Original Research Paper

Volume - 11 | Issue - 07 | July - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Biotechnology

COMPARATIVE PRODUCTION OF MULTIPLE-MILLETS VINEGAR AND PHYSICO-CHEMICAL AND ANTIBACTERIAL PROPERTIES STUDY

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ABSTRACT Vinegar is a liquid obtained after alcoholic fluid is exposed to oxygen in controlled conditions. Millet vinegar was produced basically to find a replacement for the commonly used and less economical apple cider vinegar. Initially, four millets were selected as whole grains and heated with 5 folds of water at 90 to 100°C for 2 to 3 hours. Further the liquid obtained was used as reaction mixture for alcoholic and acetic acid fermentation. Vinegar so obtained after 7 days of alcoholic fermentation and 15 days of acetic acid fermentation is measured for its various physicochemical parameters. Vinegar showed significant acidity of (5.5%) as acetic acid and various vitamins and minerals with low energy value. Vinegar also showed notable antimicrobial property with respect to selected bacterial species. Overall attempt for vinegar production using millets was successful. Further investigation on other properties and by improving vinegar production with controlled parameters could produce better vinegar.

KEYWORDS : Acetic acid bacteria, Fermentation, Millets, Nutrients, Vinegar, Yeast.

INTRODUCTION

Food demand and food supply is increasing parallel with the increase in population [1]. Agriculture has succeeded in combating the major problems related to food demand and food supply. Food demand is also influenced by the local traditions, urbanization and the services such as dining out and supermarket chains [2]. The pattern of the food consumption is determined by the gender structure, physical activity and metabolic requirements [3].

The increased food demand caused by the increased population is responded by agriculture and succeeded [4]. Food supply has increased three times since the 1960s and has continued to rise [5] (FAO, 2011). Millets are small seeded grasses. Millets are highly tolerable to extreme weather conditions and high drought [6]; hence it is largely produced. The 100 g of millets will provide 20% or more of the protein value that is consumed daily and is a rich source of various minerals and vitamins [7]. Millets are rich in dietary fibers and hence help in the digestion process and generally, help in lowering blood cholesterol, maintaining blood glucose levels, boosts liver and kidney functions. The healthy life can be maintained by the inclusion of millets in our daily diet [8].

In the present study four different species of millet has been used for the production of vinegar. The selection of millets was done based on availability, nutritional composition and economical aspect [9].Proso millet is one of the most important millets grown in India. It has relatively low water requirements and hence is suitable for less water and dry land areas. Generally, 100 gm of Proso millet consists of 1.02 g of protein, 4.22 g of lipid fat, 6.6 g of ash, 73g of carbohydrate and 8.5g of totally dietary fiber [10].Second important millet used in vinegar production is Finger millet, commonly called as Ragi [11]. Finger millet contains 5–8% protein, 65–75% carbohydrates, 15–20% dietary fiber and 2.5–3.5% minerals.100 grams of Finger millet have roughly on an average of 336 kcal of energy [12]. Little millet is one of the smallest millets. Typically, 100 gm of little millet contains around 67g of carbohydrate, 7.7g of protein, 4.7g of fat and around 341 kcal of energy [13]. Barnyard millet is also called as Japanese millet. It grows in cold areas and is not suitable for any other crops. Typically, 100g of this millet contains 65.5g carbohydrate, 6.2g of protein, 2.2g fat and provides around 307 kcal of energy [14].

There are various health benefits of these millets which can prevent diseases and also serve as a remedy for many fast life diseases [15]. Proso millet is effective against type 2 diabetes due to the rich content of magnesium. Magnesium reduces heart attacks and the rate of migraine headaches, and it is also helpful in heart related diseases and atherosclerosis. The rich content of fiber plays important role in maintaining cholesterol level in the body. Finger millet consists of highest amount of potassium and calcium comparatively. The cereal has very low-fat content and mainly contains unsaturated fat [16]. Finger millet helps in strengthening of bones in aged people and growing children since it is an excellent source of calcium. It possesses an excellent antioxidant activity and helps in regulating the blood sugar level in diabetic patients. It also helps in the recovery of Anemia as it is a very good source of natural Iron [17]. Little millet is a low glycemic index food and contains large amount of fiber which plays a significant role in controlling the blood sugar level. It is also rich in magnesium that is important for the heart health. It has vitamin B3 that is useful in maintaining cholesterol level. It contains phosphorus that aids in weight loss and tissue repair, also detoxifies body and is a protein rich source which is highly digestible and is least caloric dense [18]. The glycemic index of barnyard millet is low and hence can be used for cardiovascular patients. It is also gluten free and contains large amount of dietary fibers [19].

