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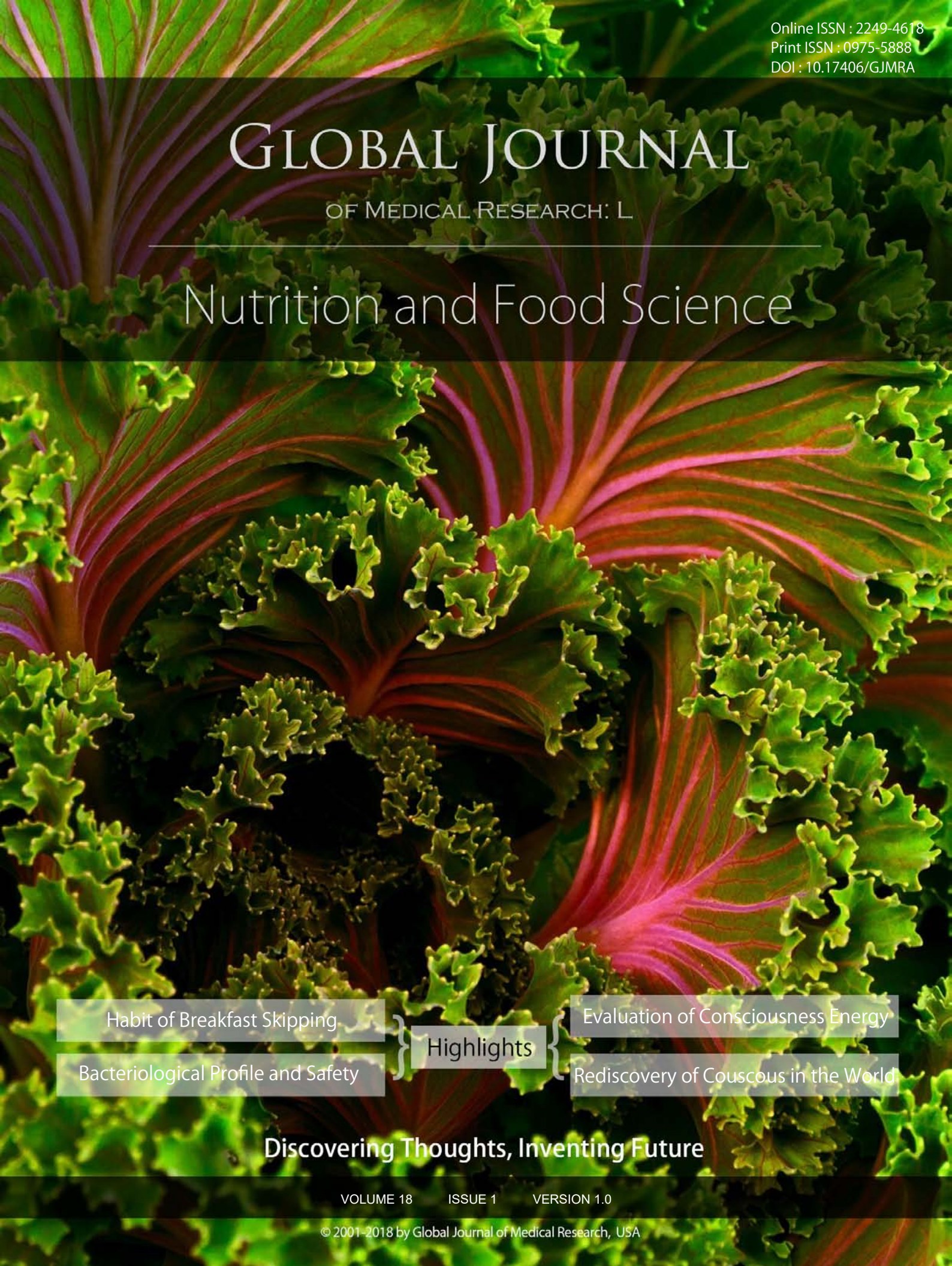

Habit of Breakfast Skipping

Bacteriological Profile and Safety

Highlights

Evaluation of Consciousness Energy

Rediscovery of Couscous in the World

Discovering Thoughts, Inventing Future

VOLUME 18 ISSUE 1 VERSION 1.0

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Bacteriological Profile and Safety of Soured Milk in Uganda

By Ivan Kamurasi, Frank Kiwanuka & Lwanira Catherine
International Health Sciences University)

Abstract- *Background:* Soured milk has an outstanding nutritional quality and highly consumed by people in Kampala and Uganda. However, it is an excellent medium for pathogen bacterial growth and an important source of bacterial infections when poorly handled. A cross sectional study was carried out to assess for occurrence of pathological bacterial species in soured milk sold in retail dairy shops in Makindye, Kampala, Uganda. A total of 174 soured milk samples were purposively collected and analyzed using by standard bacteriological methods. 89.1% of the samples showed significant bacterial contamination. The most commonly isolated organisms were E coli (47.1%), Klebsiella spp (28.4%) Shigella Spp (11.6%), Salmonella Spp (7.1%) and Enterobacteria faecalis (5.8%). Factors associated with contamination included; age, level of education, working experience, source of information on milk handling, longevity of soured milk, presence of pests at facility, availability of hand washing equipment and general cleanliness.

Keywords: *soured milk, bacterial species, food safety, contamination, bacteriological profile.*

GJMR-L Classification: *NLMC Code: WX 218*

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Bacteriological Profile and Safety of Soured Milk in Uganda

Ivan Kamurasi [α], Frank Kiwanuka [σ] & Lwanira Catherine [ρ]

Abstract- ***Background:*** **Soured milk has an outstanding nutritional quality and highly consumed by people in Kampala and Uganda. However, it is an excellent medium for pathogen bacterial growth and an important source of bacterial infections when poorly handled. A cross sectional study was carried out to assess for occurrence of pathological bacterial species in soured milk sold in retail dairy shops in Makindye, Kampala, Uganda. A total of 174 soured milk samples were purposively collected and analyzed using by standard bacteriological methods. 89.1% of the samples showed significant bacterial contamination. The most commonly isolated organisms were E coli (47.1%), Klebsiella spp (28.4%) Shigella Spp (11.6%), Salmonella Spp (7.1%) and Enterobacteria faecalis (5.8%). Factors associated with contamination included; age, level of education, working experience, source of information on milk handling, longevity of soured milk, presence of pests at facility, availability of hand washing equipment and general cleanliness.**

Keywords: soured milk, bacterial species, food safety, contamination, bacteriological profile.

I. Introduction

Globally, 550 million cases and 230,000 deaths happen every year due to consumption of fresh agricultural products and dairy products contaminated by non Typhoidal salmonella, pathogenic Escherichia coli, Campylobacter and Norovirus.

Diarrheal diseases account for more than half of the global burden of food-borne diseases more especially among children below 5 years of age (WHO, 2015). Milk has for long been a delicacy for many communities, however, its complex biochemical composition, high water activity and nutritional value makes it an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exist (Parkh and Subhush, 2008).

Humans may be infected with milk- borne pathogens through consumption of infected raw or unpasteurized milk and milk products (Belli et al., 2013).Unpasteurized milk sold to the public enters either directly from producers, via informal markets or through dairy farmer cooperatives and resources are extremely limited, yet retail production is underdeveloped with low levels of hygiene (Knight-jones et al., 2016).

Some of the pathogenic microorganisms commonly solated from milk and milk products posing a serious threat to human health include Escherichia coli, staphylococcus aureas, Salmonella species (spp), Listeria monocytogen, Brucella abortus, Mycobacterium spp, campylobacter spp, Lyptospira spp, Clostridium spp, Pseudomonas arginosa and Proteus spp (Kanyeka, 2014). According to a study that was done by Abel, 2016, Escherichia coli were found to be the dominating coliform in soured milk. Specifically, a strain of Escherichia coli (E coli O157:H7) (CDC, 2016).

Cow's udder, barns, milk collection equipment and materials, various ingredients added to the dairy products and dairy farm workers are some of the sources of bacterial contamination in soured milk (Abera et al., 2016). Coliforms which are the target of the present study have been reported to contaminate soured milk mainly as a result of using poorly sanitized utensils during milking, transportation and storage processes (Garedew et al., 2012).

In countries with poor milk production and distribution practices, one can expect a higher percentage of bacterial contamination which poses a health hazards such as Typhoid fever by salmonella and bloody diarrhea by E coli (Garedew et al., 2012). In the current study area in particular and Uganda in general, there is lack of published information on the extent of soured milk contamination by Salmonella species, and Enterohaemorrhagic Escherichia coli, yet soured milk is favored over fresh milk in this setting (Knight-Jones et al., 2016).

In many of the low income countries, raw milk and its products is found to be contaminated with potentially pathogenic E coli strains which impose a serious public health threat to humans (Ombarak et al., 2016). In a recent study done in Uganda by the Diary Development Authority (DDA) in Kasese district, milk sold to the public was found to be adulterated and contaminated with pathogenic bacteria (DDA, 2017). If nothing is done to improve the quality of dairy products sold in public centers, consumers will continuously be predisposed to potentially pathogenic organisms that are usually present in contaminated milk.

The dairy market in Uganda is mainly dominated by small-holder or retail dairy business holders with low levels of hygiene. The information generated by this study will be used by relevant regulatory bodies such as the Uganda Diary Development Authority to scale up monitoring and evaluation of operations of dairy businesses in Uganda.

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In addition, design interventions that can help to improve the quality of dairy products sold out to the public. The study sought to assess for occurrence of selected common bacteria species associated with contamination of soured milk sold out in retail dairy shops in Kampala city, Uganda.

II. Methods and Materials

Study Design and Setting: This was a cross sectional study that was carried out during the months of May to July, 2017. The study was conducted in Makindye Division. Makindye division is one of the divisions that make up Kampala capital city of Uganda.

Sampling Method: The study population was soured milk and milk retailers. Soured milk sellers in retail daily shops who sell soured milk at the time of sampling, and were residing in Makindye division were included in the study. Sellers who declined informed consent were excluded.

Purposive sampling technique was used. In this case, only retail milk sellers were selected for participation in study until the sample size was reached.

a) Data Collection Method and Tools

This study employed quantitative data collection. A Questionnaire and an Observational checklist was developed using some of recommended general principles codex of food hygiene (FAO, 2003) for the general hygiene of the milk retailers and premises, washing processes, their knowledge and attitude towards proper handling of soured milk, sources of water used and general upkeep of the soured milk. The organization of the questionnaire and observation check list was in accordance with the specific objectives of the study.

b) Sample Collection

This involved collection of soured milk and performing laboratory analysis on the samples. A total of 174 soured milk samples were collected from the diary retail shops in the selected markets and areas of Makindye division.

c) Soured Milk Sample Handling

Soured milk was collected into sterile containers, given lab numbers and immediately transferred in a courier cold box with ice before being delivered to the school laboratory at International Health Sciences University. A guided questionnaire/ observation check list was also checked during the process of collecting samples. Samples were then aliquoted into sterile glass containers in the laboratory. Samples were then examined for the presence of selected common bacterial species with main emphasis on Coliforms and specifically Enterohaemorrhagic Escherichia coli O157:H7.

d) Microbiological Analysis

Laboratory analyses were carried out at the International Health Sciences University microbiology laboratory. In the Laboratory, serial dilutions were made for each sample for coliform counting on MacConkey agar, and also for identification lactose and non-lactose fermenters.

e) Coliform Counting

A total of 10 tubes were dispensed with 9 ml of sterile normal saline. Tenfold serial dilution of the sample from 10-1 to 10 -10 in sterile normal saline solution was done. Then, 1 ml of soured milk sample was added into the 9 ml normal saline (10-1 dilution); the 1 ml of resulting solution was transferred into a second tube containing 9 ml of normal saline (10-2 dilution) and the procedure was repeated for more dilutions as shown in figure 1. The dilutions were mixed using a vortex mixer for 10 seconds.

0.1ml of each dilution was poured on a MacConkey agar plate, and spread with a sterile glass spreader on the media. Plates were allowed to dry with their lids on and then they were inverted and incubated at 35^0c under aerobic conditions for 24 hours to allow bacterial growth.

A new sterile pipette was used for each dilution and the procedure was repeated as above with further dilutions up to the last dilution and for the remaining test samples, where consecutive critical dilution steps were chosen for the inoculation on plates. After incubation period, coliforms on MacConkey agar were counted manually and findings were recorded (ISO4833-1:2013).

