Journal of Advances in Microbiology

Volume 23, Issue 10, Page 27-37, 2023; Article no.JAMB.105835
ISSN: 2456-7116

Immunological Impact of Fermented Soybean Condiments Produced from Indigenous Fermenters

C. M. Iheukwumere [a*], I. H. Iheukwumere [b], U. O. Okoli [a] and C. H. Ugwu [a]

[a] Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[b] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2023/v23i10754

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105835

Original Research Article

Received: 03/07/2023
Accepted: 08/09/2023
Published: 15/09/2023

ABSTRACT

Several studies have shown that most condiments consumed in Nigeria today are fortified with chemicals that alter the nature and stripped the natural immunological adjuvants designed by nature to protect the body system. Several condiments have been developed from fermented foods in order to control this ugly situation but there is still controversial thought in the choice of microorganism to be used in order to produce a non-toxic condiment with an improved immunological function. The aim of this study was to evaluate the toxicity, nutritive value and immunological impact of fermented soybean condiments. Soybean (*Glycine max*) sample was fermented with indigenous microorganisms isolated from 7 days old fermented soybean sample; this was oven-dried, pulverized and packaged in a cleaned sterile screw capped container. The immunological impact, pro-inflammatory and anti-inflammatory activities were determined using *in vivo* and instrumentation techniques. *Lactobacillus plantarum* strain ZS 2058 (L), *Bacillus subtilis* strain 168 (B) and *Saccharomyces cerevisiae* strain YJM555 (Y) were the indigenous microbes used both singly and in

**Corresponding author: E-mail: cm.iheukwumere@unizik.edu.ng;*

consortium for the production of light to dark brown condiments with water activity ranging from 0.27 – 0.37 for the fermented soybean in the plate and 0.22 - 0.36 for the fermented soybean wrapped with *Thaumatococcus danielli* leaves (called *Uma* in Igbo and *Ewe eran* in Yoruba). There was pronounced delay type hypersensitivity (DTH) and significant ($P<0.05$) elevation of lymphocyte population, rosette counts, antibody titers, phagocytic indices, total lymphocytes and malondialdehyde (MDA) values, of which pronounced activities were mostly seen on those condiments prepared with consortium of the organisms (BLY). Therefore condiments produced from fermentation of soybean using indigenous B, L, Y, BL and BLY are recommended as this would enhance its immunological impact and also safe, and those fermented in plastic plates using BLY were most efficient, preferable and acceptable.

Keywords: Immunological; condiments; fermented.

1. INTRODUCTION

"Soybean (*Glycine max* leguminosae) is one of the highly nutritional natural vegetable foods known to humans" [1]. "Soybean contains approximately 35%, protein, 31% carbohydrate, 17% fats, 5% mineral and 12% moisture" [2]. "The soybean protein contains acceptable amount of essential amino acids vis a viz histidine, Isoleucine, leucine, lysine, phenylalanine, tryrosine, tryptophan and valine which is recommended for daily intake as a balanced diet" [2]. "In addition to essential nutrients, soybean products, especially fermented soybean products contain various functional components including peptides, isoflavonoids and saponin" [3]. "Soybean has been reported to impact several health benefits such as lowering of plasma, choresterol, prevention of cancer, improvement in bone mineral density and provide protection against bowel and kidney disease" [3] Kreijkamp-Kaspers et al*.,* 2004; [4,5,6]. "These health benefits are caused by the presence of Isoflavone, Saponins, protein and peptide in soybean" [5,7,8].

"A condiment is a substance applied to food in the form of a sauce, powder, spread or anything similar, to enhance or improve the flavor. In Nigeria and most Africancountries, condiments such as fermented locust bean (Iru), fermented melon seed (Ogiri), fermented soybeans (Dadawa), fermented cotton seed (Ogiri) and fermented pigeon pea were widely used to season food. The production of condiments is largely on a traditional small-scale, household basis under highly variable conditions" (Odunfa 1981) [9]. "Condiments are also known to contribute to the calorie and protein intake and are generously added to soups as low-cost meat substitute by low-income families in parts of Nigeria" [10].

"Traditionally, fermentation was a method to preserve foods for a longer time; however, this process recently has attracted great attention due to the increase in the nutritional value of foods and the production of health-promoting components" [11]. "Over fermentation, the microorganisms responsible for this process generate bioactive compounds by metabolizing fermentable carbohydrates and proteins" [2]. "Generated metabolic compounds play a significant protective role against chronic disorders, including obesity, diabetes, cancer, cardiovascular disease, and allergies" [3,12, 6,13].

Moreover, fermentation increases the peptides, amino acids, vitamins, minerals, and antioxidant contents of food. Nowadays, a wide range of fermented products is produced and consumed world-wide.