Vinegar is an aqueous solution of acetic acid (4-8%) and fewer other acids. Vinegar is produced in two step process; initially alcoholic fermentation and then acetic acid fermentation [20].The following reactions represent the conversion of carbohydrate into ethanol and ethanol into acetic acid.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \text{ and } C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$$

Acetic acid bacteria are commonly used for the production of vinegar. It is produced from different sources like Apple, Rice, Barley, Grapes etc. Vinegar is known for its health and therapeutic effects like antimicrobial, anti-diabetic, antioxidant, antitumor effect etc.[21].

Apple cider vinegar is made from apple as a source. It is rich in various types of minerals and vitamins. It is useful in weight loss, to improve heart health, helps to fight obesity and also helps in preventing cancer [22].

Those diseases in which the occurrence is primarily based on the regular and daily habits of people are characterized as lifestyle diseases [23]. Food habits, disturbed biological clock, physical inactivity are the main factors responsible for the lifestyle diseases. The ever-growing cases of diabetes can be attributed to unhealthy diets and busy lifestyles with no proper supplements available especially for middle and low-income population. According to the reports around 7-9% of the total population is suffering from coronary heart disease. All these can be attributed to unnourished food with no proper supplements. Food supplements are the dietary material whose purpose is to supplement the normal diet and is present as the concentrated form of vitamins, minerals and other dietary fibers [24].

MATERIALS AND METHODOLOGY

Materials

All four millets Finger millet (*Eleusinecoracana*), Barnyard millet (*Echinochloa esculenta*), Proso millet (*Panicum miliaceum*) and little

millet (*Panicum sumatrense*) were purchased from retailer.The botanical identities of millets were determined and authenticated at Taralabalu Krishi VignanaKendra (KVK), Davangere,nearto Bapuji Institute of Engineering and Technology,Davangere. Apollo apple cider vinegar which has been used as mother culture for vinegar production was bought from Apollo medical shop near BIET campus. Double distilled water, Sucrose, Dry yeast (Saccharomyces cerevisiae) from Hi Media was used.

Methodology

The basic protocol for the conversion of millets into vinegar is figured out based on the literature study. The juice extraction from raw materials with carbohydrate composition need to be done by powdered raw material, further inoculated with 10% S.cerevisiae of total volume wherein alcohol fermentation is carried out. The ethanol produced is subjected to acetic acid fermentation by inoculating with Acetobacter aceti culture to produce fermented vinegar as the final product.

Extraction Of Millet Juice:

Initially 100g of each millet (Proso, Barnyard, Finger, Little millets) are grounded into coarse powder containing husk. This coarse powder is then mixed with distilled water in the ratio 1:5 and incubated in water bath at the temperature of 90˚C- 100˚C for2 to 3 hours. The obtained extract is filtered using muslin cloth and used for the further process of fermentation [25].

Preparation Of Yeast Inoculum For Alcoholic Fermentation:

The sugar (sucrose) of 4g (4%) is dissolved in 100 ml of distilled water and the Dry yeast powder of 500mg (0.1%) are added to the solution and kept for incubation for 60minutes, this solution is used as inoculum for alcoholic fermentation.

Alcoholic Fermentation:

The extracted millet juice with inoculum of 10% of the total volume is added to the fermenter and all the other required controlled conditions such as temperature (27°C), pH (3.45), impeller rotation speed of 100 rpm and anaerobic environment which is inevitable condition for fermentation needs to be maintained. Initially aerobic fermentation takes place where the yeast cells gets adapted to the fermentation conditions and multiplies in the limited supply of oxygen. Further the anaerobic fermentation takeover the process where the yeast cells utilize the carbohydrates and convert it into ethanol. The process is carried out in the fermenter for 5 to 7 days to convert complete reaction mixture to alcohol [25].

