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RESEARCH ARTICLE

Bacteriological Evaluation of Locally Produced Soybean Milk

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Abstract

Soybean milk is a regular beverage commonly sold across the streets and markets in Nigeria and beyond. The demand for soybean milk is fast growing due to its affordability and similarity to dairy milk, including its health benefits. However, there is a growing concern about the public health-related issues associated with this artisanal product's production, storage, and distribution. The primary objective of the present investigation was to evaluate the bacteriological composition of the locally produced soybean milk commonly consumed in the Kogi state of Nigeria. The samples for the study were collected from vendors in different locations of the state. The analysis was conducted on the samples using established standard procedures. The result found certain microorganisms such as *Micrococcus spp*, *Lactobacillus spp, Streptococcus spp, Enterobacter spp, Klebsiella spp*, and other fungi, which comprises *Aspergillus spp* and *Saccharomyces*. The study concludes that the soybean milk consumed in the study parameter was mainly contaminated with varying bacteria.

Introduction:-

Soybean milk is a well-known derivative product from soybean popularized by its functionality effects on humans (Sunarti et al., 2015). It is a commonly consumed health beverage across the globe(Ge et al., 2021). Soybean milk is an alternative beverage milk because it is an inexpensive and significant source of protein (Al-Saedi et al., 2020; Chaiwanon et al., 2000) and very similar to diary milk in compositions and physical appearance (Giri &Mangaraj, 2012). Thus, soybean milk is commonly used to substitute dairy milk (Cui et al., 2021; Kundu et al., 2018). The popularity of soybean milk is increasing rapidly worldwide (Peng et al., 2016; Vanga et al., 2020), attributable to its essential health functions (Hatanaka et al., 2014). Also, Vagadia et al. (2018)noted that soybean milk's calorie content is much lesser than cow's milk (Vagadia et al., 2018). The nutritional value is limited by soybean trypsin inhibitors (Ge et al., 2021).

Soymilk is consumed globally as a healthy protein drink and used as raw material to produce soy gel foods, such as tofu and soy yogurt (Peng et al., 2016). It is an excellent food item with numerous functional substances with antioxidant effects (Yamamoto et al., 2019). Soymilk has recently been applied in the dairy industry as a valuable ingredient to expand various products' texture, flavor, and nutritional value(Peng et al., 2016). According to Jimoh and Kolapo (2007), soymilk is a traditional oriental food-beverage growing in popularity in the United States and the world. Soymilk, a watery extract of the whole soybean, is rich in water-soluble protein, carbohydrates, and oil (Adebayo- Tayo et al., 2008).

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It is commonly characterized as having a beamy, grassy, or soy flavor, which reportedly can be improved by lactic acid fermentation as in yogurt-like products (Iwe, 2003). The increasing popularity of soymilk as a beverage worldwide is credited to health benefits, e.g., low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention of heart disease, and certain cancers (Akpan et al., 2007). The health benefits of soymilk have been widely studied (Ali et al., 2017; Apostolidis et al., 2007; Fukuda et al., 2017; Itakura et al., 2019; Li et al., 2016; Niyibituronsa et al., 2019; Oboh, 2006; Stojanovska et al., 2016; Vij et al., 2011; Zhu et al., 2020). However, research has suggested that the regular consumption of soy products is associated with an inverse incidence of type 2 diabetes (Camps et al., 2018) endocrine disrupters (Brando et al., 2013).

The market for soybean milk in Nigeria is on the increase in recent years. Ugwu and Nwoke (2011) noted that many people had been trained for soybean milk processing and distribution through various means, including skills acquisitions and virtual training. However, there is a growing concern about the production and distribution hygiene of the product, including processing instruments, water content, storage(Chuku & Akani, 2015), and the surrounding environment(Ahmed et al., 2017; Fasoyiro et al., 2010). Although the growing trend of street-vendor food in underdeveloped countries has a positive economic impact (Afreen et al., 2019), it has been associated with foodborne diseases. Over the years, foodborne pathogens are still causing many intestinal diseases in humans, resulting in substantial health and economic burdens(Gao et al., 2019; Gourama, 2020). The rapid and precise monitoring and detection of foodborne pathogens are the most effective ways to control and prevent human foodborne infections(Zhao et al., 2014).Nwaiwu et al. (2020) emphasized the importance of monitoring and characterizing the microbial flora of artisanal beverages. Accordingly, the microbial quality of soybean milk has attracted research attention in Nigeria. For example, Agwa and Ossai-Chidi (2016) examined the microbial quality of locally and industrially soybean products sold in Port Harcourt Metropolis, Nigeria. Their results revealed significantly higher bacteria and fungi counts in locally prepared samples compared to industrially prepared samples. They reported that Pseudomonas sp. and Staphylococcus sp. were the most common bacteria in locally processed samples. Thus, their findings associated locally produced soybean milk with foodborne illnesses.

Agboke et al. (2012) evaluated the microbiological content of soybean milk using samples of soybean milk locally produced by various producers. The study utilized standard techniques to determine the microbial load and identity of the microorganisms in the samples. The study found pathogenic organisms, including *S.aureus* and *E. coli*, and several fungi in the soybean products. Similarly,Agboke et al. (2012)noted that the microbial population detected in terms of number and types of organisms reflected poor hygienic standard of production, constituting a public health hazard among the populace. The study conducted by Madukwe et al. (2013)evaluated the nutrient content and microbial quality of soymilk fortified with carrot powder. The study adopted a standard procedure and found that the fortified soymilk contains higher microbial loads.Ozoh and Umeaku (2016)assessed the microbial analysis of soymilk and soymilk yogurt in Anambra State, Nigeria. Their result shows that almost all the samples bought from different markets were contaminated with *E. coli*and *Staphylococcus sp*.