"Chronic inflammatory diseases are the leading cause of mortality worldwide" [11]. "Inflammation is part of the host's complex defense mechanism. It is the immune system's biological response against different infectious or non-infectious stimuli. These stimuli may activate inflammatory signaling pathways such as nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways underlying the pathology of many chronic diseases" (Shahbazi et al., 2021) [11]. "Exploring the potential role of natural bioactive components in preventing and treating chronic inflammatory disorders such as cancers, obesity, diabetes, rheumatoid arthritis, atherosclerosis, ischemic heart disease, and inflammatory bowel disease (IBD) is now the subject of intense research" [11]. Historically, natural products have been known to exert significant biological and pharmacological properties and play a valuable role in drug discovery and treating many diseases. Due to

significant anti-infective, antioxidative, anti-inflammatory, antiangiogenic, and anticarcinogenic properties [11] many natural compounds have been applied as preventive and therapeutic agents against many ailments [11] "Fermented plant products are highly popular foods worldwide, and are a rich source of natural compounds such as probiotics and phytochemicals with known biological properties" (Obafemi et al., 2022). Studies have shown that "polyphenolic compounds found in fermented products are beneficial in microbiota metabolism and growth and can inhibit the production of inflammatory cytokines and suppress inflammatory responses" [11]. Furthermore, neutralizing free radicals, regulating antioxidant enzyme activities, reducing oxidative stress, and enhancing immune system activity are other potential mechanisms by which plant-based fermented foods exert health benefits. In this review, we will discuss the immunological impacts of fermented condiments produced from soybean.

2. MATERIALS AND METHODS

2.1 Sample Collection

This was carried out using the modified method of Suleiman and Omafe [14]. Soybean seeds were collected randomly from different shops and open markets in Eke Awka, Awka South LGA, Anambra State. Sampling was performed manually from different bags and basins, such that soybean seeds were collected from different parts of the bags and basins. The samples were aseptically pooled and mixed properly to form a bowl and placed in sterile nylon bag, the soybean seeds were properly labeled and taken to the laboratory for analysis.

2.2 Transportation

"A sterile polythene bag containing ice blocks placed inside a cooler was used for the transportation of the sample. The temperature of the cooler was carefully checked and adjusted to 28ºC -30ºC in order to prevent or reduce microbial shock by reducing the quality of the ice inside the cooler. The samples were aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was covered properly with packing tape to prevent accidental opening of the cooler. The cooler was taken to the laboratory safely for the analysis" [Iheukwumere and Iheukwumere,2022].

2.3 Preparation and Local Fermentation of the Soybean

"Two hundred and fifty grams (250g) of cleaned soybean seeds were weighed using an analytical weighing balance and steeped in 500ml bucket of water overnight, after which the seed coat were removed by rubbing between the palms and then the chaff were removed using sieve. The soybean seed were then thoroughly washed and placed inside cleaned *Thaumatococcus danielli* leaves (called "uma" in Igbo and "ewe eran" in Yoruba) and wrapped properly and then kept inside 500ml bucket that was well covered with the lid for fermentation to take place for 7 days at room temperature" [15].

2.4 Processing of the Fermented Soybean

"After the fermentation the fermented soybean were prepared for culturing and the diluents used was peptone (BIOTECH) water which was prepared according to the manufacturers instruction, then was sterilized by autoclaving at121ºC for 15min at 15psi. Ten grams of the fermented soybean was aseptically weighed using analytical weighing balance into a 200 ml beaker (G.G) and little amount of the diluent was added and homogenized and then make upto 100 ml, part of these preparations was transferred into 100 ml beaker (G.G) and boiled for 10-15 min using a pressure pot" and [15].

2.5 Isolation of the Test Sample

The media used for this isolation includes Sabourand dextrose agar (SDA), de Man Rogosa and Sharpe broth (MRS) and Nutrient agar (BIOTECH). A 0.1ml of the preparation/inoculum collected using a sterile pipette and aseptically plated onto solidified Sabourand dextrose agar plate (90 mm x 15 mm) which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough (2010) supplemented with chloramphenicol (0.05%) and spread using a spreading rod, 0.1 ml of the boiled preparation/inoculums was collected and plated unto solidified nutrient agar plate also 1 ml of the inoculums was collected using sterile pipette and aseptically inoculated into sterile 100 ml conical flask (Glassco) containing MRS broth (Oxoid) which was prepared according to the manufacturers instruction and the conical flask were incubated in a microaerophic environment (containing candle used to evacuate all traces of oxygen thereby creating an environment having

only carbon iv oxide). The incubation was done for 24 – 72 h at (30±2ºC). The SDA and NA were incubated in an inverted position for 24 h at 35±2ºC (for NA) and 30±2ºC (for SDA) in an incubator (STXB128). The isolates were sub cultured and characterized appropriately.

2.6 Preparation of Soybean Condiments

2.6.1 Processing of soybean for fermentation

This was carried out using the modified method of Farinde et al. [16]. One kilogram of soybean were carefully picked and weighed using analytical weighing balance and steeped in 200ml bucket of water overnight for fermentation to take place, after the soybean were dehaulled by rubbing between the hands to remove seed coat, after the chaff/seed coat were properly removed using a clean sieve, the soybean was then properly washed and placed inside a beaker and then autoclaved at 121ºC for 15 min at 15psi.