Acetic Acid Fermentation:

The alcohol obtained from fermentation is used as reaction mixture for acetic acid fermentation. Further the Apollo apple cider vinegar is used as mother vinegar which is inoculated as starter culture. Before inoculation, Apollo Apple cider vinegar is used for culturing of Acetobacter aceti in laboratory by maintaining necessary culture conditions and same is confirmed by bacterial biochemical identification test. Diluted alcohol is inoculated with mother vinegar by $1/10^{th}$ of the total volume of alcohol. Excess oxygen is supplied externally along with other conditions like temperature (28°C), pH (3.45) and rotation of 100rpm is maintained. The process of acetification is carried out for 15 days. The process was carried out with regular monitoring of acetification process by recording and analyzing pH and acidity values [26].

Antimicrobial Assay

For antimicrobial assay, the disc diffusion technique was followed; common pathogenic and strongly resistant bacteria species are selected such as (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia*) for assay. Nutrient agar plates were prepared and the spread plate method was followed for the even distribution of bacterial culture samples on a solidified nutrient media. The discs of 6mm diameter were punched from a sheet of Whatman filter paper and sterilized. These were made to absorb millet vinegar and air dried at 37ºC for 48 h and thereafter, the discs were placed on the surface of inoculated nutrient agar plates. Further, the nutrient agar plates were incubated at 37 °C for 14 -24 hours to observe the formation of inhibition zones around the discs.

Determination Of pH Value And Total Acidity

pH was measured using Digital pH meter (Model Eutech-GC7352901B). pH and acidity were recorded for 15 days with consecutive interval of 2 days each. Total acidity was determined by 4-fold dilution of vinegar sample by using distilled water. Further titrating with 0.1 N NaOH, phenolphthalein as end point indicator and was expressed as the acetic acid equivalent.

The parameters such as carbohydrate, fats, protein, total energy, specific gravity, residual alcohol, total ash, total solid, total Soluble solid, Vitamin B3, Vitamin C, Minerals such as Sodium, Potassium and Calcium were calculated with reference to FSSAI and AOAC standard methods [27].

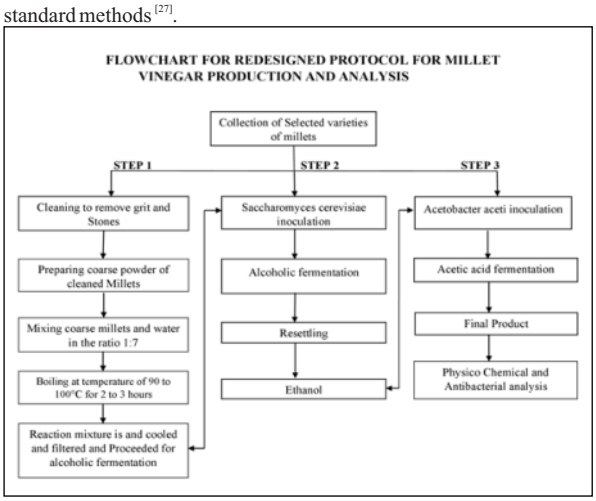

Figure 1: Flow Chart Showing Step Wise Process Involved In Vinegar Production

RESULTS

Physicochemical Parameters Of Alcohol Obtained After Alcoholic Fermentation.

The physicochemical parameters were tested for the alcohol obtained after alcoholic fermentation at the end of 7th day. The pH was measured using digital pH meter which was recorded as (3.17). The specific gravity obtained was (1.07) at 27˚C which was measured using specific gravity bottle method and the Total acidity measured was 0.8g/100ml (Table1).

pH and Acidity

The pH of acetic acid fermented millet juice was recorded for 15 days and found to be gradually decreasing each day and lowest reading of (2.97) was recorded on 15th day (Figure 2). The acidity of acetic acid fermented millet juice was recorded for 15 days and found to be gradually increasing each day with the highest of (5.5g/100ml) was recorded on 15th day (Figure 3)

Table 1: pH And Acidity Values For Every 2 Consecutive Days For Total Of 15days Of Analysis

`Parameter	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
pH	3.67±0.1206	3.62±0.0612	3.53±0.0509	3.49±0.0349	3.38±0.0571	3.35±0.0701	3.16±0.0790	2.97±0.0874
Acidity	0.83±0.0380	1.24±0.03807	2.41±0.0384	3.23±0.0412	4.31±0.0349	4.82±0.0414	5.17±0.0514	5.53±0.0796