The production and street vending of soybean milk in Kogi State is widespread. However, little is known about the microbial quality of the products consumed in the state, hence, justifying this study. Perhaps, a closer observation of the vendors and environment raises health-related safety issues. Indeed, soymilk consumption could threaten human health if harmful microorganisms are not adequately guided during production, storage, and distribution. Thus, contamination is possible following an unhygienic preparatory. Therefore, this study intends to evaluate the bacteriological composition of the locally made soybean milk in Kogi state.

Materials and Methods:-

The relevant materials and glassware were purchased from a reliable merchant and effectively sterilized. The reagents used in the study include crystal violet, Lugol's iodine, Safranin, Kovac's reagent, Lactophenol cotton blue, Hydrogen peroxide. Soybean milk was purchased from local vendors from different sites in Kogi state. They were immediately conveyed to the microbiology laboratory for analysis. The study followed the standard procedures outlined in Stanley et al. (2014) and (Agboke et al., 2012).

Result:-

Table 1:- Table showing the morphological characteristics and gram reaction of bacterial isolate.

Code no	Morphological characteristics	Gram Reaction	Isolates
A	Creamy round colonies	Gram-positive coccus in clusters	Micrococcus spp

	on nutrient agar		
B	Creamy and small round shape colonies in nutrient agar	Gram-positive cocci in chain	Streptococcus spp
C	Pale green and convex opaque colonies on cled agar	Gram position cocci in chain	Aerobacter spp
D	Pale green and creamy colonies on cled agar	Gram-negative cocci in chain	Klebsiella spp
E	Creamy and round in the shape on Mrs agar	Gram-positive rods in chain	Lactobacillus

Table 2:- Table showing the biochemical character of Gram-positive bacteria present.

Sample code	Gram reaction	Catalase test	Oxidase	Indole	Sucrose	Glucose	Lactose	Motility	Presumptive organism
A	+	+	-	+	AG	A	AG	-	Micrococcus spp
B	+	+	-	+	A	A	A	-	Streptococcus spp
C	+	+	-	+	A	AG	AG	-	Aerobacter spp
D	-	+	-	+	AG	AG	AG	-	Klebsiella spp
D	+	+	-	+	AG	A	A	-	Lactobacillus spp

Key - = Negative, + = Positive, A = Acid, AG = Acid & Gas

Table 3:- Table showing the identification of fungi isolates based on their reactions with lactophenol cotton blue.

Characteristics	Identification
Presence of septate hyphae long and smooth conidiophores, long unbranded sporoging with large, round head Black and brownish at the edges with dark mycelium spores on the surface	Aspergillus spp
Creamy, oval shape budding cell with rounded shape the end resembling barrel shape	Saccharomyces spp

Table 4:- Table showing the percentage distribution of each isolate.

Isolates	Numbers of organism	Percentage distribution
Micrococcus spp	96	36.5
Streptococcus	81	30.7
Aerobacter spp	28	10.6
Klebsiella spp	16	6.1
Lacto bacillus spp	24	9.11
Aspergillums spp	10	4.0
Saccharomyces spp	8	3.0
Total	263	100

Discussion:-

The present study was intended to evaluate the bacteriological composition of the locally made soybean milk in Kogi state. The investigation conducted on the samples demonstrated the existence of certain microorganisms such as *Micrococcus spp, Lactobacillus spp, Streptococcus spp, Aerobacter spp,* and *Klebsiella spp*, as indicated in table 2. Table 1 shows the morphological characteristics and Gram reaction of the isolates. The result is consistent with studies that found similar microorganisms in soymilk (Agboke et al., 2012; Akinola et al., 2015; Brooks et al., 2004; Edet & Peter, 2017; Mbaeyi et al., 2013; Ozoh&Umeaku, 2016). Lactobacillus spp, as observed above, has been associated with soymilk spoilage and an increase in acid production (Stanley et al., 2014). These organisms thrive in fermentable substrates as sugar, which can be reduced by acid. The presence of streptococcus spp indicates a high level of exposure and negligence occurring at any stage of the production process(Brooks et al., 2004). All the isolated organisms in the study have been linked with health-related concerns. Evidence has shown that bacteriological pathogens may find their way into food production, including soymilk processing, due to inadequate hygienic practices, insufficient decontamination, and mishandling of raw materials(Simangunsong & Susanna, 2019)

Furthermore, table 3 shows Aspergillus spp and Saccharomyces spp as the fungi isolated based on their lactophenol cotton blue reactions. Aspergillus spp is a toxigenic mold capable of producing aflatoxin (Brooks et al., 2004). Thus, it is a public health concern. On the other hand, Saccharomyces spp has been shown to cause spoilage at the fermentation stage, probably due to high sugar levels. However, the role of Saccharomyces spp in the spoilage of soymilk is unclear.

Conclusion:-

The bacteriological evaluation of the locally manufactured soymilk commonly found across different areas of Kogi state indicates that the artisanal beverage may be contaminated with varying bacteria. The study concludes that microorganisms present in the commonly available soybean milk in the study parameter could be attributed to manufacturers 'inadequate hygiene, unsanitary conditions of processing equipment, and raw materials. Pathogenic bacteria in soymilk can be either infectious or toxin-producing. Although most pathogens that contaminate soymilk grow only slowly or not at all. Perhaps, soymilk provides a safe place for microorganisms to grow. Thus, it is recommended that a robust precautionary approach be adopted in the production, storage, and distribution of the product to effectively mitigate the contamination of microorganisms in soybean milk. The present study contributes to disease control literature by further supporting the contamination of locally produced soybean milk. Thus, the study recommends that research broaden the probable measures to lessen the prevalence of consuming contaminated soymilks in Nigeria.

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