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# Proximate Analysis And Antibacterial Effects Of Tempe On Campylobacter Jejuni And Escherichia Coli O157:H7

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***Abstract:*** ***The proximate analysis and antibacterial effects of tempe on Campylobacter jejuni and Escherichia coli O157:H7 was carried out. The proximate analysis showed that tempe from soybean fermented with Rhizopus oligosporus (tempe B) has a significantly higher protein content (39.83%) than the other tempe not fermented with Rhizopus oligosporus (tempe A(38.92%) and tempe C (38.90%) ). The carbohydrate content of the tempe fermented with Rhizopus oligosporus was lower than its corresponding tempe fermented with Rhizopus oryzae and the roasted soybean without fermentation. The in vitro effects of tempe extracts showed antibacterial activity against E.coli O157:H7 and Campylobacter jejuni. The mean of antibacterial activity against E.coli O157:H7 ranged from 3.6 ± 2.48 to 22.0± 2.48 for the tempe used. Tempe inoculated with Rhizopus oligosporus (Tempe B) showed the highest antibacterial activity with mean value of 22.0 ± 2.48 followed by tempe inoculated with Rhizopus oryzea (Tempe A) with a mean value of 15.0 ± 2.48 and the least antibacterial activity was recorded for soybean without inoculation (Tempe C) with a mean value of 3.6 ± 2.48. For the antibacterial activity of tempe against Campylobacter jejuni, tempe inoculated with Rhizopus oligosporus (Tempe B) showed the widest zones of inhibition with a mean value of 20.62 ± 1.59 and Soybean without inoculation (Tempe C) had the least antibacterial activity against Campylobacter jejuni with a mean value of 1.91 ± 1.59 indicating there is a significant difference between the three tempe at p value ≤0.05.***

## I. INTRODUCTION

Tempe is a fermented soy meal using the mould Rhizopus oligosporus. Generally, tempe has white to cream appearance caused by growth of the mould's mycelia. These mycelia connect the soybeans and therefore the compact texture of tempe is formed (Nout et al., 2005). There has been a lot of scientific evidence showing the functional properties of tempe in human health. This condition will make it possible for tempe to be developed as a functional food. Tempe is a good source of nutrients, such as protein, essential amino acids, essential fatty acids, vitamin B and dietary fibre in adequate amounts. Tempe has been found to contain phytoestrogens called isoflavones. In the human body, isoflavones may act as antioxidants, anticancer, anti-osteoporosis and hypocholesterolemic agents (Roubos-van de Hil, 2010). Isoflavones may also act as an anti aging agent and help post menopausal women through problem caused by estrogen imbalance. Isoflavones in tempe have higher bioavailability than isoflavones in soybeans which might be as a result of the fungus used in the fermentation of the tempe (Shurttleff and Aoyayi 2001).

Tempe is made by a natural culturing and controlled fermentation process that bind beans particles into a cake form. The microflora in tempe is complex, as tempe is a result of a mixed culture fermentation by moulds, yeasts, lactic acid bacteria and various other bacteria. The major genus of importance is the mould Rhizopus with different species such as R.microsporus, R. oligosporus and R. oryzae (Nout and Kiers, 2005). Lactic acid bacteria play a role in the

acidification of the soy beans during soaking, thereby preventing the growth of spoilage microorganisms (Nout et al., 1996; Ashenafi and Busse, 1991). Tempe fermentation process and its retention of the whole bean give it a higher content of protein, dietary fiber and vitamins compared to tofu, as well as firmer texture and stronger flavour. Tempe is used worldwide in vegetarian cuisine; some consider it to be a meat analogue. Even long ago before people found and realized the rich nutrition fact of tempe, it was referred to as" Javanese meat" (Dubey, 2010).

Tempe is a versatile and tasty ingredient, rich in vegetable protein and vitamins, with an assertive and characteristic flavour midway between nuts and mushrooms (Nout et. al., 1996).

In developing countries like Nigeria where there is high level of malnutrition and diarrhoea especially in internally displaced persons' camps, tempe could play a role as a source of high protein food (Aderibigbe and Akindele, 2004).

E.coli is a gram negative rod. However E.coli O157:H7 is an emerging disease pathogen which has the ability to cause diarrhoea. The incubation period of E.coli O157:H7 diarrhoea is usually 3- 4 days; it may be longer than that: 5- 8 days or shorter than that: 1- 2 days (Ekundayo et. al., 2014).

Campylobacter jejuni is the leading bacterial cause of food borne illness worldwide and a major cause of Guillain Barre paralysis. Studies have shown that the ability of C. jejuni to invade the host intestinal epithelium is an important essential step in the pathogenesis of Campylobacter jejuni mediated enteritis (Cheesbrough, 2006).