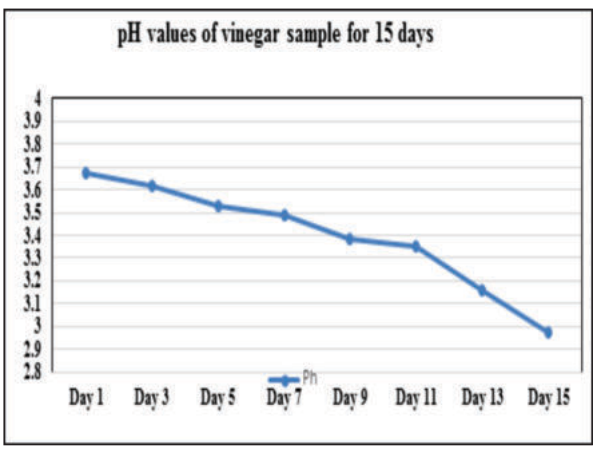

Figure 2: Graph Showing pH Values Of Vinegar Sample For 15days

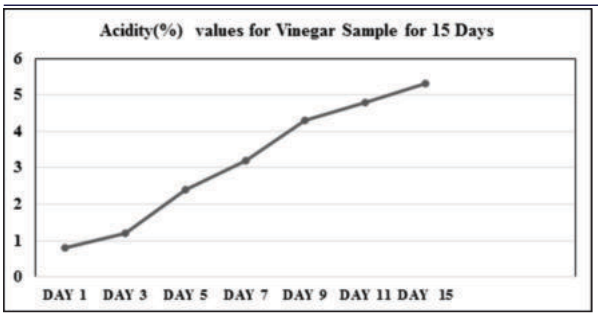

Figure 3: Graph Showing Acidity Values For Vinegar Samples For 15 Days

Table 2: Physico-chemical Parameters Of Millet Vinegar After 15days Of Fermentation

PARAMETER	UNIT	OBSERVED VALUE
pH	--	3.08±(0.1456)
Carbohydrates	g	0.06(±0.0339)
Of which sugar	g	Nil
Fat	g	Nil
Protein	g	Nil
Total Energy	Kcal	0.024±0.0356
Total acidity content as Acetic Acid	%	5.55±0.0486
Specific Gravity	--	1.0122±0.0071
Total Ash	%	0.48±0.0543
Total Soluble Solids	%	0.16±0.0474
Vitamin B3	mg/Kg	4.39±0.0316
Vitamin C	mg/100g	17.99±0.0320
Potassium	mg/Kg	862.91±0.01478
Sodium	mg/Kg	50.97±0.0308
Calcium	mg/Kg	116.1±0.0148

Table3: Antibiotic Inhibition Zone For Vinegar Sample Tested For Selected Bacterial Species

Micro-organism	Inhibition zone of samples in(mm)
Staphylococcus aureus	8 (+0.1395)
Pseudomonas aeruginosa	12 (+0.1334)
Escherichia coli	10 (+0.2121)
Klebsiella pneumonia	11 (+0.1570)