The cfu/ml of sample was determined using the formula below (Ogot, Ochoudho and Machoka, 2015).

$$\text{cfu/ml of Sample} = \frac{\text{Number of Colonies}}{\text{Amount Plated x Dilution Factor}}$$

f) Identification of Bacteria on Different Media

Samples were incubated at 35°C-37°C for 24 – 48 hrs, and colonies that appeared pink/red on MacConkey were acknowledged to be lactose fermenters while the colorless colons were registered as non-lactose fermenters. Isolated colonies where then sub-cultured on different bio-chemicals. These included the catalase test, coagulase test, triple sugar iron agar (TSI), Cled, Citrate, Indole, BA for identification of Shigella spp, Enterobacter spp, Klebsiella spp, Salmonella spp and E coli.

g) Organism Confirmation

This was done in compliance with standard biochemical tests including TSI, Sulfide Indole Motility (SIM), Citrate agar and methyl red Voges Proskauer (MRVP), Urea and Cled agar (Pizarro et al., 2013). The presumptive EHEC colonies were then confirmed using commercial E coli O157:H7 strains anti-sera (Oxiod Basingstoke, UK) based on detection of EHEC antigens. Salmonella species were confirmed by sub culturing them on XLD, where the colonies present with black centers.

h) Data Analysis

Samples collected, were given identification numbers, recorded and later stored in both soft and hard copies in the laboratory manual. Data was cleaned, coded and entered into the statistical package for social sciences (SPSS) version 20. All statistical tests were two-tailed and a P-value of less than 0.05 was considered significant.

i) Quality Control for Laboratory Methods

Prepared media was sterilized by autoclaving at a temperature of 121°C for 15 minutes. Complete sterilization was confirmed by using autoclave tapes attached to conical flasks containing media for sterilization. Aseptic methods were used during casting of culture media onto petri dishes to minimize contamination and the agar was incubated for 16 to 18 hours to check for cases of contamination. The experiment was carried out in duplicates and positive control strains of Salmonella species and Escherichia coli were obtained from Lancet laboratory Uganda and used.

j) Ethical Considerations

The research protocol was cleared by the research and ethics review committee of the Institute of Allied Health Sciences of International Health Science University. Permission was then granted by the Makindye division mayor to proceed with data collection. The research was conducted while upholding the moral, tradition and customary rules and regulations of the community in a manner that did not compromise the scientific inclinations of the research. Scientific standards in the methods employed in the collection and analysis of data were maintained. All study participants provided informed consent.

III. Results

A total of 174 samples of soured milk were examined to assess the occurrence of selected common bacteria species and risk factors associated with contamination of soured milk sold out in retail dairy shops in Makindye division, Kampala district- Uganda.

a) Common Bacterial Isolates in Soured Milk

Amongst the 174 samples, 89.1% (n=155) showed bacterial growth with total plate counts greater than 25 cfu/ml, the limit acceptable by the East African standards (EACs, 2008). Only 10.9% (n=19) showed no bacterial growth even after 48 hours of incubation. Of the 155 samples that had bacterial growth, the most commonly isolated were E coli (47.1%); 44 samples (28.4%) had Klebsiella spp and 18 (11.6%) had Shigella spp. The other bacterial species that were found in soured milk included Salmonella spp (7.1%) and Enterobacteria faecalis (5.8%).

Notably, Enterohaemorrhagic E coli strains were not found in all the soured milk samples analyzed. Some samples had more than one type of bacterial isolate, however the common ones where E coli and Klebsiella spp.

b) Demographic Factors Associated with Contamination of Soured Milk

Majority (41.4%) were in the age group of 21-30 years; 81.6% were female while the rest were male. Kisugu had the highest number of respondents (29.3%) and at least 125 (71.8%) of all respondents had attained secondary education. Majority (98.3%) reported to have had formal training on milk handling mainly through radios (51.1%) and many of the respondents (85.7%) had an experience of 1 year and above in milk handling (Table I).

Age (χ^2=55.3; P value <0.001), level of education (χ^2= 30.9; P value<0.001), sources of information on milk handling (χ^2=4.5; P value=0.001) and work experience (χ^2=21.6; P value <0.001) were significantly associated with microbial contamination of soured milk. There was no statistically significant difference in the level of bacterial contamination of soured milk obtained from female or male retail milk sellers. Also, there was no statistically significant difference in bacterial contamination of soured milk collected from milk sellers who reported having been formerly trained in milk handling and those that never received formal training (χ^2=0.35; P value=0.110) (Table I).

Notably, milk sellers of age 21-30 years and those between 31-40 years were 18 and 31 times respectively more likely to have bacterial contamination of soured milk than those of 41 years and above.

With respect to the level of education, retail milk sellers who had reached the primary and secondary levels were 1.6 and 13.5 times respectively more likely to have bacterial contamination of soured milk than those who had reached the university level.

The level of contamination of soured milk collected from milk sellers who obtained knowledge about milk handling from radios and those trained by community health workers was almost similar (OR=1.03; 95% CI=0.3-3.4); while working experience had a significant impact on the level of bacterial contamination of soured milk. In this study, milk sellers who had 1 to 5 years of working experience were 6.5 times more likely to have contamination of soured milk than those whose working experience was more than 5 years (Table I).

c) Hygienic Related Factors Associated with Contamination of Soured Milk

All respondents had at least a refrigerator for storage of soured milk. The main source of water for soured milk preparation reported by the majority (88.5%) was tap water followed by wells (9.2%). Most of the soured milk was sold off between the second and third day after preparation. Majority of the respondents

(89.7%) didn't have hand washing equipment and didn't wear protective gears (97.7%). There was also presence of pests (house flies) in some areas. All in all the general cleanliness was regarded as poor (Table II).

In this study, longevity of soured milk (χ^2=10.2; P value=0.004), availability of hand washing equipment (χ^2=2.3; P value <0.001), presence of pests at facility (χ^2=2.8; P value=0.001) and general cleanliness (χ^2=12.6; P value=0.005) were significantly associated with contamination of soured milk. There was only slight association between wearing protective gears and contamination of soured milk (χ^2=0.5; P value=0.052); whereas no statistically significant differences in the level of contamination of soured milk were observed when tap water or other source of water was used in soured milk preparation (Table II).

Notably, for most of the hygienic related factors under consideration, it was not possible to derive an odds ratio because some of the cells included in the 2x2 contingency tables had zero integers. The only odds ratio that was calculated was for estimating the relation between having pests at the facility and contamination of soured milk. Surprisingly, milk sellers who had pests at the retail facility were less likely than those who did not have pests at the facility to have had contamination of their soured milk (OR=0.38; 95%CI=0.14-2) (Table II).

IV. Discussion

80% of milk sold in Uganda goes through informal markets and such milk may pose a health hazard due to contamination with pathogens. Generally, it was found that soured milk was highly contaminated with pathogenic bacteria exceeding the recommended 25 cfu/mL of total aerobic count as per the East Africa community standards (EACs, 2008) This calls for an urgent need for improvement of milk handling protocols among the milk distributors and handlers in Uganda. Soured milk is popular delicacy in Uganda. Therefore, the high bacterial contamination pose a considerable threat to public health.

Findings of this study could reflect poor levels of hygiene displayed in preparation and handling of soured milk, since coliforms are mainly of faecal origin. This contamination could occur during harvesting, storage or during distribution of milk to the customers at the diaries. Microbial quality of food is very important measure of the safety of the food for human consumption. The presence of Salmonella spps, E coli, Shigella spps, Entrobacteria, Klebsella pneumonae indicate that the food is hazardous and is not fit for human consumption. On the other hand, presence of non-pathogen bacteria in food doesn't necessitate unfitness for consumption, but may indicate the hygiene status of the preparation and processing. However, certain levels may indicate a serious case of poor hygiene and the food becomes unfit for consumption.

These findings are similar with findings from many others studies that were done in Africa and Uganda that reported exceedingly high bacterial contamination (Knight-Jones et al., 2016; Belli et al., 2012; Kanyeka, 2014). Elsewhere, similar studies have also demonstrated that milk products such as soured milk commonly contain similar bacterial isolates to the ones identified in this study (Lu et al., 2013). however percentages of bacteria isolated was not given. Only in a longitudinal study that was carried out in Khartoum state in South Sudan during the dry and rainy seasons in April 2008 to February, 2009 where more than half of the milk (51.3%) sold for direct consumption by vendors and shops in Khartoum state conformed to the internationally accepted standards for total coliform counts (<100 cell/ml). The difference in the level of contamination in comparison to the present study could relate to the season in which samples were collected since samples that were used in the present study were collected during the dry season. High bacterial contamination rates of milk are usually observed more in dry season than in rainy or wet seasons (Adjlane-Kaouche et al., 2014).

Although few studies have investigated the relationship between demographic factors and bacterial contamination of milk, the findings from this study show that age, level of education, source of information on milk handling and work experience were statistically associated with contamination of soured milk. Majority of the soured milk samples were obtained from the informal milk sector that is to say from diaries which employ persons with low education and usually in family businesses. Notably, milk sellers in the age bracket of 21-30 years and 31-40 years were more likely to have contamination of soured milk than those above 40 years. The effect of age on milk contamination may correlate to the level of training and working experience. Usually young people have worked for fewer years and probably have achieved little training on the aspects of their work yet learning and attaining expertise may come with experience. This may explain why more contamination of soured milk was evident in samples that were collected from the young retail milk sellers. This is also in line with the findings from the present study in which milk sellers with fewer years of working experience (1-5 years) reflected more bacterial contamination than those who had working experience of more than five years.

Regarding the level of education, milk sellers of lower academic levels (primary and secondary) were more likely to have contamination of their soured milk than those who had reached university level. Again this observation may relate to the level of knowledge regarding milk handling and hygiene demonstrated by the University retailers as compared to the primary and secondary level milk retailers. More likely, those with lower education levels may not have additionally obtained sufficient training on milk handling, thus the higher chances of milk contamination. Even though majority of

the respondents reported to have received some formal training in milk handling and had knowledge about bacterial contamination of milk, given the fact that they elaborated that "they boil the milk to kill of the bacteria after purchasing the milk", the high level of microbial contamination found reflects poor practices of handling milk leading to bacterial contamination. Presumably, the procedure that they use to make soured milk, the utensils and water used could be the source of contamination. This is also in line with a study by Salman and Hamad (2011) in which the lack of knowledge regarding clean milk production, and potable water for cleaning purposes were some of the factors which contributed to the poor hygienic practices that led to contamination of milk. Another study that was done in Mbarara district also reported a significantly high proportion of farmers who had never received formal training on best milk handling practices (Kagoro et al., 2016).

In this study, there was no difference in the level of soured milk contamination sold by female or male retail sellers. Even though relationship between sex and contamination of milk in the Mbarara study was not investigated, it was noted that majority of the milk handlers (74.9%) were female and rest being male (Kagoro et al., 2016).

Notably, the fact that the present study noted significant relationships between demographic factors and soured milk contamination, yet there is very limited information on the subject; the present findings highlight the need for thought and further exploration of the influence of demographic factors on soured milk consumption. This may provide more insights into understanding the risk factors of contamination of milk besides the hygienic related factors.

In this study, longevity of soured milk, availability of hand washing equipment, presence of pests at facility and general cleanliness were significantly associated with contamination of soured milk. Unsafe practices such as not washing hands, sell of expired milk and access of pests to containers of soured milk leads to introduction of microorganisms to the milk. Food borne pathogens can be carried by the hands of the milk handlers, pests or they may develop over time as the milk is kept longer in the storage (WHO, 2015).

In the current study, respondents were observed preparing and storing soured milk in Jerri cans and polyethene bags, a similar case that was reported by Tumushabe, (2013). Similar findings were also reported from studies that were done in Gambia, Senegal and Guinea in which raw and soured milk were found to be highly contaminated. In these studies, the high microbial contamination was attributable to the poor hygiene arising from poor handling at the farm, at collection centers, during transportation and at retail points and more often by faeces (of animal or human origin), personnel, water and containers that are not properly sterilized. More often milk is packaged in discarded vegetable oil jerry cans for easy transportation; yet such jerry cans are difficulty to clean since the opening is too small to enter with hands or cleaning tools. Additionally, it was observed that some milk men and milk traders use cloth to strain out the flies and dirt, but those cloths are not thoroughly washed. Usually, milk vendors at the markets use spoons or cups as measuring tool, which also are not cleaned properly during the day but rather simply wiped with some piece of cloth (Hempen et al., 2004), a similar practice seen in the present study.

