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Volume 55

October 2023

Previous vol/issue Next vol/issue

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Article 103116

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Article preview

Review article ○ Abstract only

Bioconversion of food industry waste to value added products: Current technological trends and prospects

Manpreet Kaur, Ashish Kumar Singh, Ajay Singh
Article 102935

Article preview

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Activity, structural features and *in silico* digestion of antidiabetic peptides

Carmen Berraquero-García, Fernando Rivero-Pino, J. Lizeth Ospina, Raúl Pérez-Gálvez, ... Emilia M. Guadix
Article 102954

View PDF Article preview

Review article ○ Abstract only

Promising mung bean proteins and peptides: A comprehensive review of preparation technologies, biological activities, and their potential applications

Dianzhi Hou, Qiqian Feng, Zhitao Niu, Li Wang, ... Sumei Zhou
Article 102972

Article preview

Review article *Open access*

Food Bioscience

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UPU structured lipids and their preparation methods: A mini review

Ke Yue, Hang Yang, Jian Li, Yanlan Bi, ... Wen-Yong Lou
Article 103009

Article preview

Review article ○ Abstract only

Phytochemical composition, biological activities and antioxidant potential of pomegranate fruit, juice and molasses: A review

Leina El Hosry, Christelle Bou-Mitri, Mira Bou Dargham, Maya Abou Jaoudeh, ... Elias Bou-Maroun
Article 103034

Article preview

Review article ○ Abstract only

Recent developments in sources, chemical constituents, health benefits and food applications of essential oils extracted from medicine food homology plants

Wenqi Yin, Mengshan Shang, Xiaojing Li, Shangyuan Sang, ... Chao Qiu
Article 102997

Article preview

Review article ○ Abstract only

Fortified Jiuqu of the Chinese Baijiu: A review on its functional microorganisms, strengthening effects, current challenges, and perspectives

Jiabin Zhang, Yaochuan Hou, Qingsong Liu, Yujun Zhang, ... Kaizheng Zhang
Article 103045

Food Bioscience

Linyu Zhang, Qi Yu, Min Zhang, Chung Lim Law, Yamei Ma
Article 103047

Article preview

Review article ○ Abstract only

Fruit and vegetable polyphenols as natural bioactive inhibitors of pancreatic lipase and cholesterol esterase: Inhibition mechanisms, polyphenol influences, application challenges

Xu He, Luyao Chen, Yijing Pu, Hongxuan Wang, ... Weibo Jiang
Article 103054

Article preview

Research articles

Research article ○ Abstract only

Preservative paper containing phenolic acid grafted on chitosan for post-harvest peach preservation

Linyun Mou, Jin Zhang, Ya Lu, Muhammad Bilal, ... Ganpeng Li
Article 102925

Article preview

Research article ○ Abstract only

Gallic acid inhibits filament formation and promotes the disassembly of superoxide dismutase 1, a protein involved in the pathogenesis of amyotrophic lateral sclerosis

Yeongjin Baek, Soo-Jeong Lee, Jaekun Ryu, Soyeon Jeong, ... Nam-Chul Ha
Article 102942

Article preview

Food Bioscience

Article preview

Research article ○ Abstract only

Bioactivity evaluation of microbial exopolysaccharide and its use as an encapsulating agent for *Lactobacillus acidophilus* (LA-3)

J.H.P. Lopes Neto, S. Soares, K.S. Leite, M.I.F. Campos, ... H.R. Cardarelli
Article 102946

Article preview

Research article ○ Abstract only

A novel type of sprout food development: Effects of germination on phytic acid, glucosinolates, and lipid profiles in rapeseed

Guannan Liu, Lihua Yan, Shufang Wang, Huidong Yuan, ... Runqiang Yang
Article 102893

Article preview

Research article ○ Abstract only

Effects of butyrylated lotus seed starch on small intestinal bacteria and short-chain fatty acid production in mice

Xin Li, Jiachen Liang, Wenjie Gao, Yi Zhang, ... Baodong Zheng
Article 102931

Article preview

Research article ○ Abstract only

Food Bioscience

Research article ○ Abstract only

Effect of cryoprotectants on physicochemical and structural changes in repeated freeze–thawed egg white protein

Gaopeng Zhang, Chunyan Zhu, Noman Walayat, Wei Tang, ... Jianhua Liu
Article 102913

Article preview

Research article ○ Abstract only

Unraveling the core bacterial community responsible for quality and flavor improvement of the radish paocai during spontaneous fermentation

Qian Zhou, Zimeng Zheng, Yanping Wu, Xiaolei Zhang, ... Hong Gao
Article 102956

Article preview

Research article ○ Abstract only

Cloning and characterization of phenolic acid decarboxylase responsible for aromatic volatile phenols production in Paocai based on metatranscriptomics

Yajiao Zhao, Bofeng Zhu, Ziyi Zhou, Zhengyun Wu, Wenxue Zhang
Article 102953

Article preview

Research article ○ Abstract only

Quality characteristics and moisture migration of refrigerated bullfrog *(Lithobates catesbeiana)* under slightly acidic electrolyzed water combined with composite preservative treatment

Food Bioscience

Synergistic protection of combined *Aronia melanocarpa* Elliot anthocyanins with Aloe Polysaccharides inhibits alcoholic liver injury in mice

Jie Wei, Chenjuan Zhang, Xian Tang, Jinpeng Huang, ... Jun Gao
Article 102938

Article preview

Research article ○ Abstract only

Cleavage of macromolecule (protein/polysaccharide)-phenolic bond in soybean cell wall through *Lactobacillus casei* and *Lactobacillus helviticus* mixed culture solid-state fermentation for chlorogenic acid extraction

Nelson Dzidzorgbe Kwaku Akpabli-Tsigbe, Juliet Osabutey, Benjamin Kumah Mintah, Kwaku Tano-Debrah, Yongkun Ma
Article 102903

Article preview

Research article ○ Abstract only

Sequential fermentation with indigenous non-*Saccharomyces* yeasts and *Saccharomyces cerevisiae* for flavor and quality enhancement of Longyan dry white wine

Xiaodi Wang, Jiawei Chen, Xiaoxin Ge, Xiaofang Fu, ... Yaqiong Liu
Article 102952

Article preview

Research article ○ Abstract only

Effects of polyvinyl alcohol incorporation on the physical and antioxidant properties of soy protein isolate/*Xanthoceras sorbifolia* husk extract active films

Wentao Yan, Yingying Han, Yuping Hou, Dongmei Wang, Miao Yu

Food Bioscience

Binbin Li, Dandan Yuan, Shengguo Song, Mingwei Yang, ... Zhuhong Ding
Article 102948

Article preview

Research article ○ Abstract only

Virtual screening of quorum sensing inhibitors for *Salmonella* Typhimurium and their application as preservatives in chicken breast

Zixuan Xu, Zhenyang Hu, Zhilong Yu, Lijun Huang, ... Yunfei Xie
Article 102957

Article preview

Research article ○ Abstract only

Revealing release patterns and availability of phenolics in Tartary buckwheat under simulated digestion

Yong Zhu, Likang Qin, Yue Chen, Shaoqi Zhou
Article 102939

Article preview

Research article ○ Abstract only

Butyric-lauric acid structural lipid relieves liver inflammation and small intestinal microbial disturbance: In obese male C57BL/6 mice

