Original Research Article

Egg Shell and Snail Shell Powder Potentials in Sorghum Beer Clarification

ABSTRACT

Aim: Turbidity is one of the integral parameters used to ascertain beer quality and clarity. This has been achieved through a combination of filtration processes and utilization of kieselghur as filter aid. Kieselghur on the other hand is expensive and not readily available; therefore there is need to find potential filter aids from locally available materials. This research is aimed at determining the effectiveness of egg shell powder and snail shell powder in sorghum beer clarification.

Study design: This study was made to fit into using a combination of T-test and one way Analysis of Variance.

Place and Duration of Study: The research was carried out at laboratory of Department of Food Science and Technology, Federal University of Technology, Owerri, Nigeria, between May 2018 and November 2018.

Methodology: Temperature programmed infusion mashing method was used to produce wort from sorghum malt that was malted for 2 days and 3 days. The resulting Worts were fermented using *Saccharomyces carsbergensis* to obtain sorghum beer and the beer was clarified with kieselghur, egg shell powder and snail shell powder. Turbidity and other quality analyses were carried out on the sorghum wort, sorghum beer and clarified sorghum beer.

Results: The results showed that beers clarified with Kieselghur had better clarity than egg shell powder and snail shell powder clarified sorghum beers. Egg shell powder showed better clarification potentials than snail shell powder. There is a correlation between total solids and turbidity. Germination period significantly influenced the turbidity of sorghum beer.

Conclusion: This study showed that egg shell powder can serve as potential filter aid in beverage clarification.

Comment [K1]: carlsbergensis

Keywords: beer quality, egg shell, filter aid, kieselghur, sorghum beer, snail shell, turbidity

1. INTRODUCTION

Beer is an alcoholic beverage made from cereal grains especially malted barley [1]. Beer may be produced from the following principal ingredients such as; water, barley, hops and fermenting yeasts. Among several malts, barley malt is the most widely used starch source owing to its high enzyme content (which facilitates the breakdown of starch into sugars), less problem in filtration being a covered caryopsis as well as its composition which contributes to the distinct beer characteristic quality. Due to the wide use of barley malts in brewing, it has placed serious burden on the grain and as a result, high amount of revenue or expenditures are used for the importation of this raw material in this region since it is not grown in the tropics. An alternative towards solving this can be by the use of other cereal grains especially sorghum malt in place of barley malt in brewing since it is one of the prominent crops grown in the tropics.

Sorghum is a staple crop grown in Nigeria and in many African countries like Benin, Ghana, Zimbabwe, South Africa, etc [2]. It is cheap, locally available and can grow in the tropics. Sorghum has an endosperm structure similar to maize, though it gives nearly same qualities in beer but it has a problem in filtration due to its naked caryopsis, single aleurone layer and the pericarp contains more polyphenols than that of barley [3].

Comment [K2]: Arabinoxylans and beta-glucans can cause problems during filtration, also. What are their concentrations in sorghum?

However, it is worthy to note that the first visual impression of beer quality is determined by its turbidity. Beer quality and clarity on the other hand, can be improved through clarification which can be achieved by the use of certain filter aids or clarifying agents. These agents help to trap impurities and/or suspended particle to form clogs or sediment [4]. Hence, such clarifying agents among these categories include Isinglass, Kieselghur, Irish moss, Kappa carrageenan, gelatins, etc. In addition, due to more demand for these commercial filter aids, it also places more burden on foreign exchange which are used to import these raw materials especially kieselghur as they are relatively expensive and are not readily available. Hence, the need to divert some of the revenue or foreign exchange used for the importation of these clarifying agents into other useful areas has to be made. This can be actualized by finding substitutes for these commercial filter aids from some of the sources that are locally available such as egg shell powder and snail shell powder.

Egg shell and snail shell have different components aside the mineral contents which composed mainly of calcium compounds especially $CaCO_3$ [5] unlike kieselghur which is diatomaceous earth that contains mainly silica from the remains of diatoms. Calcium compounds also helps to precipitate the suspended particles in the solution making them to form lumps and acquire more density which enable them to settle under gravity, thus reducing turbidity. Hence, the use of some of these locally available filter aids in brewing may yield products of desired qualities that can be comparable to those obtained from the use of commercial filter aids, while reducing the overall cost of importation of other commercial filter aids. Therefore the objective of this research is to evaluate the potentials of utilizing egg shell powder and snail shell powder in sorghum beer clarification.