Additionally, one of the respondents kindly explained that to quicken the process of making soured milk, "a left over soured milk is added to fresh milk so that it can ferment faster''. Similar techniques have also been reported by Tumushabe, (2013) in ghee processing in western Uganda. This method is so hazardous since the fresh milk will also be contaminated by bacteria carried from the previous batch of soured milk. In this regards use of artificial acidifier such as citric acid would be recommended.

Also in the recent study done in Kasese by DDA similar observations where reported (DDA, 2017) however they never went into the details to confirm which common pathogen was predominate in milk.

Many of the selling points had a lot of pests in particular house flies which aid in the contamination of soured milk and food in general and of recent, studies demonstrated that adults house flies that feed on food contaminated with various levels of food borne bacteria were able to transmit those pathogens to their eggs and in the process mitigate the spread of food borne pathogens (Monica et al., 2015).

Furthermore, other studies in Zimbabwe, Tanzania and Ghana reported that unhygienic practices along the milk value chain predisposed milk to high bacterial load and recently it was noted by Knight-Jones et al., (2016) upon arrival at point of sale, poor hygiene led to high bacterial load with almost all samples culture positive for Stapylococcus aureas and E coli.

This is also in line with some other studies that demonstrated poor hygiene practices such hand washing and pests in the milk stores as compared to those at the production levels (Abera et al., 2016). Disassa (2017) observed that in Asosa town Ethiopia milk samples were produced and handled under poor hygienic conditions and Bonaventure et al., (2015) found that milk samples had added water to them in a study done in Zambia (Bonaventure et al., 2015).

Overall, the risk of food borne diseases is most severe in low- and middle income countries and is linked to preparing food with unsafe water, poor hygiene and inadequate conditions for food production and storage, lower levels of literacy and education and insufficient food safety legislation and implementation of such legislation (WHO, 2015) and in the current study the poor levels of hygiene were found to

predispose the soured milk to bacterial contamination; the water used in preparation, the selling site/ residence and storage container among others were shown to have a direct relationship with the bacterial status in the soured milk.

This study had some limitations that need to be mentioned; sampling of milk was not done at farm level, during transportation, from milk collectors and whole sellers and thus the probable bacterial contamination at all these check points was not evaluated. This has a limitation on determining the point of contamination by selected common bacteria spieces, since some of the milk might be contaminated before it reaches the distribution point. The authors recommend routine mandatory assessment of milk quality in order to safeguard the public from milk-borne infections. In addition, periodic surveillance visits to dairy retail shops and health education on milk handling practices is needed to prevent milk contamination. Milk supply chains should be regulated in a way that certified, trained and licensed suppliers by DDA should distribute milk to the retail shops.

Acknowledgement

We would like to thank the Institute of Allied Health of International Health Sciences University for their assistance.

Areas of Further Research

There is limited information regarding assessment of risk factors for soured milk contamination; thus other studies investigating the risk factors here in mentioned in other settings is highly recommended.

Conflicts of Interest

The authors declare that they have no competing interests.

Funding Disclosure

The Authors received no funding for this project.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Practical Application

All the bacteria isolated were coliforms indicating probable faecal contamination of the soured milk as a result of poor hygiene. This indicates that soured milk sold by small retail diary shops is highly contaminated with pathogenic bacteria. There is a need for improvement in soured milk handling so as to ensure that the quality of soured milk sold out to the public is acceptable and safe for human consumption.

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Table 1: Demographic Information of the Study Respondents Characteristics of the Respondents and Occurrences of Isolated Bacterial Species in Makindye, Kampala, Uganda (N=174)

Variable		Bacterial Growth n (%)		P-Value
		Yes (n=155)	No (n=19)	
Age	< 20 Years	17 (10.9)	10 (21)	< 0.001
	21-30 Years	68 (43.6)	2 (10.5)	
	31-40 Years	58 (37.8)	6 (31.6)	
	> 41 Years	12 (7.7)	2 (10.5)	
Gender	Male	27 (17.4)	9 (47.4)	0.062
	Female	128 (82.6)	10 (52.6)	
Level of Education	Primary	16 (10.3)	5 (26.3)	< 0.001
	Secondary	118 (76.1)	8 (42.1)	
	College	16 (10.3)	4 (21)	
	University	5 (3.3)	3 (15.8)	
Formal Training on Milk Handling	Yes	152 (98.0)	14 (73.7)	0.110
	No	3 (2)	5 (26.3)	
Sources of Information on Milk Handling	Radio	77 (49.6)	15 (78.9)	0.001
	Television	4 (2.6)	0 (0)	
	News Papers	4 (2.6)	0 (0)	
	Veterinary Officers	1 (7.7)	0 (0)	
	Community Health Workers	25 (16.1)	4 (21.1)	
	Others	33 (21.3)	3 (15.7)	
Work Experience	<12 Months	25 (16.1)	0	< 0.001
	1 to 5 Years	91 (58.7)	5 (26.3)	
	More than 5 Years	39 (25.2)	14 (73.7)	

Table 2: Hygiene Related Characteristics of the Respondents and Occurrences of Isolated Bacterial Species in Makindye, Kampala, Uganda (N=174)

Variable		Bacterial Growth n (%)		P-Value
		Yes n=155	No n=19	
Use a Refrigerator as Storage Equipment	Yes	117 (74.5)	19 (100)	0.008
	No	38 (24.5)	0 (0)	
Longevity of Soured Milk at the Shop	0 to 1 Day	52 (33.5)	19 (100.0)	0.004
	2 to 3 Days	94 (60.6)	0 (0)	
	1 Week	9 (5.8)	0 (0)	
Water used for Preparing Soured Milk	Tap Water	135 (87.1)	19 (100)	0.148
	Spring Wells	16 (10.3)	0 (0)	
	Others	4 (2.6)	0 (0)	
Availability of Hand Washing Equipment	Yes	18 (11.6)	0 (0)	<0.001
	No	137 (88.4)	19 (100)	
Presence of Pests at Facility	Yes	80 (51.6)	14 (73.7)	0.001
	No	75 (48.4)	5 (26.3)	
Wearing Protective Gears	Yes	4 (2.6)	0 (0)	0.052
	No	151 (97.4)	19 (100)	
General Cleanliness Condition	Fair	58 (37.4)	0 (0)	0.005
	Poor	88 (56.8)	19 (100)	
	Very Poor	9 (5.8)	0 (0)	

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Habit of Breakfast Skipping is Associated with a Higher Risk of Hypertension and Increased Level of LDL

By Zeshan Ali & Shakeel Ashraf
Jiangsu University

Abstract- *Background:* The link between breakfast skipping and cardio-metabolic disorder is well studied. Though, there are very rare studies describing the association between habit of eating breakfast and hypertension. The existing study aimed to assess the association among the habit of breakfast skipping and hypertension along with low density lipoprotein (LDL) level in university going adults.

Methods: Two hundred university going adults were enrolled for this study. The habit of breakfast skipping was assessed from self-reported questionnaires and categorized into three groups: (rare, often and regular breakfast eaters). Three days' dietary consumption over one weekend day and two week days were gathered from each participant using a 2-day daily record and a 24-hour recall.

Results: For the occurrence of hypertension the crude odds ratio of skipping breakfast was 0.359. Though, after adjusting for all substantial confounding aspects (sex, age, current smoking, regular exercise, diastolic and systolic blood pressure, waist circumference, body mass index, and red blood cell counts), not intake breakfast was linked with a higher risk of HTN (OR=1.045; 95% CI=1.036-1.053; p-value $<$ 0.001).

Keywords: *breakfast skipping, LDL, systolic and diastolic blood pressure, hypertension.*

GJMR-L Classification: *NLMC Code: WG 340*

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Habit of Breakfast Skipping is Associated with a Higher Risk of Hypertension and Increased Level of LDL

Zeshan Ali [α] & Shakeel Ashraf [σ]

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Conclusion: The habit of breakfast skipping was related with a higher risk of hypertension and increased level of LDL among university going adults.

Keywords: breakfast skipping, LDL, systolic and diastolic blood pressure, hypertension.

I. Introduction

Breakfast consumption is a well-studied topic. The initial documented researches were from a cereal companies and producers of pork in the 1800s and 1920s respectively[1]. Latest studies provide data connecting the habit of breakfast consumption with a reduced risk of obesity, weight gain and metabolic syndrome[2,3]. Furthermore, various studies showed that the habit of breakfast intake was linked with reduced cardio- metabolic risk, comprising lower risk of hypertension and type 2 diabetes mellitus[4,5]. However, few studies have inspected the connection between the habit of eating breakfast and cardio-metabolic syndrome among university going adults. In 2014, Yoo et al. reported that eating a dairy- cereal breakfast or a high energy and fiber breakfast was linked with a decreased risk of metabolic syndrome, although they did not find a substantial association among breakfast eating and hypertension[6].

Skipping breakfast can have unhealthy consequence on quality of diet and enhance in metabolic ailment risks. People who took breakfast rarely, have deprived nutritive values throughout the day. They get most of their energy through unhealthy food source (fat containing foods) which can lead to different metabolic syndrome[7]. Every day diet quality greatly reflects the type of breakfast eaten. If breakfast comprises mostly of cereals can be linked with reduced fats and higher concentration of carbohydrates, proteins and fibers. And if anyone skipped his all breakfast frequently displayed J-shaped association with enhancement in BMI. Skipping breakfast because of late night dinner can consequences in substandard diet quality and customary skipping can lead to metabolic disorder[8].

We hypothesized that there is an association between hypertension and the habit of breakfast eating, Current study was planned to assess the effects of skipping breakfast on risk of hypertension and LDL level. The objective of this study was to determine the relationship of skipping breakfast in Pakistani population on various metabolic measures especially hypertension as there is lot of variance in diet quality and eating pattern.

II. Study Population and Design

Two hundred students of University of Veterinary and Animal Sciences Lahore Pakistan from diverse departments and their acquaintances were involved in this study. The current study was a cross-sectional study. Data were directly collected by interviews and study staff performed standardized physical examinations[9]. Nutritional status, including dietary information and medical history, was measured using a 24-hour recall method. Regular exercise was indicated as "yes" when the subject exercised for more than 20 minutes at a time, more than three times per week. We disqualified subjects for the following reasons: incomplete answers about medical and social histories, or missing waist circumference, height, weight, blood

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pressure, or laboratory marker data, or age < 20 years. The participants were between 20 and 25 years of age, were not taking any medicines recurrently and did not have any family history of hypertension, diabetes and heart attack (Table 1). All participants provided written informed consent, and the data provided were anonymous. Ethical standards of the Helsinki declaration were followed to conduct study.

III. Measurements

Blood pressure, anthropometric measurements and biochemical analysis were carried out at University of Veterinary and Animal Sciences Lahore lab. After eight hours fasting blood samples were collected. Biochemistry analyzer (Micro lab 300, Merck; Darmstadt, Germany) was used to determine serum LDL-C and liver enzyme profile along with serum fasting glucose. Sphygmomanometer was used to measure systolic and diastolic blood pressure. Height and height were analyzed by using extensometer. At the tapered portion of torso (i.e., a location amid the hipbone crest and lower rib) waist circumference was calculated twice. Measurement of body weight and height (nearest to 0.01kg and 0.1cm respectively) was done in fasting condition. Body weight was divided by height square to determine BMI.

IV. Estimation of Eating Breakfast

a) Breakfast Definition and Assessment

Breakfast was defined as the meal consumed in the morning, and any beverages or food taken in the morning were categorized as breakfast. Participants were alienated into three subgroups deliberating to the occurrence of breakfast skipping over the three-days dietary intake data collecting period. Group one encompassed subjects who skipped breakfast on two or more of the three days were characterized as a rare breakfast eater as breakfast was skipped on more than 50% of the days assessed. Group 2 were encompassing subjects who skipped breakfast on one of the three days were placed in the often breakfast eater group, and group 3 comprise those who did not skip breakfast on any of the three days of the test period and characterized in the regular breakfast eater group. Similar trials have been conducted in some other researches and provided base line for present research[10].

V. Statistics

The Statistical Package for the Social Sciences (SPSS) version 21.0.0 (SPSS Inc., IBM) was used to examine the data. Variations in continuous variables like laboratory biomarkers, blood pressure and age were determined by the students t-test. Adjusted odds ratios of the habit of breakfast eating were analyzed by using multiple logistic regression analysis for hypertension. All statistical outcomes with a p value of less than 0.05 were reflected as statistically significant. The values were demonstrated as Mean ± standard deviation.