Wangxin Liu, Xianliang Luo, Ying Huang, Fengqin Feng, Minjie Zhao
Article 102944

Article preview

Food Bioscience

Article preview

Research article ○ Abstract only

Structural characterization of exopolysaccharide produced by *Streptococcus thermophilus* and rheology of mixed protein-polysaccharide systems

Puyu Li, Ying Bai, Yuejiao Yang, Mengyuan Li
Article 102961

Article preview

Research article ○ Abstract only

Enhanced storage stability and *in-vitro* digestibility of powdered-solid lipid nanoparticles with high-algae oil-load

Jiahe Zhao, Yuanyuan Liu, Cai Shen, Oi-Ming Lai, ... Ling-Zhi Cheong
Article 102810

Article preview

Research article ○ Abstract only

Direct phoxim sensing based on fluorescent metal-organic framework of Nu-1000 induced FRET

Wenhui Hao, Gengli Huang, Guoyong Jiang, Sa-adu Abiola Dauda, Fuwei Pi
Article 102967

Article preview

Research article ○ Abstract only

Food Bioscience

Research article ○ Abstract only

Assessing the effectiveness of peanut diacylglycerol oil-ethylcellulose/monoglyceride-based oleogel in sponge cake as a margarine replacer

Xiaohan Chen, Siliang Ding, Ying Chen, Dongming Lan, ... Yonghua Wang
Article 102959

Article preview

Research article ○ Abstract only

Hyaluronic acid applied as a natural flavor enhancer and its mechanism exploration

Jinhua Hu, Zhuangzhuang Chen, Xueyao Huang, Zhuying Yan, ... Peng Zhou
Article 102969

Article preview

Research article ○ Abstract only

Phenotypic screening of novel probiotics with potential anti-neuroinflammation activity based on cell and zebrafish models

Bao-Lin He, Teng-Gen Hu, Hong Wu
Article 102949

Article preview

Research article ○ Abstract only

Campomanesia guazumifolia infusion decreases human platelet aggregation by reducing cyclooxygenase 1 activity and its underlying mechanisms

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Fabrication of cell cultured meat by hydrogel with topographic microstructures

Yichun Chen, Anthony Pius Bassey, Haozhe Zhu, Guanghong Zhou
Article 102910

Article preview

Research article ○ Abstract only

Biomacromolecule based water-in-water Pickering emulsion: A fascinating artificial cell-like compartment for the encapsulation of *Lactobacillus plantarum*

Mengjiao Ruan, Yunxiao Xie, Chaoyi Zhou, Yan Li, ... Shilin Liu
Article 102916

Article preview

Research article ○ Abstract only

Protective effects of fermented blueberry juice with probiotics on alcohol-induced stomach mucosa injury in rats

Bin Li, Haikun Li, Baoge Song, Jinlong Tian, ... Chi Shu
Article 102974

Article preview

Research article ○ Abstract only

Gamma irradiation inhibited non-enzymatic browning of *Dongbei Suancai* during storage

Chen Wang, Shuang Zhang, Chenyu Fang, Yanqiu Han, ... Zhaojun Ban
Article 102976

Article preview

Food Bioscience

Article preview

Research article ○ Abstract only

The effect of fresh or frozen black truffle *Tuber aestivum* on ripening and sensory quality of semi hard cheese

Zorica Radulovic, Dusanka Paunovic, Jelena Miocinovic, Ana Satric, ... Nemanja Mirkovic
Article 102979

Article preview

Research article ○ Abstract only

Nutritional, metabolic and genetic profiling of 'Cerato' and 'Curniciello' bean landraces from Caserta, Southern Italy

Nicola Landi, Laura Alberico, Angela Clemente, Stefania Peddio, ... Antimo Di Maro
Article 102975

Article preview

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4D printing of edible insect snacks: Color changes with external pH stimulation

Zixuan Kang, Zun Wang, Wei Zhang, Zhongxiang Fang
Article 102971

View PDF Article preview

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Characterisation of *Diospyros kaki* (persimmon) vinegars produced with different microorganisms

Matteo Bordiga, Raffaele Guzzon, Marcello Manfredi, Elettra Barberis, ... Jean Daniel Coisson

Food Bioscience

expeller processed with different proteases *in silico* and *in vitro*

Jinghui Chen, Ling Zhu, Qiming Wu, Yiling Chen, ... Hui Zhang
Article 102781

Article preview

Research article ○ Abstract only

Genome and functional diversity of *Leuconostoc mesenteroides* from different habitats and geographic locations

Xin Su, Lixia Zhao, Qin Liu, Weicheng Li, ... Wenjun Liu
Article 102834

Article preview

Research article ○ Abstract only

Novel molecular mechanism of high ribonucleic acid yield in *Saccharomyces pastorianus* revealed by transcriptomics

Hao Chen, Xin Xu, Qi Li, Jinjing Wang, ... Chunfeng Liu
Article 102985

Article preview

Research article ○ Abstract only

Development of environmentally friendly composite packaging films from safflower (*Carthamus tinctorius* L.) plant wastes

Arzu Yalçın Melikoğlu, İdil Tekin, Nergiz Hayatioğlu, Seda Ersus
Article 102991

Article preview

Food Bioscience

Article preview

Research article ○ Abstract only

Impact of biodynamic viticulture on the occurrence of fungi and mycotoxins, antioxidant activity, volatile and phenolic profile of Chardonnay grapes

Athos Tópor, Flávio Fonseca Veras, Rafaela Diogo Silveira, Fernanda Cortez Lopes, ... Juliane Elisa Welke

Article 102978

Article preview

Research article ○ Abstract only

Effects of cellulose-degrading fungus *Penicillium griseofulvum* on the structure characteristics and adsorption properties of soluble dietary fiber from *Citrus aurantium* L

Jie Tang, Chaoran Yang, Xiaoting Qin, Jingyu Si, ... Qiang Yu

Article 102999

Article preview

Research article ○ Abstract only

Two-component system *virS/virR* regulated biofilm formation of *Listeria monocytogenes* 10403S

Qian Guo, Yu Zhang, Xiaowei Fang, Yuying Yang, ... Chun Fang

Article 102973

Article preview

Research article ○ Abstract only

Food Bioscience

Research article ○ Abstract only

Evaluation of quality attributes and *in vitro* characteristics of synbiotic legume-based beverage during storage

Smriti Chaturvedi, Snehasis Chakraborty
Article 103000

Article preview

Research article ○ Abstract only

Preparation of natural complex waxy structure for the evaluation of preservation performance of blueberry

Xuedong Gu, Juan Li, Lin Yang, Lei Liu, Lan Xiao
Article 102990

Article preview

Research article ○ Abstract only

In vitro studies on the inhibition of microbial pathogens by PPDHMP synthesized by *Bacillus* sp.; an endophyte of *Citrus limon* (Kaji nemu)

Tinamoni Buragohain, Parry Dey, W. Jabez Osborne
Article 103003

Article preview

Research article ○ Abstract only

Effect of pH treatment on physical stability and antioxidant activity of buckwheat protein/soybean polysaccharide nanocomplex embedded pterostilbene