## II. MATERIALS AND METHODS

The soybeans (Glycine max) were purchased from uchi market in Auchi, Edo State, Nigeria. The starter culture inoculums (Rhizopus oligosporus) was obtained from the Indonesian Embassy at No. 5 Salt Lake street Maitama - Abuja. Rhizopus oryzae was cultured from rice. Five strains of E.coli O157: H7 were collected from microbiology laboratory at Irrua Specialist Teaching Hospital, Irrua, and fifteen strains of Campylobacter jejuni were obtained from Food Technology Department, Auchi Polytechnic, Auchi, both in Edo State, Nigeria.

In the production of tempe in the laboratory, the method of Aderibigbe, et al., 2010; Adams and Moss, 2011 was adopted. The proximate analysis of the tempe produced was done using the methods of AOAC (2016). Susceptibility of E.coli 0157:H7 and Campylobacter species to the tempe extract was determined using the agar well diffusion method. The test organisms were standardized by using 0.5 McFarland standards ($1.5\times10^8$ Cfu/ml) as a reference to adjust the turbidity of the microbial suspension. 1 ml of the peptone water containing the organism each was used to flood already prepared Mueller Hinton agar. A sterile Durham tube was used to punch two wells of 5mm in diametre on each Mueller Hinton agar medium. Each well was filled with 60µl of the tempe extract. These were incubated at a temperature of 37ºC for 24 hours. Zones of growth inhibition after 24 hours were measured.

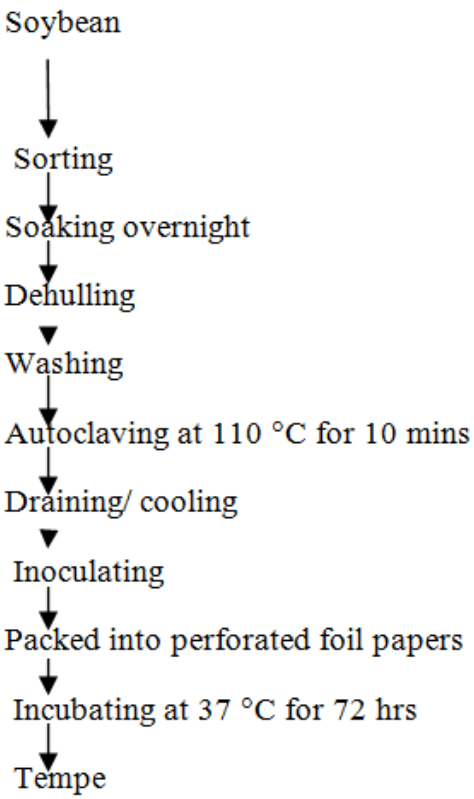


*Figure 1: Flow chart for the production of tempe in the laboratory (Source: Aderibigbe et. al, 2010)*

### STATISTICAL ANALYSIS

The statistical analysis used was two way analysis of variance and Duncan multiple range test where significant differences was at 5% level ($p \leq 0.05$).

## III. RESULTS

Figure 1 shows the flow diagram for the production of tempe in the laboratory. Table 1 shows the proximate composition of tempe produced from soy bean. Table 2 shows antibacterial susceptibility of tempe against *E.coli* O157: H7 and *Campylobacter jejuni*.

| Parameters | Value (%) | | |
|---|---|---|---|
| | Tempe A | Tempe B | Tempe C |
| Moisture | 5.13± 0.17$^{a}$ | 5.13±0.17$^{b}$ | 5.10 ± 0.15$^{c}$ |
| Ash | 3.51± 0.14$^{a}$ | 3.16±0.13$^{a}$ | 2.98 ± 0.15$^{a}$ |
| Crude fibre | 5.26± 0.14 $^{a}$ | 5.43±0.16$^{a}$ | 5.35 ± 0.14$^{a}$ |
| Fat | 10.36 ±0.16$^{a}$ | 10.74±0.11$^{a}$ | 10.56 ± 0.13$^{a}$ |
| Protein | 38.92± 0.12$^{a}$ | 39.83±0.18$^{ab}$ | 38. 90 ± 0.11$^{b}$ |
| Carbohydrate | 36.85±0.16$^{a}$ | 35.71± 0.17$^{ab}$ | 35.80 ± 0.12$^{a}$ |

*Mean within the column with the same superscript are not significantly different at p≤ 0.05.*
*"±" represent SD*
*KEY: Tempe A = Tempe inoculated with Rhizopus oryzae.*
*Tempe B = Tempe inoculated with Rhizopus oligosporus.*
*Tempe C = Roasted soybean without fungus inoculation.*