VI. Results

a) Patient Characteristics

The demographic and clinical characteristics of the patients were classified into three groups according to the habit of skipping breakfast, as shown in Table 1. Out of the two hundred studied participants one hundred nine (51.9%) were male and most of them fall in the class of rare breakfast eaters (68%). Frequency of male in regular (56%) and often (44%) breakfast eaters were almost equivalent to women. It was also noted that young males are regular breakfast eaters as related to other groups. While, rate of females was higher in the often (58%) breakfast eaters. Distribution of females in regular (31%) and rare (10) breakfast eaters was less as related to the male (Figure 1). There was no significant variance in engagement in regular exercise between the breakfast eating group and the not breakfast eating group (Table 1).

b) Prevalence of Hypertension According to Habit of Skipping Breakfast

The crude odds ratio of skipping breakfast for the prevalence of hypertension was 0.366, which specified a negative linkage among hypertension and breakfast eating. Though, after adjusting for confounding factors, such as age and sex, the odds ratio (OR) reversed (Table 2; OR = 1.125; 95% confidence interval [CI] = 1.121_1.129; p-value < 0.001). Furthermore, after amending for all measured confounding factors (regular exercise, sex, current smoking, age, systolic and diastolic blood pressure, red blood cell counts, body mass index, and waist circumference), breakfast skipping was connected with a higher risk of hypertension (Table 2; OR = 1.065; 95% CI = 1.057_1.073; p-value < 0.001).

VII. Discussion

In the present study, we found that the habit of eating breakfast was independently linked with a reduced risk of hypertension. To the best of our knowledge, this is the first university going adults-based study in Pakistan investigating the association between hypertension and breakfast. Few studies acknowledged a relationship between eating breakfast and the occurrence of some ailments. Smith et al. stated that breakfast skipping for long time may be linked with cardio-metabolic health[11]. Similarly, another researcher exposed that the habit of breakfast intake regularly contributed to the preclusion of weight gain, unlike breakfast skipping[12]. Moreover, Yoo et al. showed that breakfast intake on regular basis was linked with a decreased risk of metabolic syndrome in a Korean

population when categorizing the Korean breakfast into two kinds, a customary Korean breakfast pattern a dairy-cereal breakfast pattern. However, outcomes of their trial did not show an association between breakfast intake and the prevalence of hypertension[6]. In our study the level of LDL reduced in group of regular breakfast eaters as related to other groups. Similarly, Smith et al. showed that the higher LDL and total cholesterol values were observed in breakfast skipping group[11]. These outcomes might be due to a higher consumption of saturated fat in the breakfast skipping group. Another potential description is a higher insulin stimulus of hydroxyl methyl glutaryl Co-A (HMG-CoA) reductase. Compared with university going adults who take breakfast regularly, those who skipped breakfast had higher concentrations of fasting insulin and, thus, might have higher HMG-CoA reductase[11]. Through these conceivable mechanisms, breakfast skipping might persuade higher LDL cholesterol and, consequently, atherosclerosis. Likewise, latest studies showed that breakfast skipping clusters were linked with risk factors of hypertension, such as reduced levels of physical activity and smoking[13].

The present study was the first evidence based study in Pakistan among university going adults to observe the association between the habit of breakfast eating and the occurrence of hypertension. Our research included approximately 200 participants, so it might reflect considerable number of Pakistani university going adults. Our study has several limitations. First, since the current study was cross-sectional rather than longitudinal, a causal association among breakfast eating and hypertension could not be conclusively established. Moreover, we did not evaluate all confounding aspects, though we endeavored to embrace as many as possible, including, medication, exercise, smoking, age and alcohol consumption.

Conclusively, the habit of breakfast eating was linked with a reduced risk of hypertension between Pakistani university going adults. Further large-scale prospective experiments are required to confirm the possible effect of regular breakfast intake on hypertension and identify the physiologic mechanisms underlying this association.

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Table 1: Baseline Characteristics of Subjects According to the Breakfast Skipping Habit

Subjects	Regular	Often	Rare
Age	27.70 ± 7.5	26.04 ± 8.7	27.03 ± 6.4
Male / Female	64 / 54	23 / 27	22 / 10
SBP (mmHg)	120.1 ± 2.1	121.1 ± 2.0	119.1 ± 2.1
DBP (mmHg)	74.6 ± 1.4	75.6 ± 1.5	73.6 ± 1.6
Height (cm)	160 ± 7.4	159 ± 7.8	160. ± 7.2
Weight (kg)	58 ± 9.3	60.9.6	59.9.4
BMI kg/m2	22 ± 2.6	23 ± 2.3	22 ± 2.4
WC (cm)	82 ± 9.4	81 ± 9.6	79 ± 9.3
RBC (Mil/uL)	4.74 ± 0.49	4.72 ± 0.51	4.69 ± 0.48
Hemoglobin (g/dL)	14 ± 1.38	14 ± 1.42	13.98 ± 1.54
FBS	98 ± 21.4	101 ± 19.8	99 ± 20.6

All values are means ± SD. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, WC: Waist circumference, RBC: Red blood cell. FBS: Fasting blood sugar.

Table 2: Multivariate Logistic Regression Models of Skipping Breakfast for Hypertension

	Odds Ratio	95% CI Interval	p Value
Model 1*	0.354	0.353 - 0.356	< 0.001
Model 2**	1.121	1.117 - 1.126	< 0.001
Model 3***	1.045	1.036 - 1.053	< 0.001

**Crude odds ratio of skipping breakfast for hypertension. **Adjusted by Model 1+, sex, age and hypercholesterolemia. ***Adjusted by Model 2+ systolic blood pressure, diastolic blood pressure, regular exercise, body mass index, waist circumference, current smoking CI: confidence interval.*

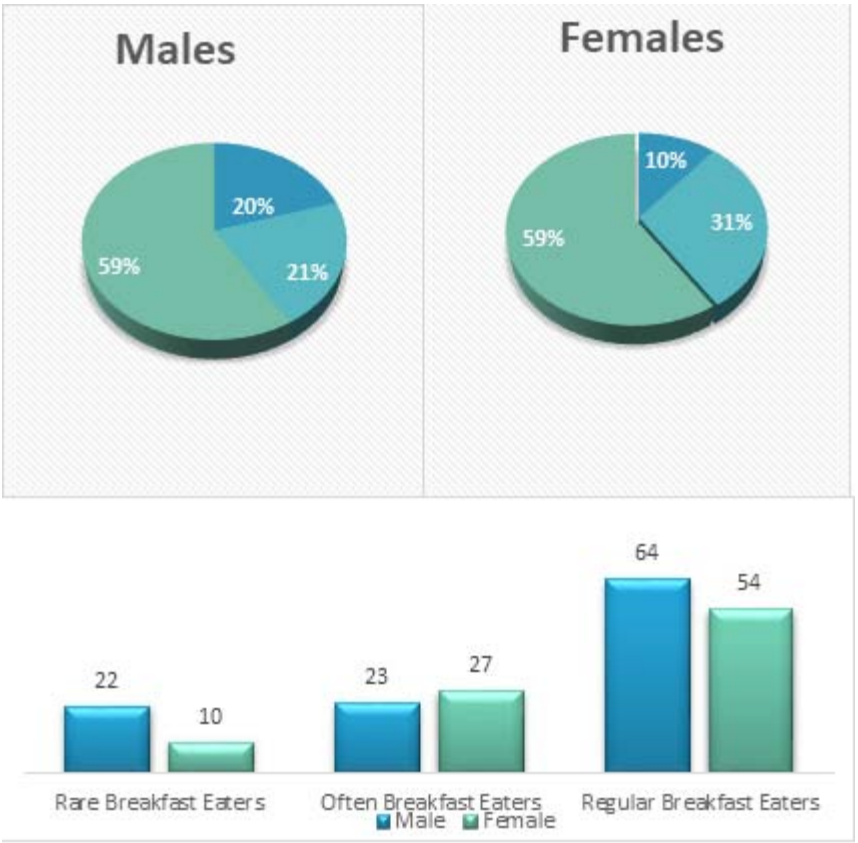

Fig. 1: Males and Females Breakfast Eating Distribution and Trends

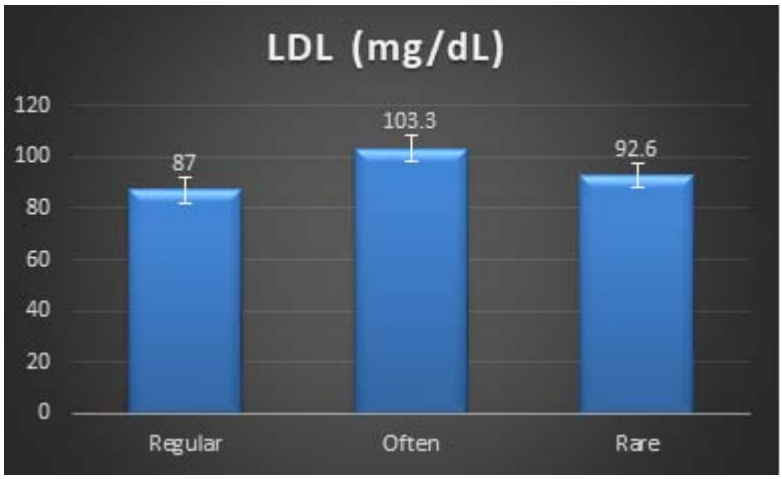

Fig. 2: Average LDL of Different Breakfast Frequency Groups

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Evaluation of Consciousness Energy Healing Treated Pyridoxine (Vitamin B_6)

By Dahryn Trivedi, Mahendra Kumar Trivedi, Alice Branton, Gopal Nayak & Snehasis Jana

Trivedi Global, Inc. and Trivedi Science Research Laboratory Pvt. Ltd.

Abstract- The aim of the research work to evaluate the impact of the Consciousness Energy Treatment on the physicochemical and thermal properties of pyridoxine using the modern analytical technique. The sample was divided into control and treated part. Only the treated sample was received the Trivedi Effect®-Consciousness Energy Treatment remotely by a renowned Biofield Energy Healer, Dahryn Trivedi. The particle sizes were significantly decreased by 17% (d_{10}), 6.2% (d_{50}), 0.54% (d_{90}), and 0.3% {D(4,3)}; thus, the specific surface area was significantly increased by 13.72% in the treated pyridoxine compared with the control sample. The PXRD peak intensities and crystallite sizes were significantly altered ranging from -40.45% to 154.76% and -57% to 51.33%, respectively; however, the average crystallite size was decreased by 6.52% in the treated sample compared with the control sample. The heat requires to melt (fusion) the treated pyridoxine was increased by 3.82% compared with the control sample. The total weight loss was significantly decreased by 5.14%; however, the residue amount was significantly increased by 8.34% in the treated sample compared with the control sample.

Keywords: pyridoxine, the trivedi effect®, consciousness energy healing treatment, particle size, surface area, PXRD, DSC, TGA/DTG.

GJMR-L Classification: NLMC Code: QU 145

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Dahryn Trivedi [α], Mahendra Kumar Trivedi [σ], Alice Branton [ρ], Gopal Nayak [ω] & Snehasis Jana [¥]

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Keywords: pyridoxine, the trivedi effect®, consciousness energy healing treatment, particle size, surface area, PXRD, DSC, TGA/DTG.

I. Introduction

Pyridoxine is a water-soluble vitamin also known as vitamin B_6. The active form of vitamin B_6 is pyridoxal 5′-phosphate, which functions as a coenzyme in many enzyme reactions in the proteins, lipids, carbohydrates metabolism, synthesis of neurotransmitters, and steroid hormones function[1]. Along with this, it also plays a significant role in the normal function of the nervous system, endocrine system, immune system, red blood cell, and also maintain the blood glucose level in the body[1-3]. Pyridoxine can be inter-converted into pyridoxal (aldehyde) and pyridoxamine (amine) form. Pyridoxine, pyridoxamine, and their phosphorylated forms are the major forms of vitamin B_6 in plant food, while pyridoxal and pyridoxal-5'-phosphate are obtained from animal food. The hydrochloride salt of vitamin B_6 is pyridoxine HCl[4]. Vitamin B_6 is commonly used as vitamin supplement or as a component of multivitamin preparations to prevent and treat the vitamin B_6 deficiency, sideroblastic anaemia, pyridoxine dependency seizures, metabolic disorders, Alzheimer's disease, pulmonary tuberculosis, cardiovascular disease, hyperhomocysteinemia, anxiety, asthma, attention deficit hyperactivity disorder, cancer, depression, dysmenorrhoea, diabetes, post-partum lactation suppression, McArdle's disease, osteoporosis, etc.[2,5-8]. Vitamin B_6 is light sensitive and degrades slowly when exposed to light. It is soluble in water and alcohol; sparingly soluble in acetone; insoluble in ether and chloroform. When it heated to decomposition, it emits very toxic fumes of oxides of nitrogen and hydrogen chloride[9].