2. MATERIALS AND METHODS

2.1 Source of materials

Grains of white sorghum variety (*Sorgum bicolour L. Moench*) that is less than one year old and live snails were both purchased at Nkwo Nnewi main market Nnewi, Anambra state, Nigeria. The egg shell were obtained from Fast food restaurant at Umuchima, Ihiagwa in Owerri, Imo state, Nigeria. Malted barley and brewer's yeast – *Saccharomyces carlsbergences* was obtained from PABOD Breweries in Port Harcourt, Rivers State, Nigeria. Hop pellets were obtained from Nigerian Breweries in Uyo, Akwa Ibom State, Nigeria. Kielselghur was obtained from Department of Food Science and Technology laboratory, Federal University of Technology, Owerri, and the processing of samples and experiments were carried out using the facilities available at the laboratory of Department of Food Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria.

2.2 Sample preparation

<u>2.2.1 Production of Sorghum Malt</u>

The sorghum malt was produced using the method of Kunze [6] which has similar protocols for malting of barley. The weighed and sorted sorghum grains were suspended in 10 litres of water and steeped for 18 hours at a temperature of 25-30 ^{o}C with 6 hours wet steep period and 45 minutes of air rest. The steeped grains were drained and heaped on a moistened jute bags previously sterilized with steam and allowed to germinate at 25-30 ^{o}C for 2 and 3 days. Water was being sprinkled on the grains throughout the germination period to avoid local overheating during germination. Kilning of the germinated grains after 2 and 3 days was done in a hot air oven at temperatures between 60-70 ^{o}C for about 2-3 hours. The sorghum malt was continuously stirred to aerate and achieve uniform heat distribution. The rootless were removed and winnowed (cleaned) to remove dust and other particles. The malted sorghum was milled (crushed) into different particle sizes – coarse, medium and fine grist using attrition mill.

Comment [K3]: Pay attention to the units.

<u>2.2.2 Production of Egg Shell Powder</u>

Egg shells from restaurant were cleaned and washed followed by the removal of the shell-membrane with the aid of hot water in order to prevent or minimize the interference of protein molecules with the final product and the shells were later oven dried at 105 ^{o}C for 3 hours followed by cooling. The dried egg shells were milled into powder using attrition mill and then packaged in an air tight container.

2.2.3 Production of Snail Shell Powder

Live snails obtained from the market were washed and the boneless body (flesh) was removed to separate them the shells. The resultant shells were cleaned and effectively washed and oven dried at 105 ^{o}C for 3 hours followed by cooling. The resultant material was crushed into powder and packaged in an air tight container.

Comment [K4]: This seems as lot of work and not very efficient in a sense of time consuming. Also, killing this amount of snails for shells is very sad.

Figure 1: Plate of steeped sorghum grains

Figure 2: Germinated sorghum grain

Figure 3: Dried egg shell

Figure 4: Dried Snail shell

2.3 Mashing of Sorghum Malt

Temperature programmed infusion mashing was used to mash the sorghum malt as described by the method of Ofoedu [7] as well as Osuji and Anih [8] with slight modification. Six hundred (600) gram of malted grains comprising of 75% of malted sorghum and 25% of malted barley was dissolved in 6,600ml of portable water that was previously made to a pH of 11.0 using $Ca(OH)_2$ solution. This step was made twice for different mash water for day 2 and day 3 sorghum malts. The mixture was stirred continuously as the temperature was raised to 45^{o}C (for protein rest) and maintained at this temperature for 20 minutes after the addition of bacterial α-amylase (0.4ml). With constant stirring but not vigorous, the temperature of the sample mixture was raised to 55^{o}C and maintained at this temperature for 10 minutes. The temperature of the content of the mashing vessel was raised to 65^{o}C and maintained at this temperature for I hour with the addition of fungal α-amylase (0.4 ml).

Conversion (hydrolysis) of starch in the medium was tested by pipetting 2 drops of iodine solution on a white ceramic tile. The mash was rested again at 70-75^{o}C until complete saccharification by fungal α-amylase was achieved which was confirmed with another iodine test. Thus, a negative test for starch indicated that all starch molecules has been hydrolyzed.