Dongze Li, Ling Zhu, Qiming Wu, Yiling Chen, ... Hui Zhang

Food Bioscience

Hua Huang, Weidi He
Article 102998

Article preview

Research article ○ Abstract only

Development of composite nanoemulsion gels as carriers for co-delivery of wheat germ oil and probiotics and their incorporation in yoghurt

Asmaa G. Abu-El Khair, Tarek N. Soliman, Ayat F. Hashim
Article 103001

Article preview

Research article ○ Abstract only

Spatial and temporal distribution of environmental microbiota in Chinese rice wine (Huangjiu) natural fermentation wineries

Qi Peng, Xueping Chen, Huajun Zheng, Kai Meng, ... Hefeng Yu
Article 102929

Article preview

Research article ○ Abstract only

Feeding effect of yellow wine lees fermented with *Candida utilis* and *Bacillus subtilis* in the cow diet on milk composition

Kaiyong Yao, Qile Xia, Yan Cao, Bindan Chen, ... Chenxing Liu
Article 103005

Article preview

Food Bioscience

Research article ○ Abstract only

Microbiological diversity of fermented food Bhaati Jaanr and its antioxidant and anti-inflammatory properties: Effect against colon cancer

Sandeep Jaiswal, Tarun Pant, Mangesh Suryavanshi, Usha Antony
Article 102822

Article preview

Research article ○ Abstract only

Isolation, identification, and structure-activity relationship of novel ACE inhibitory peptides from earthworm protein *in vitro* gastrointestinal digestion product

Zhengli Lin, Hui Wu, Mengmeng Zhang
Article 103010

Article preview

Research article *Open access*

A novel microbiological approach to impact the aromatic composition of sour loquat beer

Nicola Francesca, Antonino Pirrone, Ignazio Gugino, Rosario Prestianni, ... Raimondo Gaglio
Article 103011

View PDF Article preview

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Improved curcumin bioaccessibility in Pickering emulsion fabricated by rice glutelin fibrils

Food Bioscience

for improved shelf-life of bananas

Sandhya Alice Varghese, Danaya Phothisarattana, Atcharawan Srisa, Yeyen Laorenza, ... Nathdanai Harnkarnsujarit

Article 102993

Article preview

Research article ○ Abstract only

Total ginsenosides promotes intestinal epithelial proliferation via affecting polyamine-mediated HuR on post-transcriptional control

Anrong Wang, Xinyi Liang, Wanxia Chen, Yiting Liu, ... Ruliu Li

Article 102970

Article preview

Research article ○ Abstract only

Effects of citrus pomace on mechanical, sensory, phenolic, antioxidant, and gastrointestinal index properties of corn extrudates

Muhammad Asif, Muhammad Kashif Iqbal Khan, Muhammad Issa Khan, Abid Aslam Maan, ... Jozef L. Kokini

Article 103012

Article preview

Research article ○ Abstract only

Changes in the physicochemical properties of grouper (*Epinephelus coioides*) fillets stored under vacuum packaging at chilly temperature contributing with the spoilage bacteria

Zhijun Yang, Yuanming Chu, Chenchen Zhang, Jun Yan, Jing Xie

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temperature on bacterial community and related metabolism in fermented skates

Hye In Ko, Jong-Bang Eun
Article 103015

Article preview

Research article ○ Abstract only

Determination of ATP-related compounds by HPLC to study the effect of cell-free supernatants of *Lactiplantibacillus plantarum* on the shelf life of sliced dry-cured ham

Jing Tao, Bilian Yu, Franks Kamgang Nzekoue, Xiao Zhou, ... Yanhong Bai
Article 102984

Article preview

Research article ○ Abstract only

The effects of selenium on the growth and bone development in the weaned rats

Hongan Li, Linlin Jia, Zeyuan Deng, Xiaomao Sun, ... Hongyan Li
Article 103018

Article preview

Research article ○ Abstract only

C-Glycosidic flavone-rich *Passiflora incarnata* L. leaf extracts decrease body weight and fatty liver in obese mice

So-Hyun Park, Hang Yeon Jeong, Pyeong Geun Choi, Min Jung Kim, ... Chang Hwa Jung
Article 103028

Article preview

Food Bioscience

Article preview

Research article ○ Abstract only

Fabrication of polyphenol-pumpkin seed protein isolate (PSPI) covalent conjugate microparticles to protect free radical scavenging activity of polyphenol

Chen Yang, Jieqiong Liu, Yaqian Han, Bingyi Wang, ... Jianming Wang

Article 102982

Article preview

Research article ○ Abstract only

Preparation and physicochemical characterization of debranched rice starch nanoparticles from mono- and dual-modification by hydrothermal treatments

Yen-Chun Koh, Hung-Ju Liao

Article 103004

Article preview

Research article ○ Abstract only

Physicochemical changes and comparative proteomics analysis of hairtail (*Trichiurus lepturus*) fish muscles during frozen storage

Bowen Yan, Wenlu Bai, Yuan Tao, Weijian Ye, ... Daming Fan

Article 103021

Article preview

Research article ○ Abstract only

The facilitation between *Staphylococcus carnosus* M43 and *Zygosaccharomyces rouxii* Y-8, and as starter on the quality of broad bean paste

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containing limonene and α-terpineol stabilized with Tween®20: Formation and physicochemical stability along with time

Lorena de Oliveira Felipe, Juliano Lemos Bicas, Teetach Changwatchai, Mitsutoshi Nakajima, Marcos A. Neves

Article 102989

Article preview

Research article ○ Abstract only

Inactivation action of ultrasound-assisted cinnamaldehyde on planktonic and biofilm methicillin-resistant *Staphylococcus aureus* and its application in beef system

Zhenyang Hu, Jing Zhang, Yingying Sun, Jiang Xu, ... Yunfei Xie

Article 103031

Article preview

Research article ○ Abstract only

Effect of silicon dioxide nanoparticle on microstructure, mechanical and barrier properties of biodegradable PBAT/PBS food packaging

Harikrishnan Pulikkalparambil, Danaya Phothisarattana, Khwanchat Promhuad, Nathdanai Harnkarnsujarit

Article 103023

Article preview

Research article ○ Abstract only

Protective effect on pancreatic β cells of α-glucosidase inhibitors screened from *Rehmannia glutinosa*

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intended as starter cultures

Vesna Milanović, Federica Cardinali, Ana Boban, Jasenka Gajdoš Kljusurić, ... Irena Budić-Leto
Article 103033

Article preview

Research article ○ Abstract only

Engineering *Escherichia coli* for the efficient biosynthesis of 6′-sialyllactose

Chenchen Li, Mengli Li, Miaomiao Hu, Wei Gao, ... Tao Zhang
Article 103040

Article preview

Research article ○ Abstract only

Changes of rheological behavior, thermal and microstructural properties of myofibrillar protein-κ-carrageenan mixed sol as mediated by NaCl concentration

Chuanai Cao, Yining Xu, Baohua Kong, Xiufang Xia, ... Qian Liu
Article 103035

Article preview

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Article 103027

View PDF Article preview

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Article preview

Research article ○ Abstract only

The distinctive hepatoprotective activity of turmeric kombucha (*Curcuma longa*) induced by diethylnitrosamine in Balb/C mice

Elok Zubaidah, Ike Susanti, Hidayat Sujuti, Erryana Martati, ... Ihab Tewfik
Article 103043