2.6.2 Fermentation process

This was carried out using the modified method of Hu et al. (2010) and Chukeatirote et al [17]. After autoclaving the soybean, a 100g of soybean was weighed using analytical weighing balance and placed inside 6 different *Thaumatococcus danielli* leaves (called “uma” in Igbo and “ewe eran” in Yoruba) which was properly sterilized using electric oven at 180ºC for 2 h, each of the leaves containing the soybean were inoculated with the fermenters prepared and diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.6mL of 1% $BaCl_2.2H_2O$ and 99.4 mL of 1% Conc. H_2SO_4, 10ml of suspension *Bacillus* was added and labeled as “B”, 10ml of suspension of *Lactobacillus* was added and labeled as “L”, 10ml of suspension of yeast was added and labeled as “Y” , consortium of suspensions 5ml of *Bacillus* and 5ml of *Lactobacillus* was added and labeled as “BL”, consortium of suspensions of 3ml of *Bacillus*,3ml of *Lactobacillus* and 4ml of yeast were added and labeled as “BLY” consecutively and one of the leaves containing only soybean was set aside as the control. These leaves were carefully wrapped. This same method was repeated using sterile plates. The wrapped leaves and the plates containing the soybean were kept at room temperature for fermentation to take place for 7days.

2.7 Storage and Packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80ºC for 7days**.** After drying water activity of the fermented samples was determined, after which it was grinded into powder and stored in a sterile screw capped container for subsequent analysis.

2.8 Immunomodulatory Effects of the Prepared Condiments

2.8.1 Antigen preparation

This was carried out using the method described and published by Nfambi et al [18]. Fresh blood sample was collected from healthy sheep from NgorOkpala in Imo State, and this was mixed with sterile Alsever's solution (1:1). The sample was centrifuged at 2000xg for 5 min to enable the red blood cells (RBCs) settled at the bottom of the test tube. Then the supernatant was discarded and the sediment was collected as the sheep red blood cells (SRBCS). The SRBC was then washed three times with pyrogen- free phosphate buffered saline (PH 7.2). This was then kept under refrigeration for the study.

2.8.2 Experimental protocols for the *in vivo* models

A total of 272 albino Wistar rats were used for this study. The albino Wistar rats were grouped (16 rats in each based on the number of condiments (12 condiments), three control groups (maggi, okpeyi

and normal control), dexamethasone group and levamisole group making it total of 17 groups. The experimented rats were sensitized intraperitoneally with O.1 mL SRBC containing $1x10^8$ cells. The control groups were giving normal saline (0.85% Nacl), dexamethasone (200 mg / kg bwt) and levamisole (50 mg/kg bwt), maggi star (0.5 g/kg bwt), “Okpeyi”(0.5 g/kg) for 7 days, and the remaining groups (test groups) were fed with the respective condiments (0.5 g/kg) for 7 days. The immunomodulatory activity of set- up was determined using the parameters below.

2.8.3 Delayed type hypersensitivity

This was carried out using the modified method described and published by Anartlhe et al. [19]. On the 7th day prior to injection, right hand footpad thickness of the albino rats (4 rats of 16

in each group) were measured with micrometer screw gauge (MitutoyoDigimatic). Then the rats were challenged by injecting 20ml of 1% SRBC into the right hind foot pad. On the 8th and 9th days, the footpad thickness of the experimented rats were again measured and the mean differences of the pretreated and post treated rats were calculated and recorded in millimeters

2.8.4 Humoral activity of the condiments

This was carried out using the modified methods described and published by Anarthe et al [19] and Nfambi et al 18]. On 7th day before challenge, blood samples were drawn from retro-orbital plexus of the rats (4 rats in each group, mainly those used for the DTH). The blood samples were centrifuged for 2000xg for 5 min, and the sera were collected. Two -fold serial dilution was carried out on each of the serum (ie 50µl of serum was added in each microtiter well dilution in order to get up to the 24th well. Then 50µl of 1% SRBC prepared using normal saline was added into each well, and the plates were incubated at 37°C for 1h. The reciprocal of the highest dilution that showed visible agglutination was considered as the hemagglutination antibody titer (HA units /NL).

2.9 T-Cell Population

This was carried out using the modified method described and published by Anarthe et al [19]. On the 11th day, blood samples were collected from retro-orbital plexus of the rats (another 4 rats in each group) and these were mixed with Alsever's solution in test tubes. These were kept, in sloping position (45°) and incubated at 37°C for 1h. The RBCs were allowed to settle at the bottom of the test tubes, and supernatant was collected from each test tube using micropipette and this contains the Lymphocytes.

Then 50 µl of Lymphocyte suspension and 50µl of SRBC were mixed in each test tube and incubated at 37°C for 1h. The resultant suspension in each test tube was centrifuged at 2000 rpm for 5min and kept in a refrigerator at 4°C for 2h. The supernatant was removed and one drop was placed on clean grease - free slide. Total Lymphocytes were counted and a lymphocyte binding with three or more erythrocytes was considered as rosette and number of rosettes was counted.