The converted medium was filtered across a triple layer muslin cloth and the spent grains were sparged with 1000ml of hot sparge water. The spent grains which also include husk, seedlings and other insoluble materials were separated from the wort to recover as much extract as possible from the spent grains. The flow process for the production of sorghum wort is described in figure 4 below.

2.4 Sorghum Beer production

The procedure used for beer production was a method described by Kunze [9]. The recovered wort was boiled using classical method involving atmospheric boiling at 100^{o}C for 45 minutes. Hop extracts were added to the boiling wort so as to impart unique characteristic flavor and herbal aroma to the product. The boiled wort was cooled and the hot trubs and other undissolved particles (debris) were removed. The filtered wort was transferred into a fermenting vessel with each vessel containing wort from 2nd day and 3rd day malted grains. An already activated yeast (200ml) was pitched into each of the vessel at a temperature of 20°C and the wort was allowed to ferment for 8 days at 10° - 20°C.

2.5 Beer Filtration and Clarification

After fermentation, the beer was filtered and transferred into another vessel for maturation and clarification. During the lagering process, the beer was made in triplicate for each of the brew from the different germination periods (day 2 and day 3). Kieselghur in the ratio of 2:1 (Male:Female), egg shell powder and snail shell powder were added to each of the three separate vessels containing beer from day 2 and day 3 germination period for clarification and gently stirred. The mixture was allowed to stand undisturbed for 2 weeks followed by bottling of the clarified beer in a clean sterilized glass bottle and sealed with manual crowning machine.

2.6 Quality Evaluation of sorghum malt, wort and beer

2.6.1 pH Determination

The wort and beer pH was determined using a method of A.O.A.C. [10] with the aid of a hand-held pH meter.

2.6.2 Apparent Brix Determination

The apparent brix (^{o}B) of the wort and beer samples was determined according to the method described by Montanez-Soto *et al.,* [11] using a Milwaukee Digital refractometer.

2.6.3 Determination of Specific Gravity

Specific gravity (original and final gravity) of the wort and beer was determined using specific gravity bottle as described by A.O.A.C [10].

2.6.4 Determination of Alcohol

The percentage alcohol by volume of beer was determined by the method of A.O.A.C. [10] through distillation technique.

2.6.5 Determination of Total Solids

Total solids were determined using the method of Osuji and Anih [8].

2.6.6 Determination of Turbidity

Turbidity of the beer samples was determined by photometric method using HACH DR/21010 spectrophotometer at a wavelength of 860nm and programme number 750 according to A.O.A.C [12].

<u>2.6.7 Determination of Total Dissolved Solids</u>

The total dissolved solid of the beer sample was determined using a method of A.O.A.C. [10].

2.7 Statistical Analysis

Data obtained from these analyses were subjected to T-test and one-way analysis of variance. Means obtained were separated using Fisher's Least Significant Difference at *P<0.05*.

3. RESULTS AND DISCUSSION

3.1 Quality Evaluation of Sorghum wort and Sorghum beer: The sorghum wort and beer quality before clarification is shown in Table 1.

<u>3.1.1 pH</u>:

The pH of the sorghum wort from day 2 and day 3 sorghum malt are 5.60 and 5.50 respectively while the pH of sorghum beer recorded 4.10 for day 2 malt and 3.90 for day 3 malt. There was no significant difference (P>0.05) between the pH of day 2 & day 3 sorghum wort and the pH of day 2 & day 3 sorghum beer. pH is used to denote the degree of the alkalinity or acidity of a substance. The pH of sorghum wort obtained in this study is in agreement with the range of 5.40 – 5.70 reported by Kunze [6]. The sorghum beer pH was significantly lower than its corresponding sorghum wort. However, previous research works have reported a pH range of 3.70 – 4.50 for lager beer and the pH of sorghum beer obtained in this study falls within this range. This indicates that beer pH tends to be more acidic after fermentation. The fall in pH of sorghum wort after fermentation must be the action of yeast activity. It was reported by Coote and Kirsop [13] that yeast may be envisaged as acting to reduce pH of wort by altering the wort buffering capacity, absorbing bases (amino acids) and excreting organic acids such as pyruvic, malic, lactic, succinic, etc; thereby giving beer its unique pH in acidic region.