Physicochemical properties of the pharmaceutical or nutraceutical compounds play a verycrucial role in its dissolution, absorption, bioavailability, and therapeutic profile in the body[10]. In this regards, the Trivedi Effect®-Consciousness Energy Healing Treatment has a significant impact altering the physicochemical properties such as crystal structure, particle size, surface area, thermal behaviour, and bioavailability profile of pharmaceutical and nutraceutical compounds[11-14]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which an expert can harness this inherently intelligent energy from the Universe and transmit it anywhere on the planet through the possible mediation of neutrinos[15]. The Biofield is a unique, infinite, and para-dimensional electromagnetic energy field exists surrounding the body of every living organism, which is generated from the continuous moment of charged particles (i.e., ions, cells, blood flow, etc.) inside the body. The Biofield based Energy Healing Therapies have significant beneficial outcomes against various disease conditions[16]. The National Institutes of Health/National Center for Complementary and Alternative Medicine (NIH/NCCAM) recommend and included the Energy therapy under Complementary and Alternative Medicine (CAM) category along with other therapies, i.e., homeopathy, naturopathy, Ayurvedic medicine, acupuncture, acupressure, Qi Gong, Tai Chi, Reiki, healing touch, Rolfing, hypnotherapy, etc., which have been accepted by most of the USA people[17,18].The Trivedi Effect®-Consciousness Energy Healing Treatment also has the significant impact on the properties of metals, ceramics, and polymers, organic compounds, microorganisms, cancer cell, and improve

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the overall productivity of crops[19-28]. All these outstanding results inspired the authors to evaluate the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on the behavioural, physicochemical, and thermal properties of pyridoxine HCl using powder particle size analysis (PSA), X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA)/differential thermogravimetric analysis (DTG).

II. Materials and Methods

a) Chemicals and Reagents

Pyridoxine HCl was purchased from Tokyo Chemical Industry Co. Ltd., Japan. All other chemicals utilized in the experiments were of analytical grade available in India.

b) Consciousness Energy Healing Treatment Strategies

The pyridoxine powder sample was divided into two equal parts. One part of pyridoxine was treated with the Trivedi Effect®-Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes by the renowned Biofield Energy Healer, Dahryn Trivedi, USA, and known as a treated sample. However, the second part of the test sample did not receive the Biofield Energy Treatment and termed as a control sample. The control sample later received the treatment from a "sham" healer, whereas the "sham" healer was ignorant about the Biofield Energy Treatment. After the treatment, the Biofield Energy Treated and untreated samples were kept in sealed conditions and characterized using PSA, PXRD, DSC, and TGA analytical techniques.

c) Characterization

The PSA, PXRD, DSC, and TGA analysis of pyridoxine were performed. The PSA was performed using Malvern Master sizer 2000, from the UK with a detection range between 0.01 μm to 3000 μm using the wet method[28,29]. The PXRD analysis of pyridoxine powder sample was performed with the help of Rigaku MiniFlex-II Desktop X-ray diffractometer (Japan)[31,32]. The average size of crystallites was calculated from PXRD data using the Scherrer's formula (1):

$$G = k\lambda/\beta\cos\theta \quad (1)$$

Where G is the crystallite size in nm, k is the equipment constant (0.94), λ is the radiation wavelength (0.154056 nm for Kα1 emission), β is the full-width at half maximum, and θ is the Bragg angle[33].

Similarly, the DSC analysis of pyridoxine was performed with the help of DSC Q200, TA instruments[28,29]. The TGA/DTG thermograms of pyridoxine were obtained with the help of TGA Q50TA instruments[28,29].

The % change in particle size, specific surface area (SSA), peak intensity, crystallite size, melting point, latent heat, weight loss and the maximum thermal degradation temperature (T_{max}) of the Biofield Energy Treated sample was calculated compared with the control sample using the following equation 2:

$$\%\ \text{change} = \frac{[\text{Treated} - \text{Control}]}{\text{Control}} \times 100 \quad (2)$$

III. Results and Discussion

a) Particle Size Analysis (PSA)

The particle size analysis of both the control and the Biofield Energy Treated pyridoxine powder samples were performed, and the data are presented in Table 1. The particle sizes in the Biofield Energy Treated sample were significantly decreased at d_{10}, d_{50}, d_{90}, and D(4,3) by 17%, 6.2%, 0.54%, and 0.3% respectively compared to the control sample. However, the particle size value in the Biofield Energy Treated pyridoxine was significantly increased at by compared to the control sample. The SSA of the Biofield Energy Treated pyridoxine (0.456 m^2/g) was significantly increased by 13.72% compared to the control sample (0.401 m^2/g). From the results, it can be assumed that the Biofield Energy Healing Treatment acting as an external force for breaking the larger particles to smaller one in size, hence increased the surface area. The particle size, shape, and surface area have their impact on the solubility, dissolution rate, absorption, bioavailability, and therapeutic efficacy of a drug[34,35]. Thus, the Biofield Energy Treated pyridoxine would assume to enhance the therapeutic efficacy of the nutraceutical and pharmaceutical formulations.

Table 1: Particle Size Distribution of the Control and Biofield Energy Treated Pyridoxine

Parameter	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	D(4,3) (μm)	SSA (m^2/g)
Control	8.22	40.38	138.77	60.44	0.401
Biofield Treated	6.82	37.88	138.02	60.26	0.456
Percent Change* (%)	-17.00	-6.20	-0.54	-0.30	13.72

*d_{10}, d_{50}, and d_{90}: particle diameter corresponding to 10%, 50%, and 90% of the cumulative distribution, D(4,3): the average mass-volume diameter, and SSA: the specific surface area. *denotes the percentage change in the Particle size distribution of the treated sample with respect to the control sample.*

b) Powder X-ray Diffraction (PXRD) Analysis

The PXRD diffractograms of the control and treated pyridoxine showed sharp and intense (Figure 1) indicated that both the samples were crystalline. The control and Biofield Energy Treated pyridoxine powder samples showed the highest peak intensity at 2θ equal

to 25° (Table 2, entry 7). The peak intensities of the Biofield Energy Treated pyridoxine sample were significantly altered ranging from -40.45% to 154.76% compared to the control sample.

Table 2: PXRD Data for the Control and the Treated Pyridoxine

Entry No.	Bragg Angle (°2θ)		Peak Intensity (%)			Crystallite Size (G, nm)		
	Control	Treated	Control	Treated	% Change [a]	Control	Treated	% Change [b]
1	10.28	10.33	157.00	129.00	-17.83	344.00	307.00	-10.76
2	15.49	15.56	22.00	20.00	-9.09	362.00	443.00	22.38
3	16.86	16.93	65.00	57.00	-12.31	353.00	301.00	-14.73
4	18.61	18.65	39.00	30.00	-23.08	393.00	444.00	12.98
5	20.72	20.75	962.00	899.00	-6.55	413.00	440.00	6.54
6	24.10	24.09	63.00	94.00	49.21	386.00	498.00	29.02
7	25.00	25.04	1301.00	1292.00	-0.69	429.00	437.00	1.86
8	25.77	25.78	117.00	110.00	-5.98	306.00	317.00	3.59
9	27.77	27.81	1025.00	1075.00	4.88	431.00	422.00	-2.09
10	28.86	28.91	125.00	121.00	-3.20	149.00	143.00	-4.03
11	31.34	31.44	25.00	16.00	-36.00	529.00	277.00	-47.64
12	33.63	33.71	42.00	107.00	154.76	380.00	223.00	-41.32
13	37.09	37.20	89.00	53.00	-40.45	261.00	338.00	29.50
14	37.63	37.74	28.00	51.00	82.14	393.00	169.00	-57.00
15	38.55	38.49	42.00	39.00	-7.14	134.00	78.00	-41.79
16	47.32	47.36	41.00	44.00	7.32	150.00	227.00	51.33
17	53.26	53.30	137.00	160.00	16.79	259.00	238.00	-8.11
18	Average Crystallite Size					333.65	311.88	-6.52

[a] denotes the percentage change in the peak intensity of the Biofield Energy Treated sample with respect to the control sample; [b] denotes the percentage change in the crystallite size of the Biofield Energy Treated sample with respect to the control sample.

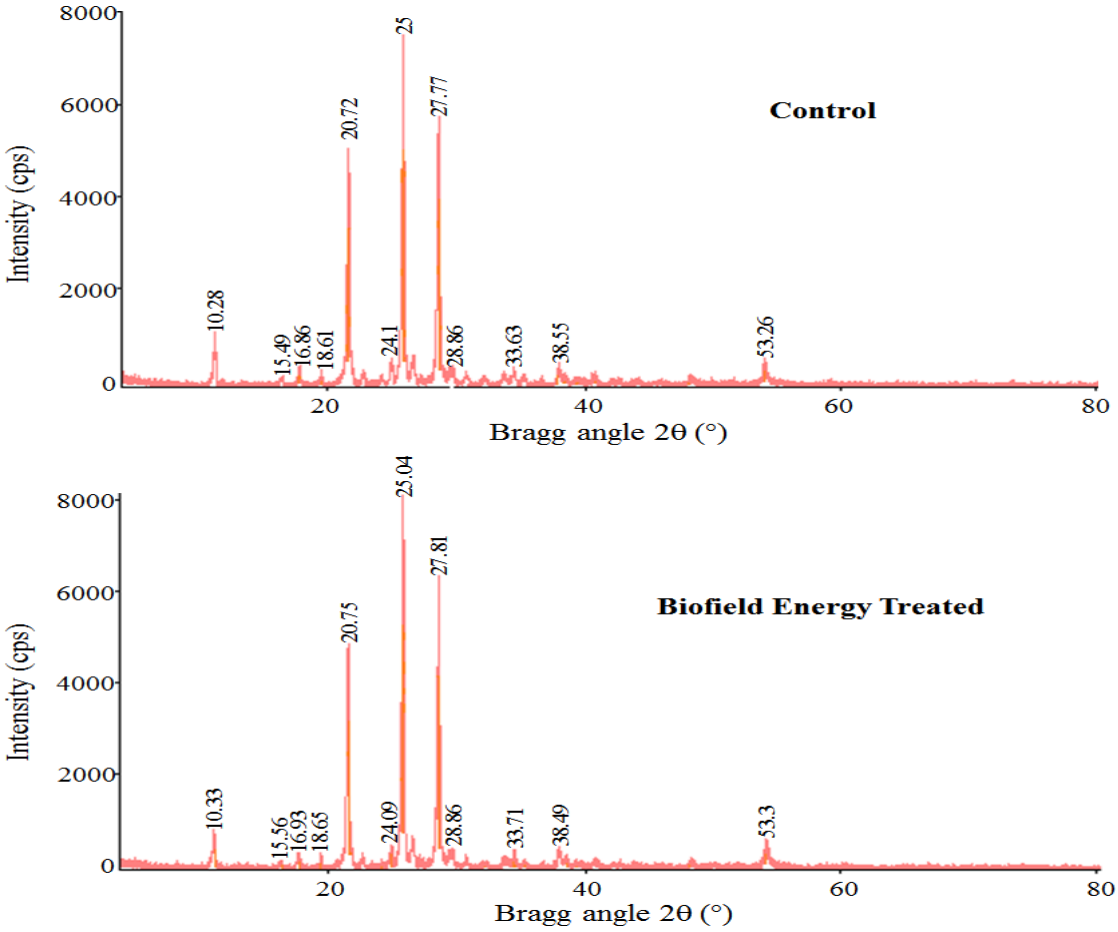


Fig. 1: PXRD Diffractograms of the Control and Biofield Energy Treated Pyridoxine

Similarly, the crystallite sizes of the Biofield Energy Treated pyridoxine powder sample were significantly altered ranging from -57% to 51.33% compared to the control sample. Overall, the average crystallite size of the Biofield Energy Treated pyridoxine powder sample (311.88 nm) was significantly decreased by 6.52% compared with the control sample (333.65 nm).