2.9.1 Carbon clearance assay (phagocytic activity)

This was carried out using the modified method described and published by Anarthe et al [19]. After 7days, the experimented rats (another 4 rats from each group) were stabilized for 2 days. On the 11th day, the selected rats were intravenously (through the tailvein) injected with carbon suspension (1:50 dilution of Indian ink) in a dose of 0.5ml/100g bwt. Blood samples were withdrawn from the retro-orbital venous plexus before injection, at 5min and 15 min after injection of the carbon suspension. Then 0.05 ml of each blood sample was lyzed with 4 ml of 0.1% Na_2CO_3 and the optical density was measured spectrophotometrically at 650nm wavelength. The phagocytic index (K) was calculated using the equation below:

$$K = \frac{\log(ODa) - \log(ODt)}{t}$$

ODa = Optical density at 0 min
ODt= Optical density at 5 and 15 min
t = time (5 and 15 min)

2.10 Pro-inflammatory and Anti-inflammatory Activities of the Condiments

2.10.1 Malondialdehyde (MDA) Analysis

This was carried out using the method described in the study published by Mao et al [20]. Test tubes A contained 1.0mL serum from the experimented rats, 1.0ml of 17.5% trichoroacetic acid (TCA), 1.0ml of 0.6% thio barbituric acid (TBA), and test tubes B contained all the reagents as contained in test tubes A but contained distilled water in place of serum. The test tubes were mixed well and incubated in water bath at 100°C for 15min. these were allowed to cool, then the test tubes were also allowed to stand at room temperature for 20min. the test tubes were centrifuged at 2000 rpm for 15 min and then the supernatants were collected in cuvette and the absorbance's were read at 534nm using UV-visible spectrophotometer.

$$MDA(Nmol/ml) = \frac{AT - AB}{156} x1000$$

Where AT = Absorbance value of the test serum
AB = Absorbance value of the blank
$156mm^{-1}CM^{-1}$ = Extinction coefficient of MDA-TBA abduct at 534nm

3. RESULTS

3.1 Delayed Type Hypersensitivity (DTH) Response for the Prepared Condiments

The study recorded pronounced activity of delayed type hypersensitivity (DTH) response from those samples collected from the rats fed with the condiments as shown in Table 1. The maximum (DTH) response after 24 h were recorded majorly on those samples collected from the rats fed with condiments prepared majorly with Lev, BLYL, BLYP and BL, followed by LL, BLL, BP and LP and least was seen among those fed with Dex. Then the maximum (DTH) response after 48 h were recorded majorly on those samples collected from the rats fed with condiments prepared majorly with Lev, BL, BLYL and BLYP, followed by LL and BLL, and least was seen among those fed with Dex. After 24 h Delayed type hypersensitivity (DTH) response generated from Lev, BLYL, BLYP, BL, LL, BLL, BP, LP, okpeyi, CP, YP, BLP were significantly ($p<0.05$) higher than that of Dex. After 48 h Delayed type hypersensitivity (DTH) response generated from Lev, BL, BLYL, BLYP, LL, BLL, LP, BP, okpeyi, CP, BLP and CL were significantly ($p<0.05$) higher than that of Dex.

Table 1. Delayed type hypersensitivity (DTH) response for the prepared condiments

Treatment	Dose (mg/g)	DTH Response	
		24h	48h
Bp	100	7.00±0.00	6.00±0.00
BL	100	8.33±0.47	7.67±0.57
Lp	100	7.00±0.00	6.33±0.47
LL	100	7.67±0.57	7.00±0.00
Yp	100	6.00±0.00	5.00±0.00
BLP	100	6.00±0.00	5.33±0.47
BLL	100	7.67±0.00	7.00±0.00
BLYP	100	8.33±0.47	7.33±0.47
BLYL	100	8.67±0.00	7.67±0.57
Maggi	100	6.00±0.00	5.00±0.00
Okpeyi	100	6.67±0.57	6.00±0.00
CP	100	6.67±0.57	6.00±0.00
CL	100	6.00±0.00	5.00±0.00
Dex	200	4.00±0.00	3.33±0.47
Lev	50	9.00±0.00	8.00±0.00
Control	-	5.00±0.00	4.00±0.00

Dex = Dexamethasone ; Lev=Levamisole

3.2 Effect of the Condiments on the Humoral Response to SRBC in Albino Rats

The study showed significant mean antibody titre with the maximum and minimum activities of 512 and 128 HA unit/NL respectively for the prepared condiments (Table 2). The humeral response was seen most among the samples collected from those rats fed with condiments prepared with BLYL; BLYP and Lev, followed by BL and BLL and least were seen among those fed with maggi. The humoral response elicited from BLYL, BLYP, BLP, BLL, BL, BP, LP, LL, YP and YL were significantly ($p<0.05$) higher than that of control group and Dex. Also the humoral response elicited by Bp, BL, LP, LL, YP, BLP, BLL, BLYP and BLYL were significantly ($P<0.05$) higher than that of CP, CL and Okpeyi.