Article preview

Research article ○ Abstract only

Rapid and sensitive detection of cyhalofop-butyl in foods using a gold nanoparticle-based lateral-flow strip

Huihui Wu, Xinxin Xu, Aihong Wu, Chuanlai Xu, ... Hua Kuang
Article 102986

Article preview

Research article ○ Abstract only

Induction of mitophagy by green tea extracts and tea polyphenols: A potential anti-aging mechanism of tea

Sarah Auguste, Bing Yan, Maolin Guo
Article 102983

Article preview

Research article ○ Abstract only

Food Bioscience

Research article ○ Abstract only

Easy-process nanoemulsions: Obtaining thymol nanodroplets with high shear speed systems

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Article 103048

Article preview

Research article ○ Abstract only

Antimicrobial activity and stability of *Satureja khuzestanica* essential oil pickering emulsions stabilized by starch nanocrystals and bacterial cellulose nanofibers

Mahdiyeh Amrani, Sunoor Pourshamohammad, Mahnaz Tabibiazar, Hamed Hamishehkar, Maryam Mahmoudzadeh

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Article preview

Research article ○ Abstract only

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The distinctive hepatoprotective activity of turmeric kombucha (*Curcuma longa*) induced by diethylnitrosamine in Balb/C mice

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Diethylnitrosamine

ABSTRACT

This study aims to investigate the potential hepatoprotective activity of turmeric kombucha before and after fermentation and to compare such distinctive activity in turmeric kombucha versus turmeric essence beverage (turmeric beverage without fermentation). Liquid chromatography-mass spectrometer (LC-MC) analyses revealed the presence of bioactive compounds in turmeric kombucha and turmeric essence beverages. *In vivo* tests appraised the levels of alanine transaminase (ALT), aspartate transaminase (AST), malondialdehyde (MDA) in Balb/C mice and the histology of their livers was determined. Upon successful fermentation process new compounds such as: tetrahydrocurcumin, ferulic acid, glucuronidated curcumin, cyclofenil, acetic acid, glucuronic acid, and D-saccharic acid-1,4-lactone were produced in turmeric kombucha, which were not found in non-turmeric kombucha. The positive effect of fermentation has boosted the hepatoprotective activity of turmeric kombucha through the release of compounds and the production of new bioactive compounds. Therefore, fermented turmeric kombucha had a greater effect on the hepatoprotective activity compared to turmeric essence beverage in Balb/C mice.

1. Introduction

The liver is the main organ that plays a part in the metabolism of drugs and toxic chemicals. Excessive exposure to toxins can cause hepatotoxicity (Maran et al., 2022). Several factors that contribute to liver toxicity include genetic, carcinogenic, and interactions with drugs and alcohol (Malaguarnera et al., 2012). Exposure to chemicals such as diethylnitrosamine (DEN) can induce liver damage and cause oxidative stress, inflammation, and deoxyribonucleic acid (DNA) destruction (Al-Rejaie et al., 2009). Liver damage is triggered when enzymes in the liver undergo lysis and are released into the blood. Compounds that can maintain and repair liver damage are called hepatoprotectives (Maran et al., 2022).

Turmeric is a medicinal plant with functional biological properties and benefits for human health. The bioactive compounds contained in turmeric are curcuminoids, essential oils, tannins, and minerals. It was reported that 2%–5% of turmeric essential oils consisted of phenylpropane turmerone derivatives (aryl-turmerone, alpha turmerone, and beta turmerone) (Goenka et al., 2021). Curcumin has been known to have antioxidant activity, as a radical scavenger, and as a catalyst for the formation of hydroxyl radicals (Bimonte et al., 2013). However, the bioavailability of active compound in curcumin is relatively low due to binding to other compounds.

The fermentation process is one of the food processing methods in which large substrates are broken down into simpler ones assisted by the action of microorganisms. Kombucha is a traditional drink from the fermentation process of sweet tea with a mixed culture of bacteria and yeast. The mixed culture is commonly known as SCOBY (symbiotic culture of bacteria and yeast) which produces a floating biofilm known as microbial cellulose layer or 'nata' (Zailani & Adnan, 2022). The substrate often used is steeped tea, so 'nata' is also known as "tea mushroom" or tea fungus" (Battikh et al., 2012). Zubaidah et al. (2021) has explored the chemical, microbiological, and antibacterial characteristics of turmeric kombucha, concluding that turmeric can be

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Table 1
List of formulations to test the hepatoprotective property of turmeric kombucha and turmeric essence beverages.

Treatment Group	Description
Control negative (P0)	Normal mice by feeding healthy mice/mice
Control positive (P1)	Mice Normal diet + DEN
P2	Mice non-DEN + Turmeric essence beverage dose 0.3 mL/20 g BW
P3	Mice DEN + Turmeric essence beverage dose 0.1 mL/20 g BW
P4	Mice DEN + Turmeric essence beverage dose 0.3 mL/20 g BW
P5	Mice DEN + Turmeric essence beverage dose 0.5 mL/20 g BW
P6	Mice non-DEN + Turmeric kombucha dose 0.3 mL/20 g BW
P7	Mice DEN + Turmeric kombucha dose 0.1 mL/20 g BW
P8	Mice DEN + Turmeric kombucha dose 0.3 mL/20 g BW
P9	Mice DEN + Turmeric kombucha dose 0.5 mL/20 g BW

processed as a kombucha with notable microbiological and antibacterial activity. There have been no research on turmeric kombucha as a hepatoprotective by the time this article was written. This study was conducted to determine the potential hepatoprotective property of turmeric kombucha.

2. Materials and methods

2.1. Materials

Turmeric (*Curcuma longa*) was obtained from a local traditional market in Malang, East Java, Indonesia. Commercial kombucha starter (SCOBY), sugar, and chemicals were obtained from local distributors. SCOBY consists of acetic acid bacteria (AAB) Acetobacteraceae and osmophilic yeast (Filippis et al., 2018). DEN was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ketamine HCl injection (Bernofarm; anesthesia) was obtained from Bioscience Institute Universitas Brawijaya (Malang, East Java, Indonesia). Thirty male Balb/c mice as the experimental animals (6 wks old, 20–30 g). Water and feed were given ad libitum during 1-week period of acclimatization.

2.2. Kombucha turmeric and turmeric essence beverage solution preparation and analysis

Kombucha preparation and analysis was done according to previous research by Zubaidah et al., 2021. Turmeric was peeled and washed, sliced to ±1 cm thick, dried in a dry cabinet at 70 °C for 12 h, and grinded using a blender (Philips, Amsterdam, Netherlands). Turmeric powder was brewed in hot water with a ratio of 1:10 (5 g of powder in 500 mL of water) for 5 min, 10% of sugar was added, and after cooling, 10% (v/v) of SCOBY starter was added. The mouth of the jar was covered with a cloth and tied. The jar was placed in a room that was not exposed to direct sunlight and at room temperature (30 °C) to ferment for 12 d. Non-fermented turmeric essence beverage was prepared with a concentration of 1.2% (6 g of powder in 500 mL of water) and was run through the same procedures as turmeric kombucha, without the addition of a kombucha starter.