The peak intensities and crystallite sizes of the Biofield Energy Treated pyridoxine powder samples were significantly altered compared to the control sample. The peak intensity of each diffraction face of a compound changes according to the change in the crystal morphology[36] and alterations in the PXRD pattern provide the proof of polymorphic transitions[37,38]. The Biofield Energy Healing Treatment probably produced the new polymorphic form of pyridoxine through the Biofield Energy *via* neutrino oscillations[14]. Different polymorphic forms of the pharmaceuticals have significant effects on the drug performance, because of their different thermodynamic and physicochemical properties from the original one[39,40]. Therefore, it is assumed that the Trivedi Effect®-Consciousness Energy Healing Treated pyridoxine would be better in designing novel pharmaceutical formulations for more drug performance.

c) Differential Scanning Calorimetry (DSC) Analysis

The DSC thermograms of both the control and Biofield Energy Treated pyridoxine samples showed a very sharp endothermic peak at 215.42°C and 215.2°C, respectively (Figure 2).The experimental results closely matched to the reported data[41]. The melting point of the Biofield Energy Treated pyridoxine did not alter much compared to the control sample (Table 3).

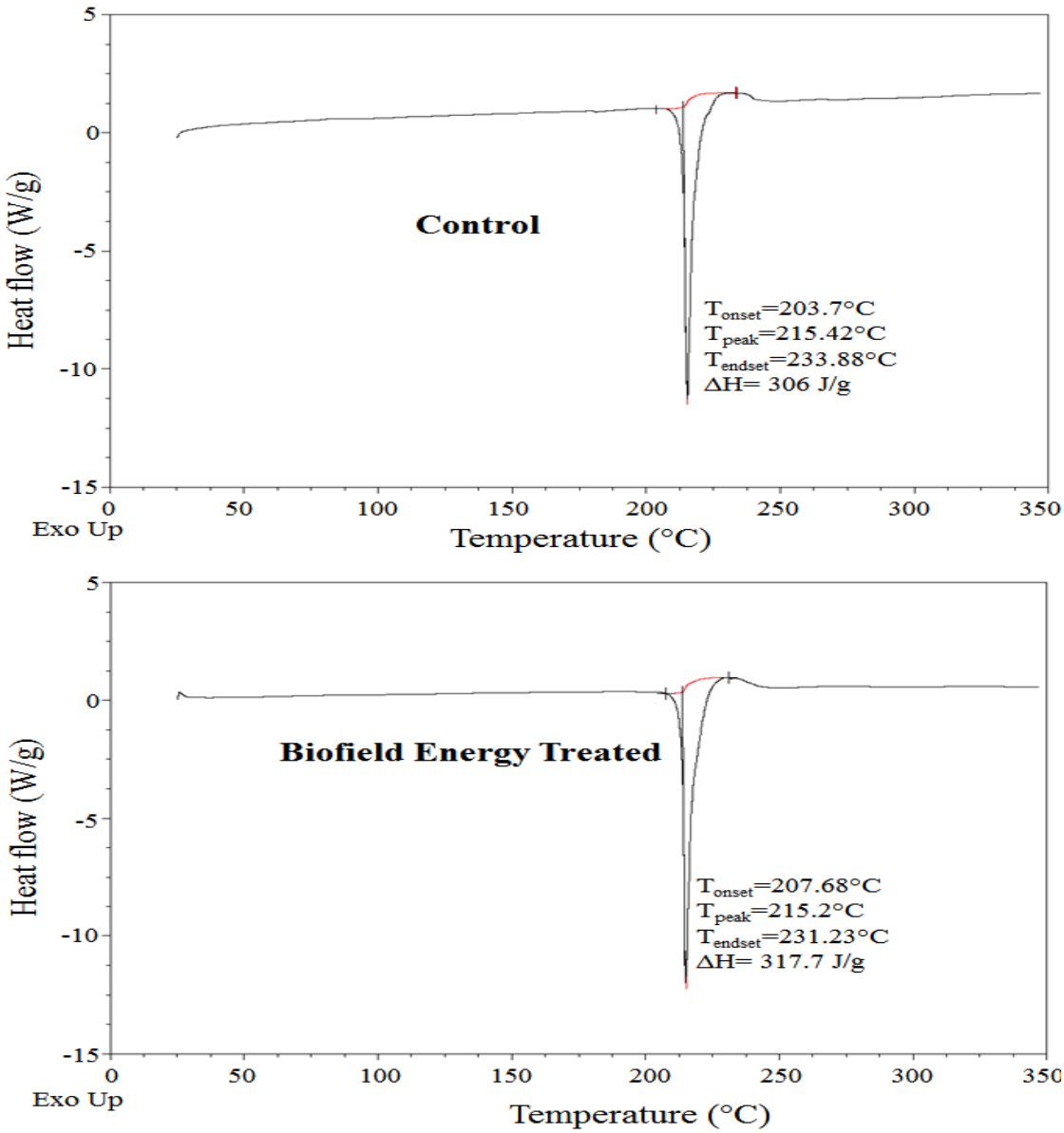


Fig. 2: DSC Thermograms of the Control and Biofield Energy Treated Pyridoxine

Table 3: DSC Data for Both Control and Biofield Energy Treated Samples of Pyridoxine

Sample	Melting Temp (°C)	ΔH_{Fusion} (J/g)
Control Sample	215.42	306.00
Biofield Energy Treated	215.20	317.70
% Change*	-0.10	3.82

*ΔH: Latent heat of fusion, *denotes the percentage change of the Biofield Energy Treated pyridoxine with respect to the control sample.*

The latent heat of fusion (ΔH_{fusion}) of the Biofield Energy Treated sample (317.7 J/g) was increased by 3.82% compared with the control sample (306 J/g) (Table 3). Any change in the latent heat of fusion can be attributed to the disrupted molecular chains and the crystal structure of that compound[42]. Therefore, the Trivedi Effect® - Consciousness Energy Healing Treatment assumed to have a significant impact on the molecular chains and crystal structure of the treated pyridoxine responsible for the elevation of thermal stability compared to the control sample.

d) Thermal Gravimetric Analysis (TGA)/ Differential Thermogravimetric Analysis (DTG)

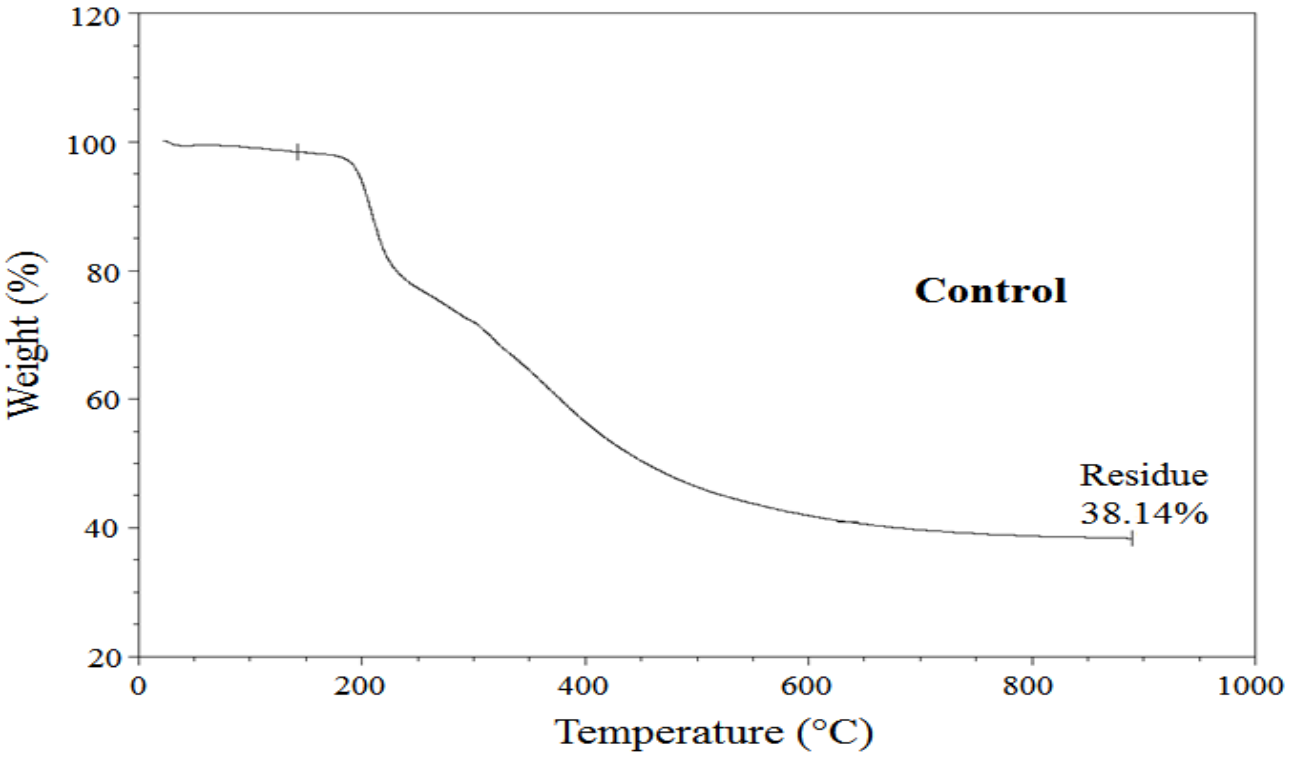
The TGA thermograms of both the control and Biofield Energy Treated pyridoxine samples showed two steps of thermal degradation (Figure 3). The total weight loss of the Biofield Energy Treated sample was significantly decreased by 5.14% compared to the control sample. Thus, the residue amount was significantly increased by 8.34% in the Biofield Energy Treated pyridoxine compared to the control sample (Table 4).

Table 4: TGA/DTG Data of the Control and Biofield Energy Treated Samples of Pyridoxine

Sample	TGA		DTG; T_{max} (°C)	
	Total Weight Loss (%)	Residue %	1st Peak	2nd Peak
Control	61.86	38.14	208.75	381.08
Biofield Energy Treated	58.68	41.32	214.94	373.16
% Change*	-5.14	8.34	2.97	-2.08

** denotes the percentage change of the Biofield Energy Treated sample with respect to the control sample, T_{max} = the temperature at which maximum weight loss takes place in TG or peak temperature in DTG.*

The DTG thermograms of the control and Biofield Energy Treated pyridoxine also reported two peaks in the thermograms (Figure 4). The T_{max} of the 1st peak of the Biofield Energy Treated sample was increased by 2.97%, whereas T_{max} of 2nd peak was decreased by 2.08% compared to the control sample (Table 4). Overall, TGA/DTG analysis revealed that the thermal stability of the Biofield Energy Treated sample was significantly increased compared with the control sample.