Table 2. Effect of the condiments on the humoral response to SRBC in albino rats

Treatment	Dose (mg/g)	Antibody Titre (HA units/UL	
		Mean	Max
Bp	100	213.33	256
BL	100	341.33	512
Lp	100	213.33	256
LL	100	213.33	256
Yp	100	170.67	256
YL	100	85.33	128
BLP	100	213.33	256
BLL	100	341.33	512
BLYP	100	426.67	512
BLYL	100	426.67	512
Maggi	100	26.67	32
Okpeyi	100	85.33	128
CP	100	85.33	128
CL	100	85.33	128
Dex	200	21.33	32
Lev	50	426.67	512
Control	-	26.67	32

Dex=Dexamethasone; Lev=Levamisole

3.3 Lymphocyte Population Associated With the Rats Fed With Prepared Condiments

The study recorded significant number of lymphocytes from those samples collected from the rats fed with the condiments as shown in Table 3. The maximum lymphocyte counts were recorded majorly on those samples collected from the rats fed with condiments prepared majorly with BLYL and BLYP. The lymphocyte counts recorded from BLYL, BLYP, BL, LL, BLP

and BLL were significantly (p<0.05) higher than that of the control group, maggi, okpeyi, CP and CL. Generally, the lymphocyte counts from the samples drawn from those rats fed with the prepared condiments were higher than that of CP, CL and maggi, but the counts recorded from okpeyi were higher than that of YP and YL. Also the counts recorded from BLYL, BLYP and BLL were slightly higher than that of Lev, but there were statistically non-significant (p>0.05).

Table 3. Lymphocyte Population associated with the rats fed with the prepared condiments

Treatment	Dose (mg/g)	Mean lymphocytes
Bp	100	194.16±1.58
BL	100	264.38±1.22
Lp	100	198.52±1.39
LL	100	209.41±1.64
Yp	100	176.48±1.91
Yl	100	182.06±1.23
BLP	100	248.91±2.03
BLL	100	271.08±1.96
BLYP	100	271.12±1.83
BLYL	100	278.31±2.11
Maggi	100	154.28±1.62
Okpeyi	100	199.23±1.68
CP	100	154.21±2.12
CL	100	157.16±1.72
Dex	200	122.46±1.32
Lev	50	269.96±2.01
Control	-	149.14±1.82

Dex = Dexamethasone; Lev = Levamisole

3.4 Mean Rosettes Counts of the Blood Samples Collected From Rats with the Condiments

The study showed elevations in rosettes counts from the blood samples drawn from the rats fed with the prepared condiments as shown in Table 4. The counts were higher for BP, BL, LP, LP, BLP, BLL, BLYP, BLYP, CP, CL and Lev when compared with the control group, and statistically significant (P<0.05) for BLYP and BLYL. Also BP, BL, LP, LL, BLP, BLL, BLYP and BLYL were higher than that of maggi, opei, CP and CL, and statistically significant (P<0.05) forBLYP and BLYL. The rosettes counts for BLYL and BLYP were slightly lower than those group administered Lev, and these were statistically non-significant (P>0.05).

3.5 Phagocytic Indices of the Prepared Condiments in the Experimented Rats

The studied condiments were able to elicit significant activities of phagocytes as shown in Table 5. Phagocytic indices of the samples drawn from the experimented rats fed with the prepared condiments were higher than those drawn from the normal control group, CP, CL, Maggi and Dex, and this was statistically significant (P<0.05) for BLP, BLL, BL, BLYP and BLYL. It was also observed that the phagocytic index of the sample drawn from rats fed with "okpeyi" was similar to that from YP and YL. The phagocytic indices were recorded most among the samples drawn from the rats majorly fed with BLL, BLYP and BLYL. Also the phagocytic indices of Lev is slightly higher than that of BLL and BLYP but slightly lower than that of BLYL, and these values were statistically non-significant (P<0.05).

Table 4. Mean rosettes counts of the blood samples collected from rats fed with the condiments

Treatment	Dose (mg/g)	Mean Rosette Count
Bp	100	17.00±0.00
BL	100	23.33±0.47
Lp	100	19.47±0.57
LL	100	19.33±0.47
Yp	100	13.00±0.00
YL	100	14.00±0.00
BLP	100	21.33±0.47
BLL	100	23.00±0.00
BLYP	100	26.00±0.00
BLYL	100	27.33±0.47
Maggi	100	15.00±0.00
Okpeyi	100	15.67±0.57
CP	100	16.00±0.00
CL	100	16.33±0.47
Dex	200	8.00±0.00
Lev	50	28.00±0.00
Control	-	14.33±0.47

Dex = Dexamethasone ; Lev=Levamisole

3.6 Pro Inflammatory and Anti-Inflammatory Activities of the Condiments

3.6.1 Malondialdehyde (MDA) values from serum samples of experimented rats

The study revealed that the studied condiments were able to produce normal and significant level of MDA (Table 6).