3.1.2 <u>Apparent Brix (^{o}B):</u>

The apparent brix of the sorghum wort from day 2 and day 3 sorghum malt are 15.4^{o}B and 16.0^{o}B respectively while the apparent brix of sorghum beer recorded 4.60^{o}B for day 2 malt and 4.70^{o}B for day 3 malt. There was no significant difference (P>0.05) between the apparent brix of day 2 & day 3 sorghum wort and the apparent brix of day 2 & day 3 sorghum beer. The major solids present in wort are mainly hydrolyzed sugars which include monosaccharide, disaccharides, dextrins as well as amino acids, polypeptides, vitamins, minerals, etc [11]. Brix refractometer measures the degree to which a solution refracts or bends light as it is normally used to measure the amount of sucrose in a solution [14]. The apparent brix of sorghum beer was significantly lower than its corresponding sorghum wort probably because of fermentation. During fermentation, soluble extracts in form of hydrolyzed sugars and soluble proteins are absorbed by the yeast, thus reducing the final brix of the beer after soluble extracts utilization is complete.

RESULTS AND DISCUSSION

Table 1: Quality Evaluation of Sorghum wort and beer before clarification

	pH		**Apparent Brix (^{o}B)**		**Specific Gravity**		**% Alcohol**	
Samples	**Wort**	**Beer**	**Wort**	**Beer**	**Wort** (Original Gravity)	**Beer** (Final Gravity)	**Wort**	**Beer**
Day 2 Malt	5.60*±0.141	4.10±0.141	15.4*±0.282	4.60±0.071	1.076*±0.001	1.020±0.014	-	6.00^{a}±0.00
Day 3 Malt	5.50*±0.007	3.90±0.0212	16.0*±0.141	4.70±0.141	1.076*±0.003	1.020±0.011	-	4.60^{b}±0.03
LSD	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	-	**0.086**

Values are the means of duplicate determinations

a,b….means with the same superscript along a column for each treatment is not significantly different (P>0.05)
*….means with asterisk (*) within a row and within pH, Apparent Brix and Specific gravity is significantly different (P>0.05)

KEY:

Day 2 Malt = Sorghum grains germinated for 2 days

Day 3 Malt = Sorghum grains germinated for 3 days

NS = Not Significant

3.1.3: Specific Gravity of the Wort:

The original gravity of the sorghum wort from day 2 and day 3 sorghum malt are 1.076 and 1.076 respectively while the final gravity of sorghum beer recorded 1.020 for day 2 malt and 1.020 for day 3 malt. The original gravity obtained in this work is in line with the range of 1.000-1.130 as reported by Kunze [6]. However, there was no significant difference (P>0.05) between the apparent brix of day 2 & day 3 sorghum wort and the apparent brix of day 2 & day 3 sorghum beer but significant difference (P<0.05) exists between sorghum beer and its corresponding sorghum wort. The lower specific gravity in sorghum beer could be as a result of the complete utilization of the soluble extracts (hydrolyzed sugars and soluble proteins) of sorghum wort by yeasts to yield alcohol and other by-products. The variation is principally due to varying levels of solid contents in the wort and beer which contributed to its density [7].

3.1.3 Alcohol content:

The alcohol content of sorghum beer from day 2 malt and day 3 malt was recorded as 6.00% and 4.60% respectively. There was significant difference (p<0.05) between sorghum beer from day 2 malt and sorghum beer from day 3 malt. The percentage alcohol content in the sorghum beer is predominantly ethanol. The higher alcohol content in sorghum beer from day 2 malt when compared to the beer from day 3 malt could be as a result of varying concentrations of sugars produced during malting and mashing operation. However, it could be stated that a correlation exists between duration of germination and hydrolysable sugar concentration as well as alcohol content. In other words, it could be that the alcohol yield decrease with increase in malting loss or decrease in malt yield [6].