DISCUSSION

The millet vinegar developed in present work showed significant acidity calculated as acetic acid of (5.55%) and total ash content of (0.48%) (Table 2) which holds well with the brewed vinegar standards mentioned in FSSAI food standards regulations as minimum acidity calculated as acetic acid should be (3.75%) and minimum total ash content is (0.18%) [27]. various studies reported, the acidity was found to be 4 to 8%, same results were seen with respect to acidity values recorded for this product. Comparatively this was achieved with the short period of fermentation of 15 days where as studies from other research works reported signify that the normal time period of 3 to 4 weeks is needed to achieve acidity of 4 to 8% [28]. The usage of millet as raw material in vinegar production is notably innovative where most of the research work is been reported by apple, fruits, malt and molasses as the source [29,30]. Vinegar produced showed good composition of various minerals and vitamins pose good probability of its constituents with elements of antioxidants which will aid in preventing and controlling heart related diseases and various kinds of cancers. Carbohydrate composition of vinegar obtained was 0.06g, which gives energy value of 0.24 Kcal for every 100ml of vinegar. This can be attributed to the overall hydrolysis of starch and sugars present in the millets and usage by alcoholic and acetic acid bacteria for production of vinegar. Low energy value of vinegar can also be attributed to its low glycemic index which further helps in controlling and reducing blood lipid profile also with proper diet obesity can be reduced [31]. Multiple studies [32,33] have reported that continuous consumption of apple cider vinegar as part of diet could reduce HDL levels and improves the serum lipid profile by reducing plasma levels of total cholesterol and other lipids [34]. Vinegar obtained also showed notable amount of Vitamin-B3 (4.39 mg/Kg) and significant amount of Vitamin-C (17.99 mg/100gm). Vitamin B3 is an important nutrient it helps to boost the brain functions and also helps in lowering the cholesterol [35]. Niacin in vinegars plays a role in blood vessel expansion and cholesterol excretion [36]. Comparing Vitamin C content in vinegar of studies has been reported by that showed (217 ± 6.3 mg/L) relatively similar amount of vitamin C content was seen in vinegar produced in present study. Vitamin C is a strong antioxidant which can boost the blood antioxidant levels and can also prevent and control the cancer [37]. The obtained product comprises of 116 mg/Kg of Calcium. Calcium helps in building bones and keeping them healthy. It is also necessary for maintaining healthy communication between the brain and other parts of the body [38]. It also contains 72mg/Kg of Phosphorus which also helps in building of the strong bones. It filters out waste in kidneys and helps in growth, maintain, repair tissue and cells, it produces DNA and RNA - the body's genetic building blocks [39]. The product contains notable amount of sodium (36gm/Kg). Sodium is an essential electrolyte for the body to function normally and it also helps in maintaining fluid and blood volume in the body. A low sodium diet has numerous health benefits [40]. It also contains a very good amount of Potassium (860mg/Kg). It decreases the risk of stroke, preserves bone mineral density, lowers blood pressure and reduces the kidney stones [41].

The results given under (Table 3) showed notable antimicrobial effect with respect to selected bacterial species comparatively vinegar showed better results with respect to Pseudomonas aeruginosa and Klebsiella pneumonia. Further concentration of vinegar and time of exposure can possibly enhance the antimicrobial property. Multiple studies have [42] also attributed possible reasons for antimicrobial activity of vinegars would be, cause of microbes undergoing significant impairment following vinegar treatment which damaged cell integrity, structural and metabolic proteins as well as nuclear material. Acetic acid is the dominant flavour compound in vinegar and has a long history as an important direct food additive to acidulate food for preservation [43, 44]. Various other studies such as Bornemeier et al and Lukasik et al. [45, 46, 47] also describe potential property of vinegar as antimicrobial agent in various other species of bacteria and fungi.

CONCLUSION

Vinegar is rich in acetic acid and various organic acids, with potent biological effects. Millets are beneficial to health as it contains different nutrients such as carbohydrates, vitamins, minerals and etc. The final product obtained contained 5.55% of acetic acid. It can be a very good replacement for the commercially available product as it is economical. The attempt to convert the millet water extract into vinegar was successful for initial stage. Further, it has a great scope of future work as a health drink which can solve many health-related issues which are acquired through fast moving life style. The millet vinegar might help in lowering blood sugar levels and fights diabetes. It may also aid in losing weight and reducing belly fat. Further it might lower cholesterol and improves heart health and cancer related conditions.

REFERENCES:

1. Lawless HT, Heymann H. Sensory evaluation of food: principles and practices. Springer Science & Business Media; 2010 Sep 27.
2. Kearney J. Food consumption trends and drivers. Philosophical transactions of the royal society B: biological sciences. 2010 Sep 27;365(1554):2793-807.
3. Valin H, Sands RD, Van der Mensbrugghe D, Nelson GC, Ahammad H, Blanc E, Bodirsky B, Fujimori S, Hasegawa T, Havlik P, Heyhoe E. The future of food demand: understanding differences in global economic models. Agricultural Economics. 2014 Jan;45(1):51-67.
4. Richards P. Indigenous agricultural revolution: ecology and food production in West Africa.
5. Gebbers R, Adamchuk VI. Precision agriculture and food security. Science. 2010 Feb 12;327(5967):828-31.
6. Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ. Crop production under drought and heat stress: plant responses and management options. Frontiers in plant science. 2017 Jun 29;8:1147.
7. Kumar A, Tomer V, Kaur A, Kumar V, Gupta K. Millets: a solution to agrarian and nutritional challenges. Agriculture & food security. 2018 Dec;7(1):1-5.
8. Sarita ES, Singh E. Potential of millets: nutrients composition and health benefits. Journal of Scientific and Innovative Research. 2016;5(2):46-50.
9. Hunt HV, Badakshi F, Romanova O, Howe CJ, Jones MK, Heslop-Harrison JP. Reticulate evolution in Panicum (Poaceae): the origin of tetraploid broomcorn millet, P. miliaceum. Journal of Experimental Botany. 2014 Jul 1;65(12):3165-75.
10. Habiyaremye C, Matanguihan JB, D'Alpoim Guedes J, Ganjyal GM, Whiteman MR, Kidwell KK, Murphy KM. Proso millet (Panicum miliaceum L.) and its potential for cultivation in the Pacific Northwest, US: A review. Frontiers in plant science. 2017 Jan 9;7:1961.
11. Bastola BR, Pandey MP, Ojha BR, Ghimire SK, Baral K. Phenotypic diversity of Nepalese finger millet (Eleusine coracana (L.) Gaertn.) accessions at IAAS, Rampur, Nepal. International Journal of Applied Sciences and Biotechnology. 2015 Jun 25;3(2):285-90.
12. Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review. Journal of food science and technology. 2014 Jun;51(6):1021-40.
13. Padulosi S, Mal B, King OI, Gotor E. Minor millets as a central element for sustainably enhanced incomes, empowerment, and nutrition in rural India. Sustainability. 2015 Jul;7(7):8904-33.
14. Sood S, Khulbe RK, Gupta AK, Agrawal PK, Upadhyaya HD, Bhatt JC. Barnyard millet–a potential food and feed crop of future. Plant Breeding. 2015 Apr;134(2):135-