The values of MDA significantly (P<0.05) increased in every 1 week interval but decelerated after two weeks of the experiment. The concentration of MDA was moderately higher than that of control on those experimented rats fed with condiments prepared using BP, BL, LP. LL, BLP, BLL, BLYP and BLYL whereas deviation from normal increase were notably Observed among those rats fed with mostly maggi. The MDA value is a biomarker for oxidative stress, activation of free radicals and inflammatory processes. The study revealed a significant (P<0.05) normal increase in the level of MDA among those rats fed with the condiments with deviation from normal increase among those rat fed with maggi. Also the increase in MDA values among those rats fed with condiments prepared with Lp, LL, BLP, BLL, BLYP and BLYL were more safe when compared with normal control rats (i.e. those rats that were not giving the condiments but also giving distilled water).

4. DISCUSSION

The significant increase in the delayed type hypersensitivity (DTH), antibody titer, population of lymphocytes, mean rosettes counts and phagocytic indices of the rats and samples drawn from the rats fed with prepared condiments indicate immunostimulatory activities of the condiments and these agree with the findings of Dashputre and Naikwode [21] Yapo et al [22] Sumalatha et al [23] Tripathiet al [24] Anarthe et al [19] Ramesh et al [25] and Obi et al [26] but disagrees with the findings of Ahirwal et al. (2013) and John et al [27].

Anarthe et al [19] reported that DTH involved initial sensitization phase and effecter phase. In initial sensitization phase TH^1 cells are activated and clonally expand by antigen presenting cell APC) with class II MHC molecule. In effecter phase subsequent exposure to the SRBC antigen induces DTH response, where TH^1 cells secrete a variety of cytokines and other non-specific inflammatory mediators. The above explanation was also made by Dashpute and Naikwode [21] and Tripathi et al. [24].

Table 5. Phagocytic indices of the prepared condiments in the experimented rats

Treatment	Dose (mg/lg)	Phagocytic Index
Bp	100	0.068±0.002
BL	100	0.081±0.003
Lp	100	0.071±0.001
LL	100	0.074±0.002
Yp	100	0.058±0.001
Yl	100	0.061±0.002
BLP	100	0.077±0.001
BLL	100	0.082±0.003
BLYP	100	0.084±0.001
BLYL	100	0.086±0.003
Maggi	100	0.049±0.001
Okpeyi	100	0.058±0.001
CP	100	0.051±0.001
CL	100	0.053±0.002
Dex	200	0.032±0.001
Lev	50	0.085±0.001
Control	-	0.048±0.001`

Dex=Dexamethasone; Lev=Levamisole

Table 6. Pro Inflammatory and anti-inflammatory activities of the prepared condiments via the effect on the production of malondialdehyde (MDA)

Sample	Week 1		Week 2		Week 3	
	ABS Conc (NM/ml)		ABS Conc (Nm/ml)		ABS Conc (NM/ml)	
Bp	0.0075	0.048	0.0094	0.060	0.0097	0.062
Bl	0.0081	0.051	0.0104	0.067	0.0108	0.069
Lp	0.0058	0.037	0.0081	0.052	0.0084	0.054
LL	0.0066	0.042	0.0086	0.055	0.0090	0.058
Yp	0.0088	0.056	0.0101	0.065	0.0112	0.072
Yl	0.0092	0.059	0.0112	0.072	0.0114	0.073
BLP	0.0048	0.031	0.0075	0.048	0.0080	0.051
BLL	0.0054	0.035	0.0080	0.051	0.0084	0.054
BLYP	0.0037	0.024	0.0059	0.038	0.0062	0.040
BLYL	0.0044	0.028	0.0066	0.042	0.0067	0.043
Cp	0.0098	0.063	0.0111	0.071	0.0114	0.073
CL	0.0102	0.065	0.0115	0.074	0.0119	0.076
Maggi	0.0154	0.099	0.0178	0.114	0.0243	0.156
Okpei	0.0109	0.070	0.0120	0.077	0.0126	0.081
Control	0.0031	0.020	0.0037	0.024	0.0036	0.023

The significant increase in antibody titer is an indication that antibody production to T-dependent antigen SRBC requires cooperation of T and B lymphocytes and macrophages and these evolved immunostimulation through humoral immunity. Similar deduction was made by Anarthe et al [19] In the present study, the condiments were able to influence the roles of immunoglobulins in order to activate pre B cells and dendritic cells, and these result produce antibodies. Thus the titer values against SRBC increases. Similar observation was reported by Anarthe et al [19].

The increase in the lymphocyte population and rosette formation in the present study supported the findings of Yapo et al [22] Anarthe et al 19] and Obi et al [26] but disagrees with the findings of Johnson et al., (2017).[27] This increase is an indication of cell mediated immunity. Also the condiments have the capability to activate CD 4 and CD8 cells and this influences the population of T cells. Similar report was presented by Anarthe et al [19].