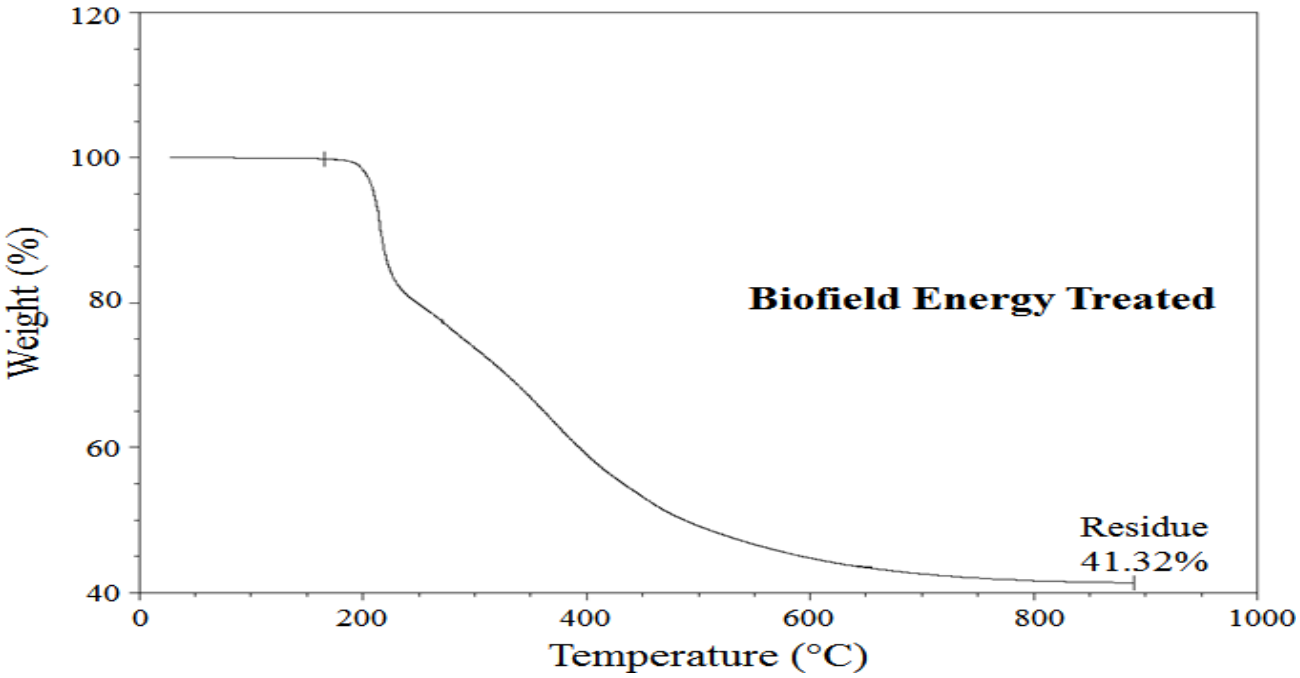


Fig. 3: TGA Thermograms of the Control and Biofield Energy Treated Pyridoxine

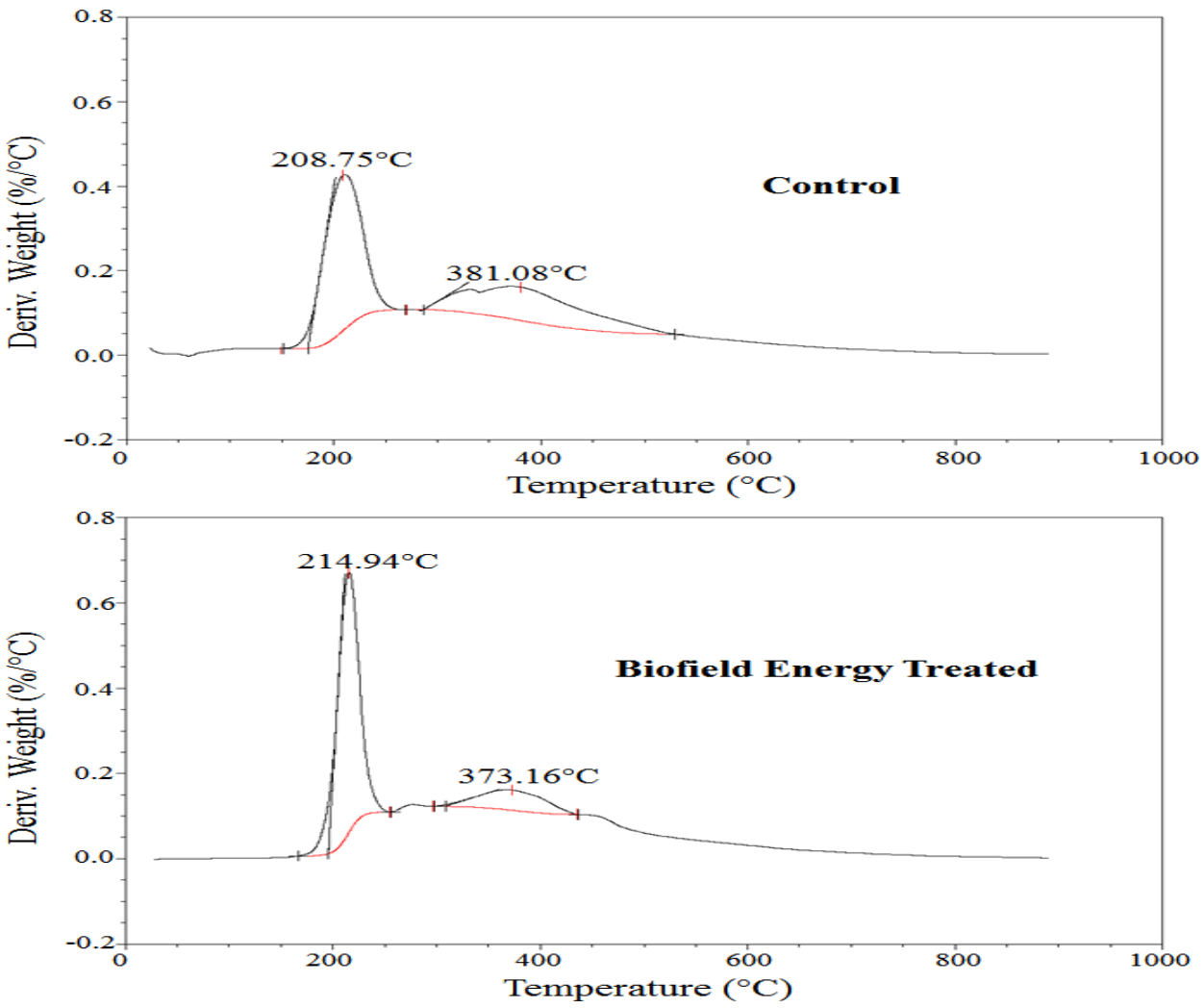


Fig. 4: DTG Thermograms of the Control and Biofield Energy Treated Pyridoxine

IV. Conclusions

The Trivedi Effect®-Consciousness Energy Healing Treatment has shown a significant effect on the crystallite size, particle size, SSA, and thermal behavior of pyridoxine. The particle sizes in the Biofield Energy Treated powder sample was significantly decreased by 17%, 6.2%, 0.54%, and 0.3% at d_{10}, d_{50}, d_{90}, and D(4,3) respectively, compared to the control sample. Therefore, the SSA of the Biofield Energy Treated sample was significantly increased by 13.72% compared to the control sample. The PXRD peak intensities and crystallite sizes of the treated pyridoxine were significantly altered ranging from -40.45% to 154.76% and -57% to 51.33%, respectively compared to the control sample. The average crystallite size of the Biofield Energy Treated pyridoxine was significantly decreased by 6.52% compared with the control sample. The ΔH_{fusion} was increased by 3.82% in the Biofield Energy Treated sample compared with the control sample. The total weight loss was significantly decreased by 5.14%; hence the residue amount was significantly increased by 8.34% in the Biofield Energy Treated sample compared with the control sample. From the results, it is concluded that the Trivedi Effect®-Consciousness Energy Healing Treatment might have generated a new polymorphic form of pyridoxine which may offer better solubility, bioavailability, and therapeutic efficacy compared with the control sample. The Trivedi Effect®-Consciousness Energy Healing Treated pyridoxine hydrochloride would be very useful to design novel pharmaceutical and nutraceutical formulations that may offer better therapeutic response against vitamin B_6 deficiency, pyridoxine-dependency seizures, sideroblastic anemia, Alzheimer's disease, metabolic disorders, diabetes, pulmonary tuberculosis, cardiovascular disease, hyperhomocysteinemia, cancer, anxiety, asthma, depression, attention deficit hyperactivity disorder (ADHD), dysmenorrhoea, post-partum lactation suppression, McArdle's disease, osteoporosis, etc.

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Abbreviations

DSC: Differential scanning calorimetry,
FWHM: Full width half maximum.
G: Crystallite size.
T_{onset}: Onset decomposition temperature.
T_{peak}: Peak decomposition temperature.
T_{endset}: Endset decomposition temperature.
ΔH: Enthalpy of fusion/decomposition.
PSA: Particle size distribution analysis.
PXRD: Powder X-ray diffraction.
TGA: Thermal gravimetric analysis.
SSA: Specific surface area.
DTG: Differential thermogravimetric analysis.
NIH: National institutes of health.
NCCAM: National center for complementary and alternative medicine.
CAM: Complementary and alternative medicine.

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Rediscovery of Couscous in the World

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Abstract- World food industry is changing by re-discovering traditional food tastes and techniques. Due to increase in curiosity on gastronomy, new tastes and "Geographical Indication-food origination", local foods are started to re-designing by food industry. Couscous is one of them, which is the world-wide known traditional cereal product, today. Its popularity has been increasing, recently. Especially, in the Western market, it is prepared due to its taste, rapid preparation and usage in salads (tabbouleh). There are three couscous types such as Turkish, African and pasta-like depending on the formulation, processing technique and usage. Pasta-like couscous is widely produced by pasta companies by using same pasta production line by changing the die of press. Couscous commonly produced by using semolina and sorghum in Africa and Asia, however in Turkey, traditional Turkish couscous is generally prepared by coating of bulgur granules with semolina, wheat flour; egg and water or milk. In the literature, studies have recently been made for enrichment of couscous by either substitution of semolina with legume flours and other grain flours or adding nutritious ingredients to the composition. Worldwide, total exportation and importation quantity of couscous are 124, 481 and 126, 799 tons in 2016, respectively. Nowadays, the need for easy prepared meals increased due to the fast lifestyles and people are more aware about the importance of nutritionally valued products and its benefits to health.

Keywords: *couscous, pasta-like, african, enriched.*

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Rediscovery of Couscous in the World

Ayse Nur Yüksel [α], Mehmet Durdu Oner [σ] & Mustafa Bayram [ρ]

Abstract- World food industry is changing by re-discovering traditional food tastes and techniques. Due to increase in curiosity on gastronomy, new tastes and "Geographical Indication-food origination", local foods are started to re-designing by food industry. Couscous is one of them, which is the world-wide known traditional cereal product, today. Its popularity has been increasing, recently. Especially, in the Western market, it is prepared due to its taste, rapid preparation and usage in salads (tabbouleh). There are three couscous types such as Turkish, African and pasta-like depending on the formulation, processing technique and usage. Pasta-like couscous is widely produced by pasta companies by using same pasta production line by changing the die of press. Couscous commonly produced by using semolina and sorghum in Africa and Asia, however in Turkey, traditional Turkish couscous is generally prepared by coating of bulgur granules with semolina, wheat flour; egg and water or milk. In the literature, studies have recently been made for enrichment of couscous by either substitution of semolina with legume flours and other grain flours or adding nutritious ingredients to the composition. Worldwide, total exportation and importation quantity of couscous are 124, 481 and 126, 799 tons in 2016, respectively. Nowadays, the need for easy prepared meals increased due to the fast lifestyles and people are more aware about the importance of nutritionally valued products and its benefits to health. Therefore, further studies should be made to produce nutritionally enriched products with fast cooked property to improve the quality properties without the forgetting old tastes.

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I. Introduction

UNESCO started a new indication to preserve the tradition and taste comes from ancient. Therefore, "UNESCO-Creative City Network" based on gastronomy is a new issue to protect the traditional foods. Additionally, there is a big trend for "Geographical Indicated" of foods and other products overall the World. Each culture starts to re-discover their products. Bordeaux wine, Antep bulgur, bread, baklava and some other most popular products are some of "Geographically Signed" food products, and day by day the number of products dramatically increases.

Couscous is a world-wide known traditional cereal product, which is a staple food of North Africa (Aboubacar and Hamaker, 2000; Rahmani and Muller, 1996) and Middle East cuisines. It can be consumed as salad (tabbouleh) and side dish with chicken and meat meals, as an alternative for pilaf. Depending on the formulation, processing technique and usage, there are three couscous such as Turkish, African and short-cut pasta-like. Turkish and African couscouses are produced traditionally by hand, which are different from pasta-like. But, preference of pasta-like couscous in market is lower than the others.

Pasta-like couscous is generally produced mechanically by using pressing technology (Çelik et al., 2004). Also, pasta-like couscous is widely produced by pasta/macaroni companies in same pasta production line by changing the die of press. The basic industrial and traditional African couscous processing steps are: a) mixing and agglomeration of *Triticum durum* semolina with water, b) steaming to precook, c) drying to preserve (Aboubacar et al., 2006; Debbouz and Donnelly, 1996), d) cooling, e) grading to separate by size and f) storage or packaging (Dick and Matsuo, 1988). Wheat flour, semolina, sorghum, millet, maize (Galiba et al., 1988), bulgur flour (Yuksel et al., 2017; Yüksel et al., 2017; Yüksel et al., 2018) and barley (Kaup and Walker, 1986) can be used in the couscous production.

Couscous commonly produced by using semolina and sorghum in Africa and Asia, however in Turkey, traditional Turkish couscous is generally prepared by coating of bulgur granules with semolina, wheat flour, egg and water or milk (Demir et al., 2010). Sometimes egg and milk mixture is used to produce Turkish couscous. In order to increase the functional properties of regular couscous, consumed widely in the world, a new formulation and production technique should be developed. According to field and literature searches, it was found that Turkish type is especially special in Minor Asia. When the recipes of African and Turkish couscous are compared, it can be said that nutritional value of Turkish couscous are higher than the others due to raw materials used in the composition. In African and pasta-like couscous, water and semolina are only used. However, in Turkish couscous, milk, egg, bulgur and semolina are used. Pasta-like couscous is not preferred in the market due to its shape, size and properties. If Turkish type production is developed, it will be a good opportunity for the industry to produce couscous.

Substitution of bulgur with different flours in production of Turkish couscous was studied by Demir et al. (2010), Çelik et al. (2004) and Demir and Demir (2016). They investigated the effects of addition of chickpea flour, soy, oat, lupine, lentil and common bean flours, buckwheat and wheat germ on quality and sensory properties of couscous.