2.3. Identification of the components of turmeric kombucha bioactive compound

LC-MS analysis was carried out using a high-performance liquid chromatography-mass spectrometer (LC-20 A, Shimadzu Corporation, Kyoto, Japan) equipped with a Waters 2695 preconditioner pump (Waters Corporation, MA, USA). The MS calibration used was Kromtekindo PRO\ACQUDB/Mass. MS scan was carried out with an initial mass of 50.0/s and final mass of 1200.0, scan time was 5.00, interscan time was 0.10 s, start time was 0.0 min, and end time was 50 min. The storage volume used was 50 L, flow ramp was 0.10, flow was 0.20 mL/min, stop time was 35 min, column temperature was 40 °C, column temperature limit was 10 °C, minimum pressure was 0.0 Bar, maximum pressure was 300 Bar, pre-column volume was 0 L, column type 2, with a size of 1 mm × 100 mm. Solvent 'A' was 10% methanol, solvent 'B' was 90% water, solvent 'C' was 0 formic acid, and solvent 'D' was 0 acetonitrile. Draw speed; needle depth was 1/mm, sample temperature was 20 °C, and sample limit temperature was 20 °C.

2.4. Animal experiment and analysis

Testing of hepatoprotective activity was carried out using the *in vivo* method with 30 male Balb/c mice (6 wks old, 20–30 g). The research was approved by Brawijaya University Research Ethics Committee (Ethical Clearance No. 104-KEP-UB-2021). Grouping of the mice was carried out according to the experimental design with 10 treatments (Table 1). Turmeric kombucha and turmeric essence beverage were given daily for 3 wks, with the induction carried out only after then. DEN with a dose of 100 mg/kg was given through an intraperitoneal injection process at the rate of 1 injection/wk for 2 wks. During the DEN injection treatment, turmeric kombucha and turmeric essence beverage were still being given, with an incubation period of 1 wk. Mice without DEN injections were treated according to the grouping. On the 49th day, surgery was performed after fasting for 24 h from the last day of treatment. An anesthesia process was used during the induction of 0.2 mL ketamine (50 mg/kg). During surgery, blood serum samples were taken from the heart and liver. Parameters observed were alanine transaminase (ALT) activity, aspartate transaminase (AST), malondialdehyde (MDA), and liver histology (Fig. 1).

2.4.1. ALT and AST enzyme (modification Devaraj et al., 2014)

The clotted blood samples were centrifuged at 3000 rpm (3461×*g* in a EBA 200, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at room temperature (30 °C) for 15 min to separate the cell nucleus from the blood serum. Blood serum was then taken and biochemically tested for the amount of AST and ALT enzymes.

2.4.2. MDA enzyme (modification Devaraj et al., 2014)

As much as 10% of the liver homogenate was mixed into 0.1 M Tris-HCl buffer pH 7.4 at 40 °C. The sample was homogenized (VELP Scientifica Srl, Usmate, Italy) with at 1000 rpm for 2 min. The homogenate was centrifuged at 1000 rpm at 40 °C for 10 min to separate the nucleus and cell solids. The supernatant was tested for the amount of MDA to see the level of liver oxidation.

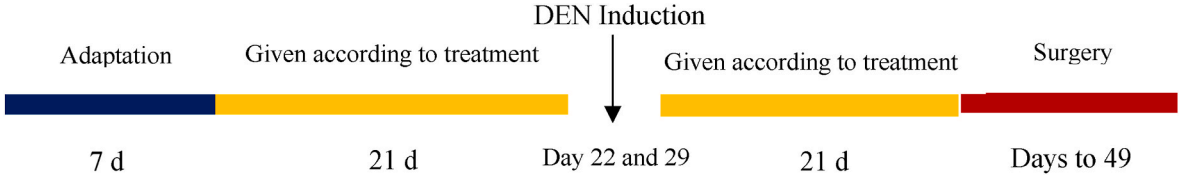

Fig. 1. Experimental animal treatment procedures, modified (Zheng et al., 2018).

Table 2
Turmeric kombucha and turmeric essence beverage characteristics (Zubaidah et al., 2021).

Parameter	Turmeric kombucha	Turmeric essence beverage
pH	3.8	7.4
Total titratable acid	1.24%	Not detected
Total phenolic content	137.28 mg GAE/mL	94.25 mg GAE/mL
IC_{50} antioxidant activity	76.16 ppm	106.59 ppm
Total microbial cells	2.70×10^7 CFU/mL	Not detected

2.4.3. Histopathological observation (Modification Jantararussamee et al., 2021)

Histopathological observations of mice were carried out by taking some liver samples from each group by dissection. The results of the dissection were dehydrated with 50%–100% ethanol and given paraffin with a thickness of 5 cm. The paraffin-treated sections were then stained with hematoxylin and eosin (HE) to color the cell parts and observed under a light microscope (Olympus Corporation, Tokyo, Japan) with magnifications of 40×, 100x, and 400x.

2.5. Statistical analysis

The statistical analysis was carried out by comparison of all the groups. Analysis of variance (ANOVA) was used and followed by Fisher's exact test at $p < 0.05$. All statistical analyses were carried out using Minitab software (17.0 version, Minitab, LLC).

3. Result and discussion

3.1. Turmeric kombucha and turmeric essence beverage characteristics

Turmeric kombucha and turmeric essence beverage were used as treatments to mice. Physicochemical and microbiological analysis were conducted prior to the *in vivo* procedures. The higher the concentration of turmeric, then the lower the microbe total and acid total obtained. The higher the total phenol concentration of turmeric, then the higher the antioxidant activity. The best treatment results were obtained with 1% of turmeric kombucha concentration (Zubaidah et al., 2021).

The characteristics of turmeric kombucha and turmeric essence beverage found by Zubaidah et al. (2021) are shown on Table 2. Turmeric kombucha showed higher total phenolic content and antioxidant activity elevation compared to turmeric essence. Turmeric kombucha also had a higher total of titratable acid, lower pH, and an increase of the AAB total. This was due to the addition of kombucha starter. Kombucha starter mainly comprised of bacteria and yeast, which led to them influencing the microbial characteristics of turmeric and black tea kombucha. According to Zubaidah et al. (2021), black tea kombucha recorded 1.3×10^8 CFU/mL of total microbes on day-14, higher than the turmeric kombucha with 2.0×10^7 CFU/mL. Microbial activity results in the breakdown of turmeric bioactive compounds. Turmeric kombucha showed and increase of total titratable acid, decrease of pH, higher total phenolic content, and lower IC50 value which enabled better free radical degradation than turmeric essence beverage. This was due to the existence of organic acids produced by microorganisms during fermentation. This proved that kombucha fermentation increased total phenolic content and antioxidant activity of turmeric.

3.2. Components of bioactive compounds in turmeric kombucha and turmeric essence beverage

Identification of chemical compounds contained in turmeric kombucha and turmeric essence beverage using LC-MS (Table 3) revealed that they contained phenolic compounds, curcumin,

Table 3
Identified chemical compounds in turmeric kombucha and turmeric essence beverage using LC-MS.