The significant increase in the phagocytic indices of the samples drawn from the rats fed with the condiments could be attributed to the ability of the condiments to stimulate the reticulo endothelial system (R.E.S). Anarthe et al. [19] reported that the rate of removal of carbon particles by the reticulo endothelial system (liver, spleen), from the blood stream is a measure of R.E.S. phagocytic activity.

Malondialdehyde (MDA) is one of the final products of poly unsaturated fatty acids perioxidation in the cells. An increase in free radicals causes over production of MDA. Raghavan et al [28] reported that MDA was produced due to poly unsaturated lipid peroxidation and this induces pro-inflammatory activities and thus increases the population of lymphocytes as the level of IL-6, IL-8 and IL25 increases. In the present study, there was increased in blood MDA, and this could be due to degradation of nuclear factor NF-kB subunit due to reactive oxygen species (ROS), thus allows the promotion of the expression of genes that could lead to pro-oxidant and inflammation.

The increase in the lymphocytes and red blood cells (RBCs) associated with the present study supported the findings of Yapo et al [22] Sumalatha et al [23] Anarthe et al [19] and Obi et al. (2019) but disagree with the findings of Johnson et al. (2017). Variations in the values of immune cells/blood cells observed in the present study could be attributed to the variation in the ability of the condiments to argument the hematopoietic processes in the cells of the experimented rats [29-32].

5. CONCLUSION

This study has shown that condiment produced from fermentation of soybean using indigenous *Bacillus subtilis* strain 168 (B), *Lactobacillus plantarum* strain ZS2058 (L) and *Saccharomyces cerevisiae* strain YJM555 (Y) were safe and the blood samples drawn from rats fed with the condiments showed elevation in T cell population, phagocytic indices, total lymphocytes, malondialdehyde value and pronounced activity DTH. Therefore condiments produced from fermentation of soybean using indigenous B, L, Y, BL and BLY are recommended as this would enhance its immunological impact and also safe, and those fermented in plastic plates using BLY were most efficient, preferable and acceptable.

CONFERENCE DISCLAIMER

Some part of this manuscript was previously presented in the conference: 2nd International Bioscience Conference dated from 10-11 January 2023, in Anambra State, Nigeria, Web Link of the proceeding: https://bioscientistjournal.com/pre_pages/2nd_BIOSCIENCE_INT'L_CONF.%20_%202023_BOOK_OF_ABSTRACTS.pdf

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shrestha AK, Dahal NR, Ndungutse V. Bacillus fermentation of soybean: A review. Journal of Food Science and Technology. 2010;15:30–365.
2. Tidke SA, Ramakrishna D, Kiran S, Kosturkova G. Ravishankar GA. Nutraceutical potential of soybean: Review. Asian Journal of Clinical Nutrition. 2015;7: 22–32.
3. Yang HJ, park S, Pak V, Chung KR, Kwon DY. Fermented soybean products and their bioactive compounds; 2011
4. Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, Vander Schouw YT. Effects of soy protein containing isoflavones on cognitive post-