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African and pasta-like couscouses were studied by Debbouz and Donnelly (1996) whom compared home-made, commercial and extruded couscous samples. Rahmani and Muller (1996) investigated thiamin and riboflavin contents of nine couscous samples (five traditional and four commercial) during preparation. In another study, the effects of different textures and types of endosperm on the production of couscous were observed by Galiba et al. (1988). Industrial quality (manufactured in Algeria) three durum wheat semolina were used as raw materials for the agglomeration of couscous experiments by Lefkir (2017).

Different flour additions to African and pasta-like couscous were also studied. Yuksel et al. (2017) and Yuksel et al. (2017) investigated the effects of bulgur flour (undersize bulgur) addition on the quality, sensory and texture properties of couscous. The effect of different decortications levels of sorghum kernel on couscous quality was also studied by Aboubacar et al. (2006). Sidibe (1981) presented a paper in a conference about comparison of couscous yields of different varieties of sorghum grains. Couscous produced with sweet potato was studied by Kpomasse (2014). In the study of Opata (2007), fifteen varieties of water yam were used to produce fries, couscous and flour. Besides, technological feasibility to obtain gluten-free couscous based on rice - leguminous supplementation was studied by Benatallah et al. (2008).

II. Economic Value of Couscous

Pasta-like and African couscouses are very popular products in the world, where Turkish-like couscous is produced and consumed generally in Turkey, Georgia, Azerbaijan and Armenia.

According to Union of Organizations of Manufactures of Pasta Products of E.U., about 14.3 million tones pasta (including couscous) is produced worldwide in 2015 (Anonymous, 2015). In Turkey, the production of pasta quantity has been increased to 1315 thousand tons in 2015 (Anonymous, 2014) and in terms of worldwide production quantity, Turkey ranks third after Italy and United States (Anonymous, 2015). On the contrary, the consumption quantity of pasta in Turkey is lower than other countries (7.5 kg per person per year).

In terms of worldwide import quantity of couscous, Turkey ranks one hundred nineteenth between the years of 2012 to 2016. However, Turkey ranks forty-second in the list of exporters. Quantity of exported couscous decreased from 268 to 231 tons in 2016. Exportation of pasta and couscous is made from Turkey to Iraq, Japan, and United Arab Emirates etc. In Figures.1 and 2, the importer and exporter of first fifteenth countries in the world are given and the data was obtained from Trade Map (Anonymous, 2017). Worldwide, total exportation and importation quantity of couscous are 124,481 and 126,799 tons, respectively. Italy, France and Morocco exported couscous in 2016, in terms of quantity 37281, 34809 and 22113 tons, respectively. France ranks first in importers list with 31,436 tons and followed by United Kingdom, Belgium and United States of America with 16763, 8597 and 6870 tons in 2016, respectively. In the first quarter of 2017, Turkey ranks third for exported quantity of couscous after France and United States of America (Anonymous, 2017).

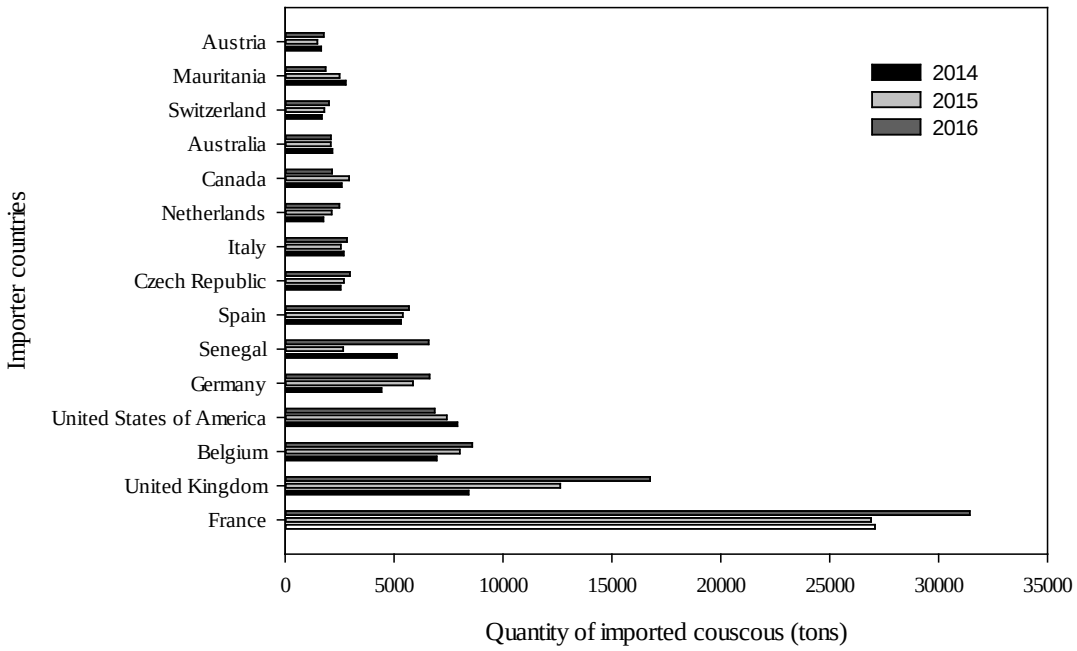

Fig. 1: Quantity of Imported Couscous by Countries

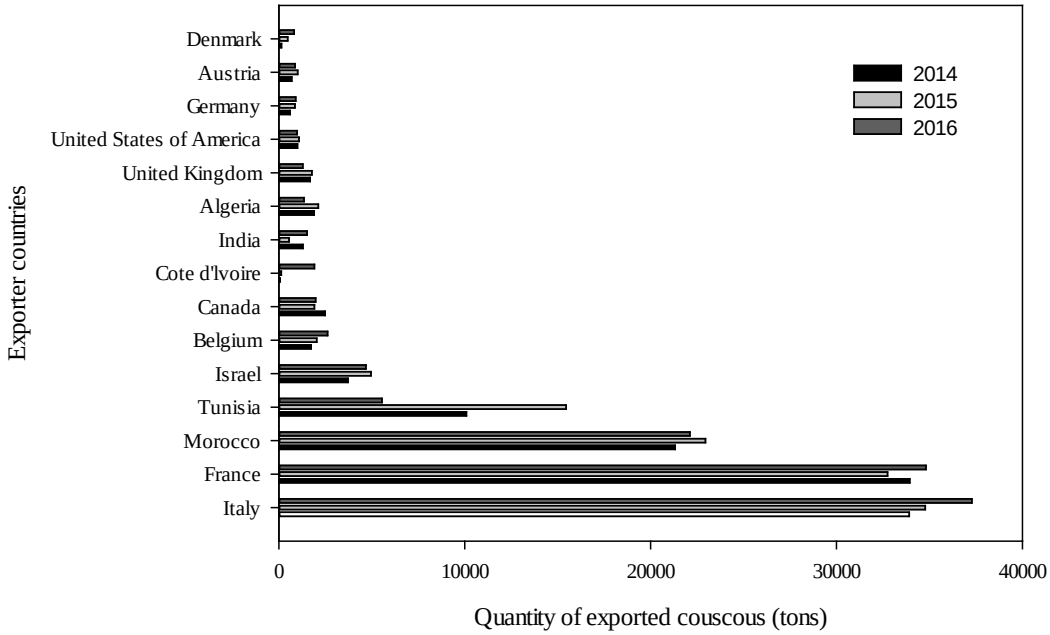

Fig. 2: Quantity of Exported Couscous by Countries

III. Nutritional Enrichment of Couscous

Wheat semolina is generally used as raw material in traditional couscous production. Durum wheat (*Triticum durum*) is the second - most widely cultivated wheat species after hard wheat (*Triticum aestivum* L.). Due to its extra-hard, translucent, light-color properties, it is mainly ground to make semolina for pasta and couscous (Gazza et al., 2011).

Ash content of durum semolina indicates the bran content. The ash in commercial durum semolina is normally ranges from 0.55 to 0.75 %. The protein content of semolina is important because it influences the functional quality of pasta. Suitable amounts of gluten protein are necessary to provide desirable attributes of mechanical strength and cooking quality of pasta. Protein level is between 11.0-13.0 % (Dick and Matsuo, 1988). According to USDA National Nutrient Database for Standard Reference (Anonymous, 2001) dry semolina couscous nutrient value is given in Table 1. Another common ingredient in production of traditional African couscous is sorghum. Protein and ash contents of sorghum are in between 8.0-11.6 % and 0.60-1.31 %, respectively (Aboubacar and Hamaker, 2000).

Table 1: Dry Couscous Nutrient Data (Anonymous, 2001)

Nutrient	Unit	Value per 100 g	1 Cup = 173.0g
Proximates			
Water	g	8.56	14.81
Energy	kcal	376	650
Protein	g	12.76	22.07
Total Lipid (Fat)	g	0.64	1.11
Carbohydrate, by Difference	g	77.43	133.95
Fiber, Total Dietary	g	5	8.7
Minerals			
Calcium, Ca	mg	24	42
Iron, Fe	mg	1.08	1.87
Magnesium, Mg	mg	44	76
Phosphorus, P	mg	170	294
Potassium, K	mg	166	287
Sodium, Na	mg	10	17
Zinc, Zn	mg	0.83	1.44
Vitamins			
Vitamin C, Total Ascorbic Acid	mg	0	0
Thiamin	mg	0.163	0.282
Riboflavin	mg	0.078	0.135
Niacin	mg	3.49	6.038
Vitamin B-6	mg	0.11	0.19
Folate, DFE	µg	20	35
Vitamin B-12	µg	0	0
Vitamin A, RAE	µg	0	0
Vitamin A, IU	IU	0	0
Vitamin D (D2 + D3)	µg	0	0
Vitamin D	IU	0	0
Lipids			
Fatty Acids, Total Saturated	g	0.117	0.202
Fatty Acids, Total Monounsaturated	g	0.089	0.154
Fatty Acids, Total Polyunsaturated	g	0.252	0.436
Cholesterol	mg	0	0

As mentioned previously, bulgur, egg and milk mixture are traditionally used as ingredients in Turkish couscous production. Bulgur is a whole grain product, which is generally produced from *Triticum durum* wheat by cleaning, cooking, drying, tempering, debraning, milling, polishing (optional) and size classification (Bayram and Öner, 2005; Bayram and Öner, 2007). Protein and ash contents of bulgur are in average 13.36 % and 1.79 %, respectively (Balcı and Bayram, 2017). It is nutritionally richer than semolina. Moreover, it has high dietary fiber content, having 18.3 g dietary fiber per 100 g. Its dietary fiber content is 3.5, 6.8, 1.8, 2.3, 1.3 and 4.3 times greater than rice, wheat flour, oatmeal, whole wheat bread, soybean and pasta, respectively (Bayram and Öner, 2007; Yıldırım et al., 2008a, 2008b). The protein contents of egg and milk are 12.17 and 3.30 g 100 g-1 (Çelik et al., 2004), respectively. In addition, they are rich in Na (sodium), K (potassium) and Ca (calcium). Turkish couscous produced with and without eggs have 11.04 and 11.27 % of protein, 0.73 and 0.79 % of ash, 3.13 and 4.16 % of dietary fiber, respectively (Çelik et al., 2004). In another study, Yüksel et al. (2017)

produced pasta-like couscous by using only bulgur flour and water. They observed higher protein and ash contents when compared to semolina couscous.

IV. Future Aspects of Couscous

In modern life, need of easy and fast prepared, precooked and ready-to-eat, or ready-to-eat with the addition of small amount of hot water foods is increased. Besides the faster preparation, people are more aware that the nutritional value of a food product should be high. Therefore, higher protein and ash contents of bulgur are highlighting the economic and possible health benefit of milk, egg and bulgur containing enriched couscous.

Further studies especially on enriched couscous are required to evaluate and improve its nutritional and sensory properties. Studies should be concentrated on the production of functional and/or gluten-free couscous, which can be a different and nutritious option for pasta or noodle.

About couscous industry and market, the production technology should also be developed. Increase in consumption of traditional couscous will force the industry to produce couscous having traditional properties. Because, pasta-like couscous do not have big interest in contrast to traditionally produced couscous. Therefore, as a recommendation; couscous in industry should be produced at high capacity by using modern technology to obtain same specification with traditional ones.

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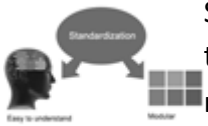

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The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

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We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

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- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

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27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

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30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

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Key points to remember:

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- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

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- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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