Component	MW (g/mol)	Retention time	Turmeric kombucha	Turmeric essence beverage	Benefit
Tetrahydrocurcumin(1,7-bis(4-hydroxy-3-methoxyphenyl)	372.4	2.563	✓	N/D	Anti-inflammation, Anti-cancer and anti-bacterial
Ferulic acid (4-Hydroxy-3-methoxy cinnamic acid)	194.18	1.277	✓	✓	Antioxidant, anti-inflammation, apoptosis, and cardioprotective (Bezerra et al., 2017; Mattila & Kumpulainen, 2002)
Acetylsalicylic acid (2-acetyl benzoic acid)	180.31	2.563	✓	N/D	Analgesic (Ashok & Upadhyaya, 2013)
Methoxyphenol	124.14	1.772	✓	✓	Anti-carcinogenic and antioxidant (Sun et al., 2015).
Eugenol	127.39	2.735	✓	✓	Anti-bacterial, antioxidant, and analgesic
Bisdemethoxycurcumin	308.3	6.127	✓	✓	Anti-inflammation and lower expression NF-Kβ (Ramezani et al., 2018).
Demethoxycurcumin	308.3	17.871	✓	✓	Anti-inflammation and anti-neoplastic (Hoehle et al., 2006)
Curcumin glucuronide	544.5	3.182	✓	N/D	Immunosuppressive, antioxidant, anti-neoplastic, cytotoxic, anti-cancer, and anti-tumor (Ming et al., 2020)
Cyclofenil	364.4	1.565	✓	✓	Ovulation induction, infertility anti-virus (Sayed & El-Kordy, 2014), and inhibition of MCF cell proliferation in breast cancer (Mughal, 2019)
Acetic acid	60.05	14.183	✓	N/D	Antioxidant, anti-microbial, toxicity (Jakubczyk et al., 2020; Jayabalan et al., 2014; Tahri et al., 2016), and anti-inflammation (Bimonte et al., 2015)
D-saccharic acid-1,4-lactone (DSL)	192.12	18.721	✓	N/D	Antioxidant, anti-inflammation, and heart damage (Ireson et al., 2002), anti-diabetes, cytotoxic, hepatoxic, and hepatoprotective (Wang et al., 2014)
Glucuronic acid	397.17	17.234	✓	N/D	Antioxidant, hepatoprotective, and anti-inflammation (Martínez-Leal et al., 2020)
Carboxylic acid	477.4	3.182	✓	N/D	Prevent liver damage (Rocha-Ramírez et al., 2017) and immunomodulator (Bauer-Petrovska & Petrushevska-Tozi, 2000)
Chloroacetyl-DL-phenylalanine	241.67	1.600	N/D	✓	Bacterial xenobiotic metabolites (Aggarwal et al., 2013; Zhao et al., 2018)
Phenyl	364.4	2.356	✓	✓	Anti-microbial (Pereira et al., 2009), cardiovascular, and anti-cancer (Sim et al., 2019)
Pyrazine	979.0	2.735	✓	✓	Analgesic, anti-inflammation, antioxidant, anti-cancer, and anti-microbial
Quinazoline	1033.2	2.941	✓	✓	Anti-inflammation, anti-cancer, anti-inflammation, and anti-microbial (Bimonte et al., 2015; Hatcher et al., 2008)

demethoxycurcumin, bisdemethoxycurcumin, several compounds derived from curcumin, and organic acids. Chemical compounds detected in the phenolic group were nitrophenol, phenol, and quinoline. Phenol compounds are secondary metabolites of plant metabolism that attach to metal ions which can fight free radicals and increase antimicrobial activity (Cavalcanti et al., 2012). Phenolic compounds have functional abilities such as cardiovascular inhibition, anticancer, and chronic disease prevention (Soto-Quintero et al., 2019).

Chemical compounds detected in the curcuminoids group were curcumin, bisdemethoxycurcumin, and demethoxycurcumin. The derivative components of the curcumin compounds consisted of ferulic acid, acetylsalicylic acid, guaiacol, eugenol, licopyranocoumarin, and phenyl. Ferulic acid is an acid consisting of *trans*-cinnamic acid which has methoxy and substitution of hydroxyl on the phenyl ring. Ferulic acid has bioactivities as an antioxidant, anti-inflammatory, inhibitor of apoptosis, and cardioprotective prevention. Ferulic acid is a chemical compound that is commonly found in plants, belonging to a group of secondary metabolites that bind to esters, glycosides, components of lignin, and tannins (Mattila & Kumpulainen, 2002). Based on the chemical structure, it can be divided into benzoic acid derivatives by substitution of hydroxyl and methoxy groups and phenolic acids. Ferulic acids such as caffeic, *p*-coumaric, sinapic acid, and vanillin acid are cinnamic acid derivatives (Bezerra et al., 2017).

Acetylsalicylic acid is a chemical compound that functions as an analgesic drug or pain reliever. Acetylsalicylic acid can bind and acetylate serine residues in cyclooxygenase (COX), resulting in decreased prostaglandin synthesis, platelet aggregation, and inflammation. Acetylsalicylic acid has analgesic, antipyretic, and anticoagulant properties. Research conducted by Purpura et al. (2018) reported that curcumin significantly reduced pain in the legs of experimental rats. Prostaglandins are known to reduce pain receptors through the COX and lipoxygenase (LOX) pathways. Conditions like this can suppress COX-2 and 5-LOX which are enzymes that cause pain. Curcumin showed a significant antipyretic effect with decreasing rectal temperature. The decrease in temperature can be caused by the presence of acetylsalicylic acid which can inhibit prostaglandins (Hatcher et al., 2008).

3.3. Hepatoprotective activity

3.3.1. Alanine transaminase

ALT is an enzyme present in the cytosol of liver parenchyma cells and thus is a more specific parameter to analyze liver damage. If there was damage to the liver, the cell would undergo lysis and ALT enzymes would come out of the cells and be carried in the blood circulation. This indicated that the ALT enzyme was detected in the analysis of blood serum (Jilkova et al., 2019). Treatment with DEN can affect the activity of ALT in the blood serum of mice. The blood serum of the positive control group (normal diet + DEN) showed higher values than the negative control group (normal diet). ALT activity decreased after the administration of turmeric essence beverage and turmeric kombucha of various concentrations. The administration of turmeric kombucha with a concentration of 0.5 mL/20 g BW showed the largest decrease among the DEN-induced groups, which was 20.851 U/L (Table 3). The normal diet group with DEN induction had the highest ALT value, where there was an increase in the ALT value to 41.147 U/L. DEN damages liver cells, causing lysis and triggering liver cell death. DEN can be metabolized in dysenterylabular hepatocytes followed by oxidative DNA damage reactions (Jilkova et al., 2019). After DEN induction and administration of turmeric essence beverage at a dose of 0.5 mL/20 g BW, the ALT value was reduced to a value of 30.451 U/L. The treatment with turmeric kombucha had lower ALT activity than the turmeric essence beverage treatment. Turmeric kombucha with various concentrations had a higher ability to reduce ALT activity in mouse blood serum. The difference in dosages of turmeric kombucha and turmeric essence beverage showed a significant difference in decreasing ALT activity ($p < 0.05$). The reduction of the ALT enzyme in blood serum was because the ability of curcumin to fight free radicals and induce arachidonic acid metabolism through the COX and LOX pathways (Ak & Gülçin, 2008). The results of research conducted by Bimonte et al. (2013) showed that curcumin can prevent liver toxicity and reduce ALT levels caused by methotrexate induction.

