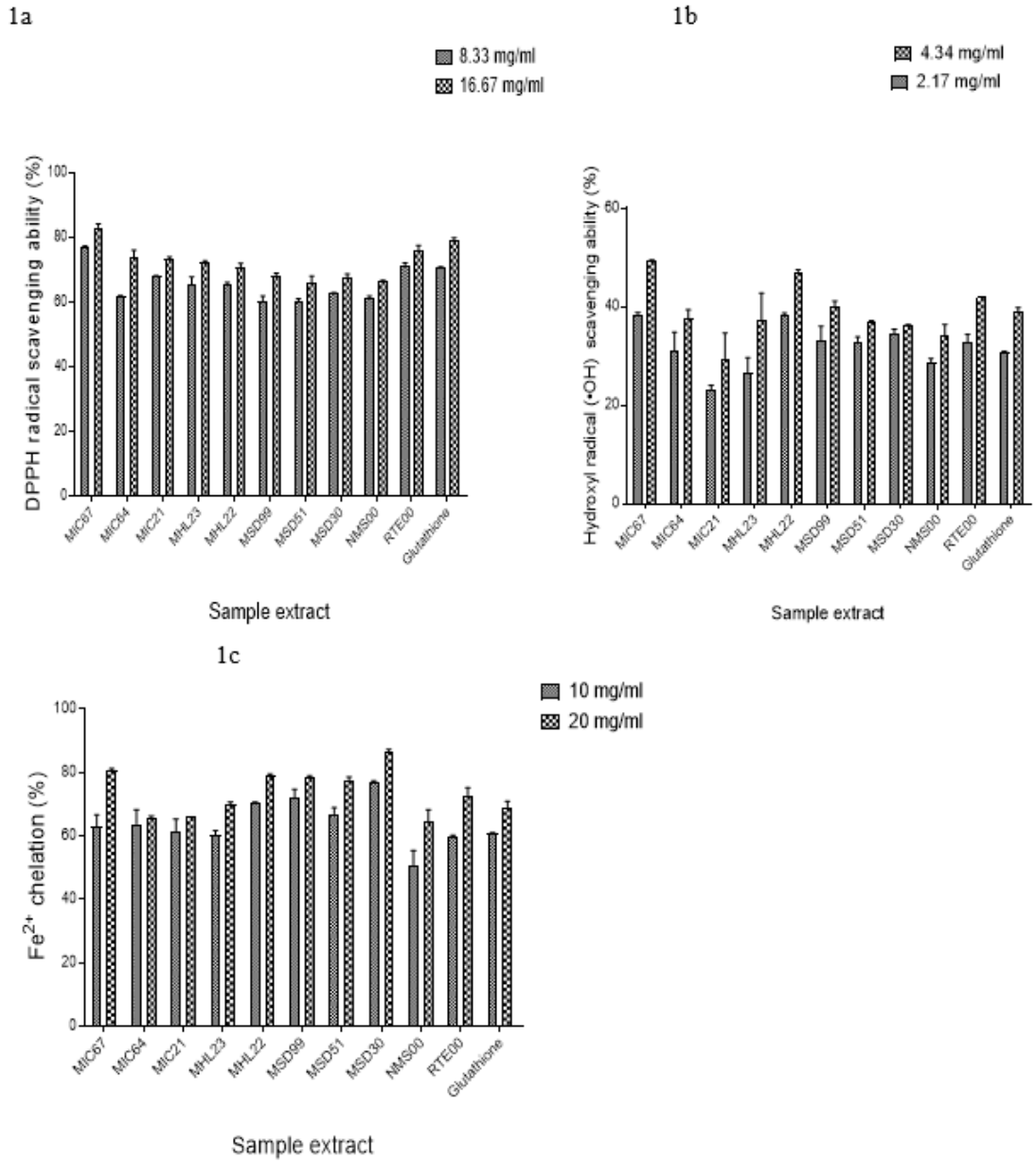

Figure 1a to 1c: Antioxidant properties of *ogiri* samples produced with mutant and non-mutant strains of *B*acillus *subtilis*

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