Table 2: Physicochemical Properties of Clarified Beer Samples

SAMPLE	Specific Gravity	Turbidity (NTU)	Total Solids (g/ml)	Total Dissolved Solids (g/ml)
A	1.001^{a} ±0.001	8.28^{d} ±0.280	0.016^{b} ±0.001	0.008^{a} ±0.003
B	1.001^{a} ±0.001	21.88^{c} ±0.140	0.017^{b} ±0.001	0.012^{a} ±0.000
C	1.002^{a} ±0.000	23.24^{b} ±0.140	0.024^{a} ±0.000	0.011^{a} ±0.003
D	1.004^{a} ±0.001	8.72^{d} ±0.280	0.016^{b} ±0.001	0.009^{a} ±0.001
E	1.003^{a} ±0.003	23.42^{b} ±0.140	0.022^{b} ±0.003	0.010^{a} ±0.001
F	1.004^{a} ±0.001	26.56^{a} ±0.000	0.026^{a} ±0.00	0.011^{a} ±0.001
LSD	**NS**	**0.47**	**0.004**	**NS**

a,b….means with the same superscript along a column for each treatment is not significantly different (P>0.05)

KEYS:

A = Day 2 beer clarified with Kieselghur
B = Day2 beer clarified with egg shell powder
C = Day 2 beer clarified with snail shell powder
D = Day 3 beer clarified with Kieselghur
E = Day 3 beer clarified with Egg shell powder
F = Day 3 beer clarified with snail shell Powder.

3.2 **Quality Evaluation of the Clarified Sorghum Beer samples:** The quality of sorghum beer clarified with kieselghur, egg shell powder and snail shell powder are shown in Table 2.

3.2.1 Total Solids:

The total solids of the clarified beer samples ranged from 0.016g/ml to 0.024g/ml. For Day 2 clarified beer, there was no significant difference (P>0.05) between sample A (Day 2 beer clarified with Kieselghur) and sample B (Day2 beer clarified with egg shell powder) but both showed significant difference (P<0.05) with sample C (Day 2 beer clarified with snail shell powder). Also, for Day 3 clarified beer, there was no significant difference (P>0.05) between sample D (Day 3 beer clarified with Kieselghur) and sample E (Day 3 beer clarified with Egg shell powder) but both showed significant difference (P<0.05) with sample F (Day 3 beer clarified with snail shell Powder). The variations in the total solids of the clarified beer samples may be attributed to the nature and/or the chemical composition of the filter aids. It could be observed that the germination period of malt used in producing these clarified sorghum beers had no significant influence on the amount of total solids in the beer sample. However, the filter aids influenced the quantity of solids present in the sorghum beer samples. For both Day 2 and Day 3 clarified sorghum beer samples, beer clarified with kieselghur had the least total solids, followed by egg shell powder clarified beer, while snail shell powder clarified beer recorded a higher total solid content. According to Anger *et al*., [15] and Ofoedu [7] total solids are largely composed of soluble and insoluble constituents of a solution such as sugars, organic acids, amino acids (nitrogenous compounds) and minerals. Comparing the egg shell and snail shell powders used as filter aids in this research work, it could be that the chemical composition of the egg shell powder tends to have more affinity for insoluble or suspended solids by trapping insoluble and/or suspended solids and precipitating them out of solution in form of flocs, thereby resulting to a lesser solid content compared to snail shell powder clarified beers.

3.2.2 Total Dissolved Solids (TDS):

The total dissolved solids of the clarified beer samples ranged from 0.008g/ml to 0.012g/ml. There was no significant difference (P>0.05) in the total dissolved solids of the clarified beer samples from Day 2 and Day 3. The total dissolved solids are the soluble solutes that were not precipitated by the filter aids. The differences in the total dissolved solids of the clarified beer samples could be as a result of variations of germination periods and some chemical reaction that occurred during mashing operation, thereby yielding different concentrations of soluble solutes. According to Ofoedu [7], total dissolved solid is the measure of the combined content of all organic and inorganic substances contained in a liquid in molecular and/or ionized form.

3.2.3 Specific Gravity:

The specific gravity (SG) of the clarified beer samples ranged from 1.001 to 1.004 and there was no significant difference (P>0.05) in the SG of the beer samples. Specific gravity of beer is a function of the density of beer and density of water at equal volumes [7]. It could be observed that the SG of the clarified beer samples from Day 2 and Day 3 were lower than the final gravity of the beer after fermentation in Table 1. The decrease in SG observed in the clarified beer samples is because of the combined action of beer maturation and clarification with the aid of filter aids. Maturation is the transformation between the end of primary fermentation and final filtration of beer [16]. During this stage of brewing process, complex biochemical, chemical and physical reactions that leads to beer clarity as well as flavour development occur.