Table 4
Hepatoprotective activity of turmeric kombucha and turmeric essence beverage.

Treatment group	ALT (U/L)	AST (U/L)	MDA (nanomole/mL)
Normal diet	$25.770^{d} \pm 1.0$	$20.199^{e} \pm 0,8$	$4.319^{ab} \pm 0,7$
Normal diet + DEN	$41.147^{a} \pm 0.4$	$40.739^{a} \pm 1,1$	$5.292^{a} \pm 0,8$
Normal diet + turmeric essence beverage	$20.445^{e} \pm 1.2$	$21.347^{d} \pm 1,3$	$3.812^{b} \pm 0,3$
DEN + dose 0.1 mL/20 g BW	$32.510^{b} \pm 0.4$	$31.958^{b} \pm 3,7$	$4.322^{ab} \pm 0,1$
DEN + dose 0.3 mL/20 g BW	$31.355^{bc} \pm 0.4$	$31.107^{bc} \pm 0,9$	$4.076^{ab} \pm 0,6$
DEN + dose 0.5 mL/20 g BW	$30.451^{c} \pm 1.1$	$30.077^{c} \pm 3,3$	$4.032^{ab} \pm 0,7$
Normal diet + turmeric kombucha	$20.884^{e} \pm 0.8$	$20.454^{de} \pm 0,5$	$3.858^{ab} \pm 0,3$
DEN + dose 0.1 mL/20 g BW	$21.962^{e} \pm 0.1$	$21.738^{d} \pm 0,5$	$4.079^{ab} \pm 0,2$
DEN + dose 0.3 mL/20 g BW	$21.040^{e} \pm 0.8$	$21.341^{de} \pm 1,2$	$3.807^{b} \pm 0,4$
DEN + dose 0.5 mL/20 g BW	$20.851^{e} \pm 0.8$	$20.110^{e} \pm 0,3$	$3.761^{b} \pm 0,1$

Note: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; MDA: Malonaldehyde; different notations show a real difference ($\alpha = 0.05$); data obtained from the average of 3 replications ± SD.

3.3.2. Aspartate transaminase

AST is an enzyme found in the cytosol and mitochondria of liver cells, cardiac muscle cells, striated muscles, and kidneys (Jilkova et al., 2019). This indicates that high AST values are not only caused by damage to liver cells but can also occur due to the presence of AST in other cells. If liver cells are lysed, the enzyme will be carried out in the blood circulation so that it can be detected in blood serum analysis (Castro et al., 2015). Treatment with DEN can affect AST activity in the blood serum of mice. The blood serum of the positive control group (normal diet + DEN) shows a higher value than the negative control group (normal diet). AST activity decreased after the administration of turmeric essence beverage and turmeric kombucha of various concentrations. The administration of turmeric kombucha resulted in a higher reduction activity than turmeric essence beverage (Table 4). The increase in the value of AST activity in the positive control group was 40.739 U/L. After the administration of turmeric kombucha and turmeric essence beverage of various concentrations, there was a decrease in AST activity in the blood serum of mice. Notwithstanding, the decrease in AST value in turmeric essence beverage was within normal limits with the lowest value at a dose of 0.5 mL/20 g BW which was 30.077 U/L, while in turmeric kombucha the lowest value was 20.110 U/L within normal limits. This indicates that the higher the dose given, the lower the value of AST activity in the blood serum of mice.

The normal AST value for mice is 8–40 U/L. Curcumin is a compound found in turmeric with functions as a hepatoprotective, such as antioxidant activity, anti-inflammatory, antimicrobial, and anticarcinogenic (Karimian et al., 2017). Curcumin and curcumin derivatives such as 5-benzo [1,3] dioxol-5-il-1-phenyl-penta-2,4-dien-1 have the ability as hepatoprotectives to protect and repair damaged liver cells. According to research conducted by Kapelle et al. (2020), the increase in turmeric hepatoprotective activity was due to microbial activity during the kombucha fermentation process. According to Acosta-Cota et al. (2019), yeast and *Gluconacetobacter* sp. Formed glucuronic acid during the fermentation of kombucha. Identification with LC-MS of turmeric

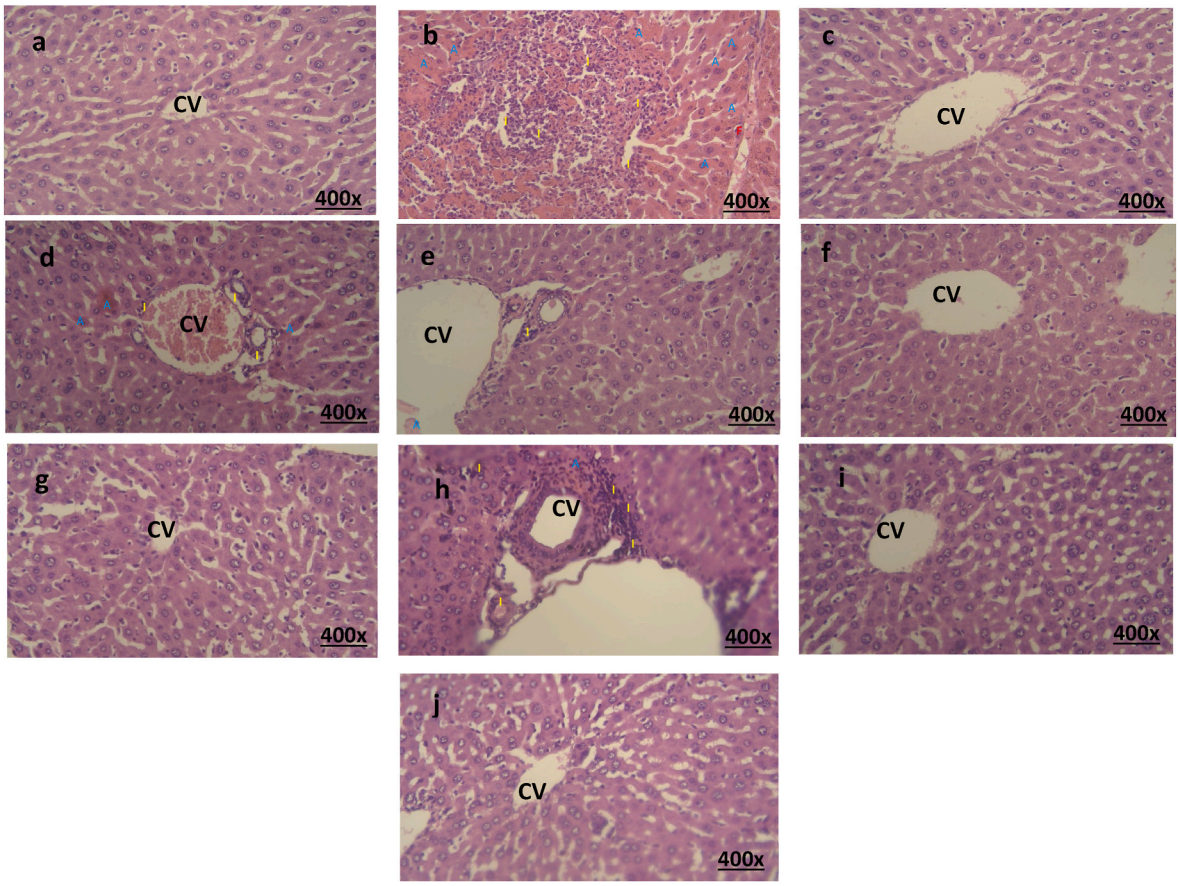

Fig. 2. Mice liver histology
(a) Normal diet; (b) Normal diet + DEN; (c) Normal diet + turmeric essence beverage; (d) DEN + 0.1 mL/g turmeric essence beverage; (e) DEN + 0.3 mL/g turmeric essence beverage; (f) DEN + 0.5 mL/g turmeric essence beverage; (g) Normal diet + turmeric kombucha; (h) DEN + 0.1 mL/g turmeric kombucha; (i) DEN + 0.3 mL/g turmeric kombucha; (j) DEN + 0.5 mL/g turmeric kombucha; magnification 400x
I: inflammation; A: apoptotic body; F: fibrosis; CV: central vein.