*Table 1: Proximate composition of tempe*

| Sample ID | Tempe A | Tempe B | Tempe C Diametres of zones of inhibition (mm) |
|---|---|---|---|
| STE A | 16 | 20 | 3 |
| STE B | 20 | 23 | 5 |
| STE C | 15 | 25 | 2 |
| STE D | 13 | 27 | 6 |
| STE E | 11 | 15 | 2 |

*KEY: Tempe A = Tempe inoculated with Rhizopus oryzae. Tempe B = Tempe inoculated with Rhizopus oligosporus. Tempe C = Roasted soybean without a fungus inoculation, STE A- STE E = E.coli O157:H7 from Irrua specialist teaching hospital.*

*Table 2a: Antibacterial activity of tempe against E.coli O157:H7*

| Diametres of zones of inhibition (mm) | | | |
|---|---|---|---|
| **Sample ID** | **Tempe A** | **Tempe B** | **Tempe C** |
| CDA | 16 | 36 | R |
| CDB | 10 | 21 | 4 |
| CDC | 9 | 16 | 3 |
| CDD | 8 | 22 | R |
| CDE | 6 | 10 | 2 |
| CDF | 9 | 17 | 3 |
| CDG | 24 | 34 | 6 |
| CDH | 17 | 32 | R |
| CDI | 18 | 28 | 7 |
| CDJ | 16 | 28 | 6 |
| CDK | 22 | 33 | 4 |
| CDL | 15 | 40 | R |
| CDM | 18 | 27 | 3 |
| CDN | 19 | 31 | 2 |
| CDO | 14 | 20 | R |
| CDP | 17 | 38 | 4 |

*KEY: Tempe A = Tempe inoculated with Rhizopus oryzae. Tempe B = Tempe inoculated with Rhizopus oligosporus. Tempe C = Roasted soybean without a fungus inoculation. CDA – CDP = Campylobacter jejuni form food technology Dept., Auchi Polytechnic, Auchi Nigeria*

*Table 2b: Antibacterial activity of tempe extract against Campylobacter jejuni*

## IV. DISCUSSION

The proximate analysis showed that tempe from soybean fermented with *Rhizopus oligosporus* (tempe B) has a significantly higher protein content (39.83%) than the other tempe not fermented with *Rhizopus oligosporus*(tempe A(38.92%) and tempe C (38.90%) ). The carbohydrate content of the tempe fermented with *Rhizopus oligosporus* was lower than its corresponding tempe fermented with *Rhizopus oryzae* and the roasted soybean without fermentation and these results are in accordance with that of Nowak *et. al*.,(1992). The *in vitro* effects of tempe extracts showed antibacterial activity against *E.coli* O157:H7 and *Campylobacter jejuni*. The mean of antibacterial activity against *E.coli* O157:H7 ranged from 3.6 ± 2.48 to 22.0± 2.48 for the tempe used. Tempe inoculated with *Rhizopus oligosporus* (Tempe B) showed the highest antibacterial activity with mean value of 22.0 ± 2.48 followed by tempe inoculated with *Rhizopus oryzea* (Tempe A) with a mean value of 15.0 ± 2.48 and the least antibacterial activity was recorded for soybean without inoculation (Tempe C) with a mean value of 3.6 ± 2.48 which confirms that of Wang *et. al*.,(1969). For the antibacterial activity of tempe against *Campylobacter jejuni*, tempe inoculated with *Rhizopus oligosporus* (Tempe B) showed the widest zones of inhibition with a mean value of 20.62 ± 1.59 and Soybean without inoculation (Tempe C) had the least antibacterial activity against *Campylobacter jejuni* with a mean value of 1.91 ± 1.59 indicating there is a significant difference between the three tempe at p value ≤0.05. The results from this study are in accordance with Roubos-van den Hil *et. al*., (2010) and Kuligowsky *et. al*., (2013).

With the parameters above, fermentation increased the protein content of the cotyledons significantly. The reduction in the carbohydrate content of tempe fermented with *Rhizopus oligosporus* is indicative of the fact that this organism help in the breakdown of the sugar content in the soybean (Mulyowidaro *et.al*.,1991)

## V. CONCLUSION

The present study showed that tempe contain significant effects against tested pathogens which implies that this fermented soy meal contains antibacterial components that may be of great use for the development of pharmaceutical industries as a therapy against diseases caused by *E.coli* O157:H7 and *Campylobacter jejuni*. Also as a plant protein rich food, it will help to solve the problems of protein calorie malnutrition especially in internally displaced persons' camps in Nigeria where carbohydrates are mainly consumed. Also it can serve as an alternative to the use of antibiotics in the control of infections caused by *E.coli* O157:H7 and *Campylobacter jejuni*. The results of the study support the folklore claim associated with the development of new antimicrobial drugs from soybean tempe.

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