3.2.4 Turbidity:

The turbidity of the clarified beer samples ranged from 8.28 to 26.56NTU (Nephelometric turbidity Unit). There significant differences (P<0.05) in the turbidity of the clarified beer samples. Sample F (Day 3 beer clarified with snail shell Powder) recorded the highest turbidity of 26.56NTU while sample A (Day 2 beer clarified with Kieselghur) had the least turbidity of 8.28NTU followed by sample D (Day 3 beer clarified with Kieselghur) with a turbidity of 8.72NTU and both showed no significant difference (P>0.05). The differences in the clarified beer turbidity could be as a result of variations in the concentration of soluble solutes and suspended particles in the beer samples. The turbidity of the beer samples could also be due to the extent of grain modification during germination/malting [6] and the degree of hydrolysis during mashing. Sorghum grain is without husk and this tends to cause filtration problem due to their naked caryopsis.

In addition, the type of filter aids used, might have partly contributed to the variations in the turbidity of the beer samples. It could be observed that for Day 2 and Day 3 clarified beer, beer samples clarified with kieselghur had a lower turbidity, followed by egg shell powder clarified beer while snail shell powder clarified beer recorded the highest turbidity and this also corresponds to the total solid content of the individual beer samples. In other words, the concentration of total solids in a beer sample or solution can be correlated to turbidity. It is important to note that Day 3 clarified beer samples had a higher turbidity than its corresponding Day 2 clarified beer samples, probably due to production of more proteins (amino acids) and other compounds that can cause haze in beer. Turbidity is simply the cloudiness or haziness of a beer. Haze in beer can be as a result of polyphenols and proteins. These micronutrients are always present in beer and can form haze when they cross-link to form insoluble particles [17]. Unlike insoluble particles and/or suspended solids, coloured compounds such as melainoidins that are produced during kilning and mashing operation and forms part of soluble solutes can also contribute to beer's turbidity. Turbidity is measured when incident light is scattered at right angles from the sample by the concentration of suspended solids. However, though melainoidin pigments may be present in low concentrations in beer, they also contribute significantly to the turbidity of the beer.

Four beer samples analyzed for turbidity by Thermoscientific [18] reported that lager beer had 16.3NTU, light pilsner had 7.2NTU, seasonal ale had 13.5NTU and stout had 5.8NTU; but the sorghum lager beer produced in this study had 8.28-8.72NTU for kieselghur clarified beer, 21.88-23.42NTU for egg shell powder clarified beer and 23.24-26.56NTU for snail shell powder clarified beer. According to literature, 1EBC = 4NTU = 69ASBC [19], and the turbidity of beer samples are graded based on their degree of haziness (Brilliant for 0.0 to 0.5EBC, Almost Brilliant for 0.5 to 1.0EBC, Very Slightly Hazy for 1.0 to 2.0EBC, Slightly Hazy for 2.0 to 4.0EBC, Hazy for 4.0 to 8.0 EBC and Very Hazy for >8.0EBC). This implies that the turbidity of kieselghur clarified sorghum may be classified as slightly hazy while egg shell powder and snail shell powder clarified sorghum beer may be classified as Hazy. This is in agreement with the work of Olu *et al.,* [20] who reported 5.25EBC lager beer from 100% barley malt and a range of 5.25EBC to 7.00EBC lager beer from blends of barley malt and sorghum adjunct.

4. CONCLUSION

The effectiveness of egg shell powder and snail shell powder as potential filter aids for brewing exhibited some clarification properties. Kieselghur clarified sorghum beer had better clarity than egg shell powder and snail shell powder clarified sorghum beer; but egg shell powder showed better clarification potentials than snail shell powder. Sorghum beer clarified with kieselghur was classified as slightly hazy while egg shell powder and snail shell powder clarified sorghum beer was classified as hazy in terms of its turbidity. Malting of sorghum improves the hydrolysis and modification of starchy endosperm. There is a direct correlation between total solid content and turbidity since the concentration of total solids in the beer samples influenced the beer quality and clarity. Germination period significantly influenced the turbidity of the beer samples. It is therefore recommended that further research be carried out on the treatment of egg shell, snail shell and other crustacean shells so as to improve their effectiveness in beer/beverage clarification for a better product quality and acceptability.

Comment [K5]: Please check the manuscript for similar mistakes.

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