kombucha found compounds of organic acids which were glucuronic acid and 1,4-lactone D-saccharic acid (DSL). Glucuronic acid can bind to toxic metabolites or compounds that will be eliminated from the body so these compounds are more water soluble and their toxic activity is reduced. DSL in kombucha tea is a hepatoprotective detoxifier and can curatively maintain liver pathophysiology. In addition to glucuronic acid, it has the potential to clear hepatotoxins caused by toxins such as acetaminophen, carbon tetrachloride, hydrocarbon carcinogens, nitrosamines, and aromatic amines (Bhattacharya et al., 2011).

3.3.3. *Lipid peroxidation level*

MDA is a product of fat oxidation. The high the levels of MDA the high the levels of fat oxidation in the body. Lipid peroxidation has a role in the pathogenesis of tissue injury, especially in damage caused by several toxic substances (Dzoyem et al., 2014). Normal diet + DEN induction treatment showed the highest MDA levels. Turmeric kombucha and turmeric essence beverage decreased MDA levels. The lowest MDA levels were found in 0.5 mL/20 g BW turmeric kombucha induced mice (Table 4). This was due to turmeric kombucha containing more bioactive compounds, organic acids, and microorganisms compared to turmeric essence beverage. There were several bioactive compounds derived from curcuminoids that have the functional properties of preventing liver damage. In addition, several organic acid compounds in turmeric kombucha could prevent liver damage such as glucuronic acid and DSL, these compounds were not found in turmeric essence beverage. The higher the dose of kombucha, the lower the MDA levels in the mice's serum. This increase in antioxidant activity reduced lipid peroxidation and prevented the formation of MDA (Sobhani et al., 2020). Organic acids such as acetic acid and glucuronic acid have high antioxidant activity. Kombucha was able to reduce liver damage caused by oxidative stress (Gharib, 2009). Glucuronic acid is a bioactive compound in kombucha with high antioxidant activity as a detoxifier in the liver through the glucuronidation process. Glucuronidation is a xenobiotic conjugation process such as; acetylaminofluorene (carcinogenic), aniline, benzoic acid, and steroid compounds. The conjugation process with glucuronyl transferase enzyme is catalyzed by UDP-glucanosyltransferase (Alvarenga et al., 2018; Coton et al., 2017).

3.4. *Liver histology*

Liver histology was performed to determine the condition of the cells in the liver, observations were made using preparations from the liver. Liver damage is characterized by the occurrence of inflammatory cell damage, fibrosis, and the formation of acidophilic bodies/apoptotic bodies. The negative control group/normal diet (Fig. 2a) shows that the liver cells looked normal, where the condition of the cells stained with HE had purple cytoplasm, the cell nucleus was clear and had a dark purplish color, the boundaries between the cells were visible, and the central blood vessels were visible. Normal histology has a brownish-red color, shiny, sharp edges, a smooth texture, good cytoplasmic conditions, a prominent nucleus, and sinusoidal spaces. It also has liver lobules and a uniform pattern around polyhedral hepatocytes from the central vein to the periphery (Jantararussamee et al., 2021; Jeyadevi et al., 2019; Mondal et al., 2019). Normal diet groups fed with turmeric

Table 5
Total cell damage.

Treatment	Total of dead cells
Normal diet	$17^{e} \pm 0.8$
Normal diet + DEN	$49^{a} \pm 0.8$
Normal diet + Turmeric Essence Beverage	$16^{e} \pm 1.2$
DEN + dose 0.1 mL/20 g BW	$32^{b} \pm 1.6$
DEN + dose 0.3 mL/20 g BW	$30^{bc} \pm 0.8$
DEN + dose 0.5 mL/20 g BW	$28^{cd} \pm 0.5$
Normal diet + Turmeric kombucha	$15^{e} \pm 0.5$
DEN + dose 0.1 mL/20 g BW	$30^{b} \pm 0.9$
DEN + dose 0.3 mL/20 g BW	$27^{d} \pm 1.2$
DEN + dose 0.5 mL/20 g BW	$26^{d} \pm 0.5$

Note: Different notations show a real difference ($\alpha = 0.05$); data obtained from the average of three replications ±SD.

essence beverage and turmeric kombucha display similar liver histology to the negative control group (Fig. 2c and g). The positive control group (normal diet + DEN) shows the histology of a damaged liver due to the toxicity of DEN (Fig. 2b). Cells had a light pink color and some cells did not have a cell nucleus. The boundaries between liver cells were not clearly visible. Liver cells underwent degradation and inflammation occurred in some cells. Induction of DEN can cause hydropic degradation, mitosis, pseudo-nucleus, apoptosis, and liver necrosis (Santos et al., 2017). The treatment of turmeric kombucha and turmeric essence beverage showed changes in liver histology for the better. The 0.1 mL/20 g BW dose from turmeric essence beverage and kombucha improved cell boundaries and nucleus prominence (Fig. 2d and h), then doses of 0.3 mL/20 g BW and 0.5 mL/20 g BW produced almost normal liver histology (Fig. 2e, f, 2i, and 2j).

3.5. Total cell damage

Turmeric is a rhizome that contains curcumin as an anti-inflammatory bioactive. Administration of turmeric kombucha and turmeric essence beverage can reduce and prevent inflammation. Curcumin can inhibit proliferation and reduce inflammation. In addition, it can also reduce levels of MDA, glutathione, nitric oxide (NO), and tumor necrosis factor (TNF) and increase catalase, superoxide dismutase (SOD), and glutathione transferase (GST) activity in the liver (Tokaç et al., 2013). Based on the histology data, the results show liver cell damage due to DEN induction through several damaged and dead cells (Table 5).

4. Conclusion

This study shows that the fermentation process can produce other compounds in turmeric kombucha that are not detected in turmeric essence beverage. Fermentation affects the hepatoprotective activity of turmeric through the release of compounds and the production of new bioactive compounds. Therefore, fermented turmeric kombucha offers greater effect on the hepatoprotective activity compared to turmeric essence beverage in experimental animal.

Author statement

We hereby declare that all the authors of "The distinctive hepatoprotective activity of turmeric kombucha (*Curcuma longa*) induced by diethylnitrosamine in Balb/C mice" have approved the newly revised manuscript to be re-submitted to Food Bioscience. There are no conflicts of interests.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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