47.
15. Dayakar Rao B, Bhaskarachary K, Arlene Christina GD, Sudha Devi G, Vilas AT, Tonapi A. Nutritional and health benefits of millets. ICAR_Indian Institute of Millets Research (IIMR), Rajendranagar, Hyderabad. 2017:112.
16. Graybosch RA, Baltensperger DD. Evaluation of the waxy endosperm trait in proso millet (Panicum miliaceum). Plant Breeding. 2009 Feb;128(1):70-3.
17. Gull A, Jan R, Nayik GA, Prasad K, Kumar P. Significance of finger millet in nutrition, health and value added products: a review. Magnesium (mg). 2014;130(32):120.
18. Srilekha K, Kamalaja T, Maheswari KU, Rani RN. Nutritional Composition of Little Millet Flour. International Research Journal of Pure and Applied Chemistry. 2019 Dec 31:1-4.
19. Verma S, Srivastava S, Tiwari N. Comparative study on nutritional and sensory quality of barnyard and foxtail millet food products with traditional rice products. Journal of food science and technology. 2015 Aug;52(8):5147-55.
20. Coelho E, Genisheva Z, Oliveira JM, Teixeira JA, Domingues L. Vinegar production from fruit concentrates: Effect on volatile composition and antioxidant activity. Journal of food science and technology. 2017 Nov;54(12):4112-22.
21. Nanda K, Taniguchi M, Ujike S, Ishihara N, Mori H, Ono H, Murooka Y. Characterization of acetic acid bacteria in traditional acetic acid fermentation of rice vinegar (komesu) and unpolished rice vinegar (kurosu) produced in Japan. Applied and Environmental Microbiology. 2001 Feb 1;67(2):986-90.
22. Amrithaa B, Rani SL, Brundha MP. Knowledge, attitude, and perception of the effects of apple cider vinegar among college students. Drug Invention Today. 2020 Apr 15;14(4).
23. Bonnet F, Irving K, Terra JL, Nony P, Berthezène F, Moulin P. Anxiety and depression are associated with unhealthy lifestyle in patients at risk of cardiovascular disease. Atherosclerosis. 2005 Feb 1;178(2):339-44.
24. Sharma M, Majumdar PK. Occupational lifestyle diseases: An emerging issue. Indian journal of occupational and environmental medicine. 2009 Dec;13(3):109.
25. Kaur S, Kocher GS, Phutela RP, Soni G. Production of malt vinegar and its antioxidant potential. Journal of Research. 2007;44(4):326-9.
26. Zahoor T, Siddique F, Farooq U. Isolation and characterization of vinegar culture (Acetobacter aceti) from indigenous sources. British Food Journal. 2006 Jun 1.
27. Lab manual 16 FSSAI-manual of methods of analysis of food (alcoholic beverages).
28. Minh NP. Utilization of ripen starfruit for vinegar fermentation. International Journal of Multidisciplinary Research and Development. 2014;1(4):82-93.
29. Roda A, De Faveri DM, Dordoni R, Lambri M. Vinegar production from pineapple wastes–Preliminary saccharification trials. CHEMICAL ENGINEERING. 2014;37.
30. Praveena RJ, Estherlydia D. Comparative study of phytochemical screening and antioxidant capacities of vinegar made from peel and fruit of pineapple (Ananas comosus l). International Journal of Pharma and Bio Sciences. 2014;5(4):394-403.
31. Dabija A, Hatnean CA. Study concerning the quality of apple vinegar obtained through classical method. Journal of agroalimentary processes and technologies. 2014;20(4):304-10.
32. Khezri, S.S., Saidpour, A., Hosseinzadeh, N. and Amiri, Z., 2018. Beneficial effects of Apple Cider Vinegar on weight management, Visceral Adiposity Index and lipid profile in overweight or obese subjects receiving restricted calorie diet: A randomized clinical trial. *Journal of functional foods*, *43*, pp.95-102.
33. Bouderbala H, Kaddouri H, Kheroua O, Saidi D. Anti-obesogenic effect of apple cider vinegar in rats subjected to a high fat diet. InAnnales de cardiologie et d'angeiologie 2016 May 18 (Vol. 65, No. 3, pp. 208-213).
34. Kongkiattikajorn J. Antioxidant properties of roselle vinegar production by mixed culture of Acetobacter aceti and Acetobacter cerevisiae. Agriculture and Natural Resources. 2014 Dec 31;48(6):980-8.
35. Diba F, Alam F, Talukder AA. Screening of acetic acid producing microorganisms from decomposed fruits for vinegar production. Advances in Microbiology. 2015 May 7;5(05):291.
36. Kulkarni SJ. Research and studies on vinegar production-a review. International Journal of Scientific Research in Science and Technology. 2015;1(5):146-8.
37. Budak NH, Aykin E, Seydim AC, Greene AK, Guzel-Seydim ZB. Functional properties of vinegar. Journal of food science. 2014 May;79(5):R757-64.
38. Ganji SH, Kamanna VS, Kashyap ML. Niacin and cholesterol: role in cardiovascular disease. The Journal of nutritional biochemistry. 2003 Jun 1;14(6):298-305.
39. Xia T, Yao J, Zhang J, Duan W, Zhang B, Xie X, Xia M, Song J, Zheng Y, Wang M. Evaluation of nutritional compositions, bioactive compounds, and antioxidant activities of Shanxi aged vinegars during the aging process. Journal of food science. 2018 Oct;83(10):2638-44.
40. Heaney RP. Calcium intake and disease prevention. Arquivos Brasileiros de Endocrinologia & Metabologia. 2006 Aug;50(4):685-93.
41. Sundbom M, Vrede T. Effects of fatty acid and phosphorus content of food on the growth, survival and reproduction ofDaphnia. Freshwater Biology. 1997 Dec;38(3):665-74.
42. Crocco SC, White PL. Public health issues and intervention programs in hypertension. Some public policy concerns in managing hypertension. Hypertension. 1982 Sep;4(5_pt_2):III160.
43. Srinivasulu C, Reddy MR, Reddy DN. Nutritive value of Gliricidia (Gliricidia maculata) leaves for sheep. Indian Journal of Animal Nutrition. 1998;15(3):225-7.
44. Yagnik D, Serafin V, Shah AJ. Antimicrobial activity of apple cider vinegar against Escherichia coli, Staphylococcus aureus and Candida albicans; downregulating cytokine and microbial protein expression. Scientific reports. 2018 Jan 29;8(1):1-2.
45. Saqib A. Antimicrobial activity of apple cider vinegar. Mapana Journal of sciences. 2017 Apr 7;16(2):11-5.
46. Zetterberg C, Öfverholm T. Carpal tunnel syndrome and other wrist/hand symptoms and signs in male and female car assembly workers. International Journal of Industrial Ergonomics. 1999 Mar 1;23(3):193-204.
47. Lukasik J, Bradley ML, Scott TM, Dea M, Koo A, Hsu WY, Bartz JA, Farrah SR. Reduction of poliovirus 1, bacteriophages, Salmonella Montevideo, and Escherichia coli O157: H7 on strawberries by physical and disinfectant washes. Journal of food protection. 2003 Feb 1;66(2):188-93.