menopausal women: A randomized control trial. Journal of America Medical Association. 2004;292: 65 – 74.
5. Friedman M. Brandon DL. Nutritional and health benefits of soy proteins. Journal of Agricultural Food Chemistry. 2001;49: 1069–1086.
6. Das S, Sharangi AB, Egbuna C, Jeevanandam J, Ezzat SM, Adetunji CO, Tijjani H, Olisah MC, Patrick-Iwuanyanwu KC, Adetunji JB, Ifemeje JC, Akram M, Moboladji BM, Onyeike PC. Health benefits of isoflavones found exclusively of plants of the fabaceae family. In: Egbuna, C. and Tupas, G-D., *Functional Foods and Nutraceuticals: Bioactive Components,* Formulations and Innovations. Springer Nature Switzerland AG. 2020;473-508.
7. Michelfelder AJ. Soy: A complete source of protein. American Fam.Physician. 2009;79: 43 – 47.
8. Xiao, C.W. (2008). Health effects of soy protein and isoflavones in humans. *Journal of Nutrition*138: 1244S – 1249S.
9. Odunfa SA. A note on the microorganisms associated with the fermentation of Africa locust bean (*Parkia filicoidea)* during *iru* production. Journal of Plant Foods. 1981; 3:245–250.
10. Eka OU. Effect of fermentation on the nutrient statu locust beans. Food Chemistry. 1980;5: 305 – 308.
11. Shahbazi R, Sharifzad F, Bagheri, Alsadi N, Yasavoli-Sharahi H, Matar C. Anti-Infammatory and immunomodulatory properties of fermented plant foods. Nutrients. 2021;13:1516–1532.
12. Srivastav VK, Egbuna C,Tiwari M. Plant Secondary Metabolites as Lead Compounds for the Production of Potent Drugs. In: Egbuna C, Kumar S, Ezzat SM, Ifemeje JC. Saravanan K. (Eds.) Phytochemicals as Lead Compounds for New Drug Discovery. Elsevier, Cambridge, USA. 2019;3-13.
13. Tijjani H, Zangoma MH, Mohammed ZS, Obidola SM, Egbuna C, Abdulai SI. Polyphenols: classifications, biosynthesis and bioactivities. In: Egbuna, C. and Tupas, G-D., Functional Foods and Nutraceuticals: Bioactive Components, Formulations and Innovations. Springer Nature Switzerland AG. 2020;389-414.
14. Suleiman, Omafe KA. Identification and characterization of hyphomycetous fungi in bean seeds. American Journal of Pathology. 2013;114:23–27.
15. Iheukwumere CM, Iheukwumere IH. Hematological indices and sensory quality of fermented soybean condiments. World Journal of Advanced Research and Reviews. 2022;14(2):435-42.
16. Farinde A, Owolarafe O, Ogungbemi I. An overview of production, processing, marketing and utilization of Okra in egbedore local government area of Osun State, Nigeria. Agriculture and Engineering. 2007;4:1– 17.
17. Chukeatitrote E, Dajanta K, Apichartssrangkoa, A. ThuaNao indigenous Thai fermented soybeans. Journal of Biological Science. 2010;10:581– 583.
18. Nfambi J, Ebosa GS, Sembajire LF' Gakunga J, Kasolo J. Immunomodulatory activity of methanolic leaf extract of *Moringaoleifera* in wister albino rats, Journal of Basic Clinical and Physiological Pharmacology. 2015;26(6):603–611.
19. Anarthe SJ, Rani SDS, Raju MG. Immunomodulatory activity of methanolic extract of Trigunellafoenumgraecum whole plant in wistar albino rats. American Journal of Phytomedicine and clinical Therapeutics. 2014;2(9):1081–1092.
20. Mao C, Yuan J, Lv Y, Gao X, Yin Z, Kraus VB, Luo J, Chei C, Matchar DB, Zieng Y, Shi X. Association between superoxide dismutase, malondialdehyde and all-cause mortality in older adults: A community-based cohort study. BMC Geriatrics. 2019;19:104–120.
21. Dashputre NL, Naikwade NS. Immunomodulatory activity of *Abutilon indicum*Linn. On Albino mice. International Journal of Pharmaceutical Sciences and Review. 2010;1:178–184.
22. Yapo FA, Yapi FH, Ahiboh H, Haubout-Attounbre ML, Guede NZ, Djaman JA, Monnet D. Immunomodulatory effect of the aqueous extract of Erigeron floribundus (kunth) sch Beep (Asteraceae) Leaf in Rabbits. Tropical Journal of Pharmaceutical Research .2011;10:187 – 193.
23. Sumalatha RBP, Shwetha RB, Sadananda A. Studies on immunomodulatory effects of *Salaciachinensis L.* on albino rats. Journal of Applied Pharmaceutical Science. 2012;2:98–107.
24. Tripathi S, Maurya AK, Kahrana M, Kaul A, Sahu RK. Immunomodulatory activity of ethanolic extract of *Trigonella foenum*

graecum. School Research Library. 2012;4:708–713.

25. Ramesh KS, Ravindranath SK, Venkatesan V. Study of immunomodulatory activity of aqueous extract of *Carica papaya* in wistar rats. National Journal of Physiology, Pharmacy and Pharmacology. 2016;6: 442–444.
26. Obi A, Egwurugwu JN, Ojefa SO, Ohamaeme MC, Ekweogu CN Ogunnaya FU. Immunomodulatory effects of hydro methanolic extract of *Moringa oleifera* leaf on male wistar rats. Nigeria Journal of Experimental and Clinical Biosciences. 2019;6:26 – 32.
27. Johnson JT, Ekpo GI, Ugwuoke JE. Immunomodulatory potentials of Ethanolic leaf extract of *Phyllantus amarus* in wistar rats. The Pharmaceutical and Chemical Journal. 2017;4:83 –88.
28. Raghavan S, subramaniyam G. Shanmugam N. Proinflammatory effects of malondialdehyde in lymphocytes. Journal of Leukocyte Biology. 2012;95:1055–1067.
29. Ahirwal L, Singh S, Dubey MK, Bharti V, Mehta A, Shukla S. *In vivo* immunomodulatory effects of the methanolic leaf extract of *Gymname sylvestre* in swiss albino mice. Archives of Biological Sciences. 2015;67:561–570.
30. Cheesbrough M. District laboratory practice in tropical countries (2nd ed.). Cambridge University Press: Cambridge, England. 2010;45–70.
31. Iheukwumere IH, Olusola TO, Chude C. Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. Journal of Natura Sciences Research. 2018:8 21–33.
32. Ihekwumere CM., Umedum CU, Iheukwumere IH. Identities and prevalence of *Aspergillus* species on *Phaseolus vulgaris* (Bean) seeds sold in Ihiala, Anambra State, Nigeria. Greener Journal of Microbiology and Antimicrobials 2020; 5(1):16–25.

© 2023 Iheukwumere et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/105835